ULTRASTRUCTURAL STUDIES OF *SARCOCYSTIS CRUZI* (HASSELMANN, 1926) WENYON, 1926 INFECTION IN CATTLE (*BOS TAURUS*): PHILIPPINE CASES

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Summary :

This paper documents the first report of *Sarcocystis cruzi* infection in domesticated cattle (*Bos taurus*) in the Philippines. Fusiform-shaped microscopic sarcocysts (183-578 μm long and 20-98 μm wide) with distinct septae were found in the skeletal, striated and heart muscle. The sarcocyst wall or parasitophorous vacuolar membrane, 1.37-2.75 μm thick consisted of closely-packed villar protrusions 80-400 nm in dm. Middle and distal segments of VP were bent approximately 90 degrees parallel to the cyst wall surface. The villar core lacked microtubules, and at some points, the distal ends of the VP collectively formed conical tufts. Primary cyst wall had numerous 70-100 nm bubble-like undulations, and the ground substance was 0.25-0.5 μm in thickness. The ultrastructure of *S. cruzi* cyst wall typifies the Type 7 sarcocyst wall, and bears close similarities with the Philippine and the Vietnam strain of bubaline *Sarcocystis levinei*.

KEY WORDS : *Sarcocystis cruzi*, ultrastructure, Philippine cases, *Bos taurus*.

MOTS CLÉS : *Sarcocystis cruzi*, ultrastructure, cas philippin, *Bos taurus*.

Résumé : ÉTUDE ULTRASTRUCTURALE DE L'INFESTATION DU BOVIN (*BOS TAURUS*) PAR *SARCOCYSTIS CRUZI* (HASSELMANN, 1926) WENYON, 1926 : UN CAS PHILIPPIN

Il s'agit de la première observation de l'infestation du bovin domestique (*Bos taurus*) par *Sarcocystis cruzi* aux Philippines. Des sarcocystes fusiformes (183-578 μm de long sur 20-98 μm de large) avec un septum distinct ont été observés au niveau des muscles striés squelettiques et cardiaque. La paroi du sarcocyste ou membrane vacuolaire parasitophore, épaisse de 1,37-2,75 μm présente en un amas de protrusions villositaires (PV) de 80-400 nm de diamètre. Les segments moyen et distal des PV sont fléchis d'environ 90° par rapport à la surface de la paroi. Le corps de la villosité manque de microtubule et, en certains endroits, les extrémités distales des PV forment des huppées coniques. La paroi des cystes présente des ondulations qui ressemblent à des bulles de 70-100 nm, et la membrane basale est épaisse de 0,25-0,50 μm . L'ultrastructure de la paroi de *S. cruzi* est de même type que celle de la paroi du sarcocyste de Type 7, et présente d'étroites similitudes avec les souches de *Sarcocystis levinei* observées chez le bubale au Philippines et au Vietnam.

INTRODUCTION

Earlier studies in the Philippines had documented infection of water buffaloes (= carabaos) with *Sarcocystis fusiformis* (Railliet, 1897) Bernard & Bauche, 1912, and *Sarcocystis levinei* (Dissanaike & Kan, 1978) Huang, Dubey & Uggla, 1997 (Tongson & Molina, 1979; Parairo *et al.*, 1988; Claveria & Cruz, 1999; 2000). Worldwide, three different species of *Sarcocystis* are known to infect cattle, *Sarcocystis cruzi* (Hasselman, 1926) Wenyon, 1926 (syn: *Sarcocystis bovicanis*), *Sarcocystis hominis* (Railliet & Lucet, 1891) Dubey, 1976 (syn: *Sarcocystis bovihominis*) and *Sarcocystis hirsuta* (Moule, 1888) (syn: *Sarcocystis bovifelis*) (Heydorn & Rommel, 1972; Heydorn *et al.*, 1975;

Bottner *et al.*, 1987; Pacheco *et al.*, 1978, Dubey *et al.*, 1989a; 1989b). In 1978, Tongson & Pelagio reported the presence of zoites in ground beef sold in some selected supermarkets and wet markets in Metro Manila. Considering the high prevalence of bubaline *Sarcocystis fusiformis* then, and the absence of records of bovine sarcocystosis locally, they conjectured that the ground beef sold could have been a mixture of water buffalo and cattle meat (= carabeef). However, infection of cattle with *Sarcocystis* parasite was not confirmed then. In a recent survey on sarcocystosis in both Philippine homegrown and imported cattle, Claveria *et al.* (1999) had found round and fusiform-shaped sarcocysts in skeletal, striated and heart muscle, and they suggested possibilities of cattle infection with at least two of the species reported worldwide. In this paper, *S. cruzi* infection in Philippine domesticated cattle (*Bos taurus*) is documented.

MATERIALS AND METHODS

Eighty-eight sample sets, each consisted of muscle tissues from the diaphragm, pharynx, esophagus, neck, upper and lower limbs, and the heart

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Figs A-D. – *Sarcocystis cruzi* in bovine cervical muscle tissue section. A. Mature sarcocyst. Bradyzoites (arrowhead); Metrocytes (arrows). Bar: 13 μ m. B, C. Young sarcocysts with cross, longitudinal and tangential sections of villar protrusions (VP). Nucleus (N); Amylopectin granules (arrowhead). Bar: B = 2.5 μ m; C = 1.7 μ m. D. Portion of sarcocyst and its cyst wall. Ground substance (G); Bubble-like undulations (arrowhead); Conical tufts (arrows). Bar = 1 μ m. Note marked damage to host muscle tissue (HT) including displacement of Z-line.

were obtained from 2-to-6 year old *B. taurus* slaughtered in an abattoir located within Metro Manila. Tissue samples were collected within six months. The animals originated from a cattle farm in the Batangas Province, Island of Luzon. The actual number of cattle heads was not determined due to some limitation during the actual tissue sample collection.

Tissue samples were collected as early as 06-00 h, and were transported inside a cooler in properly-labeled plastic bags to the laboratory. The presence of both macroscopic and microscopic sarcocysts was determined within the day of collection. Those positive for sarcocysts were fixed in 10 % formalin and processed following the standard histologic and H & E staining procedures. Tissue samples (1-2 mm²) that matched those used for histological evaluation were fixed in ice-cold 2.5 % glutaraldehyde, and were subsequently processed for transmission electron microscopy (TEM Model Joel-1010, Japan).

RESULTS

Round or globular to oval, but mostly fusiform-shaped microscopic sarcocysts were found in the cervical, esophageal, pharyngeal, diaphragm, fore- and hind limbs, and heart muscle tissues. Serial tissue sections showed the presence of 2-to-6 sarcocysts. Mature sarcocysts measured 183-578 µm long (average: 374 µm) and 20-98 µm (average: 57 µm) across, and had numerous bradyzoites and some metrocytes within well defined septae (Fig. A). The sarcocyst wall or parasitophorous vacuolar membrane, 1.37-2.75 µm thick consisted of closely-packed villar

protrusions (VP), 80-400 nm in dm (Figs. B-D). Middle and distal segments of VP were bent approximately 90 degrees parallel to the cyst wall surface (Figs. C-D). The villar core lacked microtubules, and at some points, the distal ends of the VP collectively formed conical tufts (Fig. D). Primary cyst wall had numerous 70-100nm bubble-like undulations (Fig. D), and the ground substance (= ground layer) was 0.25-0.5 µm in thickness (Fig. D). Cyst wall ultrastructural descriptions typify the Type 7 sarcocyst wall (Dubey *et al.*, 1989a).

DISCUSSION

The variation in the number of sarcocysts noted in serial tissue sections suggests a difference in the grade, and age of *Sarcocystis* infection. While we noted some differences in the size of *S. cruzi* sarcocysts relative to those earlier reported, its cyst wall ultrastructure bears close similarities with that described by Mehlhorn *et al.* (1976), Pacheco *et al.* (1978), and Dubey *et al.* (1989b). *Sarcocystis cruzi* also shows resemblance with the Type 7 cyst wall of *Sarcocystis arieticanis* in sheep (Heydorn & Mehlhorn, 1987), *Sarcocystis rangi* in reindeer (Gierde, 1985), *Sarcocystis hircicanis* in goats (Heydorn & Unterholzner, 1983), and *S. levinei* infecting water buffaloes in Vietnam (Huong *et al.*, 1997) and in the Philippines (Claveria & Cruz, 2000).

The similarities in the ultrastructure of *S. cruzi* cyst wall in the present study with the Vietnam and the Philippine strain of *S. levinei* (Table I), and the utilization of dogs as definitive host of *S. cruzi* (Dubey, 1982) and *S. levinei* in Vietnam (Huong *et al.*, 1997) suggest pos-

Features	<i>S. cruzi</i> (P)*	<i>S. levinei</i> (P)**	<i>S. levinei</i> (P)***
I. Sarcocysts	Bradycoites in compartments Basophilic 183-417 µm long 20-98 µm dm Skeletal, esophageal, pharyngeal, cervical, diaphragm & heart muscle	Same Same No data 13-48 µm dm Skeletal, esophageal, tongue & diaphragm muscle	Same Same 640 µm long 95 µm dm Skeletal, esophageal, tongue & myocardium
II. Parasitophorous vacuolar membrane (= cyst wall)	Type 7	Same	Same
a. Villar protrusions	1.37-2.75 µm thick Lacks microfilaments With conical tufts 80-400 nm dm	2.3-2.75 µm thick Same Same No data	0.3-0.6 µm thick Same Same 80-500 nm dm
b. Basal cyst wall	With bubble-like undulations (70-100 nm)	Dome-shaped to expanded + base undulations in small clusters (0.33-1.6 µm long & 0.22-1.0 µm wide)	Dome-shaped + undulations (0.08-0.5 µm wide)
III. Ground substance	0.25-0.5 µm thick	0.42-0.5 µm thick	0.4-0.9 µm thick

* Present study; ** Claveria & Cruz, 2000; *** Huong *et al.*, 1997.

Table I. – Comparison of the ultrastructure of *Sarcocystis cruzi* with the Philippine and the Vietnam bubaline *Sarcocystis levinei*.

sibilities of parasite cross-transmission between cattle and water buffaloes, and the likelihood that *S. cruzi* and *S. levinei* are of the same species utilizing two different intermediate hosts. In a related study on cross-transmission, buffalo calves were not infected with the bovine *S. cruzi* (Jain & Shah, 1985). Interestingly, Wang *et al.* (1992) had reported successful cross-transmission of the bovine *S. cruzi* to buffalo calves that developed acute sarcocystosis, and subsequently died. The esophageal content used, and the dogs' status at the time of their exposure to sarcocysts however, were not established. With the detection of round or globular to oval sarcocysts in this study, we have not precluded the possibility of finding other *Sarcocystis* spp. infecting cattle. The isolation of other morphologic forms of bovine sarcocysts for ultrastructural evaluation is continuing in our laboratory. Finding the definitive host(s) of *S. cruzi* in domesticated cattle in the country is worth pursuing.

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