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Observation on Ultrastructure of Rabbit *Cysticercus pisiformis*

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Abstract: To observe ultrastructure of rabbit *Cysticercus pisiformis*. *Cysticercus pisiformis* of naturally infected rabbit was collected and prepared for Transmission Electron Microscope (TEM) examinations. Under TEM, cysticercosis pisiformis appeared to be ovoid form which comprised cyst wall, cyst fluid, scolex and cervical segment from outer to inner side. The structure of cyst wall was 3 layers (cortex, mesenchyina and parenchyma) of which the cortex was composed of 2 layers regular long strip shape cells, the mesenchyina included a large number of glycogen granules and some bunches of fiber and the parenchyma included two kinds of cells besides glycogen granules and fiber. One kind was calcareous corpuscles cell and the other was spindle cell with bigger nuclear. The scolex comprised cortex, mesenchyina and parenchyma from outer to inner side of which the outside cortex laid a large number of regular microvilli, the mesenchyina laid a large number of structureless fibrous material and glycogen granules and the parenchyma laid parenchymal cells, cortical cell, myoblast, flame cell, calcareous corpuscles cell, hamulus, collecting duct and excretory duct. The structure of cervical segment was similar with that of the scolex which included collecting duct and gather duct net. The ultrastructure of rabbit cysticercosis pisiformis was similar with that of the reported other cestode except a large number of regular microvilli in the cortex of scolex, sparser microvilli in cervical segment than scolex and no microvilli in cyst wall. Furthermore, 2 layers regular long strip shape cells were in the cortex of cyst wall which had no report in cysticercus cellulosae and sheep coenosis.

Key words: Rabbit (*Cysticercosis pisiformis*), ultrastructure, transmission electron microscope, coenosis, sheep

INTRODUCTION

Cysticercus pisiformis, metacestodes of *Taenia pisiformis*, parasitize liver capsule, greater omentum and mesenteric of rabbit and other rodent of which the final host is dog and fox. It is characterized by hepatic lesions with a worldwide distribution and often occurs at different areas of china. The prevalence rate is range from 14-100%. Serve infected rabbit will be dead and mortality rate is various from 4-23.96%. Light infected rabbit manifest as digestive disorder. Young rabbit mainly shows growth retardation and a feed conversion decrease of 16.7 and adult rabbit mainly shows emaciation. It can lead to immunity decline resulting from secondary disease easily. Therefore, the disease can make economic benefit decrease 87.01% and cause a serious threat to the development of rabbit keeping.

This disease is very popular in China and of great economic losses. However, only Sun *et al*. (2008). reported light-microscopic structure of cyst wall, scolex and cervical segment of rabbit *Cysticercus pisiformis*. At present, there is still no report about ultrastructure of rabbit *Cysticercosis pisiformis*. In view of this, the experiment observed ultrastructure of rabbit cysticercosis pisiformis to enrich and perfect its basic structure which can lay a foundation for the studies on the pathogenesis of rabbit *Cysticercosis pisiformis*.

MATERIALS AND METHODS

Reagents and equipment: About 2.5% glutaraldehyde was purchased from American Sigma Company; LKB-V ultramicrotome was purchased from Swedish LKB Company; EM-1230 Electron Microscope was purchased from Japanese LEOL Company; SONY-6210 Microscopic Imaging System was purchased from Japanese SONY Company.

Sample collection and treatment: A rabbit farm in He Nan Province of China was selected to carry epidemiological investigation, 32 positive rabbits detected with ELISA were taken blood and anatomized, mature rabbit *Cysticercosis pisiformis* adhering on greater omentum and mesenteric were collected, washed with physiological

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saline, fixed with 2.5% glutaraldehyde and then stored at 4°C. The fixed *Cysticercosis pisiformis* were taken out and separated into cyst wall, scolex and cervical segment. The cyst wall was prepared into 1×1 mm tissue blocks and scolex and cervical segment were prepared into 1×1×1 mm tissue blocks, all tissue blocks were carried prior fix with 40 g L^{-1} paraformaldehyde and 20 mL L^{-1} glutaraldehyde, washed 3 times with 0.1 mol L^{-1} two sodium cacodylate buffer, 10 min each time and then carried posterior fix with 10 g L^{-1} OsO_4.

Electron microscopy sample preparation: Fixed tissue blocks of cyst wall, scolex and cervical segment were taken out to trim as the requirement of preparation for electron microscopy sample, gradient dehydrated 10 min per time in 500, 700, 900, 1000 mL L^{-1} acetone, conventionally imbued 1 h in mixture of 1 fold acetone and 3 fix embedding medium at room temperature, embedded with 618 epoxy resin, infiltrated 1 h in oven and then polymerized 30 and 60 min at 60 and 100°C, respectively. The treated tissue blocks were repaired with routine method, cut into 5 nm section with LKB-V ultramicrotome and stained with uraninm and aluminium. The sections were observed with JEM-1230 TEM.

RESULTS AND DISCUSSION

Ultrastructure of scolex: The scolex comprised cortex, mesenchymal and parenchyma from outer to inner side (Fig. 1a). Cortex included 3 layers: villi, matrix and basement membrane. Villi were located on the surface of cortex, comparatively dense, about 2 μm length which may be divided into basal part and end part. Basal part was about 1 μm length and 0.1 μm width, end part was about 0.5 μm length and 0.05 μm width (Fig. 1a). Basal part was hollow, no core and its transsection presented on annular. The juncture of basal part and end part had high dense material which accumulated toward the center of villi and extended to the end part to form dense annular. Matrix was between the villi and basement membrane which is composed by a layer very thin, regularly arrayed fiber (Fig. 1b).

Mesenchymal was the region below the basement membrane and exhibited a fibroblast-like morphology which had no obvious boundary of distinguishing with parenchyma and dendritically extend toward parenchyma. A large number of glycogen granules were between the fibers and collecting duct, gather duct and flame cell can be observed (Fig. 1c).

Parenchyma had a large number of fiber like materials and included the parenchymal cells, cortical cell, myoblast, flame cell, hamulus, collecting duct and excretory system in parenchyma. The parenchymal cells had long cytoplasmic processes, many ribosomes, smooth endoplasmic reticulum and few reticulums and a large number of glycogen granules composed 50% of the cytoplasm and extend to the cytoplasmic processes; the shape of cortical cell was irregular, its cytoplasmic processes was very long forming channel and extending to cortical layer via basement membrane; flame cell appeared as groups of 3-5 cells, its soma had

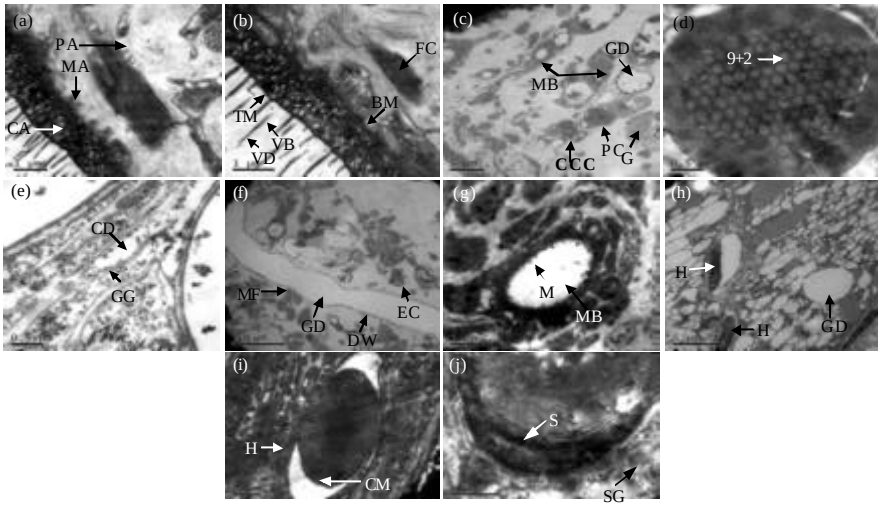

Fig. 1: Ultrastructure of scolex; a) The whole structure of scolex 20000x; b) Cortex of scolex 25000x; c) Cell and structure of parenchyma 4000x; d) Flame cell 40000x; e) Collecting duct 40000x; f) Gather duct 1500x; g) Gather duct 25000x; h) Hamulus 6000x; i) Core of hamulus 8000x; j) Wall layer of hamulus 30000x

much processes, its cytoplasm had smooth endoplasmic reticulum, polyribosome, vesicle and more mitochondria, the nucleus was bigger and the void was about 0.03 μm between nuclear membrane and circular nucleolus, the stereociliary bundles was in the one side of cell which presented a stripe-like when slitting line and appeared 70~80 branches cilia arraying together regularly when crosscutting, each bunch cilia had a outer membrane and arrayed hexagonal each other, the cilia had 2 central fibers and 9 pair of peripheral fibrils forming the structure similar as 9+2 (Fig. 1d); excretory system was very perfect and divided into collecting duct and gather duct according to its structure and diameter, the lumen of collecting duct was smaller with a irregular diameter which was stenosis in some part and even integrated together to form the net of collecting duct (Fig. 1e), the lumen of gather duct was bigger, the duct wall had internal and external layers (Fig. 1f), the internal layer had much villi-like material arraying regularly (Fig. 1g); hamulus, oblate, appeared in pairs and arrayed regularly (Fig. 1h); the core was homogeneous and presented ellipse (Fig. 1i), the great void was between the core and wall layer, wall layer included 5~6 fibrage (Fig. 1j).

Ultrastructure of cervical segment: The cervical segment may also divided into cortex, mesenchymal and parenchyma from outer to inner side. The structure of cortex was similar as that of scolex. Much villi adhered on the surface of the cortex but sparse than scolex. Massive fiber-like material was observed in mesenchymal. Parenchyma included parenchymal cells, flame cells and the net of collecting duct and gather duct (Fig. 2a, b).

Ultrastructure of cyst wall: The cyst wall included 3 layers: cortex, mesenchymal and parenchyma. The outer layer of cyst wall was cortex which was composed by two layers of long strip cells arraying regularly. The nuclear was smaller with uniform cytoplasm (Fig. 3a). Subcutaneous tissue was mesenchymal that had much bunches of fiber-like material, a number of glycogen granules distributed between fibers. Parenchyma appeared 3 types of cells, the first type of cell was irregular shape, bigger nuclear about 1/2-1/3 of whole cell, karyolobism and much smooth endoplasmic reticulum, ribosome, mitochondria and golgi complex in cytoplasm (Fig. 3c). The second type of cell was very long and stripy cytoplasmic processes, ellipse nucleus with uniform cytoplasm, more glycogen granules, few smooth endoplasmic reticulum and mitochondria in cytoplasm (Fig. 3b). The third type of cell was bigger and irregular shape. The nuclear presented irregular inverted spindle

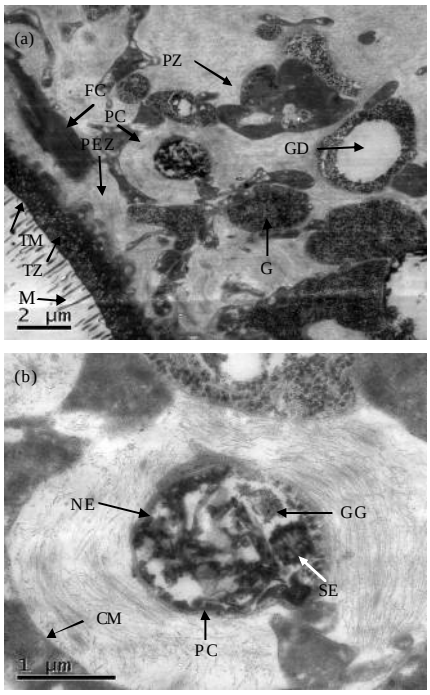

Fig. 2: Ultrastructure of cervical segment; a) The whole structure of cervical segment 12000x; b) Parenchyma cell 3000x

and the bigger end split, smooth endoplasmic reticulum, ribosome, mitochondria and so on can be observed in cytoplasm and a number of glycogen granules can be observed in cytoplasm also (Fig. 3a).

Tian *et al.* (1995a), Wang *et al.* (2009) and Yan *et al.* (1994) researched the ultrastructure of cysticercus cellulosae in 1995, the ultrastructure of cervical segment and cyst wall had been described but the concept of scolex was not put forward. Sun *et al.* (2008) and Newman *et al.* (1993) reported the microstructure of *Cysticercus pisiformis* and concluded that *Cysticercus pisiformis* can obvious divided into 3 parts including scolex, cervical segment and cyst wall. On this basis, the experiment observed the ultrastructure of scolex, cervical segment and cyst wall and found that the ultrastructure of rabbit *Cysticercosis pisiformis* was similar as that of reported taeniid metacestodes but there were some difference in ultrastructure between rabbit *Cysticercosis pisiformis* and other *Taeniid metacestodes*.

Much microvilli were located on the surface of scolex cortex of rabbit *Cysticercosis pisiformis* which enlarged contacting area between scolex and cyst wall so as to exchange material beneficially. Much dendritic fibroblast-like material distributed in the mesenchyina and extended to the parenchyma for playing a stent role. A large number

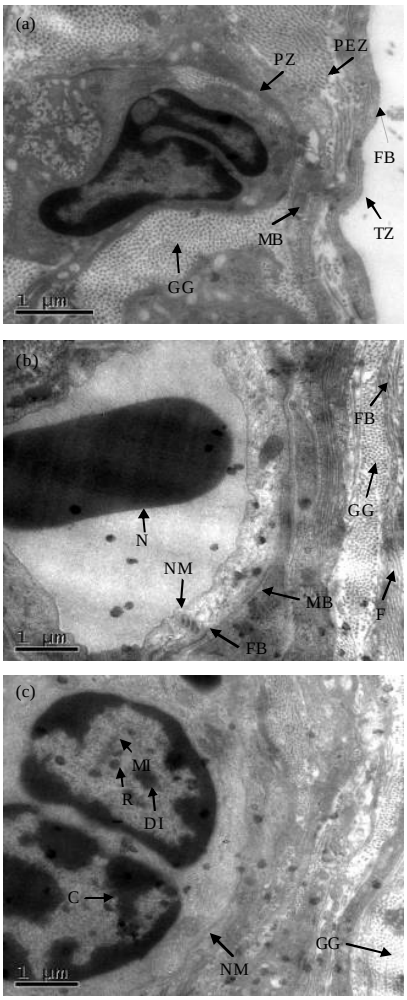

Fig. 3: Ultrastructure of cervical segment; a) The whole structure of cyst wall 25000x; b) Mesenchyina of cyst wall 20000x; c) Parenchyma of cyst wall 20000x

of glycogen granules between the fibers may provide nutrition for cysticercosis pisiformis itself. Much villi adhered on the surface of the cervical segment cortex but sparse than scolex. The parenchymal cells had long cytoplasmic processes, many ribosomes, smooth endoplasmic reticulum and smooth endoplasmic and few reticulums and a large number of glycogen granules composed 50% of the cytoplasm and extend to the cytoplasmic processes; flame cell appeared as groups of 3-5 cells and connected with excretory ducts forming perfect excretory system to excrete metabolites, regulate body fluid and reabsorb some nutrient. The internal wall of gather duct had the microvilli which increased surface area of internal wall and accelerated excretion by means of microvilli movement. Cortical cell, irregular shape, very long cytoplasmic forming channel and extending to cortical layer via basement membrane, played the regeneration role for the cortex. Similar structure of cortex, mesenchyina and parenchyma may be observed between the scolex and cervical segment so, the cervical segment may be concluded as the extension of scolex. But the excretion system ofcervical segment such as flame cells and gather duct was more perfect than that of scolex so, the scolex may mainly played excretion role (Zhang *et al*., 1994, 2012). That no microvilli can be observed in the cyst wall of *Cysticercus pisiformis* but much microvilli in the cyst wall of *Cysticercus cellulosae* and sheep coenosis was difference from the microstructure of other *Taeniid metacestodes*. A kind of cells in cyst wall, very long and stripy cytoplasmic processes, ellipse nucleus with uniform cytoplasm, more glycogen granules, few smooth endoplasmic reticulum and mitochondria in cytoplasm may be fibroblast. The long strip cells with smaller nuclear in cyst wall may be epithelioid cell but no report in cysticercus cellulosae and sheep coenosis (Tian *et al*., 1995a, b). A number of glycogen granules in mesenchyina and parenchyma of scolex, cervical segment and cyst wall may offer nutrition.

More flame cells distributed in scolex than cervical segment which appeared as groups of 3-5 cells and arrayed around the collecting duct and gather duct 9+2 cross section structure can be observed in flame cells of *Cysticercus cellulosae* which was same as other cestode that had reported. Oblate hamulus appeared in pairs and arrayed regularly in scolex of which the core was ellipse and homogeneous. The great void was between the core and wall layer, the wall included 5~6 layers but its specific structure still is unclear.

CONCLUSION

The experiment firstly observed the ultrastructure of rabbit *Cysticercosis pisiformis* and confirmed that the ultrastructure of rabbit *Cysticercosis pisiformis* was similar with that of *Cysticercus cellulosae* and sheep coenosis, except that a large number of glycogen granules distributed in cyst wall, scolex and cervical segment. Furthermore, the cyst wall outer of *Cysticercus cellulosae* and sheep coenosis had microvilli which was no found in rabbit *Cysticercosis pisiformis* but much microvilli distributed in the cortex of cervical segment and scolex. That two layers regular long strip shape cells were in the cortex of cyst wall of rabbit *Cysticercosis pisiformis* had no report in *Cysticercus cellulosae* and sheep coenosis.

NOMENCLATURE

CA = Cortical Area
MA = Mesenchymal Area
PA = Parenchyma Area
VD = The Distal part of the Villi
VD = The basical part of the Villi
TM = Tunica Matrix
MZ = Matrix Zone
BM = Basement Menbrance
MB = Myoblast
PC = Parenchchyma Cell
CCC = Calcareous Corpuscle Cell
FC = Flame Cell
CD = Collecting Duct
GDL = Gather Duct
DW = Duct Wall of collecting duct
MF = Muscle Fibe
EC = Excrete Cell
GG = Glycogen Granula
G = Granula
V = Villi
H = Hamulus
MI = Mitochondria
CM = Cell Membrane
NE = Nucler Envelope
SE = Smooth Endoplasmic reticulum
FB = Fibroblast
F = Fibrage
DI = Dictyosome
R = Ribosome
C = Chromatin
SG = Stratum Granulosum

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