قســـــــم : الميكروبيولوجيا والطفيليات ـ كلية الطب ـ جامعة أسيوط.
رئيس القسم : أ.د / عماد كامل نافع.

دراسة بالميكروسكوب العادي والاليكتروني للتركيب الدقيق لنوع من الساركوستسس المعزول مـــــن حيوان الظربان الامريكى ميفايتس ميفايتس فى ولاية أيوا بالولايات المتحدة الامريكيـــــة

عاطف سكـــــــــــــلا

درس لاول مرة التركيب الدقيق لنوع من الساركوستسس المعزول من حيوان الظربان الامريكى بولاية أيوا الامريكية.

وقد شملت الدراسة التركيب الدقيق لكل من جدار الحويصلات والزوتيات والميكروسيتات لهذا الطفيل. وقد وجد التركيب الدقيق للزوتيات تشبه الى حد كبير ماسبق وصفه وخاصة لزوتيـــات الحويصلات الدقيقة ـ ولذلك أقترح الباحث بعدم أخذ التركيب الدقيق للزوتيات فى الاعتبـــار فى تشخيص هذه الطفيليات ولكن بحـــذر.

ويقترح الباحث استمرار دراسة دورة الحياة لهذا الطفيل والذى يعتقد أنها الوسيلـــة الوحيدة والدقيقة لتصنيفف هذه المجموعة من الطفيليات.

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LIGHT AND ELECTRON MICROSCOPIC STUDIES ON SARCOCYSTIS SPECIES FROM THE STRIPPED SKUNK, MEPHITIS MEPHITIS IN IOWA, U.S.A.

(With 8 Figures)

By

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SUMMARY

Sarcocystis sp. have been found naturally in the skeletal muscle of a stripped skunk, Mephitis mephitis captured from Iowa state, U.S.A. Light and electron microscopic appearance of this parasite has been studied. Ultra-structure of the cyst wall as well as the zoites were described, compared and discussed. The present study deals, for the first time, with the fine structure of a Sareocystis species from the stripped skunk, Mephitis mephitis.

INTRODUCTION

More than seventy species of Sarcocystis have been reported and named. They are differentiated on the basis of the host, the structure of the cyst wall as well as the size of the zoites, LEVINE (1977).

So far, the ultra-structure of some species of Sarcocystis have been studied. Thus, LUDVIK (1958 & 1960) have described the fine structure of S.tenella and S.miescheriana; SENAUD and PUYTORAC (1961) and SENAUD (1965) on S.tenella; MANDOUR et al. (1965) on a Sarcocysytis sp. from harsh-furred rat, Lophuromys flavopunctatus; SIMPSON (1966) and SIMPSON and FORRESTER (1973) on S.tenella and Sarcocystis sp.; MANDOUR (1971 & 1974) on S. kortei from rhesus monkey and S.blanchardi from Egyptian buffalo; MEHLHORN and SCHOLTYSECK (1973) on S.tenella of sheep and MEHLHORN et al. (1975 a,b & c). On S.fusiformis.

Moreover, light and elctron microscopic studies on S.fusiformis were extended by GESTRICH et al. (1975) and HEYDRON et al. (1975 a & b); ZAMAN and COLLEY (1975) on S.orientalis from Rattus norvegicus.

Recently, MEHLOHORN et al. (1976) studied the ultra-structure of thirteen Sarcocystis spp. cyst wall; RZEPCZYK and SCHOLTYSECK (1976) on a Sarcocystis sp. from Rattus fuscipes; KAN and DISSANAIKE (1977) on a Sarcocystis sp. from Rattus rattus diardii, KAN (1979) on Sarcocystis spp. from Malaysian rodents; ABDEL GHAFFAR et al. (1979) on a Sarcocystis sp. from Camelus dromedarius; BEAVER and MALECKAR (1981) on S.singaporensis, S.villivillosi and S.zamani from Rattus norvegicus.

The present work deals with the fine structure of Sarcocystis sp. from stripped skunk, Mephitis mephitis.

MATERIAL and METHODS

Skeletal muscles were collected from freshly sacrified stripped skunk infected naturally with Sarcocystis. Portions of skeletal muscles were squashed between two slides and examined microscopically. For ligyt microscopic studies, the infected specimens were fixed in Bouin's fixative.Paraffin sections were stained with Haematoxylin and eosin. Impression smears form the cut surface of the infected mucles were fixed in absolute alcohol and stained with Giemsa's stain. For electron microscopic studies, the infected materials were fixed in the glutaraldehyde in Millonig's buffer, PH 7.4, for 24 hours. Specimens were placed in 2% OsO_4 in Millonig's phosphate buffer. Sections were stained in 2% aqueous uranyl acetate and lead citrate and examined with a Philips 300 electron microscope. Original copies of the photo-micrographs were obtained for description and comments.

RESULTS

Sarcocystis sp. cysts were found in the striated muscles throughout the body, including the tongue, oesophagus, and diaphragm, but not in the heart.

Light microscopic appearance:

Haematoxylin and eosin stained sections of the microscopic cysts showed the Miescher's tube with thin cyst wall and thin trabeculae dividing the cyst into numerous compartments enclosing small banana-shaped zoites. The cysts were spindle shaped with rounded ends. The largest cysts were 500 x 75 U, and cyst wall was 6 U in thickness. The wall of the large cysts in sections of well-fixed tissue, stained with H & E., appeared to have two distinct layers. The outer layer was 1.5 U thick, weakly basophilic, and provided with fine, mostly erected, cilia-like processes or villi. The eosinophilic inner layer was approximately 0.8 U thick and had the appearance of a thin line. In tangential sections of the wall, the villi were seen rounded. The Giemsa's stained smears contained a large number of minute benana-shaped zoites, approximately 7-8 x 1.5-2 U. The nuclei were slightly elongated, and nearly as wide as the widest part, but not entirely filling the posterior end. Its middle third contained deeply stained granules, while its anterior end was relatively light in colour.

Electron microscopic appearance:

The cyst wall: Large cilia-like processes or villi appeared projecting from the outer layer of the cyst wall towards the host tissue (Fig. 1). The pellicle of the villus had a mamillated or serrated appearance when highly magnified (Fig. 2, 3 & 4). In tangential sections, the number of the outer minute papillae on the outer surface of the villus was 20-30 (Fig. 5). Bundles of fine filamentous fibrils were seen running parallel to the long axis within the villus core (Fig. 1-5). These bundles, which form the central core of the villi, were found blended proximally in the stroma of the cyst wall, while they were separated from each other toward the free end of the villi (Fig. 1, 2 & 3). A clear zone was also separating these bundles from the inner surface of the villus pellicle (Fig. 1-5). Around and between the villi, they was a large number of host cell mitochondria, as well as, other elements of the host muscle cells (Fig. 4). The stroma of the inner layer of the cyst wall showed electron dense undifferentiated bodies which were darkly stained, of varying shapes and sizes (Fig. 5 & 6). Some of these bodies were subdivided into unequal portions by clefts. Thin septa of ground substance extended from the inner layer of the cyst wall into the cyst interior in which zoites were tightly packed assuming polygonal forms (Fig. 6). Metrocytes were much larger than the zoites and were seen only in contact with the ground substance of the inner layer of the cyst wall (Fig. 6).

The zoites: Mature zoites were surrounded with ultra-thin doubled-layer pellicle. Three divisible zones were the distinctive feature of the zoites stroma in longitudinal section (Fig. 7). The anterior fibrillar zone, was filled with fine convoluted microtubules, the sarconemes or micronemes. The anterior end of the fibrillar zone was occupied by the polar ring within which the conoid was detected. In addition, the anterior zone showed club-shaped portions of the paired organelle, rhoptries (Fig. 7). A micropyle was observed just under the pellicle and about the middle of the fibrillar zone (Fig. 8). The middle vesicular zone, contained a relatively large stained vesicles, as well as, large unstained vaculoes possibly filled with lipid material. Within the middle zone, and near the nucleus lied the mitochondria. The posterior nuclear zone contained the nucleus which was nearly as wide as the cell. It was an ellipsoidal vesicle and contained an endosome and chromatin granules.

DISCUSSION

The finding of the electron microscopical examination of the skunk Sarcocystis observed in the present study were compared by the ultrastructure of the previously described species in the different hosts. However, and on the basis of the ultra-mprphological structure,it was found that the parasite under discussion closely resembles other species of Sarcocystis particularly the zoites of the microcyst producing parasites. Nevertheless, the detection of the fundamental structures characterestic to the Sarcocystis zoites as the polar ring, conoid, micronemes, paired organelle, micropyle, mitochondrion, vacuoles, vesicles and nucleus were confirmed by a number of authors since the work of LUDVICK (1958). Accordingly, this study yielded evidence that some animal species may be intermediate hosts for one species of Sarcocystis. Thus far, the present author kept the identification of the present material at the generic level, because of the much controversy regarding the nomenclature of Sarcocystis parasites, a fact which was mentioned by LEVINE (1977).

As far as the ultra-structure of the cyst wall of the skunk Sarcocystis, the present work revealed quite variations when compared with the previously described other parasites. However, it could be postulated that the fine structure of the Sarcocystis cyst wall could be used with care as a criteria to identify the different species of this parsite.

Further studies on the life history of the skunk Sarcocystis will permit complete and acurate identification to the species. It is worth-while mentioning that the present study deals, for the first time, with the ultra-structure of Sarcocystis parasite from the stripped skunk, Mephitis mephitis.

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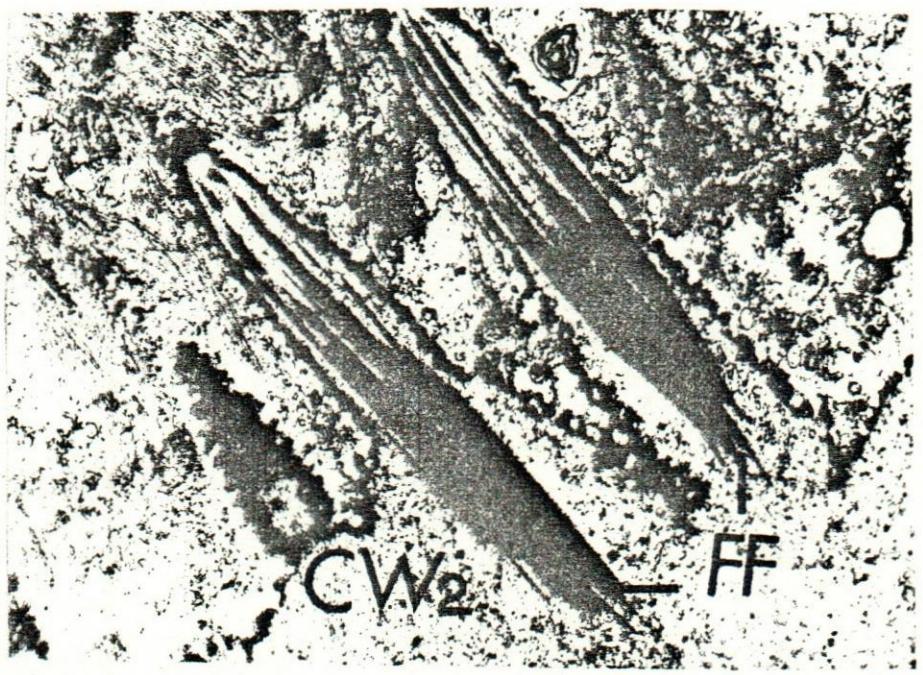
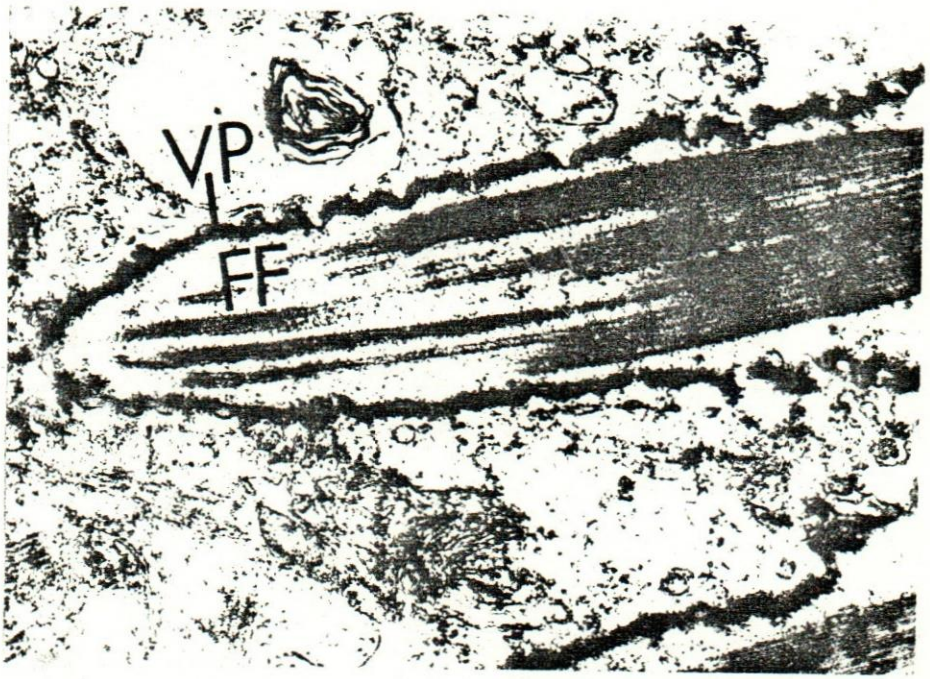
(Fig. 1): Electron micrograph of skunk Sarcocystis showing the cyst and wall villi. (X 5,000).

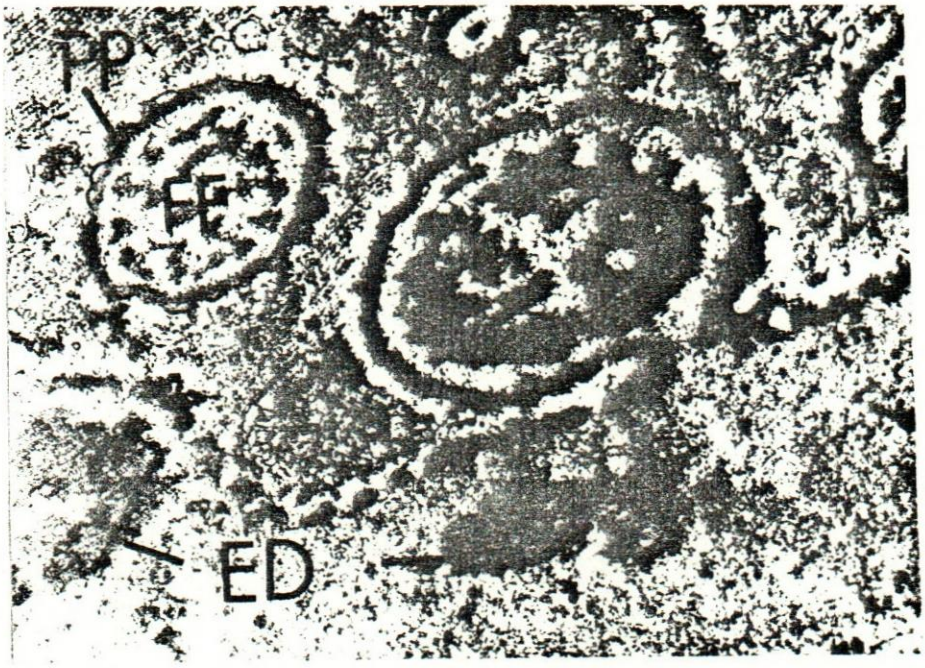


(Fig. 2): Electron micrograph of skunk Sarcocystis showing a portion of cyst wall and the villi with fine filamentous fibrils core (X 10,000).

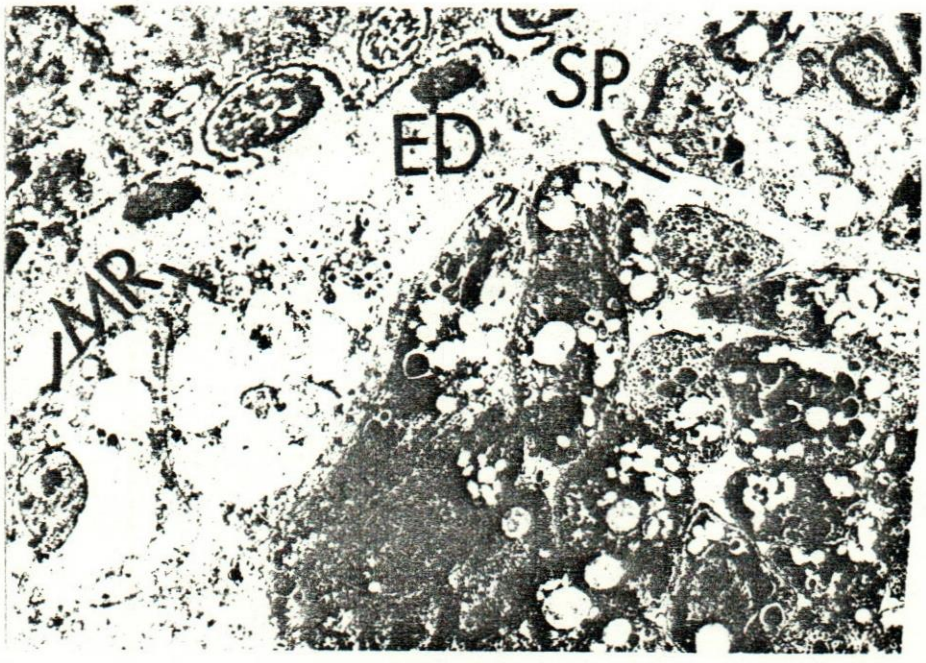


(Fig. 3): Electron micrograph of skunk Sarcocystis showing the papillated villus pellicle and the central villus core. (X 20,000).

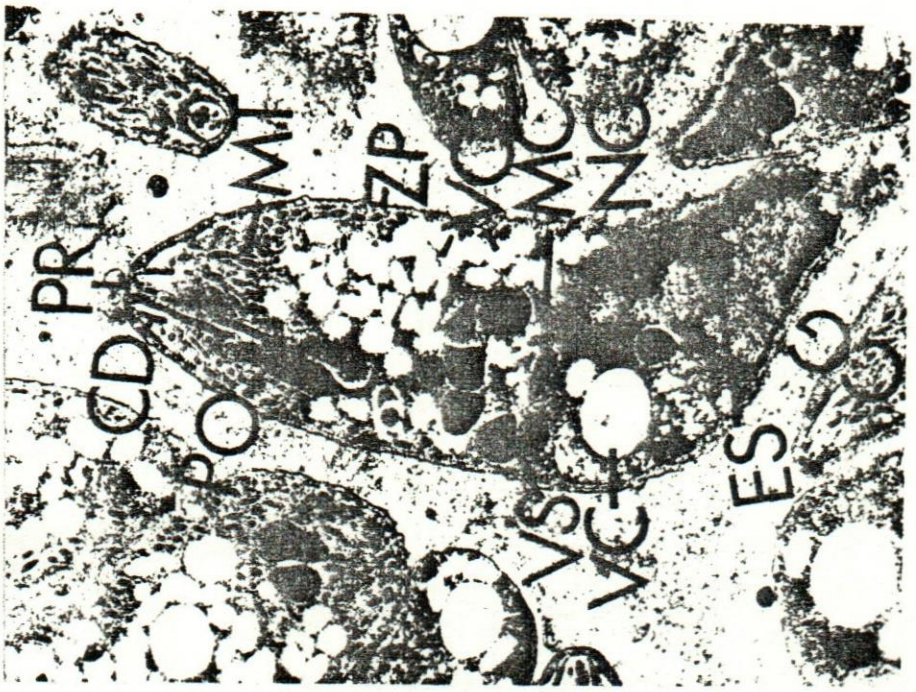
(Fig. 4): Electron micrograph of skunk Sarcocystis showing a highly magnified villus with separated fibrillar bundles. (X 50,000).



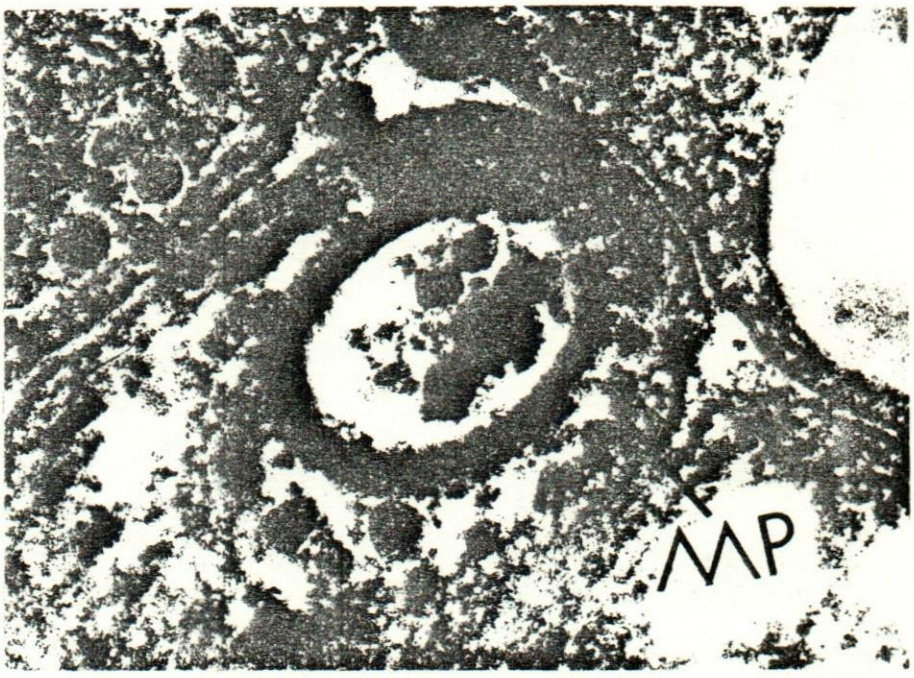
(Fig. 5): Electron micrograph of skunk Sarcocystis showing tangential sections in villus with papillae on its pellicle and fine fibrils in its core. (X 20,000).



(Fig. 6): Electron micrograph of skunk Sarcocystis showing metrocytes, septa and electron dense bodies. (X 5,000).



(Fig. 7): Electron micrograph of skunk Sarcocystis showing the fine structure of a zoite (X 10,000).



(Fig. 8): Electron micrograph of skunk Sarcocystis showing a highly magnified micropyle. (X 70,000).

ABBREVIATIONS USED ON MICROGRAPHS

Chromatin granule	CG	Mitochondrion	MC
Conoid	CD	Nucleus	NC
Cyst wall, outer layer	CW1	Paired organelle	PO
Cyst wall, inner layer	CW2	Papilla	PP
Endosome	ES	Polar ring	PR
Electron dense body	ED	Septa	SP
Filamentous fibrils bun.	FF	Vacuole, unstained	VC
Host mitochondria	HM	Vesiele	VS
Metrocyte	MR	Villi	VI
Micropyle	MP	Villus pellicle	VP
Microtubules	MT	Zoite pellicle	ZP