

INTRAVASCULAR DETECTION OF *GIARDIA* TROPHOZOITES IN NATURALLY INFECTED MICE AN ELECTRON MICROSCOPIC STUDY

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Summary :

During routine transmission electron microscopic (TEM) examination of mice naturally infected with *Giardia muris*, an intense infection with *Giardia* trophozoites was demonstrated within intestinal and renal tissues. Examination of randomly taken sections from these heavily infected tissues revealed marked deep affection with mixed pathology. Duodenal sections were found loaded with *Giardia* trophozoites in intimate contact with necrotic gut cells. Some of these trophozoites were detected within central lacteal of damaged villi and nearby blood vessels. Interestingly, and for the first time to be demonstrated, morphologically identical *G. muris* trophozoite was detected in a renal blood vessel. An intense cellular immune reaction was obviously demonstrated with remarkable interaction between giant macrophages and the trophozoites particulates. Involvement of deep tissues by *Giardia* trophozoites and their presence within vascular channels could open up questions about the possible invasive and disseminative behavior of *G. muris*, particularly in heavily and naturally infected hosts.

KEY WORDS : *Giardia muris*, giardiasis, intra-vascular, intra-lacteal, electron microscopy, naturally infected mice.

Although *Giardia* isolates from primates and rodents display the same polymorphism as human, however, there is still debate over the dramatic variation in the host susceptibility, and zoonotic potentials of these isolates (Wolfe, 1992; Garcia & Bruckner, 1997). Primarily, all *Giardia* species inhabits the intestinal epithelium of their hosts. A complex interaction between the parasite and the intestinal surface comprising number of mechanisms by which *Giardia* affect its host have been proposed (Gillon, 1984; Buret *et al.*, 1990; Katelaris & Farthing, 1992). Despite, studies of the pathological effects of *Giardia* on the host, including those of the intestinal epithelia in man and experimentally infected animals, little is known about these effects on tissues of naturally infected laboratory reared mice (EL-Shoura *et al.*, 1993).

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Résumé : DÉTECTION INTRAVASCULAIRE DE TROPHOZOITES DE *GIARDIA* CHEZ LA SOURIS NATURELLEMENT INFECTÉES, OBSERVATION EN MICROSCOPIE ÉLECTRONIQUE

À l'occasion d'un examen en microscopie électronique de souris naturellement infectées par *Giardia muris*, des trophozoites de *Giardia* en très grand nombre ont été observés au niveau des tissus intestinaux et rénaux. Un examen plus précis de sections de ces tissus a révélé une infestation pathologique très marquée à deux niveaux. Les sections duodénales étaient remplies de trophozoites en contact étroit avec des cellules digestives nécrosées. Certains de ces trophozoites ont été détectés au contact des vaisseaux sanguins et lymphatiques à l'intérieur des villosités déteriorées. Il est intéressant de noter que pour la première fois des trophozoites de *G. muris* ont été détectés dans les vaisseaux sanguins rénaux. Une réaction cellulaire intense a été observée avec d'importantes interactions entre cellules macrophagiques géantes et trophozoites. L'invasion des tissus profonds par des trophozoites de *Giardia* et la présence de ceux-ci au niveau des vaisseaux ouvre sur des questions au sujet des modalités d'invasion et de dissémination de *G. muris*, particulièrement chez des hôtes naturellement fortement parasités.

MOTS CLÉS : *Giardia muris*, intravasculaire, intralymphocytaire giardiose, microscopie électronique, souris, infestation naturelle.

Giardia muris in mice usually causes deep tissue affection (Owen *et al.*, 1981). In this respect, it has been identified in urinary tract, and recovered from the bronchoalveolar lavage (Meyer *et al.*, 1977; Osterholm *et al.*, 1981; Steven & Vermeir, 1981). However, no documented reports have been published so far for demonstration of these parasites extra-intestinally or intravascularly. Herein, we demonstrated heavy *Giardia muris* trophozoites in renal and intestinal tissues of naturally infected laboratory reared mice. Moreover, and for the first time to be demonstrated intravascular existence of these trophozoites was clearly recognized.

MATERIALS AND METHODS

ANIMALS

Laboratory reared albino mice (Tuck ordinary strain), naturally infected with *Giardia muris* as detected by screening of their fecal suspension (Robert-Thomson *et al.*, 1976), were exposed to light and transmission electron microscopic (TEM) exami-

nation. Twelve out of twenty mice examined were found heavily infected with *Giardia muris*. Five out of these heavily infected mice were randomly chosen and sacrificed, together with five uninfected mice used as controls. Mice were rapidly dissected and segments from brain, liver, kidney and duodenum were removed. Specimens were fixed in alcoholic Bouin with 10 % buffered neutral formalin for subsequent light microscopic examination. Similar sections were fixed in 2.5 % glutaraldehyde for transmission electron microscopic examination.

TRANSMISSION ELECTRON MICROSCOPY

Specimens prepared for TEM were cut into 2-3 mm² sections and immediately fixed in 2.5 % glutaraldehyde with 0.1 M sodium cacodylate buffer (pH 7.2) at 4° C for two-three hours. Specimens were postfixed in 1 % osmium tetroxid, dehydrated in an ascending dilutions of ethyl alcohol, and embedded in spurr's resin. Semi-thin sections (0.5 µm) were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Jeol 12000 EX TEM, at 80 KV (Glauret, 1980).

RESULTS

Light and TEM examination of the intestinal specimens taken from naturally infected mice revealed massive *Giardia* trophozoites at the brush border and in between the intestinal villi (Fig. 1A & B). However brain and liver specimens from the same mice were found free from any parasite (data not shown). In two of the infected mice, TEM revealed some trophozoites within central lymphatics of the intestinal villi in an intimate contact with blood capillaries. The walls of these lymphatics as well as the epithelial linings were severely necrotic (Fig. 1C & D). *Giardia* trophozoites were detected in renal specimens from the same mice at where they found within and around lumen of some proximal tubules. In one of the infected mice, *Giardia* trophozoite was clearly demonstrated inside a renal capillary. The internal fine structures of this trophozoite was obviously seen (Fig. 2A & B). Active plasma cells, monocytes with esinophils were remarkably detected nearby the intravascular trophozoite and at the interstitium of the renal tubules. Small and large macrophages (giant macrophages), were found in sizable number accumulating in renal blood vessels (Fig. 2C), and within the lumen of renal tubules (Fig. 3A & B). Other giant macrophages were seen vacuolated with *Giardia* particulates (Fig. 3C). Giant macrophages were found to fix themselves to the proximal convoluted tubules by tugging a branched

part of the basement membrane of the tubule. Remarkable mitochondrial and Golgi bodies were obviously seen at the point of fixation (Fig. 3D).

DISCUSSION

In naturally infected mice with *Giardia muris*, giardiasis is reported to be heavy with marked tissues damage (EL-Shoura *et al.*, 1993). In such infection *Giardia* has been reported to penetrate the mucosa of the intestinal epithelium (Owen *et al.*, 1981). However, not too much that has been published on the ultra-structural changes during such kind of infection.

TEM examination of naturally infected mice in our laboratory revealed a heavy duodenal and renal infection with *G. muris*, and demonstrated trophozoites within their vascular channels. The detection of these trophozoites in these uncommon sites represents the first demonstration of one of the *Giardia* species extra-intestinally and intravascularly. The lower duodenal specimens taken from these mice were loaded with *Giardia* trophozoites with mixed pathological changes ranging from mild to remarkable tissues necrosis. The detected structural alterations were similar to those occurring in experimentally infected mice (Gillon *et al.*, 1982). *Giardia muris*, however, was more invasive and destructive in the naturally infected mice. Clearly demonstrated trophozoites were seen in close contact with necrotic blood capillaries and within central lymphatic in these duodenal sections.

Moreover, *Giardia* trophozoite was located within the lumen of a renal blood capillary. Trophozoites in lacteal and in the renal capillaries, both demonstrated the main fine structures of the *Giardia muris* trophozoite as previously described in other murine models (Owen *et al.*, 1979). The intra-lacteal existence of the *Giardia* trophozoites could represent the entrance through which such trophozoites arrive at the renal tissues. The survival of *Giardia* trophozoites within central lacteal (which acts as a rich medium for nourishment), could participate in the complex mechanisms by which absorption of most of the metabolites and not only fat are impaired during giardiasis (Tandon *et al.*, 1977; Katelaris & Farthing, 1992; Ellis *et al.*, 1996).

Host defense against giardiasis is mediated by T-cell dependent antibodies and involves antibody-mediated cytotoxicity together with ingestion of the parasite by macrophages (Smith, 1985; Hill & Pohl, 1990; Chen *et al.*, 1995). A marked cellular reaction was clearly seen in close contact with the detected trophozoites in blood and in renal tubules.

TEM examination of this cellular reactions demonstrated small and large sized macrophages. Many of these cells were found phagocytosing *Giardia* parti-

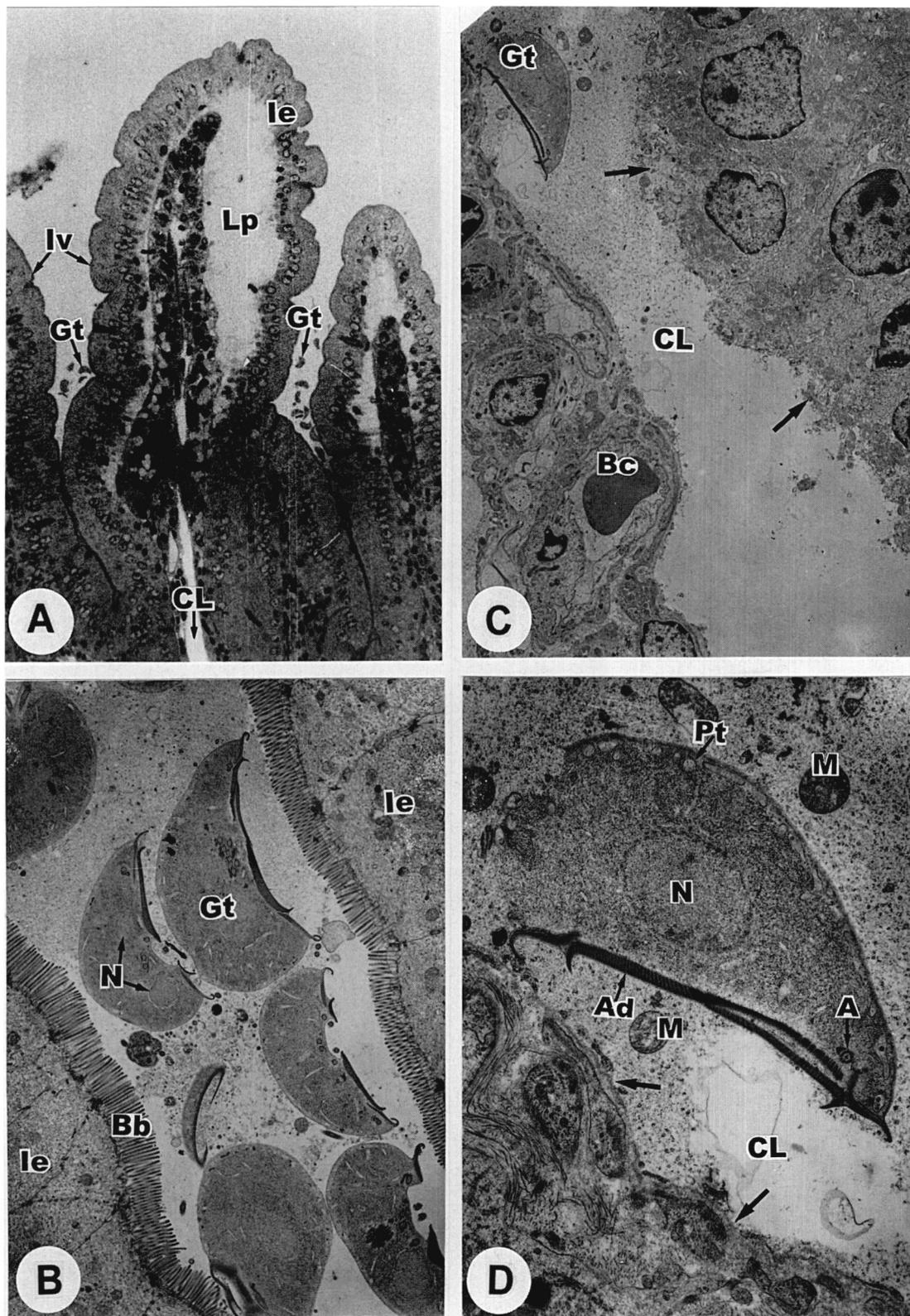


Fig. 1. – **A**: Light micrograph of semithin section from naturally infected mouse duodenum demonstrating intestinal villi (Iv), covered with the intestinal epithelium (Ie). Many *Giardia* trophozoites (Gt), are demonstrated in between the villi. Lamina propria (Lp). Central lacteal (CL). $\times 500$. – **B**: Transmission electron microscopic (TEM) picture of the same section in A, showing heavy intestinal infection with *Giardia* trophozoites (Gt). Intestinal villi (Iv). Intestinal epithelium (Ie). $\times 4,500$. – **C**: TEM of intestinal villous demonstrating *Giardia* trophozoites (Gt), within central lacteal (CL). Arrows pointed to necrotic lymphatic wall. Blood capillary (Bc). Intestinal epithelium (Ie). $\times 3,600$. – **D**: Higher magnification of the *Giardia* trophozoite seen in C. Adhesive disc (Ad). Nucleus (N). Peripheral tubules (Pt). Mitochondria (M). Axoneme (A). Central lymphatic (CL). Big arrows pointed to the lymphatic wall. $\times 10,600$.

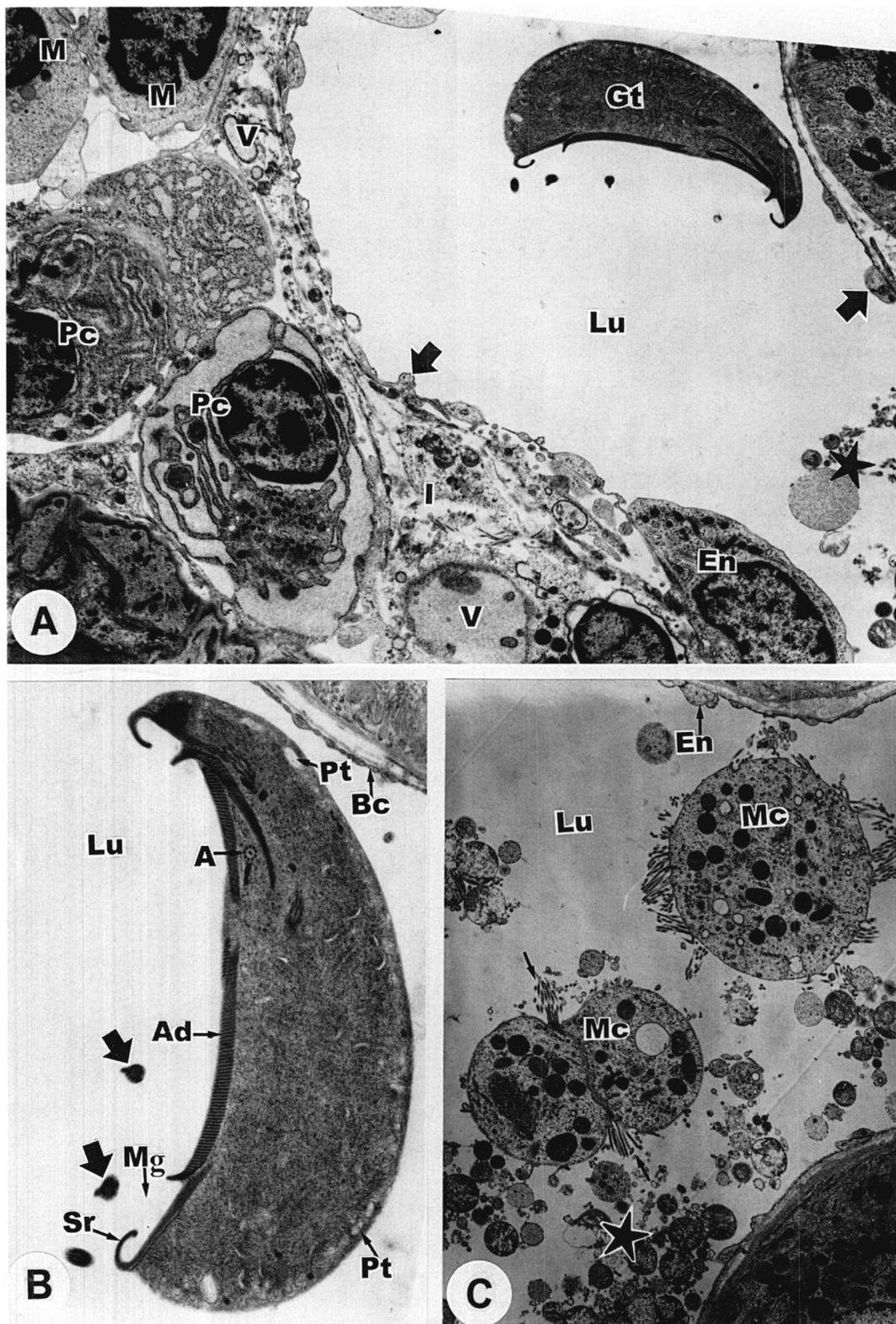


Fig. 2. – **A**: TEM photograph showing the lumen (Lu) of a blood capillary in the interstitium of renal tissue (I), with intra capillary *Giardia* trophozoite (Gt). Note accumulation of inflammatory cellular reaction including monocytes (M), active plasma cells (Pc). Endothelial cell (En) of blood capillary (thick solid arrows). Vacuoles (V). Star represents cellular organelles. $\times 5,500$. – **B**: Higher magnification showing fine ultrastructures of *Giardia* trophozoite seen in A. Peripheral tubule (Pt). Axoneme of the ventral flagellum (A). Adhesive disc (Ad). Marginal groove (Mg). Striated rim of the groove (Sr). Flagellae (thick solid arrows). Blood capillary (Bc). $\times 11,400$. – **C**: TEM photograph demonstrating two sized small and large macrophages (Mc). Small macrophage appeared dividing with two desmosomes (small arrows) inside a renal blood vessel (Lu). Endothelium of blood vessel (En). Star represents fragmented cellular organelles. $\times 4,700$.

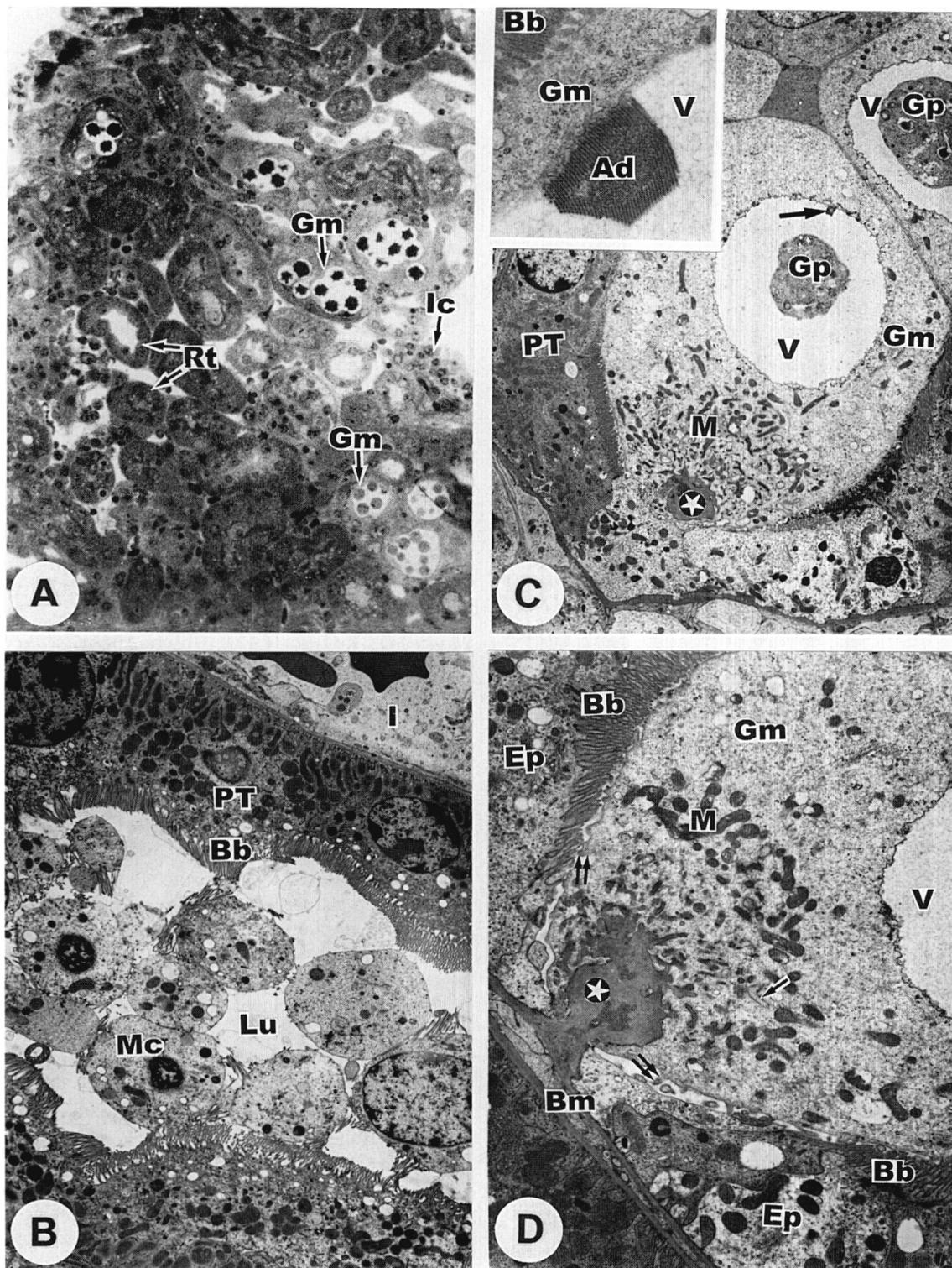


Fig. 3. – **A**: Light micrograph of semithin section of renal tubules (Rt) showing many inflammatory cells (Ic), and giant macrophages (Gm), containing many autophagic vacuoles. $\times 500$. – **B**: TEM of the lumen of renal tubule (Lu) showing many macrophages (Mc). Proximal tubule (PT). Interstitium (I). Brush border (Bb). $\times 3,100$. – **C**: TEM of one giant macrophage (Gm), in a proximal renal tubule (PT) demonstrating large autophagic vacuole (V), with *Giardia* particulate (Gp), and many mitochondrial bodies (M). Arrow points to adhesive disc of *Giardia* trophozoite. Brush border (Bb). Star points to irregular part of proximal tubular membrane and start of fixation of macrophage. $\times 5,500$. Insert: shows a magnification of the autophagic vacuole (V), with a part of *Giardia* adhesive disc (Ad). $\times 20,000$. – **D**: Higher magnification of giant macrophage (Gm) showing a branched part (star) of the tubular basement membrane (Bm). Many mitochondria (M), with few endoplasmic reticulum cisternae (single arrow). Note surface projections of macrophage (double arrows). Epithelial cell (Ep). Brush border (Bb). Autophagic vacuole (V). $\times 6,800$.

culates. Both types were found actively vacuolated with many mitochondrial and Golgi bodies. Two different types of macrophages structurally similar to those described here were found during chronic fungal infection (El-Shoura, 1993). An interesting tugging of the macrophages and fixation to the basement membrane was noticed. This phenomenon could represent an extra-cellular secretory process for degradation of various biological materials or devitalized cells (El-Shoura, 1994), or it might be a fixation process against intratubular movement. The phagocytic role of macrophages against *Giardia* has been described before in Peyer's patches (Hill & Pohl, 1990). Together with the detected active macrophages; remarkable active plasma cells were demonstrated. These cells are the main source for IgM and IgE needed for controlling giardiasis (Smith, 1985; Garcia & Bruckner, 1997).

All other cells representing the antibody-mediated cytotoxicity against giardiasis were observed e.g. eosinophils, monocytes, blood platelets, and fibroblast. The extra-intestinal, intra-vascular giardiasis detected in this report could throw light on the possible invasive behavior of these parasites. This behavior could be due to a *Giardia* toxin that helps invasion and lysis of the tissues. In this respect, cystein rich surface protein (GRP136) was previously addressed as first evidence for potential *Giardia* toxin that causes severe giardiasis (Chen *et al.*, 1995).

The demonstration of the *Giardia muris* trophozoites intravascularly and extra intestinally which has not been reported before, could be of particular significance especially with zoonotic potentials and lack of host specificity. However, the mechanism(s) by which this intestinal protozoan parasite could reach these sites, and the dramatic variation of the host susceptibility, remained to be clarified more on large scale research.

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