

The ultrastructure of the intestinal microvillous border in the common vole (*Microtus arvalis*) naturally infected with *Giardia microti*

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Abstract. The anterior jejunum from common vole naturally infected with *Giardia microti* (Kofoid et Christiansen, 1915) was examined by TEM and compared with the anterior jejunum from control (metronidazole-treated, *Giardia*-free) common voles (Pallas, 1778). *Giardia microti* infection resulted in significant diffuse shortening of microvilli and significant greater microvillous diameters. In addition, deformations of the microvilli were observed at the margin of the ventral disc. The microvilli attached to the lateral crest of the ventral disc were vesiculated with a disorganised filamentous core and contained whorled structures resembling "myelin-like figures". The findings are discussed in context of the *Giardia*-epithelial cell interaction.

Various investigators have described ultrastructural intestinal injury in human giardiasis (Morecki and Parker 1967, Gillon and Ferguson 1984, Ferguson et al. 1990) and in laboratory animal models infected with *G. muris* (Erlandsen and Chase 1974, Owen et al. 1979, Buret et al. 1990, Majewska et al. 1990) or *G. duodenalis* (syn. *G. lamblia*, *G. intestinalis*) (Buret et al. 1991, 1992). Recently, others described the ultrastructural damage induced by *G. duodenalis* trophozoites to cultured cells *in vitro* systems (Chávez et al. 1986, Magne et al. 1991, McCabe et al. 1991, Pedago and de Souza 1994).

The diplomonad flagellates belonging to the genus *Giardia* (Diplomonadina, Hexamitidae) are frequently found in the intestine of various species of voles (Kulda and Nohýnková 1978, Pacha et al. 1987). Parasitological examination in our laboratory-reared colony of common voles showed that they were infected with *Giardia microti*. The aim of the present study was to characterize the effect of the *Giardia microti* infection on intestinal microvillous border ultrastructure in the common vole, *Microtus arvalis*.

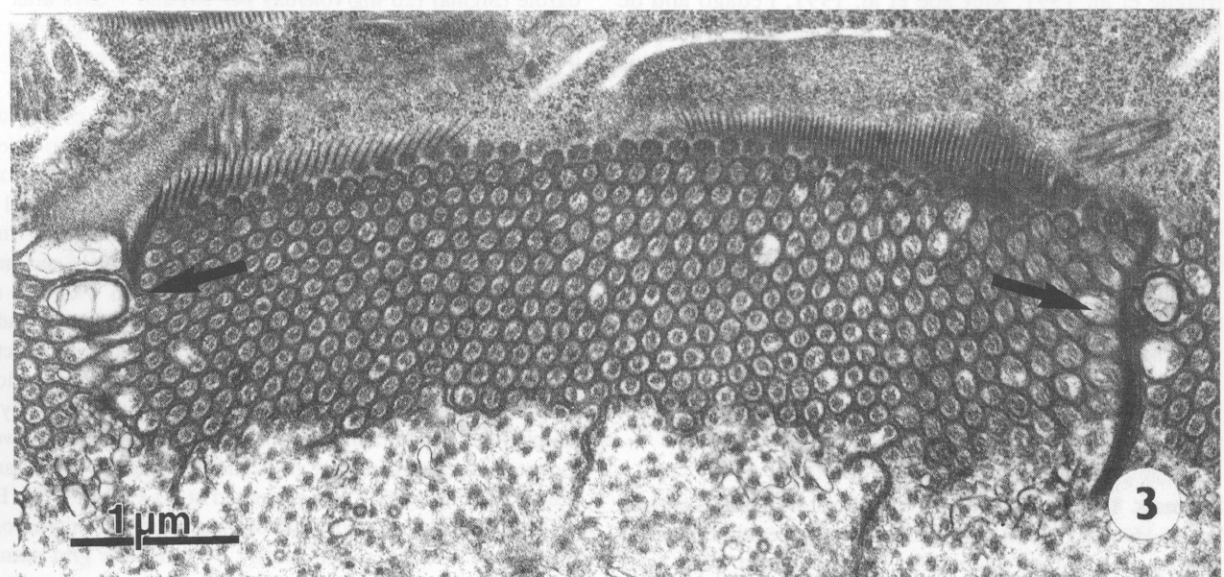
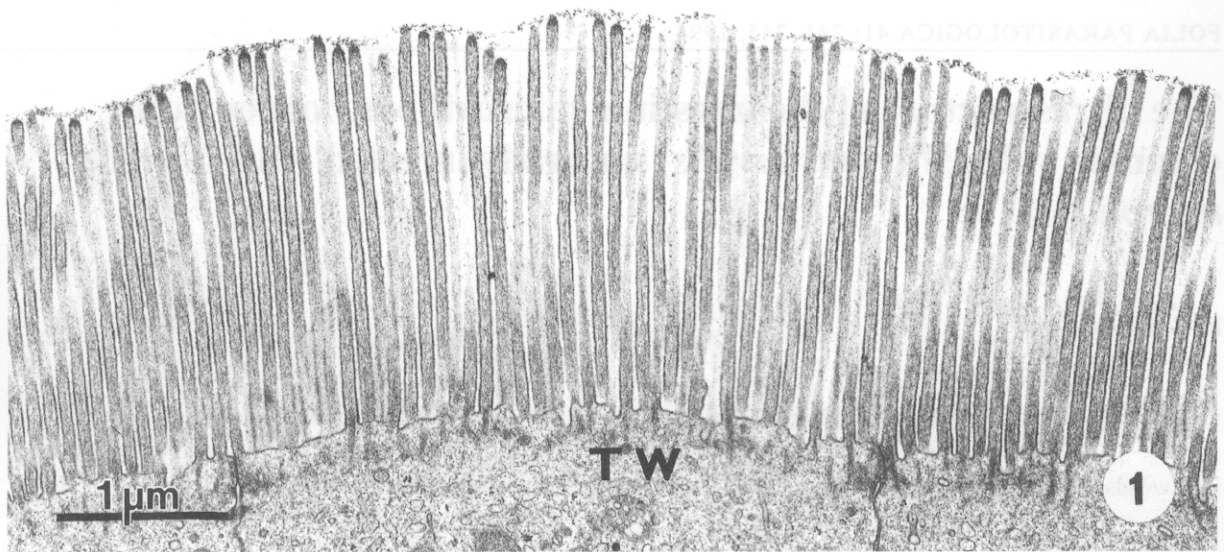
MATERIALS AND METHODS

Animals. Three to five-month-old laboratory-reared common voles, *Microtus arvalis* (second or third generation from the wild) were used in this study. These common voles were maintained at room temperature, given a commercial rodent feed *ad libitum*, occasionally supplemented with carrots and apples.

Microvillous border ultrastructure was assessed in four naturally infected common voles which excreted large

numbers of *Giardia microti* cysts, and in four control *Giardia*-free animals. *Giardia*-infected common voles also excreted *Spirotrunculus* sp. cysts. *Giardia microti* and *Spirotrunculus* sp. trophozoites counts were performed during necropsy. The entire small intestine was removed and divided in three parts. The first part (duodenum and anterior jejunum) was split longitudinally and placed in 25 ml of PBS (pH 7.2). After incubation for 2 h at 37°C and vibration for 3 minutes (Touch Mixer, Cole Parmer, Model 4721-00) trophozoites were counted with a hemocytometer. Four other common voles were treated orally for 3 consecutive days with metronidazole (Sigma) (20 mg/vole/day *per os*) and 7 days after the end of the treatment, were killed. Intestinal scrapings from the duodenum, middle jejunum and cecum were examined for presence of flagellates.

Transmission electron microscopy. Infected and control animals were killed with ether and segments of the anterior jejunum (approx. 5 cm distal to the pylorus) were excised and immediately immersed in 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2. Specimens were then cut into 1 mm pieces and stored at 4°C in the fixative overnight. Specimens were washed in cacodylate buffer, postfixed in 1% (v/v) osmium tetroxide in cacodylate buffer, dehydrated in graded ethanols and embedded in Durcupan. Five randomly selected specimens were embedded for each animal. Semithin sections were stained with basic toluidine blue for light microscopy to select fields for ultrathin sectioning. Ultrathin sections were mounted on copper support grids and then stained with uranyl acetate and lead citrate and examined at 80 kV in Philips EM 420 transmission electron microscope. Electron microscopic fields were selected systematically by using the grid corners as a convenient reference. Only fields containing the villous surface (with *G. microti* trophozoites – in infected voles) from mid-villous area were selected. To estimate the heights and diameters of microvilli, favourable fields were required. Height was estimated from microvilli sectioned along their



Figs. 1–3. Microvillous border in control common vole. Terminal web (TW) as an extension of filaments of microvillous core into the cytoplasm. TEM. **Fig. 2.** Diffuse shortening of microvilli and irregularly arranged terminal web (TW) in *G. microti* and *Spironucleus* sp. (Sp) infected common vole. TEM. **Fig. 3.** Cross section of microvilli in *G. microti* infected common vole. Vesiculation of microvilli (arrows) attached to the lateral crest of the *G. microti* ventral disc. TEM.

longitudinal axis and measured from tips to base. Diameters were measured on transverse or oblique sections across microvilli. In oblique sections, the shorted axis of the ellipse was recorded. Sets of 2–5 randomly selected favourable fields of the mid-villous surface area per section were recorded and used to estimate absolute dimensions of microvilli. For each infected and control animal, 8 randomly chosen micrographs were obtained to calculate microvillous dimensions. With the aid of a carbon grating replica (1,200 lines per mm), magnifications were calibrated and found to be $\times 9,900$, $\times 29,400$ and $\times 34,200$. Measurements of 30 microvilli were taken from each micrograph and measurements were made in a blind fashion.

All values were expressed as mean $\pm$ standard error (SE). Means were compared by one-way analysis of variance (ANOVA). Significance level was established at $P \leq 0.005$.

RESULTS

All infected common voles used for examination by transmission electron microscopy (TEM) were heavily infected with *Giardia microti*. The number of *G. microti* trophozoites in the anterior jejunum ranged from 10^4 to 10^5 and the number of *Spiroplasma* sp. trophozoites ranged from 10^2 to 10^3 . The metronidazole-treatment successfully eliminated *Giardia* and other intestinal flagellates infections in common voles which served as a control.

Dimensions of intestinal microvillous border from infected and control common voles examined by transmission electron microscopy (TEM) are summarised in Table 1.

Table 1. Dimension of microvilli in *G. microti* infected and control common voles.

Dimension	Control	Infected
Length (μm)	1.87 ± 0.32	$0.68 \pm 0.54 *$
Diameter (nm)	103 ± 12	$158 \pm 24 *$

Values are means $\pm$ SE

* Significant differences in infected animals compared to controls

TEM observation of the intestinal microvillous border from control animals revealed typical microvillous border appearance. The microvilli were remarkably regular in height, diameters and spacing. The fibres of the terminal web in the apical cytoplasm of enterocytes were regularly arranged (Figs. 1 and 5). The mean microvillous height varied in control animals from $1.7 \mu\text{m}$ to $2.2 \mu\text{m}$ (Fig. 1). In transverse sections, the intestinal microvillous border from infected animals exhibited a reduction in microvillous height as compared with controls. The decrease in height of microvilli was uniform and was observed in areas removed from trophozoite attachment as well as at sites of attachment. At the sites of *Giardia* trophozoite attachment to the microvillous

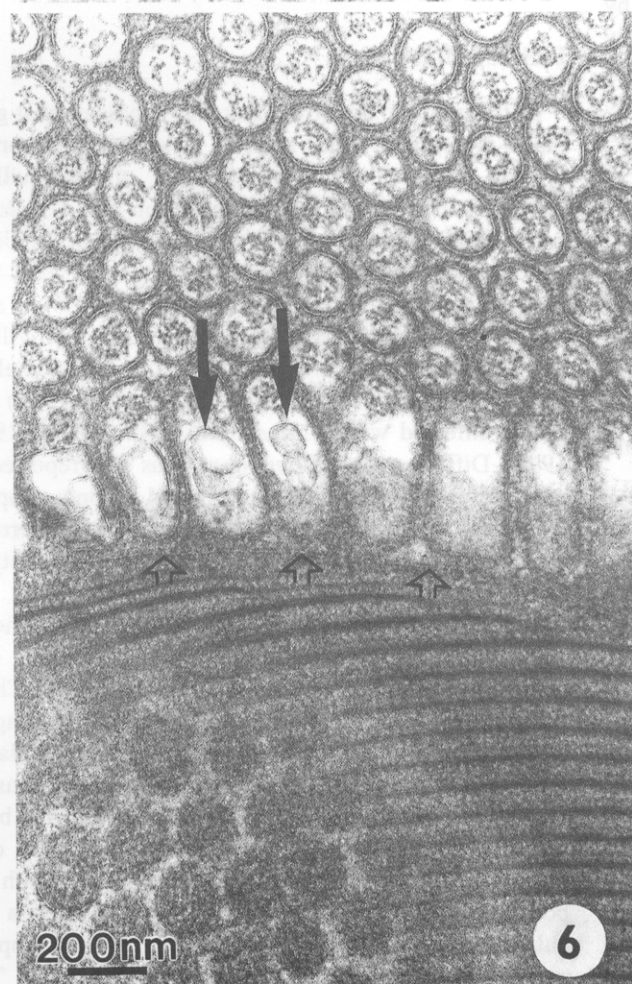
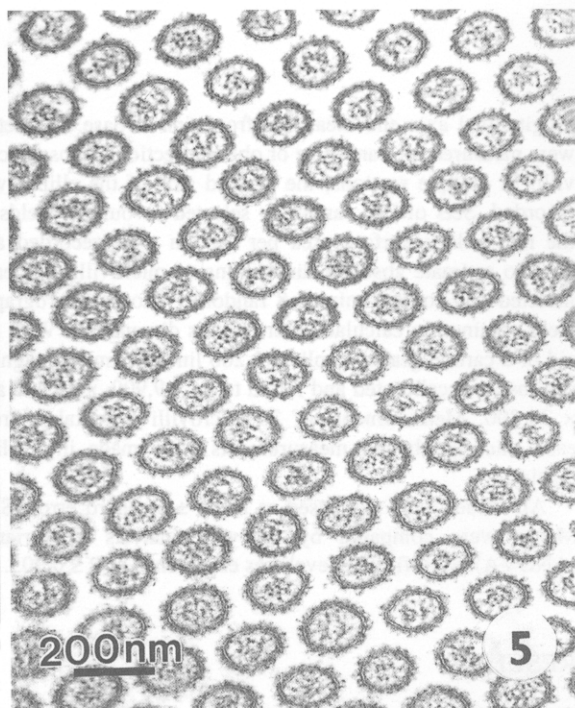
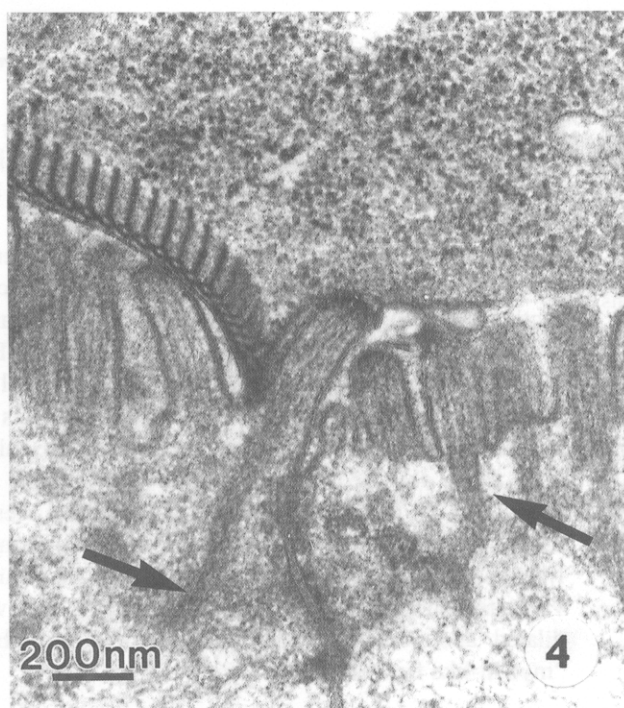
border, the fibres of the terminal web were longer and irregularly arranged (Fig. 4). The mean microvillous height in infected common voles was significantly lower when compared with controls (Table 1).

Microvilli around and beneath the body of the ventral disc of *Giardia* trophozoites appeared normal in cross section (Fig. 3). The core of the microvilli contained filaments which were arranged in an axial fashion (Figs. 6–7). However, when compared with the control the mean microvillous diameter in infected animals was significantly greater (Table 1). Deformations of the microvilli were observed at margin of the ventral disc (Figs. 3, 6–7). Microvilli attached to the lateral crest of the ventral disc were vesiculated with a disorganised filamentous core (Figs. 3–6). In some cases, the microvilli attached to the lateral crest of the ventral disc were dilated and contained whorled structures resembling “myelin-like figures” (Figs. 3, 7).

DISCUSSION

Ultrastructural data from this study indicate that giardiasis in common voles resulted in significant shortening of microvilli and the increase of microvillous diameters. It was observed that infection with *G. microti* caused diffuse injury of the microvillous border. Ultrastructural changes of microvilli were seen in areas previously occupied by trophozoites as well as at sites where trophozoites were attached. Similar microvillous border injury was recently reported in experimental infections of mice with *G. muris* (Buret et al. 1990) and gerbils infected with *G. duodenalis* (Buret et al. 1991, 1992). Diffuse microvillous injury was also reported in association with bacterial enteritis and chronic anaphylaxis. This suggests that the diffuse changes of microvilli may represent a common host response to a variety of stimuli (Hardin and Gall 1992).

Giardia trophozoites are attached to the epithelial cell surface by their ventral disc with its edges embedded in the microvillous border. Erlandsen and Chase (1974) described that the presumably firm grasping or contractile action of the adhesive ventral disc displaced the microvilli medially and produced deformations of the microvilli. Examples of fused microvilli have been encountered where the margin of the adhesive disc penetrates between the microvilli as well as beneath the body of the disc. These lesions were observed as a circular imprint on the microvillous border after trophozoite detachment (Erlandsen and Chase 1974). The present study demonstrated that only the microvilli attached to the lateral crest of the ventral disc were dilated, vesiculated and contained whorled structures resembling “myelin-like figures”. These lesions may be induced by lectins located on the surface of *Giardia*



Figs. 4. Longitudinal section of microvilli in *G. microti* infected common vole. Lateral crest of the *G. microti* ventral disc penetrated between individual microvilli and compressed them. Note the irregularly arranged fibres of the terminal web (arrows). TEM. **Fig. 5.** Cross section of microvilli in control common vole, showing unit membranes and filamentous core. TEM. **Fig. 6.** Cross section of microvilli in *G. microti* infected common vole. Vesiculation and disorganisation of the filamentous core of microvilli (arrows) attached to the lateral crest of the *G. microti* ventral disc (arrowheads). TEM. **Fig. 7.** Cross section of microvilli in *G. microti* infected common vole. Whorled structures (arrows) attached to the lateral crest of the *G. microti* ventral disc. TEM.

trophozoites (Farthing et al. 1986, Lev et al. 1986, Magne et al. 1991, Pedago and de Souza 1994). Lectins are involved in the process of *Giardia*-epithelial cell interaction (Pedago and de Souza 1994) and have been shown to produce similar type of damage to the intestinal microvillous border (Hart et al. 1988).

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