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STUDIES ON THE SCANNING ELECTRON MICROSCOPY (SEM) OF THE OOCYSTS OF VARIOUS *EIMERIA* SPECIES

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ABSTRACT

Preliminary studies on the morphology of the oocysts of several species of *Eimeria* of cattle, using electron microscopy have shown the surface structure of the oocysts of several species. The entire surface of *E. bovis* was covered with ridges and giving an overall rough appearance to the oocyst. The surface of *E. canadensis* appeared cloudy relatively smooth with small scattering papillae and the border of the wall appeared smooth. The oocyst of *E. alabamensis* contained large micropores of different sizes in its wall. At least 9 clear pores were found on the surface of oocyst. The surface of *E. ellipsoidalis* appeared smooth with no distinguishing features. While *E. brasiliensis* entire surface was covered with small, very fine papillae. The polar cap appears to be thicker at the top and thinner at its point of attachment to the wall. The oocyst of species of *E. auburnensis* appeared cloudy and the wall is covered with very tiny papillae. The papillae look to be embedded in the matrix of the wall. However, scattered papillae were also seen on the oocyst wall of *E. cylindrica.*

Keywords: Eimeria spp., oocysts, scanning electron microscopy

INTRODUCTION

A great deal of work regarding the morphological characteristics of the species of bovine coccidia has been carried out throughout the world (Lee and Armour, 1959; Soulsby, 1986; Anonymous, 1986; Parker, 1991). These species are found to be similar morphologically but can be differentiated by light microscopy and also by their surface and internal fine structures as seen by scanning and transmission electron microscopy.

Little is known concerning the fine structure of the surface of the oocyst of bovine *Eimeria* species.

Courtney *et al.* (1976) carried out an electron scanning study of the oocyst of *E. wyomingensis* and *E. bukidnonensis* to differentiate both species from each other. According to their investigation, they observed that the surface of the oocysts of *E. wyomingensis* were covered with fewer, larger and variable papillations compared with that of *E. bukidnonensis*, where oocysts were found to be covered with many smaller and more or less similar papillations. Furthermore, they described these papillations as giving the appearance of striations to the oocyst wall. In addition they also mentioned that the striations in the oocysts of *E. bukidnonensis* were more pronounced than those of *E. wyomingensis*. In contrast using light microscopy, Huizinga and Winger (1942) described the oocyst wall surface as speckled in the case of *E. wyomingensis* and in the case of *E. bukidnonensis*, because of tiny particles embedded in the matrix of the wall. Although the separation of individual species oocysts and their preparation for scanning electron microscopy is extremely difficult, this study was undertaken to determine the fine structure of the surface of the oocyst of as many of the bovine species encountered as possible.

MATERIALS AND METHODS

Separation and concentrating of oocysts from faecal samples

Fresh faecal samples were collected from Friesian calves and oocysts were isolated and purified by the McMaster flotation technique (Anonymous, 1986) and stored at 4°C. The small intestine of a freshly slaughtered mouse was removed, cleaned from internal debris and rinsed several times with saline solution. The small intestine was divided into pieces approximately 4cms in length. Before filling with oocysts the pieces were ligated from one end then filled with thousands of purified mixed oocysts of different species and ligated at the opposite end with nylon thread. The pieces of gut containing oocysts were processed as follows:

Fixation and processing of oocysts for scanning electron microscopy (SEM)

1. The pieces of intestine containing oocysts were fixed in 25% gluteraldehyde (1 part gluteraldehyde and 7 parts distilled water) for 2 hours.

2. The fixative was removed using a Pasteur pipette and the pieces of intestine were washed carefully in several changes of distilled water, each wash lasting 30 minutes.
3. The pieces of intestine were stored in 50% osmium solution (50% osmium and 50% distilled water) for 2 hours in a refrigerator at 4°C and then washed with several changes of distilled water.
4. The pieces of intestine were dehydrated in a graded series of ethanol (25, 50, and 75%) for 1 hour in each.
5. They were placed in 50% ethanol acetone solution (50% ethanol and 50% absolute acetone) for 1 hour.
6. This was followed by dehydration in 75% absolute acetone (25% ethanol and 75% absolute acetone) for 1 hour.
7. The last dehydration was carried out overnight in absolute acetone (100% acetone).
8. The intestinal pieces were dried using a critical point apparatus using CO_2.
9. The intestinal pieces were cut into small portions opened and stuck on an aluminium stub and examined using a Hitachi S-520 scanning electron microscope.

RESULTS AND DISCUSSION

Electron scanning microscopy (SEM) was carried out to identify ultrastructural differences in the wall of the oocysts of those species of bovine coccidia which were obtained in mixed form from the faeces of naturally infected cattle. It was often very difficult to recognise the species involved since mixed species were present. However, based on size and shape an attempt was made to identify the oocyst under investigation. The species which were identified on the basis of their fine structure and dimension are as follows:

Eimeria bovis

The oocyst (Plate 1-a) was covered with ridges of variable sizes. The ridges cover the entire surface of the oocyst giving an overall rough appearance. The ridges gave the edges of the wall a rough seriated appearance. A few very small pin point papilla-like structures were seen on the wall around the micropyle. The micropyle itself appeared as a rough sunken disc. Micropores were not evident in the wall of the oocyst.

E.canadensis

The oocyst (Plate 1-b) of this species appeared cloudy relatively smooth with a small scattering of papillae which were often difficult to see. The border of the wall of the oocyst appeared smooth. Micropores and ridges were not found.

E .alabamensis

During light microscopic examination, it was very difficult to separate *E. alabamensis* from *E. ellipsoidalis* and therefore both species were grouped together as a species complex. However, by scanning electron microscopy, *E. alabamensis* appeared completely different from *E .ellipsoidalis*. The oocyst of *E. alabamenisis* contained large micropores of different sizes in its wall (Plate 1-c). At least 9 clear micropores of different sizes were found on the surface of oocyst wall. These micropores varied from 10x0.5 to 30x10μm. Only a single oocyst of this species was encountered in the fine structure study. No micropyle was observed but a few papillae of variable size were evident.

E. ellipsoidalis

The surface of the oocyst (Plate 1-d) appeared smooth with no distinguishing features such as papillae, micropyle, ridges or micropores.

E. brasiliensis

The oocysts (Plate 2-a and b) of *E. brasiliensis* are typified by the obvious polar cap which is absent from other species of bovine *Eimeria*. The entire oocyst wall is covered with small, very fine papillae. The polar cap appears to be thicker at the top and thinner at its point of attachment to the wall. A very pronounced micropyle is evident in plate 2 (b) which measures 40x18μm in size. The polar cap seems to be attached by thin permeable membrane to the wall of the oocyst. It is thicker at the top and thinner at the point of attachment as described earlier for plate 2 (a). No micropores were observed in the wall of the oocyst.

E. auburnensis

The oocyst (Plate 2-c) appeared cloudy and the wall is covered with very tiny papillae. These papillae look to be embedded in the matrix of the wall. The border of the oocyst wall was found to be smooth and thin. No micropores were seen on the surface of the oocyst wall. A very thin ring like structure micropyle was observed in the wall of oocyst.

E. cylindrica

The oocyst (Plate 2-d) is cylindrical in shape and covered with scattered papillae. No micropyle or micropores were found in the wall. The depression seen in the wall of oocyst, does not seem to be a micropore, but looks more like an artefact caused by damage.

This is a preliminary study of the fine structure of the oocysts of bovine *Eimeria* species and represents the first attempt to study the bovine coccidia. This is not surprising since the separation of species obtained from natural infections is difficult and the problems of preparation and examination are

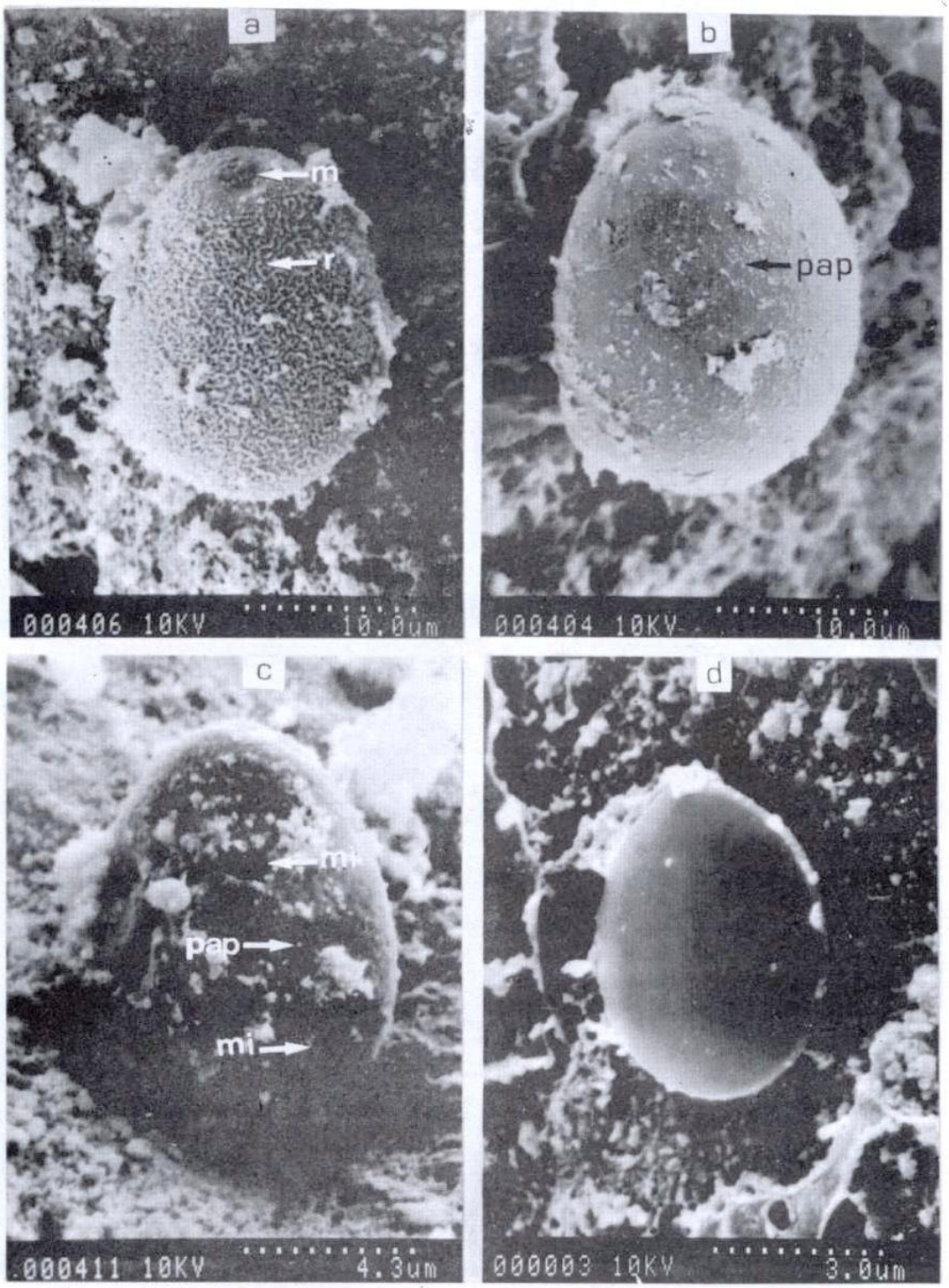

Plate 1: Scanning electron micrographs of the fine structure of the wall of unsporulated oocysts of Eimeria species a). *E. bovis*; b). *E canadensis*; c). *E. alabamensis*; d). *E. ellipsoidalis*; m = micropyle; r = ridges; mi = micropores

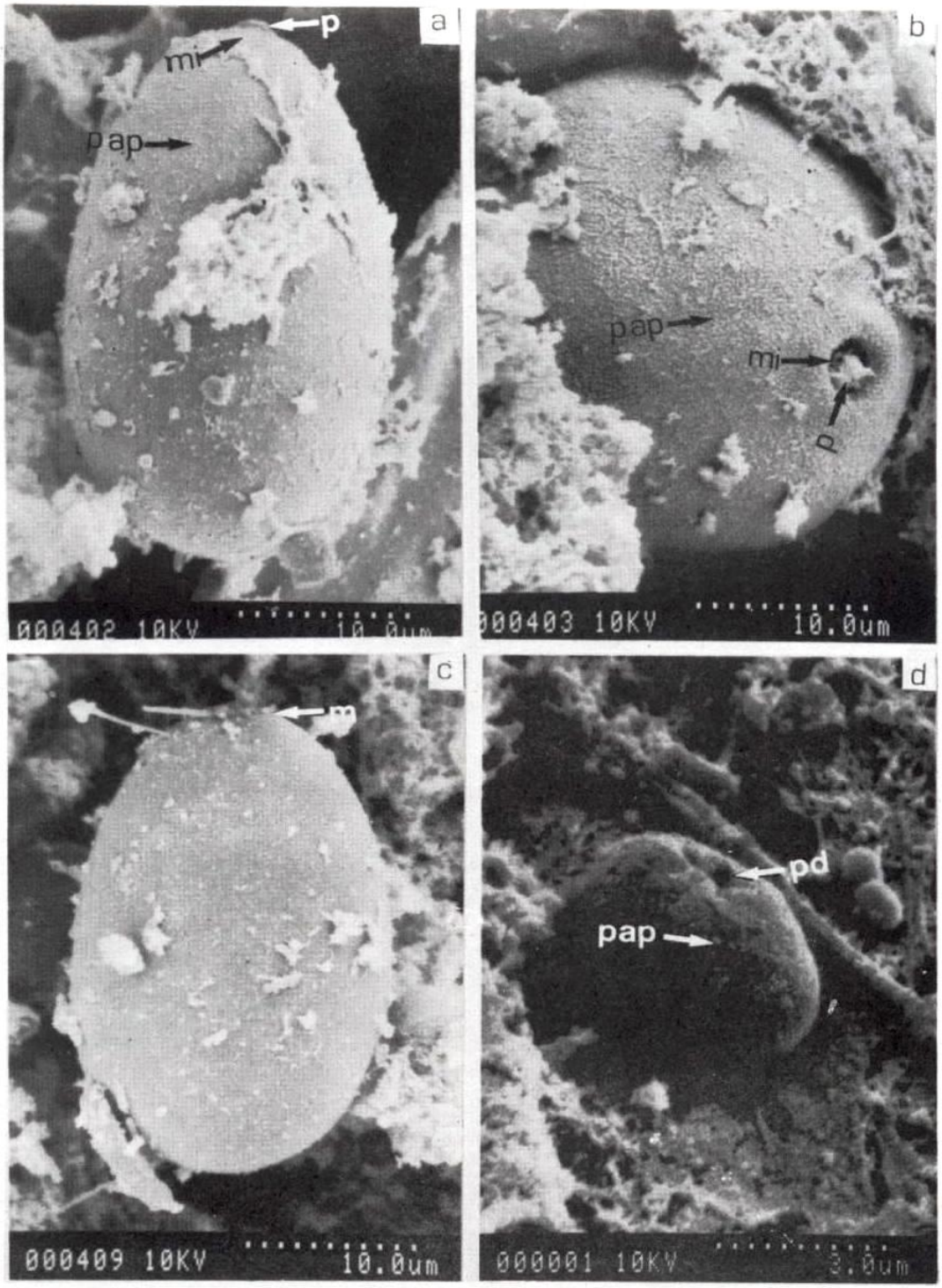

Plate 2: Scanning electron micrographs of the fine structure of the wall of unsporulated oocysts of *Eimeria* species. a-b). *E. brasiliensis*; c). *E. auburnensis*; d). *E. cylindrica*; p = polar cap; pap = papilliae; mi = micropyle; pd = possible damage.

also extremely problematical. Attempts to centrifuge the oocyst and fix them in a pellet proved to be impossible. However, the enclosure of oocyst in mouse gut proved to be a valuable technique which could be applied to the examination of other microorganisms. Fixation appears not to have been restricted by the ligation procedure. However, location of the oocysts within the mucosal folds of the intestine was not always easy.

The wall of the oocyst of *E. bovis* was covered with ridges which were embedded in the matrix of the wall. No previous reports have been published on the fine structure of the surface of the wall of *E. bovis*. Courtney *et al.* (1976), described papillae of variable sizes on the wall of the oocyst of *E. wyomingensis*. However, these papillae were completely different from the ridges found in the wall of the oocyst of *E. bovis*. The micropyle seen in *E. bovis* has not been previously described at the electron microscope level but it does appear to resemble that described in *E. intestinalis* by Cheinssin and Snigirevskaya (1965) and Garnham *et al.* (1963) in the sporozoite of *Plasmodium* sp. The micropyle is described as an opening in the coccidian oocyst which functions as a feeding mechanism in the case of avian malarial parasites acting as a cytostome during the ingestion of erythrocyte cytoplasm (Aikawa *et al.*, 1966).

The surface of the oocyst of *E. canadensis* was covered with tiny papillae similar to those described by Courtney *et al.* (1976) in the oocyst of *E. bukidnonensis*. Scattered, somewhat pronounced papillae, were also seen in the wall surface of *E. alabamensis*. Papillae on the surface clearly increase the surface area of the oocyst. It is considered by Courtney *et al.* (1976) that these papillae give the striated appearance to the oocyst wall, but nothing was described concerning their function. Micropores were evident on the surface of the oocyst of *E. alabamensis*, the majority located in the anterior half of the body of oocyst. Sampson and Hammond (1971) reported one or more typical, apparently non-functional micropores, in the extracellular sporozoites of *E. alabamensis*. These authors also located the micropores in the anterior half of the body of sporozoites. Roberts and Hammond (1970) described fewer micropores and of different sizes in the wall of the sporozoites of *E. auburnensis*.

REFERENCES

Aikawa, M,. P.K. Heplen, C.G. Huff and H. Spring, 1966. The feeding mechanism of malarial parasites. J. Cell Biol., 28: 355-375.

Anonymous, 1986. Ministry of Agriculture, Fisheries and Food. Manual Veterinary Parasitological Laboratory Techniques.Reference Book 418. HMSO London.

Cheinssin, E.M. and E.S. Snigirevskaya, 1965. Some new data on the fine structure of the merozoites of *Eimeria intestinalis* (Sporozoa, Eimeriidae). Parasitologica, 1: 121-126.

Courtney, C.H., J.V. Ernst and G.W. Benz, 1976. Redescription of oocysts of the bovine coccidia, *Eimeria bukidnonensis* Tubangui 1931 and *Eimeria wyomingensis Huizinga* and Winger 1942. J. Parasitol., 62: 372-376.

Garnham, P.C.C., R.G. Bird and R.G. Backer. 1963. Electron microscope studies of motile stages of malaria parasites IV. The fine structure of the sporozoites of four species of *Plasmodium*. The Royal Society of Tropical Medicine and Hygiene, 57: 27-31.

Huizinga H., and R.N. Winger. 1942. *Eimeria wyomingensis*, a new coccidium from cattle. Transactions of the American Microscopical Society, 61: 131-133.

Lee R.P. and J. Armour, 1959. Coccidia oocysts of Nigerian cattle. British Vet. J., 115: 6-7.

Parker, R.J., 1991. *Eimeria alabamensis* and *Eimeria brasiliensis* in cattle in Northern Queensland. Australian Vet. J., 68: 365-366.

Roberts W.L. and D.M. Hammond, 1970. Ultrastructural and cytologic studies of the sporozoites of four *Eimeria* species. J. Protozool., 17: 76-86.

Sampson, J.R. and D.M. Hammond, 1971. Ingestion of host-cell cytoplasm by micropores in *Eimeria alabamensis*. J. Parasitol., 57: 1133-1135.

Soulsby E.J.L. 1986. Helminths, Arthropods and Protozoa of Domesticated Animals. Baillier Tindall London, pp: 607-612.