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RESEARCH NOTE

Observations on oocyst development of *Eimeria stiedai* in rabbits

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Abstract

The formation of the oocyst wall was examined in *Eimeria stiedai* in the bile duct epithelium of the rabbit and was found to follow the general eimerian pattern. However from the beginning of the formation of the outer layer of the oocyst wall the parasite was surrounded by a rarely reported veil membrane. Cell damage of the bile ducts at the gamogony stage of parasite development is depicted.

Keywords

Eimeria stiedai, oocyst wall, ultrastructure

Pakandl (2009) updated the earlier list by Levine and Ivens (1972) of coccidia in the rabbit (*Oryctolagus cuniculus* L.) and considered 11 species of *Eimeria* to be valid. Ten of these develop in the intestines, the exception being *E. stiedai* (Lindemann 1865) Kisskalt and Hartmann, 1907 which is the only liver coccidium infecting the epithelium of bile ducts. *Eimeria stiedai* is considered to be one of the most important rabbit coccidia (Yakhchali and Tehrani 2007) together with the intestinal species, *E. intestinalis* and *E. irresidua* (Pakandl 2009). Experimental infections with *E. stiedai* can result in severe functional disorders and death (Hein and Lämmler 1978; Barriga and Arnoni 1979, 1981). Variable pathogenicity of *E. stiedai* infections has been recorded under natural conditions ranging from little or no reduction in performance (Licois, 2004) to showing signs of anorexia and causing death (Das Singla *et al.* 2000).

The ultrastructural development of the macrogamete of *E. stiedai* in the bile duct epithelium was recorded by Pittilo *et al.* (1980). The present study records aspects of the further development of the mature macrogametes to form oocysts and depicts the damage associated with this late stage of the life cycle.

Liver tissue was collected from two specific pathogen-free rabbits 21 and 23 days after oral inoculation with 10,000 sporulated oocysts of *E. stiedai* originally supplied by Dr M.E. Rose, and prepared by methods described by Pittilo and Ball (1979, 1984). Briefly, for transmission electron microscopy the tissue was fixed in 3% (v/v) glutaraldehyde buffered at pH 7.2 with cacodylate buffer, post fixed in 1% (w/v) osmium tetroxide, dehydrated and embedded in Epon 812. Ultrathin sections stained with uranyl acetate and lead citrate were viewed in an AEI Corinth electron microscope. For scanning electron microscopy, tissue fixed as above was dehydrated, frozen in liquid nitrogen and fractured, then hydrated, post-fixed in 1% osmium tetroxide, dehydrated to trichlorotrifluoroethane and then critical-point dried. Tissue coated with gold-palladium was examined in a Jeol JSM-35 scanning electron microscope. Separately, liver tissue for light microscopy was fixed in 4% formol saline, processed in a histokinette and sections stained with haematoxylin and eosin (H & E).

Even though all stages of oocyst wall formation were present at 21 and 23 days post infection, it was possible to postulate a sequence of events. Large wall forming bodies of type 1 (WFB1) were present in the cytoplasm around the periphery of the mature macrogametes (Fig. 1). The early sign of oocyst wall formation was the release of the contents of the WFB1 after they had assumed an irregular shape adher-

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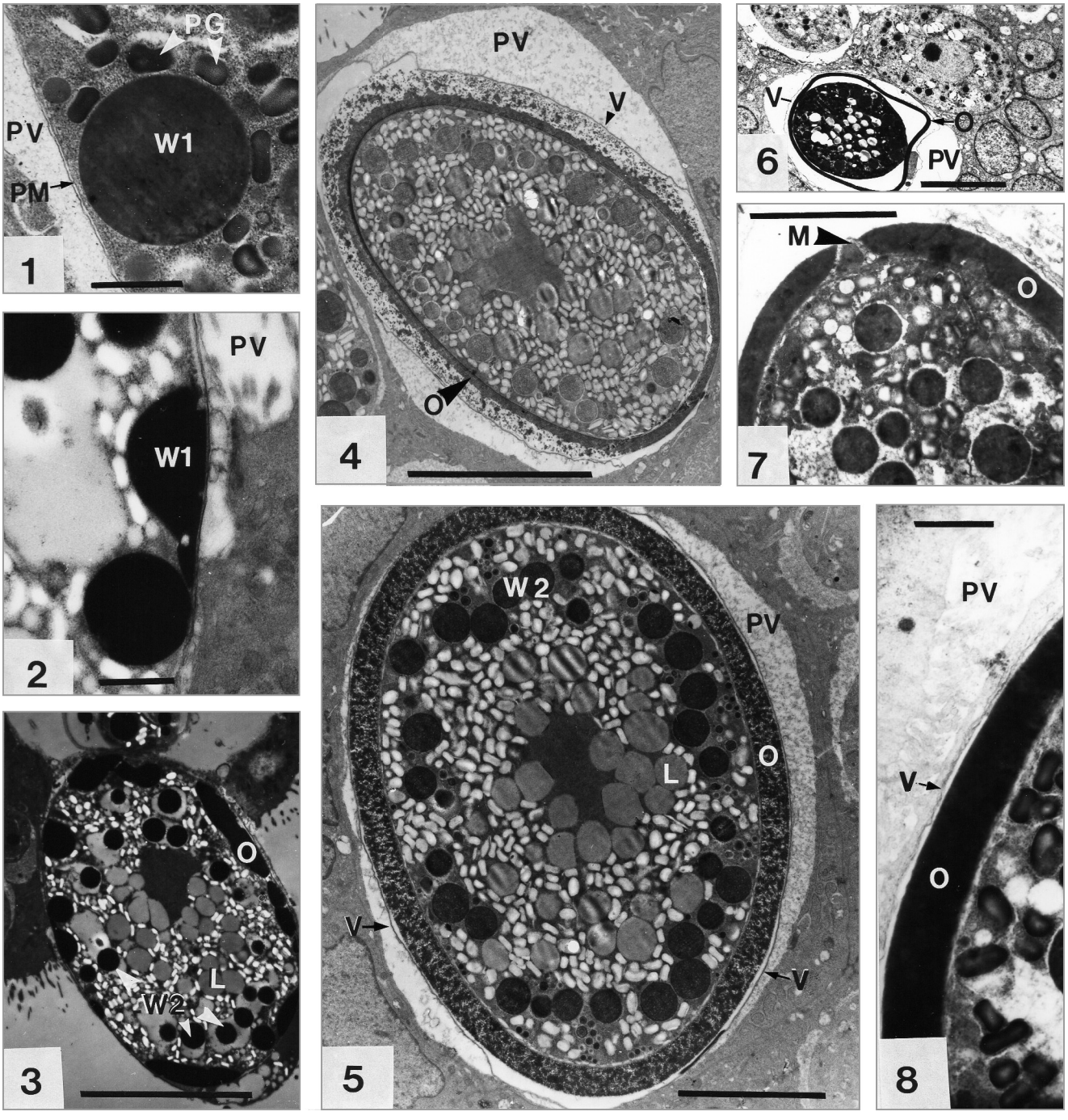
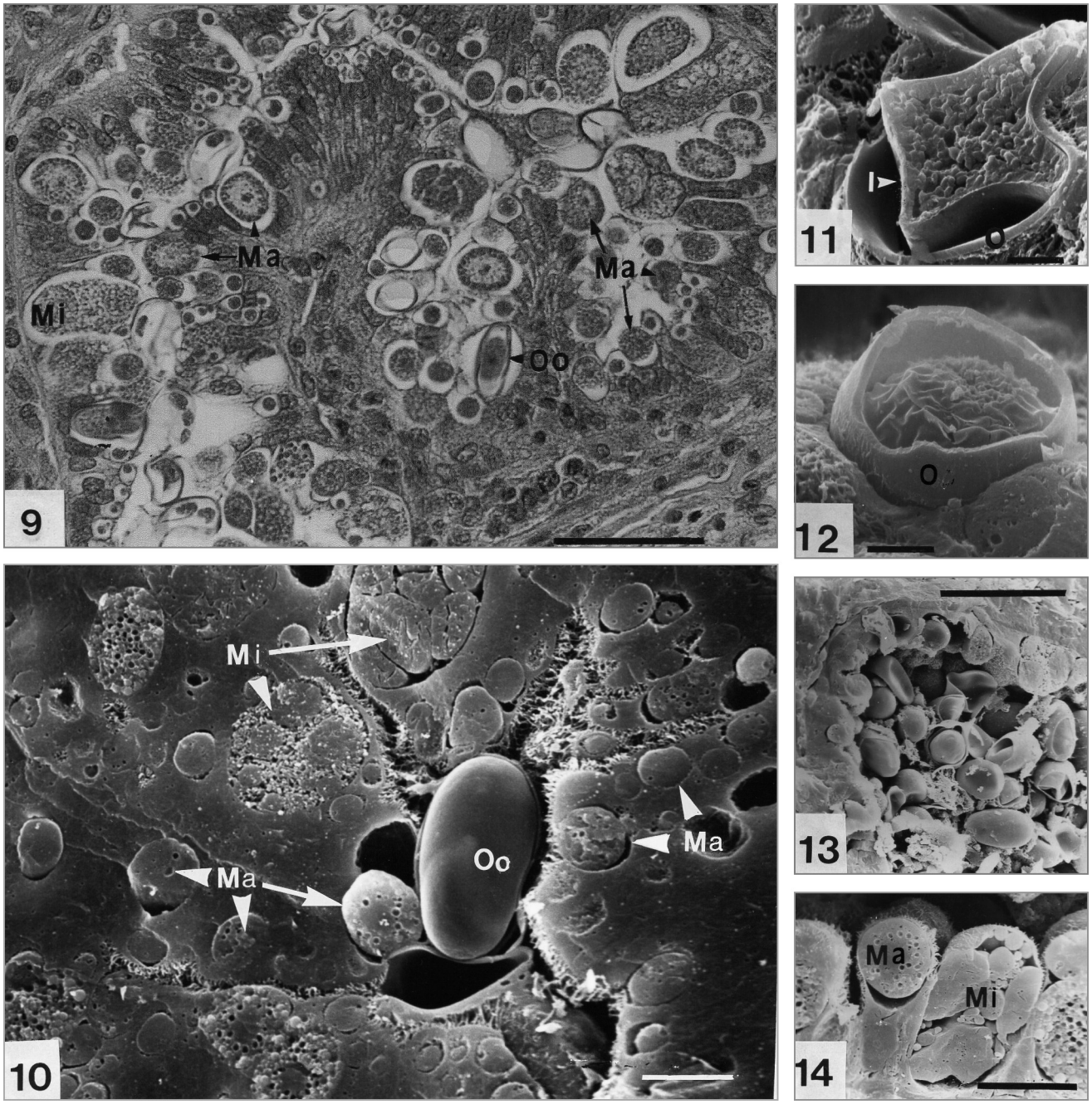
ing to the interior of the parasite outer membrane (Fig. 2). This was followed by their fusion (Fig. 3).The outer wall layer had an initial granular appearance of approximately 800 nm thick (Fig. 5), which became more electron dense (Figs 4, 6–8). The thickness varied from 330 nm (Fig. 4) to generally about 700 nm (Fig. 8) or 1 µm (Fig. 7). Lipid globules and



Figs 1–8. Transmission electron micrographs of macrogametes and early oocyst stages of *Eimeria stiedai* in the bile ducts of rabbit 21 days after infection. 1. Portion of mature macrogamete showing wall forming body type 1 (W1), a number of polysaccharide granules (PG), parasitophorous vacuole (PV) and parasite membrane (PM); scale bar = 1.0 µm. 2. Wall forming body type 1 adhering to parasite membrane to initiate outer oocyst wall; scale bar = 1.0 µm. 3. Outer oocyst wall (O) partially formed. Wall forming bodies type 2 (W2), lipid droplets (L) present; scale bar = 10 µm. 4 and 5. Showing two stages of early wall formation. V = veil; scale bars: Fig 4 = 10 µm; Fig 5 = 5 µm. 6. Fully formed outer oocyst wall layer showing veil (V) still present; scale bar = 10 µm. 7. Portion of outer wall showing possible edge of micropyle (M); scale bar = 5 µm. 8. Portion of mature outer oocyst wall with veil attached; scale bar = 1.0 µm.



Figs 9–14. Micrographs of gamogonic stages of *Eimeria stiedai* in the bile ducts of rabbit 21 days (Figs 9, 11–14) and 23 days (Fig 10) after infection. 9. Histological section stained with H & E showing macrogametes (Ma), oocysts (Oo) and microgamont (Mi) and severe destruction of parasitizes cells; scale bar = 40 μm. Figs 10–14. Scanning electron micrographs of cryo-fractured tissue. 10. Showing macrogametes, microgamonts, the release of a macrogamete and an oocyst from destroyed cells; scale bar = 10 μm. 11. Oocyst with outer wall (O) and inner wall (I) showing distortion due to inadequate fixation as a result of the resistance of the oocyst wall; scale bar = 10 μm. 12. Broken oocyst wall showing contraction of contents; scale bar = 10 μm. 13. Oocysts breaking free into lumen of a bile duct; scale bar = 40 μm. 14. Macrogamete and microgamont emerging from parasitized cells; scale bar = 5 μm.

WFB2 were still present in the cytoplasm. A small space in the oocyst wall (Fig. 7) could represent a section through the edge of the oocyst micropyle. With the progressive formation of the outer wall the oocyst became resistant to fixatives and resin penetration resulting in shrinkage of the interior cytoplasm (Figs 6, 11 and 12). A veil membrane enveloped the developing oocyst (Figs 4, 5 and 8) and this was lost when the oocyst was released from the host cell. The process of oocyst wall development was completed within the host cell. The part played by WFB2 was not followed in this study.

By 21 and 23 days post infection most epithelial cells of the bile ducts were parasitised by developing gametes and oocysts. Oocysts and some immature stages were being released into the bile duct lumens by the breakup of infected cells (Figs 10, 13 and 14), and the normal biliary architecture was severely distorted.

Discussion

The development of the oocyst wall as observed in this study followed the general pattern of other eimerians. A veil membrane was observed on the outer surface of the macrogamete of *E. stiedai* and this structure has been reported in only four other eimerian species, namely *E. brunetti* (Ferguson *et al.* 1977), *E. maxima* (Pittilo and Ball 1980; Ferguson *et al*. 2003) and *E. acervulina* (Pittilo and Ball 1984) all in the chicken, and *E. bakuensis* in sheep (Pittilo *et al*., 1988). The role of the veil in oocyst wall development is at present unknown (Mai *et al*. 2009).

A thinning of the wall at one end of the oocyst of *E. stiedai* was depicted by Kessel and Jankiewicz (1931) and Marotel and Guilhon (1941). Jolley *et al.* (1979) examined the process of excystation by chemical treatment *in vitro* and found that the wall ruptured mainly around the micropylar lid. Fig. 5 shows a section through a possible fracture area corresponding to Fig. 3 of Jolley *et al.* (1979).

Pellérdy and Dürr (1970) described six schizogonous generations in *E. stiedai*. By six days post infection, when the 3rd schizonts were present, cell proliferation of the biliary duct epithelium could fill the duct (Pellérdy, 1974). This cell proliferation and damage continues and was evident in our results at 21 and 23 days after infection.

References

Barriga O.O., Arnoni J.V. 1979. *Eimeria stiedai*: Weight, oocyst output, and hepatic function of rabbits with graded infections. *Experimental Parasitology*, 48, 407–414. DOI: 10.1016/ 0014-4894 (79) 90125-5.

Barriga O.O., Arnoni J.V. 1981. Pathophysiology of hepatic coccidiosis in rabbits. *Veterinary Parasitology*, 8, 201–210. DOI: 10.1016/0304-4017 (81) 90051-0.

Das Singla L., Juyal P.D., Sandhu B.S. 2000. Pathology and therapy in naturally *Eimeria stiedae* – infected rabbits. *Journal of Protozoology Research*, 10, 185–190.

Ferguson D.J.P., Belli S.I., Smith N.C., Wallach M.G. 2003. The development of the macrogamete and oocyst wall in *Eimeria maxima*: immuno-light and electron microscopy. *International Journal of Parasitology*, 33, 1329–1340. DOI: 10.1016/ S0020-7519 (03) 00185-1.

Ferguson D.J.P., Birch-Andersen A., Hutchison W.M., Siim J.Chr. 1977. Ultrastructural studies on the endogenous development of *Eimeria brunetti*. IV. Formation and structure of the oocyst wall. *Acta pathologica et microbiologica Scandinavica*, B85, 201–211. DOI: 10.1111/j.1699-0463.1977.tb01697.x.

Hein B., Lämmler G. 1978. Alteration of enzyme activities in serum of rabbits infected with *Eimeria stiedai. Zeitschrift für Parasitenkunde*, 57, 199–211. DOI: 10.1007/BF00928033.

Jolley W.R., Allen J.V., Nyberg P.A. 1979. Micropyle and oocyst wall changes associated with chemically mediated *in vitro* excystation of *Eimeria stiedai* and *Eimeria tenella*. *International Journal for Parasitology*, 9, 199–204. DOI: 10.1016/0020-7519 (79) 90029-8.

Kessel J.F., Jankiewicz H.A. 1931. Species differentiation of the coccidia of the domestic rabbit based on a study of the oocyst. *American Journal of Hygiene*, 14, 304–324.

Levine N.D., Ivens V. 1972. Coccidia of Leporidae. *Journal of Protozoology*, 19, 572–581. DOI: 10.1111/j.1550-7408.1972.tb 03531.x.

Licois D. 2004. Domestic rabbit enteropathies. *Proceedings 8th World Congress*, Puebla, Mexico. pp. 385–403.

Mai K., Sharman P.A., Walker R.A., Katrib M., De Souza D., McConville M.J., Wallach M.G., Belli S.I., Ferguson D.J.P., Smith N.C. 2009. Oocyst wall formation and composition in coccidian parasites. *Memórias do Instituto Oswaldo Cruz*, 104, 281–289. DOI: 10.1590/S0074-02762009000200022.

Marotel G., Guilhon J. 1941. Recherches sur la coccidiose du Lapin. *Recueil de Médicine Vétérinaire*, 117, 322–328.

Pakandl M. 2009. Coccidia of rabbit: a review. *Folia Parasitologica*, 56, 153–166.

Pellérdy L.P. 1974. *Coccidia and Coccidiosis*. 2nd Ed. Paul Parey, Berlin and Hamburg.

Pellérdy L., Dürr U. 1970. Zum endogenen entwicklungszyklus von *Eimeria stiedai* (Lindemann, 1865) Kisskalt & Hartmann, 1907. Acta *Veterinaria Academiae Scientiarum Hungaricae*, 20, 227–244.

Pittilo R.M., Ball S.J. 1979. The fine structure of the developing macrogamete of *Eimeria maxima*. *Parasitology*, 79, 259–265. DOI: 10.1017/ S0031182000053336.

Pittilo R.M., Ball S.J. 1980. The ultrastructural development of the oocyst wall of *Eimeria maxima*. *Parasitology*, 81, 115–122. DOI: 10.1017/ S0031182000055086.

Pittilo R.M., Ball S.J. 1984. Electron microscopy of *Eimeria acervulina* macrogametogony and oocyst wall formation. *Parasitology*, 89, 1–7. DOI: 10.1017/ S0031182000001074.

Pittilo R.M., Ball S.J., Catchpole J., Neal C.R. 1988. Electron microscopy of *Eimeria bakuensis* (syn *E. ovina*): Macrogametogony and oocyst wall formation. *Acta Veterinaria Hungarica*, 36, 233–237.

Pittilo R.M., Ball S.J., Hutchison W.M. 1980. The ultrastructural development of the macrogamete of *Eimeria stiedai*. *Protoplasma*, 104, 33–41. DOI: 10.1017/ BF01279366.

Yakhchali M., Tehrani A. 2007. Eimeriidosis and pathological findings in New Zealand white rabbits. *Journal of Biological Sciences*, 7, 1488–1491. DOI: 10.3923/ jbs.2007.1488.1491.

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