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Histopathological Changes in Small and Large Intestines during Hymenolepidosis in Rats

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The tapeworm *Hymenolepis diminuta* is a chronic parasite living in the small intestine of rats, mice and humans. The aim of this study was to determine histopathological changes in the rat intestine during experimental hymenolepidosis. Our results showed that in rats infected with *H. diminuta* slight changes occurred in the length of the villus and crypts in different parts of the digestive tract. The changes were most distinct in the duodenum and jejunum on the 16 days post *H. diminuta* infection.

Key words: *Hymenolepis diminuta*; experimental hymenolepidosis; gastrointestinal tract; histopathological changes; rats.

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Hymenolepis diminuta is a cosmopolitan tapeworm of rodents infecting mainly rats, mice and humans (READ & KILEJIAN 1969). At present wide is known about immunological processes from the definitive host after infection with *H. diminuta* (DWINELL *et al.* 1998). Although hymenolepidosis is often asymptomatic, the worm induces physiological changes in the hosts (PALMER & CASTRO 1986). In rodents *H. diminuta* infection results in an increase in crypt depth and number of goblet cells in the villus epithelium in the intestine (MCKAY *et al.* 1990; WEBB *et al.* 2007), and infected rats may also have musocal mastocytosis and smooth muscle hypertrophy (DWINELL *et al.* 1998). In previous studies, we observed changes in active transport of ions, passive movement of ions and altered activity of antioxidant enzymes and glutathione level as a consequence of oxidative stress in the gastrointestinal tract in rats infected with *H. diminuta* (KOSIK-BOGACKA *et al.* 2010; 2011 a and b). Those changes were observed in all parts of the small intestine and were associated with the size of the parasite and its migration in circadian rhythm in the intestine in response to the food intake of the host (PODESTA & METTRICK 1976).

Also, in other studies conducted on the same experimental model, we observed changes in both transepithelial ion transport, tight junctions, and in the indicators of oxidative stress in both small and large intestines of rats infected with *H. diminuta* (KOSIK-BOGACKA *et al.* 2010; KOSIK-BOGACKA *et al.* 2011a, b). In this context, the aim of this study was to assess the impact of *H. diminuta* on the histopathological changes of the rat digestive tract.

Material and Methods

The study was approved by the Local Ethics Committee for Scientific Experiments on Animals in Szczecin, Poland (No. 15/2006).

Male Wistar rats (4 months old) were either infected with *Hymenolepis diminuta* or uninfected, using a previous method (KOSIK-BOGACKA *et al.* 2010). *Hymenolepis diminuta* WMS il 1 (STRADOWSKI 1998) was maintained by cyclical passage through flour beetles (*Tribolium destructor*) and Wistar rats. Infected rats were dosed via a stomach tube with 5-cysticercoids in 1 ml of 0.9% NaCl solution. All cysticercoids used for the infections were isolated from *Tribolium destructor* infected with worm eggs.

The animals were housed singly, kept on a 12-hr-light-dark cycle and were given feed (Murigran, Motycz, Poland) and water *ad libitum*.

The rats (n=30) were divided into six groups:

– control group (n=5) – uninfected (0 dpi);

– group I (n=5) – 8 days post *H. diminuta* infection (8 dpi);

– group II (n=5) – 16 days post *H. diminuta* infection (16 dpi);

– group III (n=5) – 25 days post *H. diminuta* infection (25 dpi);

– group IV (n=5) – 40 days post *H. diminuta* infection (40 dpi);

– group V (n=5) – 60 days post *H. diminuta* infection (60 dpi).

The obtained results were analyzed statistically using Statistica 6.1 software. Arithmetic means and standard deviations (SD) were calculated for each of the studied parameters. A nonparametric Mann-Whitney U-test was used to check the significance of differences between experimental and control groups. The value of $p<0.05$ was taken as the level of statistical significance.

Results

In control rats, the mean length of villus and crypts was the greatest in the duodenum and the shortest in the jejunum (Table 1).

Table 1

Average villus and crypts depth in three regions of the small intestine and in the colon from uninfected and *Hymenolepis diminuta*-infected rats

Days post infection	duodenum		jejunum		ileum		colon
	villus	crypts	villus	crypts	villus	crypts	crypts
0	0.56±0.05	0.23±0.05	0.28±0.05	0.14±0.01	0.25±0.07	0.16±0.06	0.29±0.07
8	0.43±0.15	0.20±0.05	0.41±0.06	0.19±0.02	0.20±0.9	0.12±0.04	0.20±0.05
16	0.84±0.06*	0.19±0.00	0.69±0.11*	0.42±0.06*	0.22±0.07	0.17±0.04	0.37±0.05
25	0.48±0.20	0.17±0.05	0.38±0.06	0.17±0.04	0.41±0.11	0.23±0.04	0.25±0.05
40	0.54±0.22	0.24±0.08	0.38±0.09	0.22±0.07	0.26±0.08	0.21±0.07	0.25±0.07
60	0.57±0.09	0.2±0.04	0.42±0.04	0.18±0.04	0.25±0.07	0.14±0.04	0.21±0.05

The values are expressed in micrometers (μm). Data represent mean ± SD and are representative of groups of three animals in an experiment. * $P<0.05$, compared with the control value derived from uninfected rats (Mann-Whitney U-test).

Before each experiment, a coproscopic examination of the rats' faeces was performed to ascertain the presence of the parasite. Uninfected and infected rats were killed by thiopental anaesthesia (Biochemie GmbH, Austria) administered at 100 mg/kg body weight (b.w.) intraperitoneally (i.p.). The rats were weighed, and then their duodenum, jejunum, ileum and colon were removed for analysis. In the examined rats, 3 to 5 cestodes were observed (mean 4.5) which is 90% in comparison with the given cysticercoids.

Fresh sections of duodenum, jejunum, ileum and colon in rats of all examined groups were fixed overnight in Bouin's solution and embedded in paraffin. Serial 3-5 μm sections were used for staining by PAS (Periodic Acid Schiff technique according to McManus described by Totty) and immunohistochemical reactions. PAS-stained slides were morphometrically evaluated for microvilli and crypt lengths.

In rats infected with *H. diminuta* the average villus and crypts length in the duodenum in all examined groups decreased in comparison with control, but the changes were not statistically significant (Table 1). Only in rats 16 dpi did the duodenal crypt length increase by about 53%, which was statistically confirmed. In the jejunum of rats infected with *H. diminuta* the average length of crypts and villus increased in rats 8, 25 and 40 dpi compared with the control group (Table 1). In rats 60 dpi the length of villus increased and the depth of crypts decreased in comparison with the control group. The changes were not statistically significant. Only in rats 16 dpi was the length of villus and crypts in the jejunum significantly higher, by 116% and 200%, respectively. The length of villus and crypts in the ileum and colon of rats infected with *H. diminuta* did not significantly differ from data obtained in the control group.

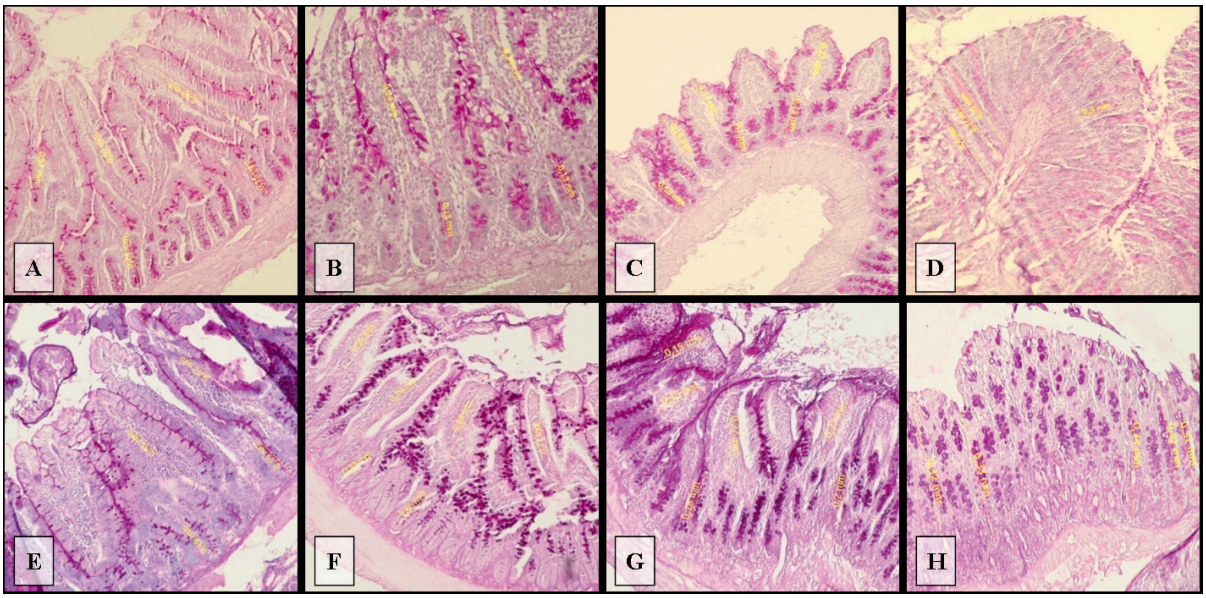

Fig. 1. Cross sections of four part of intestine of control rats (A-D) and 16 days post *H. diminuta* infection rats (E-H). Evaluation of length of villus and depth of crypts appropriate regions of intestine. Periodic Acid Schiff (PAS) staining, ×20.

Discussion

In rats infected with *H. diminuta*, histopathological changes occurred in the small and large intestine. In this study on Wistar rats infected with *H. diminuta*, only rats 16 dpi had longer villus in the duodenum and jejunum and deeper crypts in the jejunum, whereas there were no changes in the ileum and colon. The slight changes in the morphometry of the digestive tracts of *H. diminuta* infected rats could have been caused by the fact that the tapeworm does not have rostellar hooks, is non-invasive, and therefore is usually accompanied by little or no tissue damage.

Histopathological changes in the small intestine of rats 16 dpi also indicate that both the duodenum and jejunum of rats at that time are subject to the accumulated impact of all factors associated with the presence of the tapeworm, including the increase in the biomass of the parasite. As suggested by HINDSBO *et al.* (1982), the histopathological changes in the small intestine of rats infected with *H. diminuta* may also be induced by immune responses. The observed increased crypt depth and villi length may reflect functional changes in secretion or absorption during *H. diminuta* infection which may maintain host weight gain and/or prevent diarrhea (DWINELL *et al.* 1998). This is confirmed by previous observations of changes in the active transport of ions and the passive movement of ions in the ileum and colon of *H. diminuta* infected rats starting from 16 dpi (KOSIK-BOGACKA *et al.* 2010; KOSIK-BOGACKA *et al.* 2011a, b).

In contrast to the results of this paper, FAL and CZAPLICKA (1991) in Buffalo rats observed the greatest changes in the ileum, and the smallest in the duodenum. In the duodenum they observed a reduction in the height of villi, inflammatory infiltrations, and necrosis of crypt cells, and from 35 dpi the progressive atrophy of villi. In the jejunum they observed a widening or flattening and shortening of villi starting from 10 dpi, and in the ileum a widening and flattening of villi until the atrophy of villi starting from 25 dpi. DWINELL *et al.* (1998) observed a significant increase in crypt depth in all the segments of the small intestine in Sprague-Dawley rats 26 days post *H. diminuta* infection. These aforementioned various histological changes in the digestive tract in *H. diminuta* infected rats may have been associated with the strain of rats used in the experiments.

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