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Light Microscopic and Ultrastructural Studies on *Sarcocystis* spp. Infection in Philippine Water Buffaloes (*Bubalus bubalis*)

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ABSTRACT

In a survey for sarcocysts in muscle tissues obtained from 142 water buffaloes (*Bubalus bubalis*), 92 (64.8%) carcasses had sarcocysts. Macroscopic and two forms of microscopic cysts, the spindle-shaped or fusiform cysts commonly occurring in the muscles of the esophagus, throat and limbs, and the globular to oval cysts which were the dominant form in the diaphragm and cervical muscle tissue were noted. Ultrastructural analysis of macroscopic and microscopic cysts and their cyst wall revealed two distinct species of *Sarcocystis* infecting Philippine water buffaloes. These are the macroscopic species, *Sarcocystis fusiformis* which has been previously reported in the country possessing highly-dendritic cauliflower-like projections emanating from the primary cyst wall, with annulated microfibrils and numerous electron dense granules; and the newly redescribed *Sarcocystis levinei* (Dissanaike and Kan 1978; Huong, Dubey and Uggla 1997b) exhibiting a cyst wall with numerous, minute hair-like villar protrusions with expanded or dome-shaped base, an intermediate finger-like, and distal tapering segments which at some points join to form conical tufts. Our findings represent the first report of *S. levinei* in Philippine water buffaloes supported with ultrastructural analysis of the sarcocyst and its cyst wall, and likewise refute earlier published reports that all microscopic cysts in Philippine water buffaloes are developing forms of *S. fusiformis*.

INTRODUCTION

Earlier studies in the Philippines have documented the presence of

sarcocysts in muscles of water buffaloes (Arambulo et al. 1972; Manuel et al. 1983). The macroscopic species, *Sarcocystis fusiformis* commonly occurring in water buffaloes worldwide (Kan and Dissanaike 1978; Houng et al. 1997a; Dubey et al. 1989) has likewise been reported in the Philippine water buffalo, *Bubalus bubalis* (Tongson and Molina 1979; Manuel et al. 1983; Parairo et al. 1988). Light microscopic and ultrastructural studies of both macroscopic cysts of variable sizes and microscopic cysts conducted by Tongson and Molina (1979) led them to conclude that all small bubaline sarcocysts are developing forms of the macroscopic species, *Sarcocystis fusiformis*. Manuel et al. (1983), likewise, reported morphological differences between bubaline microscopic and macroscopic cysts, and opined that there are at least two or three distinct species of *Sarcocystis* infecting Philippine water buffaloes. However, ultrastructural analysis of the cysts and cyst wall has not been provided to substantiate such conjecture. This paper presents our findings of two distinct species of bubaline *Sarcocystis* that likewise lends support to our preliminary observation that not all microscopic cysts represent developing or young forms of the macroscopic species, *S. fusiformis* in Philippine water buffaloes.

MATERIALS AND METHODS

Muscle tissue samples were obtained from the esophagus, throat, cervical, diaphragm and upper and lower limbs of 142 freshly slaughtered water buffaloes from two different slaughter facilities in Metro Manila. Water buffaloes originated from various provinces of Luzon, Visayas and Mindanao. Tissue samples collected as early as 0600h were placed in properly labeled plastic bags or containers, brought in a cooler to the Zoology and Parasitology Research Laboratory, Science and Technology Research Center at the De La Salle University-Manila, and were kept in the refrigerator, and if needed in the freezer (-40°C), prior to examination for sarcocysts.

Sarcocysts were noted through gross inspection of muscle tissue samples, and examination of unstained muscle tissue smears with the aid of light microscopy. Tissue samples positive for sarcocysts were fixed in Bouin's fluid and processed following the standard histologic procedure. H & E stain was used. Likewise, 1-2 mm^2 tissue samples were fixed in ice-cold 2.5% glutaraldehyde and were subsequently processed for transmission electron microscopy (TEM) at the Biomedical Research Unit of the St. Luke's Medical Center, Quezon City. Ultrastructure of the cyst wall was analyzed using TEM (JEOL-1010, Japan). Examination of tissue sections, as well as documentation of sarcocysts was done using Nikon photomicroscope (Eclipse E400, Japan). Measurements of cysts and histopathological changes in both histological and ultrathin sections were noted.

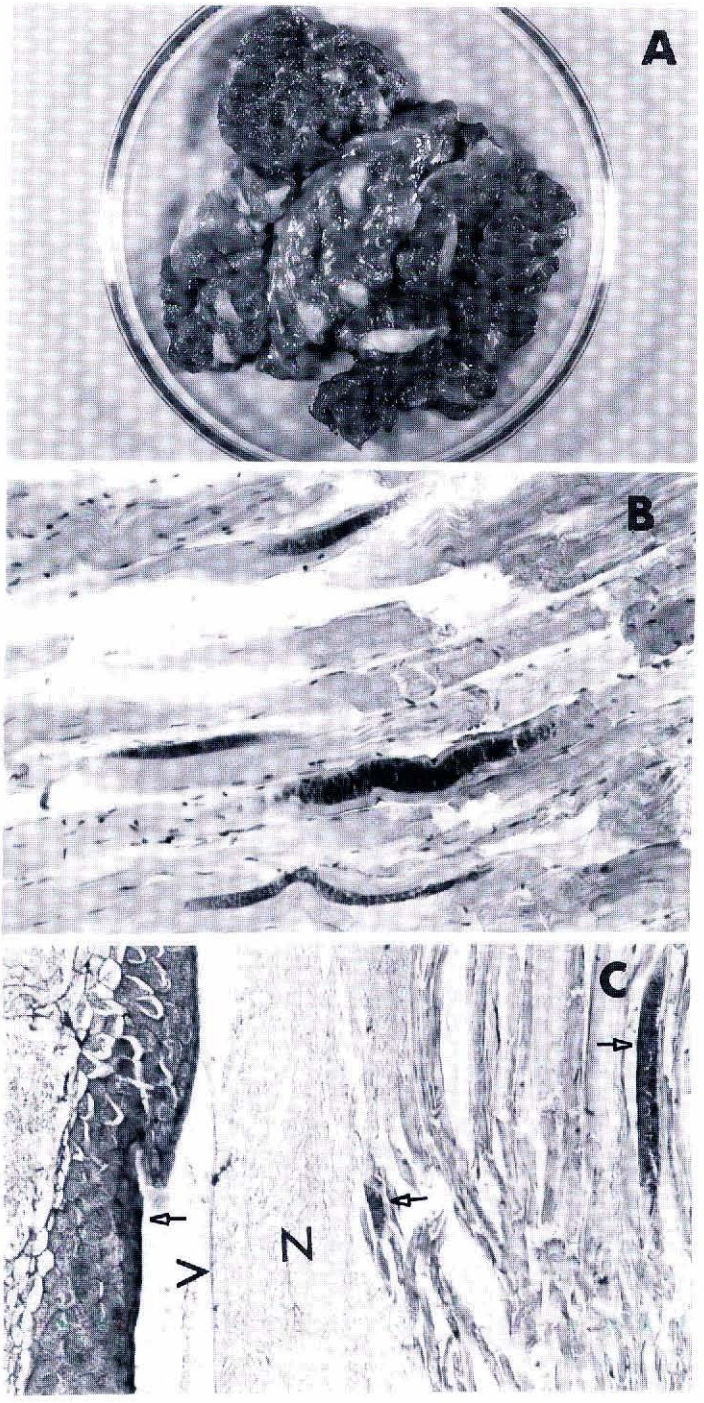

Fig. 1. Sarcocysts were detected in muscle tissues. A: Macroscopic cysts in limb muscles (1-18 mm×1-7 mm); B: Microscopic fusiform cysts in esophageal muscle tissue (70-150 μm×5-15 μm); C: Mixed infections (arrows) in the limb muscle tissue. Note: host tissue necrosis (N) and displaced host cell sarcolemma (arrow head).

RESULTS AND DISCUSSION

One hundred male and 42 female water buffaloes, age ranging between 5.5-17 years old were used in the survey for sarcocysts. Approximately 98% of the 142 water buffalo carcasses were 6-17 years of age. Ninety-two water buffaloes (64.8%) were positive for sarcocysts with infection of 72.0% and 47.6% in males and females, respectively. Infection in the 10-17 years old group was 77.8%. This seems to suggest the incidence and maturity of *Sarcocystis* spp. infection increasing with host age, an observation consistent with that reported in pigs (Omata et al. 1993).

Sarcocysts were detected in all muscle tissue types with the highest density and frequency of distribution in the esophageal (64.79%), throat (46.48%) and limb muscles (49.29%). Single infection with macroscopic cysts were observed in all muscle tissues types except in the diaphragm, and with a preponderance in the muscles of the esophagus, throat and limbs. Single infection with microscopic cysts were noted more frequently in all muscle tissue types compared to single infection with macroscopic cysts. Mixed infections with macroscopic and microscopic cysts were common occurrences, as well.

Macroscopic cysts measuring 1-18 mm long and 1-7 mm in width embedded in fresh muscle tissue are spindle-shaped or fusiform, milky white, opaque bodies lying in between muscle bundles (Fig. 1). Examination of unstained and histologically processed muscle tissues showed both globular to oval, and spindle-shaped or fusiform microscopic cysts. Spindle-shaped cysts were numerous in the esophageal, throat and limb muscles; while the globular to oval cysts were the dominant form in the diaphragm and cervical muscles (Fig. 2), with a few cysts found in the throat, esophageal and limb muscles.

Macroscopic cysts exhibit Type 23 cyst wall (Dubey et al. 1989) with highly branched or anastomosing cauliflower-like projections emanating from the primary cyst wall (Fig. 3). The dendritic branches are hollow and contain annulated microfilaments and electron dense granules. These ultrastructural features are characteristic of the macroscopic species, *Sarcocystis fusiformis* previously reported in *Bubalus bubalis* in the Philippines (Tongson and Molina 1979; Parairo et al. 1988), in Malaysia (Kan and Dissanaike 1978; Dissanaike and Kan 1978), in Vietnam (Huong et al. 1997a), and in India, Egypt, Romania, Brazil, and Turkey (Dubey et al. 1989).

With gross inspection of macroscopic cysts, there were no apparent histopathological changes on host tissues, and large cysts were easily removed from the muscle mass. In histological sections however, young and mature to old macroscopic cysts showed displacement of host tissue and sarcolemma, with apparent tissue degeneration in some areas (Fig. 1C). Inflammatory host tissue

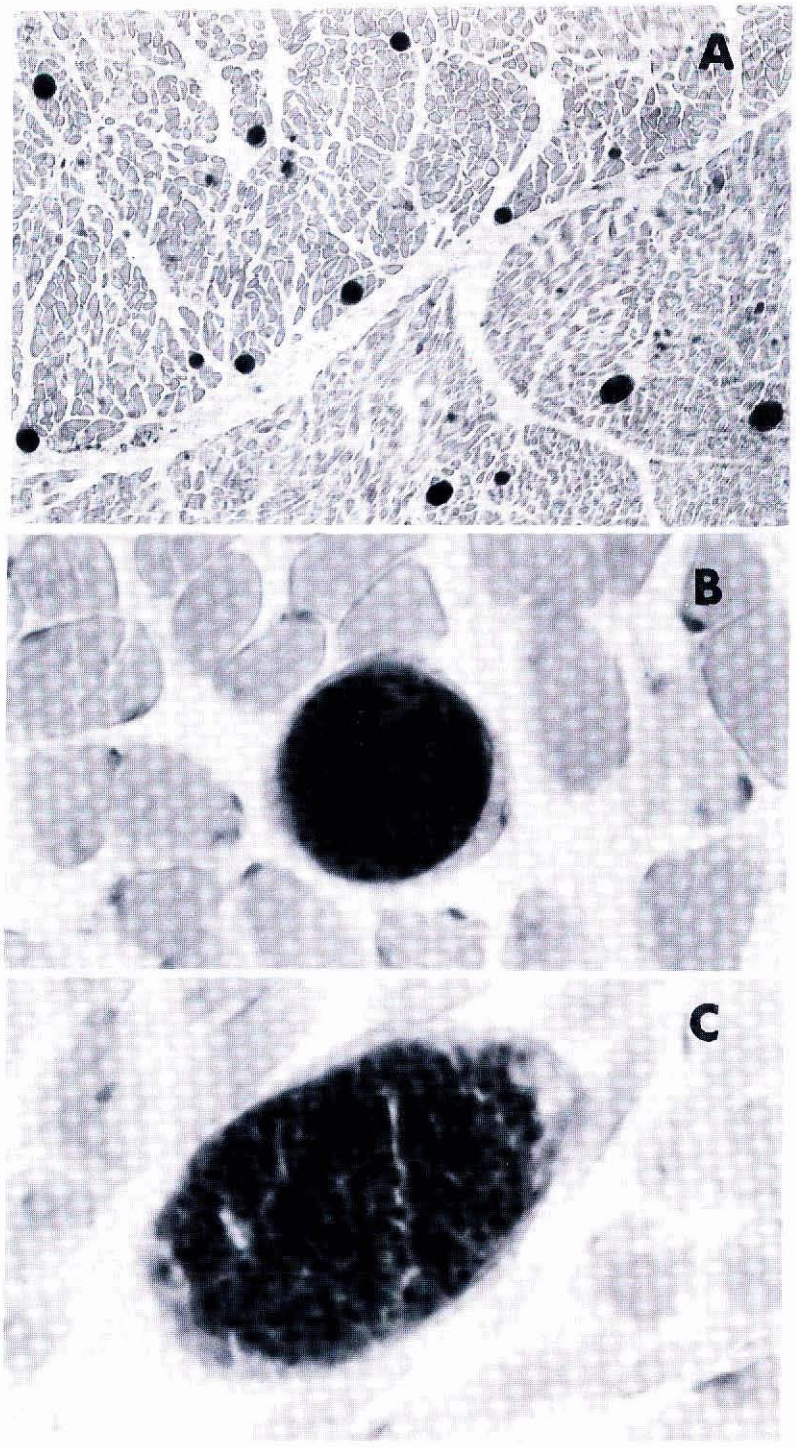

Fig. 2. Globular to oval microscopic cysts. A: Diaphragm (12.5-37.5 μm); B: Diaphragm cross section (20 μm); C: Cervical muscle tissue longitudinal section (70 μm$\times$25 μm).

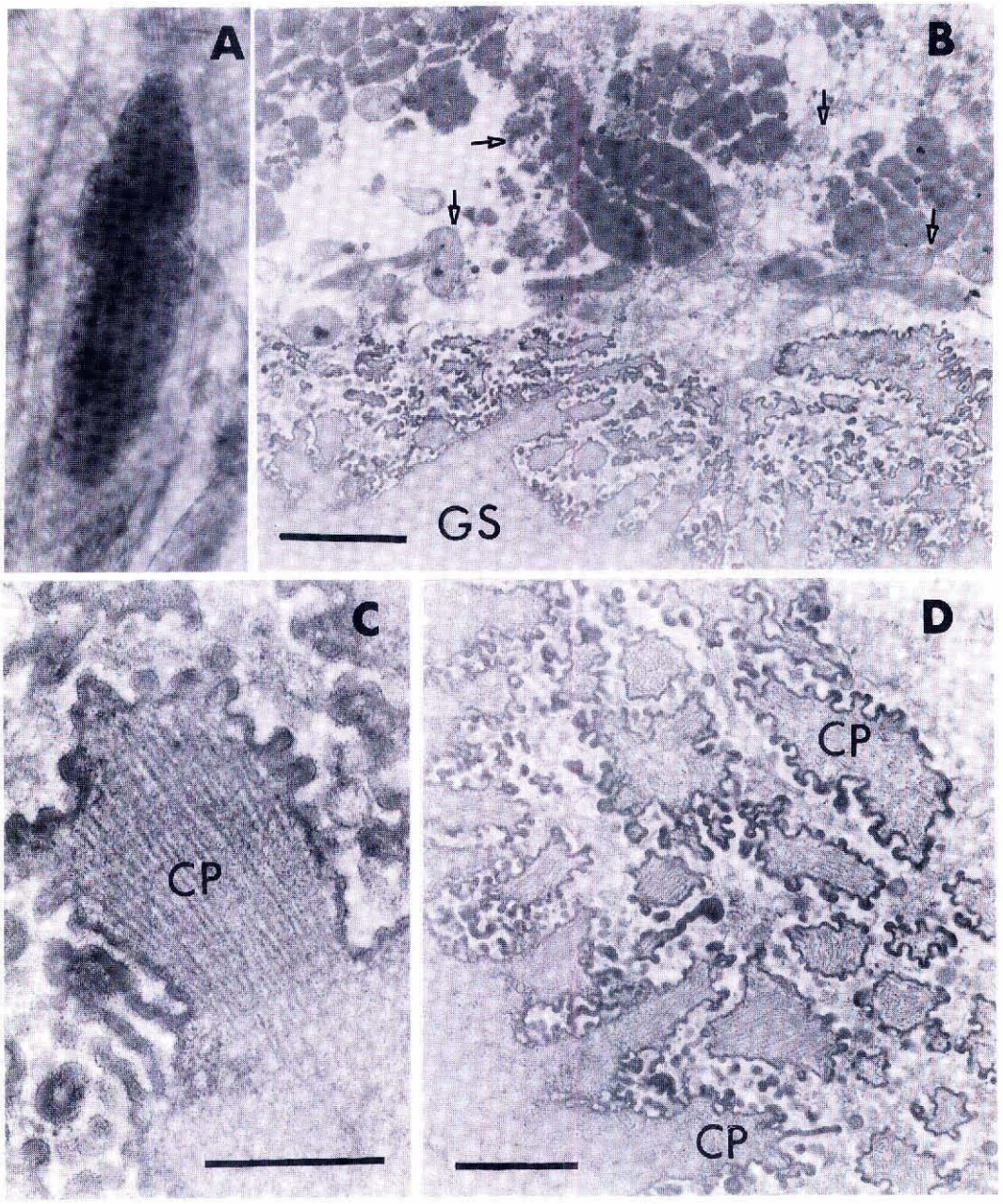

Fig. 3. A: Unstained muscle tissue smear (esophagus) with fusiform cyst (160 μm×45 μm); B, C & D: Ultrastructure of *S. fusiformis* macroscopic cyst wall showing primary cyst wall with highly anastomosing cauliflower-like projections (CP) with prominent microfibrils. Note: necrotic myofibrils and mitochondria (arrows). Ground substance (GS). Bar scale: B=2 μm; C=1 μm; D=0.5 μm.

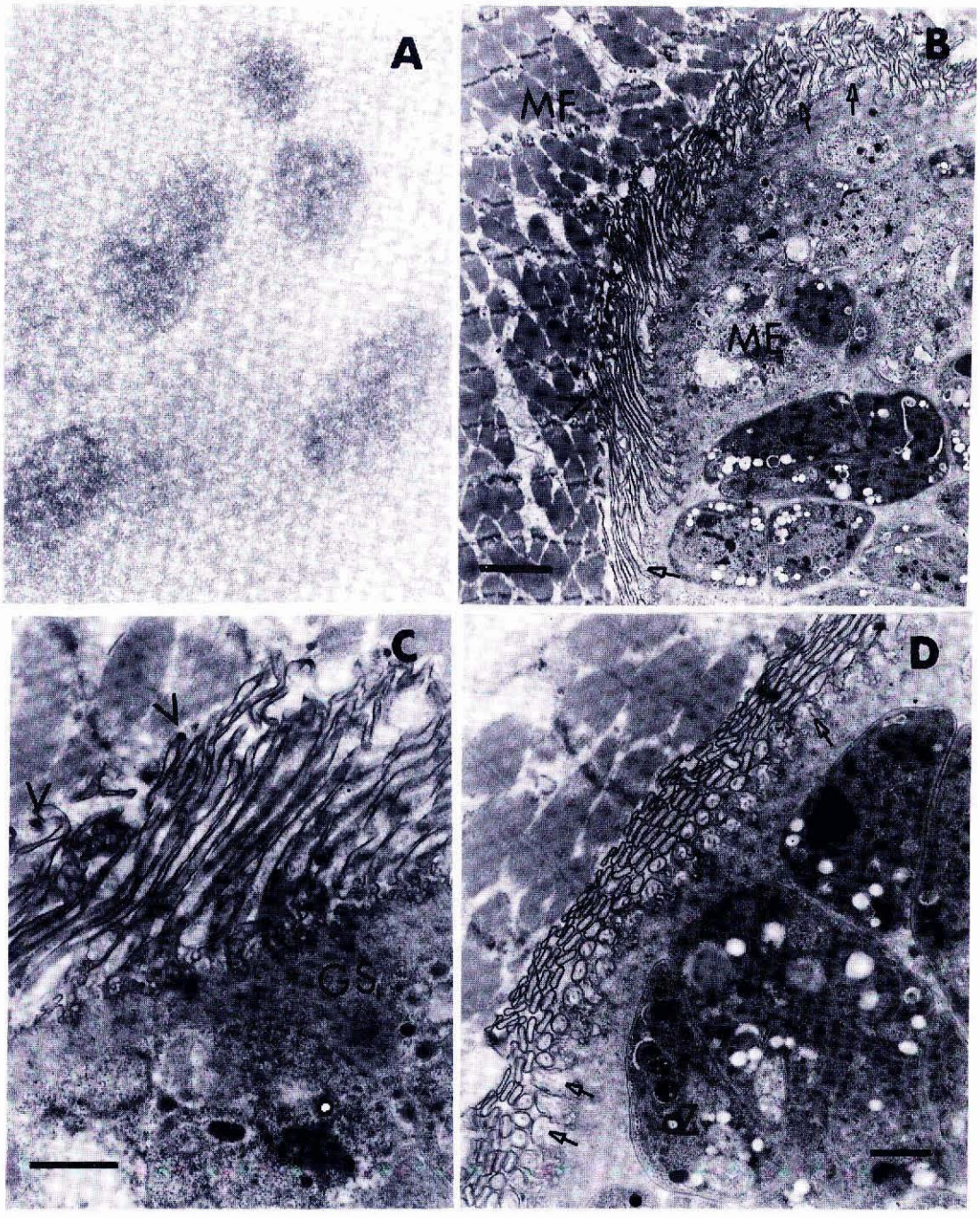

Fig. 4. A: Unstained muscle tissue smear (diaphragm) with globular to oval microscopic cysts (diameter=30-40 μm); B, C & D: Ultrastructure of the cyst wall with undulating villar protrusions bent and run parallel to the cyst surface (B) with conical tufts (arrow heads) in the distal tapering segment (C), and expanded/dome-shaped villar bases (arrows) in D. Note: zoites (Z) inside compartments, metrocytes (ME), myofibrils (MF) and ground substance (GS). Bar scale: B=2 μm; C & D=1 μm.

reaction was not evident. In ultrathin sections, damage to host cells was marked, with accompanying necrosis of both myofibrils and mitochondria (Fig. 3B).

There are striking similarities between the macroscopic and microscopic fusiform cysts as viewed with light microscopy. These include their seeming predilection to muscles of the esophagus, throat and limbs as sites of infection, sarcocysts containing zoites packed into compartments with prominent septal partitions, and possessing a cyst wall exhibiting hair-like projections. These observations suggest the likelihood of spindle-shaped microscopic cysts representing developing forms of *S. fusiformis*. In the absence however of ultrastructural analysis of spindle-shaped microscopic cysts present in all muscle tissue types examined, and in the light of another Type 23 macroscopic species, *Sarcocystis buffalonis* recently reported in water buffaloes in Vietnam (Houng et al. 1997a) showing similarities and differences in cyst wall ultrastructure with *S. fusiformis*, it may be premature to assume that they are all indeed developing cysts of *S. fusiformis*.

The globular to oval microscopic cysts show compartmentalized arrangement of zoites with septal partitions. The cyst wall consists of numerous, minute hair-like villar protrusions emanating from the primary cyst wall (Fig. 4). Villar protrusions have an expanded or dome-shaped base containing numerous prominent electron dense granules, an intermediate finger-like segment, and a tapering thin distal segment which at some points join together to form conical tufts. The intermediate and distal segments of the villar protrusions are bent about 90 degrees and run parallel to the cyst surface.

The ultrastructure of the globular to oval cysts documented in this paper is similar to that of the newly redescribed microscopic species, *Sarcocystis levinei* (Dissanaike and Kan 1978; Huong, Dubey and Uggla, 1997) occurring in the esophagus, tongue and striated muscles, and exclusively in the myocardium of water buffaloes in Vietnam (Huong et al. 1997b), and with that of *S. cruzi* in cattle (Dubey et al. 1989). In the Philippine and Vietnam *S. levinei* show differences in cyst size, which may be attributed to differences in the maturity of the cysts studied. The diaphragm of 35 carcasses showed globular to oval microscopic cysts only, and cervical muscles had mixed infections with more of the globular cyst than fusiform cysts present. These data suggest an apparent predilection of the Philippine *S. levinei* to the diaphragm and cervical muscle in contrast to the heart muscles in the Vietnam strain. This paper documents the first report of *S. levinei* in Philippine water buffaloes supported with ultrastructural analysis of the sarcocyst and its cyst wall, and likewise refutes earlier published reports that all microscopic cysts in Philippine water buffaloes are developing forms of *S. fusiformis*.

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