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Clinicopathological Studies on Spontaneous *Hymenolepis diminuta* Infection in Wild and Laboratory Rats

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Abstract

Out of 78 adult laboratory and wild rats investigated for parasitic diseases, 19.23% were diagnosed positive for spontaneous *Hymenolepis diminuta* infection. Infection was more in laboratory rats (24%) than wild rats (10.71%). Sex wise distribution of *H. diminuta* infection was also higher male laboratory rats than females while wild rat females were found free from this tapeworm. Value of hemoglobin was significantly decreased in *H. diminuta* infected laboratory rats than controls. Significant increased plasma protein values in *H. diminuta* infected wild rats than uninfected wild rats were observed. Serum values of alkaline phosphatase, SGPT and SGOT were significantly increased in *H. diminuta* infected wild rats than uninfected wild rats and other groups. Tissue enzyme studies revealed that although there were alterations of different enzymes in non-target organs of *H. diminuta* infected rats, but only lipid peroxidation, acetylcholinesterase and catalase were altered in target organ intestine. On SEM, the segments of *H. diminuta* showed width from 1120 to 1160 µm while length ranged from 120 to 150 µm. Most of segments had vertical lining and raised border on its each side of circumference. On necropsy examination, intestines were found to contain 25-40 mm long and about 1 mm wide, 3-4 or more tapeworms in each rat. Relative weight of intestine was significantly increased in *H. diminuta* laboratory rats than controls. Histopathologically, intestinal lumina showed varying number of *H. diminuta* segments with serrated borders. Occasionally, scolex of tapeworm attached with intestinal mucosa was also seen. *H. diminuta* infection caused pressure atrophy, compressed and atrophied villi, degeneration and desquamation of lining epithelium cells and excessive mucin secretion in intestinal mucosa and lumina. Occasionally, eosinophilic cellular infiltration was also observed. High prevalence of *H. diminuta* infection in rats is matter of concern as zoonosis in contact human beings.

Key Words: *Hymenolepis diminuta,* wild and laboratory rats, zoonosis.

Introduction

Hymenolepiasis caused by *Hymenolepis diminuta* (rat tapeworm) and *Hymenolepis nana* (dwarf tapeworm) is not uncommon in wild and laboratory rats. Both tapeworms are known for their global zoonotic significance (31). Although rats are the principal host of *H. diminuta* (Rudolphi, 1819) and *H. nana* (von Siebold, 1852) but both cestodes can infect humans, particularly children. *H. nana* has been found in men, rats and mice while *H. diminuta* occurs in rats, mice and has been recorded in men particularly in children. The parasitization rates of *H. diminuta* in human beings ranges from 0.001-5.5 per cent in different parts of world (28). In India, 10,000 human stool samples were tested and 23 samples were found positive for eggs of *H. diminuta* (7). Sporadic cases of *Hymenolepiasis* are also frequently reported from India (28) and different other parts of world (15). The location of large size adults of *H. diminuta* in intestine as well as eggs compared to *H. nana* make differential diagnosis. In rodents, infection can be associated with slow growth and pot-bellied syndrome (17).

The varying prevalence of *Hymenolepis*

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species is reported in brown rats from Belgium (8), UK (29), Iran (21), India (22) and Jamaica (31). In Scandinavian countries, this parasitic infection is rare *(www.ph source.us/PH/ZD/Zoonotic_Disease_Table.htm*). Macroscopic and microscopic examination of *H. diminuta* shows presence of 2-6 cm long tapeworm, proglottids and the scolex without hooks while concentrated stool samples reveals 70 micron diameter, spherical eggs, with a striated outer membrane and a thin inner membrane and containing six central hooklets but no polar filaments, (of *H. diminuta* eggs) and differentiated from *H. nana* eggs, which have a similar appearance but are smaller and have two evident polar thickenings, from each of which arise four to eight polar filaments .

Besides, reports on incidence and histopathological changed in intestine, information on hematology, serum and tissue biochemical alterations of *H. diminuta* are not available. SEM surface structure of *H. diminuta* was described (20) and it was reported (26) that SEM of the surface of *H. diminuta* particularly the scolex, indicated dense populations of microtriches which occur on the rostellum, suckers and scolex proper. Research on immunology and biochemistry of *H. diminuta* has revealed early biochemical research, its specific characteristic metabolic pathway, role of 5-hydroxy-tryptamine on worm behavior and control of its metabolism and strain variation of *H. diminuta* component in relation with immune mechanism which was earlier thought crowd behavior (1).

Therefore, present studies were undertaken to characterize morphology of segments *of H. diminuta* and comparative diagnostic clinicopathological alterations in adult laboratory and wild rats spontaneously infected with it.

Materials and Methods

Animals

A total of 78 adult rats (male 36 and female 42) were used in present study. Out of these 50 were albino laboratory Wistar rats (LR's) including 23 male and 27 female, weighing about 150-300g were obtained from Laboratory Animal Resaearch Section, Indian Veterinary Research Institute, Izatnagar, UP, India. Further, 28 wild brown wild rats (WR's) (13 male and 15 female) were caught alive using rat nests from Experimental Animal Sheds, Division of Pathology, IVRI, Izatnagar, UP. Both laboratory and wild rats were euthanized humanely using chloroform anesthesia.

Helminthic examination

Fecal samples of rats were collected in 5 per cent formal saline in air tight containers. Each sample was examined macroscopically for presence of tapeworm segments. After this samples were examined microscopically by direct smear method for eggs of *H. diminuta*.

Hematology

Approximately 2-3 ml blood was collected in Ethylene Diamine Tetraacetic Acid (EDTA) (1-2 mg/ml) in 5 ml vials. Complete hemogram, including red blood cell count (RBC), hemoglobin (Hb), erythrocytic sedimentation rate (ESR), packed cell volume (PCV), total leucocytes count (TLC) and differential leucocytes count (DLC) was determined as per standard methods (11).

Serum biochemistry

Total serum protein, albumin (modified Biuret & BCG dye binding methods), cholesterol (30), urea (DAM method), alkaline phosphate (13), Serum Glutamic-Oxaloacetic Tetraminase (SGOT) (19) and Serum Glutamic Pyruvate Transminase (SGPT) (19) were estimated using Commercial Biochemical kits manufactured by M/s Span Diagnostics Ltd. Surat, Gujarat, India. Total serum globulin was determined by appropriate calculation, while ceruplasmin was estimated as per standard procedure (25).

Tissue biochemistry

Part of pieces of brain, stomach, intestine, liver and urinary bladder were collected immediately after necropsy examination, washed in normal saline and stored at -20°C for tissue biochemical analysis. The homogenates of different tissues (10 per cent) were prepared in normal saline. Frozen tissue samples of control and *H. diminuta* positive rats were partially thawed and 1.00 g samples was weighed and taken in 10 ml of ice cold saline (1:9 volume/volume). Tissues were homogenized in Remi homogenizer for about 80 seconds. All the steps were carried out at 4°C. The homogenates were centrifuged for about 10 minutes at 10,000×g in Sorvall RC-5B refrigerated centrifuge using SS 34 rotor. In these tissues samples, Lipid peroxidation (18), Acetylcholinesterase (5), Catalase (6), Superoxide dismutase (14), Reduced Glutathione (Spectrophotometeric method) and Sorbitol dehydrogenase (27) were determined as per standard procedures.

Pathological studies

Detailed gross, Scanning Electron Microscopic (SEM) and histopathological studies of rat tissues were conducted as described below.

Necropsy examination

Dead/euthanatized animals were weighed prior to necropsy examination. Brain and visceral organs of all euthanatized animals were systematically examined. Weight of carcass, brain, lungs, heart, liver, spleen, kidneys and genitalia (testes/uterus) and visible gross lesions were recorded. After necropsy examination, representative tissue pieces from different visceral organs and intestine positive with *H. diminuta* fixed *in situ* were preserved in 10 per cent formalin for histopathological evaluation.

Scanning Electron Microscopy

Immediately after euthanasia of rats, 2-3 mm^2 pieces of segments *H. diminuta* were collected in 2.5

per cent chilled gluteraldehyde. After this tapeworms were transferred in 0.2 M phosphate buffer (pH 7.4) for 6 hr at 4°C. Then these were washed with three changes (at 2 hrs. each) of cold 0.2 M phosphate buffer (pH 7.4) and in thermos flask containing ice were taken to Electron Microscope Facility, Department of Anatomy, All India Institute of Medical Sciences, New Delhi. After fixation the samples were dehydrated in a graded series of ethanol, transferred to Freon TF, which served as an intermediate fluid and critical point dehydration. After dehydration tissue samples were mounted on stubs of colloidal graphite tissue paste (Lutel coated) with gold-palladium in a sputter coater and examined in a Leo 435 VP, 30 kV. SEM images were taken at different magnifications and stored in a computer and CD was prepared for further examination and analysis was done under Adobe Photoshop 6.0 software.

Histopathology

Tissues were made into small pieces of 2-3 mm thickness. After proper fixation, the thin tissue pieces of visceral organs were processed in ascending grades of alcohol for dehydration and cleared in xylene. The paraffin embedded tissues were cut into 4-5 micron thick sections and stained with Haematoxylene and Eosin (H&E) as per conventional procedure (9).

Statistical Analysis

The data for various parameters were subjected to statistical analysis by SPSS 7.5, software programme using analysis of variance (ANOVA).

Results

Incidence

In present investigation 15/78 (19.23 per cent) rats were found to be infected with *H. diminuta* tapeworms. Out of these, 12/50 (24 per cent) were LR's while 3/28 (10.71 per cent) were WR's. As regards sex-wise distribution of this tapeworm was concerned, higher per cent of males 10/78(12.82 per cent) were infected than females 5/50 (6.41 per cent). WR females were found free from this tapeworm.

Fecal examination

Fecal samples and intestinal contents contained numerous tapeworm eggs. Under microscope, globular oval shaped eggs were seen (Plate 1)**.** The embryophore had thickening at the poles which were provided with 4-8 polar filaments. Hexanth embryo was present within the oncosphere.

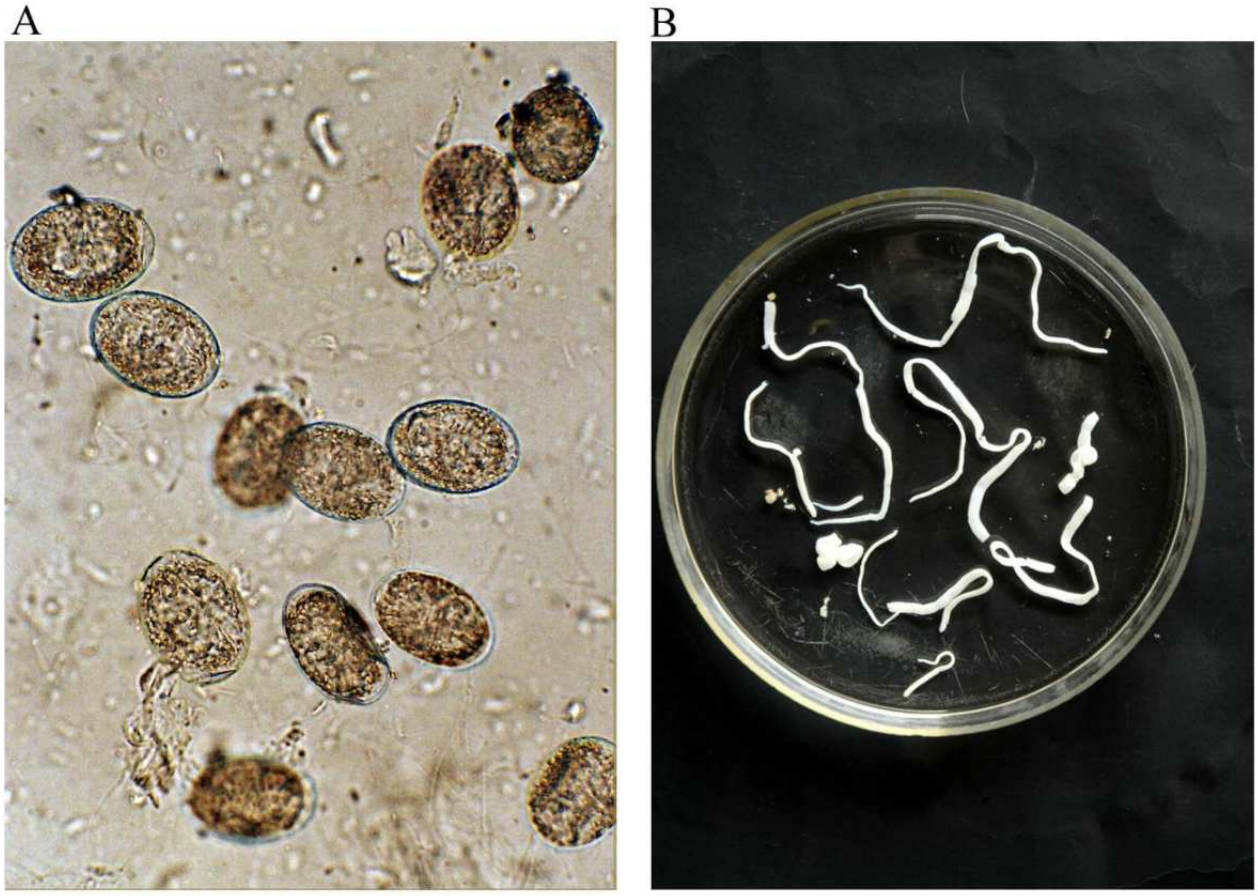


Plate 1: A - Globular oval shaped eggs of *H. diminuta* in fecal samples. B - Tapeworm from intestinal contents.

Scanning Electron Microscopy features

On SEM, the segments of *H. diminuta* showed width from 1120 to 1160 µm while length ranged from 120 to 150 µm. It showed that *H. diminuta* tapeworm segments had clear demarcations, folds, wavy borders, few un-symmetrical and/or damaged segments, vertical linings giving cellular appearance. Border of each segment was raised on all side of its circumference (Plate 2).

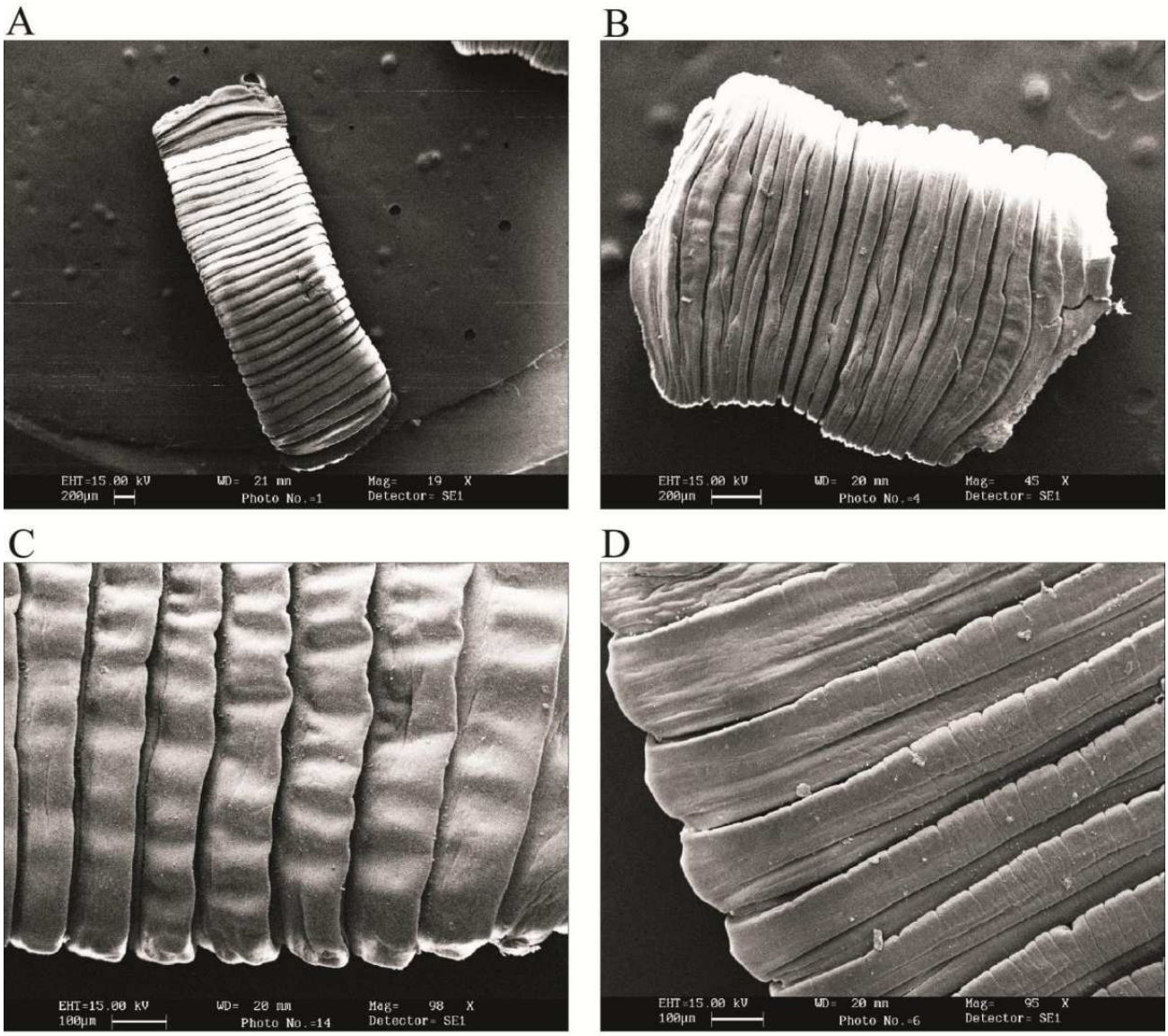


Plate 2: Scanning electron microscopic features of *H. diminuta.*

A- Segments of *H. diminuta.* Few segments were damaged. SEM X19

B- Segments of *H. diminuta.* Dissymmetry was seen in certain segments. SEM X45

C- Segments of *H. diminuta.* Borders were raised and some folds were seen. SEM X98

D- Segments of *H. diminuta.* Vertical linings and particulate material were seen. Borders had wavy pattern. SEM X95

Hematology and serum biochemistry

Results of hematology and serum biochemistry are presented in Table 1 and 2. Value of hemoglobin was significantly decreased in *Hd*LR's than CLR's. Other blood parameters failed to show any significant changes. Serum values of alkaline phosphatase, SGPT and SGOT were significantly increased in *Hd*WR's than CWR's and other groups. Significant increased plasma protein values in *Hd*WR's than WR's were observed. In addition to above, non-significant lowered total protein, albumin and globulin values in *Hd*WR's than WR's were observed. Specific trend of any value was not observed in serum biochemistry parameters in both types of rats.

Tissue biochemistry

Results of tissue biochemistry are presented in Table 3 (a, b, c, d & e).

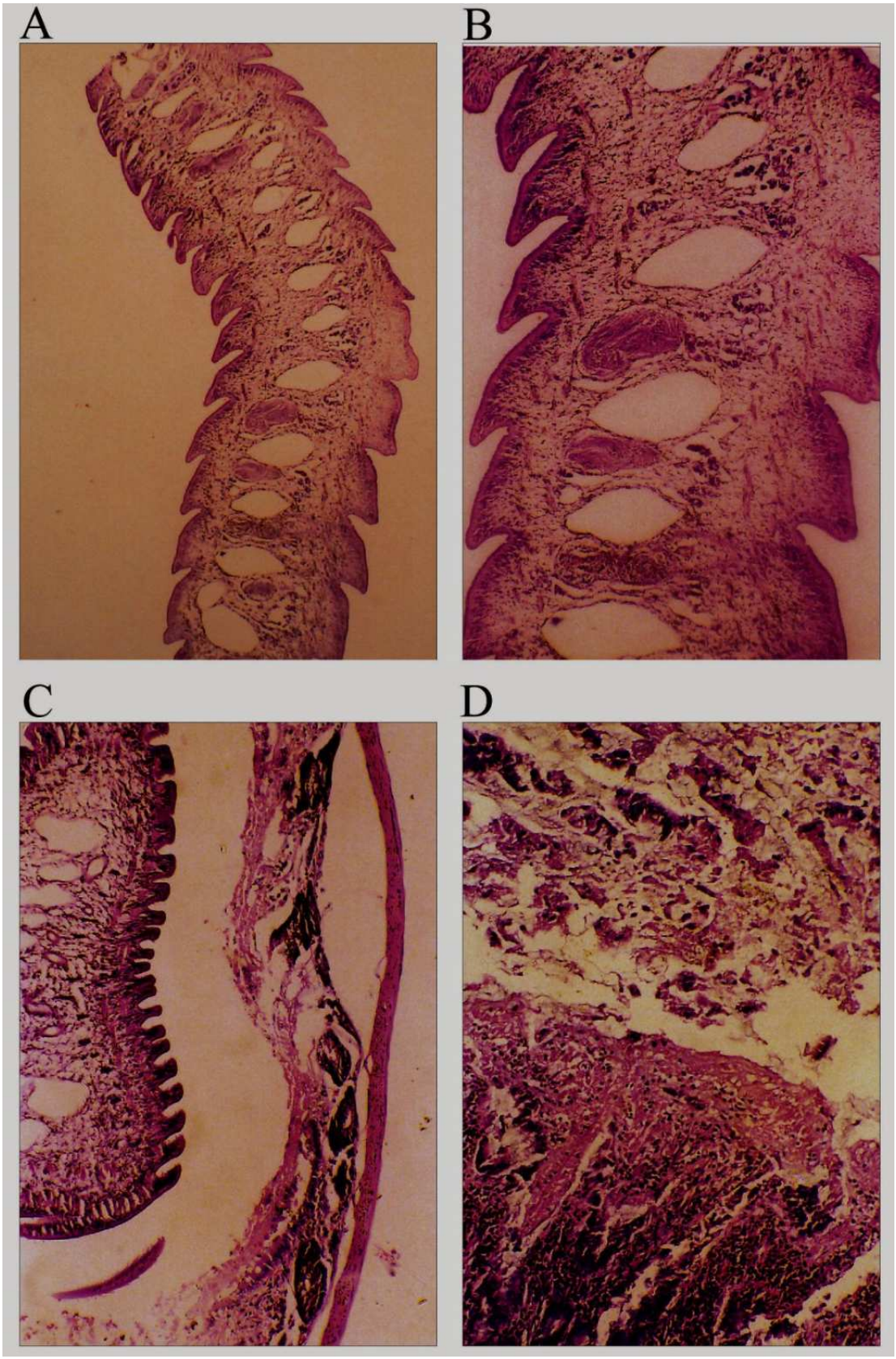


Plate 3: Light microscopic features of *H. diminuta.*

A- Segments of *H. diminuta* showing serrated borders and elliptical shaped uterus. H&E X 40
B- Segments of *H. diminuta* showing testes and uterus. H&E X 90
C- *H. diminuta* in intestinal lumina showing atrophied villi and mucosa. H&E X 90
D- Degenerated and desquamated mucosa and excessive mucin in lumina of intestine in *H. diminuta* infection. H&E X 90.

Values of lipid peroxidation was significantly increased in brain, intestine and urinary bladder in *Hd*LR's than CLR's while it was increased in liver and stomach in *Hd*WR's than CWR's (Table 3 a). Values of catalase were significantly increased in brain, liver and stomach in *Hd*LR's than CLR's while it was increased in *Hd*WR's than CWR's in brain and intestine. General increasing trend of catalase was seen in both types of *H. diminuta* infected rats (Table 3 b). Values of superoxide dismutase was significantly increased in brain and liver in *Hd*LR's than CLR's. It was significantly decreased in brain and stomach while increased in liver in *Hd*WR's than CWR's (Table-3 c). Values of acetylcholinesterase were increased in intestines in *Hd*WR's while decreased in urinary bladder in *Hd*LR's as compared to CWR's and CR's, respectively (Table-3 d). Significant decreasing trend of reduced glutathione was seen in liver and intestine in *Hd*LR's, brain, liver and stomach in *Hd*WR's than CLR's and CWR's, respectively (Table 3 e). Decreased values of Sorbitol dehydrogenase were seen in *Hd*R's (0.002 ± 0.01) than CR's (0.002 ± 0.01) in liver.

Gross changes

Clinically, most of rats had sub-clinical *H. diminuta* infection and no retarded growth and weight loss was evident. Only one LR showed stunted growth, debility and atrophied skeletal muscles on post mortem examination. No gross lesions of pathological significance were observed in visceral organs. Small intestine had 3-4 tapeworms but occasionally the number was as high as up to 5 to 7. These worms were visible as whitish-yellow structures during gross examination of intestine. In few rats, intestinal lumina were dilated due to presence of multiple numbers of tapeworms. Data of relative weight (RW) of different visceral organs are presented in Table-4. RW of brain was significantly decreased in *Hd*LR's than *Hd*CR's while RW of intestine was significantly increased in *Hd*LR's than CLR's.

Histopathological findings

Histopathologically, lumina of small intestine contained tapeworm segments indistinguishable from *H. diminuta*. It showed serrated borders, in its centre uterus, eggs, testes and other visceral organs. Occasionally, eggs and scolex of *H. diminuta* attached with mucosa were also observed. Intestinal mucosa showed pressure atrophy, compressed and atrophied villi, degeneration and desquamations of lining mucosal epithelial cells, excessive mucin secretion and its presence in luminal debris (Plate 3). Infrequently, in mucosa eosinophilic cellular infiltration was seen.

In addition to target organ small intestine, other visceral organs showed interstitial or suppurative pneumonia, mononuclear cellular infiltration in portal triads of liver, prominent red pulp in spleen, engorged blood vessels in kidneys, etc. These findings were considered as incidental and non-specific and had not much relation with pathogenesis of *H. diminuta*.

Discussion

H. diminuta infects the small intestine of several species, including the rats. The incidence of 19.23 per cent of *H. diminuta* in present study is in accordance with reports of earlier workers (21, 22, 29) from abroad and India. This tapeworm is known to found commonly in areas where large amounts of food grains or other dry feed products, which are the favorite foods for wild rats, are stored. This was reason for infection of *H. diminuta* in wild rats who visits experimental sheds for want of feed of laboratory rats kept in store rooms. The higher infection in LR's than WR's is indicative of poor hygiene in earlier ones. The LR's were maintained in polysterene cages with paddy husk as bedding material for longer period. Earlier, prevalence of *H. diminuta* and *H. nana* was detected in brown rats as 7 and 0 per cent in Belgium (8) and 11 and 22 per cent in UK (29). In Jamaica, relatively low 3.8 per cent prevalence of *H. diminuta* was detected in two species of wild rats (31). However, in India in an earlier study, incidence of *H. diminuta* was reported high (22) as 39.6% LR's and 27% WR's. Similarly, incidence of *H. nana* and *H. diminuta* was reported as 31.3 and 12.5%, respectively in wild rodents from Khuzestan, South-West Iran (21). In present preliminary survey of helminthic and protozoan diseases in wild and laboratory rats revealed that Trichosomoidiasis was most common infection followed by *H. diminuta*, *Cysticercus fasciolaris*, *Hepatozoan muris* and *Toxoplasma gondii* infections (10). High infection of *H. diminuta* in laboratory as well as wild rats are matter of zoonotic concern in contact animal attendants and farmers as wild rats are in abundance around human dwellings. Approximately, 500 cases of *H. diminuta* are reported in human beings and causes diarrhea and abdominal pain in heavy infections while in rodents this infection can be associated with slow growth and pot-bellied syndrome.

Clinically, infection of *H. diminuta* is diagnosed by fecal examination and presence of its characteristics eggs. Earlier workers (3, 23) reported that concentrated stool samples revealed 70 micron diameter, spherical eggs, with a striated outer membrane and a thin inner membrane and containing six central hooklets but no polar filaments, (of *H. diminuta* eggs) and differentiated from *H. nana* eggs, which have a similar appearance but are smaller and have two evident polar thickenings, from each of which arise four to eight polar filaments

On low SEM magnification most of segments of *H. diminuta* had vertical lining giving cellular appearance to each segment. The border of each segment was raised on its each side of circumference. SEM features of segments *H. diminuta* segments were not described and its knowledge may be utilized in differentiation of *H. diminuta* and *H. nana*. For this more SEM studies are required particularly of scolex of both these worms and segment of *H. nana*. Earlier, SEM features of the scolex of the surface of *H. diminuta* was described (26) which indicated that dense populations of microtriches is present on the rostellum, suckers and scolex proper. Microtriches are seen on 30-

35 thousand magnifications. Functionally, these microtriches have been suggested to: (1) increase the surface area for absorption and secretion, (2) aid in maintaining parasitism in host and (3) agitate the microhabitat (26). Polymorphism in the microtriches from *H. diminuta*, *H. microstoma* and *H. nana* was reported by SEM examination (4). These studies suggested that the microtriches played a role in locomotion of these organisms within the host's gut.

Significantly low hemoglobin values were seen in *H. diminuta* infected laboratory rats. Anemia (hemoglobinemia) is a common and significant finding in heavy parasitic infections. Significantly increased serum values of ALP, SGPT and SGOT in *Hd*WR's than CWR's and other groups were indicative of liver damage in this infection. ALP is found in high concentration in the liver and bile ducts and certain other tissues. SGOT is found in liver cells and metabolizes protein. SGPT in liver cells also play role in the metabolism of alanine, an amino acid. The liver histopathology revealed minor changes and mononuclear cellular infiltration in portal triads which also indicate hepatic damage in *H. diminuta* infection. Present study indicated that subclinical *H. diminuta* infection influenced liver function of rats. In different hematological and serum biochemical parameters a definite trend was not observed in LR's and WR's which may be due to varying worms load, stage of infection and variation in genetic constitution these two species of rats.

Tissue enzyme studies revealed that although there were alterations of different enzymes in non-target organs of *H. diminuta* infected rats, but only lipid peroxidation, acetylcholinesterase and catalase, were altered in target organ intestine. Lipid peroxidation is indicative of oxidative degradation of lipids. It is the process whereby free radicals steal electron from lipids in cell membranes resulting in extensive tissue damage. This process proceeds by a free radical chain reaction. The polyunsaturated fatty acids of lipids particularly are prone to oxidation in this way (2). Acetylcholinesterase (AchE) is an enzyme that degrades (through its hydrolytic activity) the neurotransmitter acetylcholine, producing choline and an acetate group. It is mainly formed at neuromuscular junctions and cholinergic synapses in the central nervous system where its activity serves to terminate synaptic transmission. AchE inhibitor blocks the function of AchE and thus causes excessive acetylcholine to accumulate in the synaptic cleft (2). Since both enzymes were altered in non-target organs also as such these changes were considered as non-specific. In this investigation common increasing trend of catalase was seen in intestine in both types of *H. diminuta* infected rats. Catalase enzyme is present in different organs but its concentration is high in liver. When it is exposed to oxygen it catalyzes decomposition of hydrogen peroxide into water and oxygen. Hydrogen peroxide is a harmful product of metabolism which needs to be converted into less harmful product as such it is having protective role (2). Increased catalase activity may be due to damage of cell membrane by superoxide and hydrogen peroxide which had some latent period ((12). Increase in catalase activity may be due to greater production of reactive oxygen species due to lipid peroxidation.

Increased RW of intestine in *H. diminuta* infected rats was due to presence of multiple numbers of tapeworms in the intestine. The present histopathological findings are in accordance with our earlier report (22). Excessive mucin secretion and desquamation of epithelial cells may be due to irritation caused by serrated border of segments of tapeworm. Further, these findings are in accordance with the SEM features of intestinal mucosa reported earlier (16). Hemorrhagic enteritis in bandicoot rats associated with *H. diminuta* was observed (24). In principal, no basic differences were seen in nature of histopathological lesions in wild and laboratory rats.

Keeping in view wide spread population distribution and zoonotic significance of *H. diminuta* in both type of rats and man further investigations and public educational control programmes are desired in developing countries.

Conclusions

The spontaneous prevalence of *Hymenolepiasis* was higher in laboratory rats than wild rats. It caused sub-clinical infections without causing any mortality. The infection of *H. diminuta* (in ascending to descending order) was only second followed by *T. crassicauda*. Haemato-biochemical studies failed to reveal any specific trends in alteration in hematological, serum and tissue biochemistry parameters in both types of rats. Conventional fecal, gross and histopathological methods were better tools in study of pathogenesis and diagnosis of *H. diminuta* infections than clinical haematological and biochemical parameters. Higher *H. diminuta* infection in both types of rats is matter of zoonotic concern in contact persons.

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