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Morphotaxonomy, Histopathology and Population Dynamics of Natural Enoplid Infections in Wild Rat, *Rattus rattus* of Eastern Uttar Pradesh, India

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Abstract: Rodents are shared primary or intermediate or paratenic hosts for number of helminthes fauna and may serve as valuable indicators for assessing the occurrence, level of environmental contamination and infection pressure of the nemic parasites. During ten years (2008-2018) extensive study regarding the epidemiology of nemic infestation in population of wild rats *Rattus rattus* (Mammalia: Rodentia) from urban areas of eastern Uttar Pradesh, India, adult enoplid roundworms, *Trichuris* spp. (Nematoda: Enoplea) were recovered from intestine. However, bunch of barrel-shaped bipolar eggs and considerable pathological changes observed in hepatic tissue. The worms were characterized as *Trichuris* spp. by typical whip like body, cuticle with fine transverse striations, narrow cephalic end, indistinct oral papillae and dorsally curved caudal end with subterminal anus. The male worms with slender well cuticularized, distally sharp and pointed spicule, however, females had non-protrusible muscular vulva and long monodelphic uterus. The bioecological investigations by the application of modern numerical tools reflected that about 68.3% of the rat populations were found to be infected and had female to male nemic ratio (FMR) 7.38 in spring and 5.11 during winter season. The spatial distribution dynamics of *Trichuris* spp. in rodent hosts was over dispersed and found to be significant on Poisson series ($p<0.50$). The present study also described younger rats to be more resistant than the older one to infections by *Trichuris* spp. The correlation of feeding habits with size of the host played a major role in heavier infection due to greater intake of resources, potentially capable of exposing these hosts which played a major role to more infectious stages of parasites through stochastic ingestion.

Keywords: Morphotaxonomy, *Trichuris* Spp., Histo-Pathology, Infection Distribution, *Rattus rattus*

1. Introduction

The outbreak of diseases due to parasitic roundworms infections are of magnificent importance to humans and domestic animals, consequential to great monetary losses. The developing countries and Indian sub-continent are in the main list, which is sitting on the pinnacle of mass of parasites. Even though the animals of all ecosystem are parasitized to a larger or minor degree by a variety of endoparasites. Among all, the roundworms impersonate more grim threat by emerging as a relatively more prevalent parasites invading the live stock particularly rodents. [1] The competence of gut factors to kindle the discharge of eggs are governed by the status of infection in mice. The advance recent farming practices, enhanced shipping of animals, foodstuff, population, and climate change have fashioned environments which smoothen the progress of the swift and widespread broadcasting of water and food borne zoonotic pathogens. [2] The defenselessness against infections is usually augmented due to immune deficiency and process is also allied to some types of allergies and autoimmune diseases. The lifelong anxiety under parasitological invasions might also be one of the causes ensuing into immune deficiency in addition to virus infection, intake of certain mycotoxins, peroxides of rancid feed and corticosteroid treatment. [3] Consequently immune deficiency correlated to nutrition indirectly, namely if the primary cause of disease leads to chronic diarrhoea, malabsorption may lead to significant fecal blood, protein, mineral and vitamin losses. [4] The active or passive transmissions of endo-parasites are critical to conclude unpredictability in population ecology and distribution dynamics. As an upshot, therefore, the portrait of ecto and endo-parasitic communities in a model environment is the function of the influence of host intrinsic factors such as body size, diet, abundance, territory behavior and host phylogeny. [5]

There are 23 species of *Trichuris* was cited in mammals so far. *Trichuris* spp. (Nematoda: Enoplea) is commonly known as the whipworm which refers to the shape of the worm like a whip with wider handles at the posterior end. [6] The study of contact, interaction and relationship between the host and the parasite is known as host parasite relationship and with pathological study of host tissue is known as histopathology. The mechanism of entry and the establishment of a parasite with in a particular host vary widely from species to species in different animals. The degrees of response by each host to parasites, which making tissue contact are related to nature of invasive tissue and also the intimacy of the stages of development of living organism, either an adult or larva. [7] At cellular level when parasites make contact with a host, the host reacts by bringing in to action as cellular and serological reactions. It is thought that the host is able to distinguish between self and non self or foreign material at molecular level, but the mechanism of recognition may take place at the surface of the susceptible cells or recognizing cells. [8] Rodents as pests are associated with unhygienic environments and inadequate disposal of refuse, characteristic of major cities, increased rat human contact. The *Trichuris* spp. infection in the house rat reflect a possibility that either rodents could contribute an active role in the transmission of worms to human beings or worms itself could infect human. Therefore, these worms are of zoonotic significance and as a shared indicator of zoonoses. [9] Linnaeus was the first to describe the worm, *T. trichiura.* Trichuriasis or trichocephaliasis or trichocephalosis or whip worm infection, a neglected tropical disease afflicts about 1 billion people throughout the world, which is a major problem in the area where the wild animals have such nemic infection. [10] Therefore, the present investigation was taken in consideration to work out morphotaxonomy, histopathological and distribution aspects of *Trichuris* spp. in the urban localities for health, mankind and eco-societal welfare.

2. Materials and Methods

The wild rats, *Rattus rattus* (Mammalia: Rodentia) were trapped by rat trap kits from trapping sites of urban localities of the Gangetic geographical areas of eastern Uttar Pradesh, India (81°49’06.28”E (Lon), (25°24’53.24”N (Lat), 74m (Alt) (Figure 1). All the hosts were brought to laboratory, separated sex and size-wise. Viscera of hosts were teased by dissecting needle and examined microscopically for developing stages of helminthes parasites, while intestines, liver and gonads were carefully examined for helminth parasites. [11] The worms recovered from the gut of hosts were washed in lukewarm water and fixed in hot dehydrated ethanol and glycerine (95:5 *v/v*) for overnight and cleared in lactophenol (Lactic acid: ethanol: Glycerol: Phenol:: 1:1:1:1 *v/v* respectively). [12] Nematodes were temporarily mounted in glycerol or lactophenol for morphotaxonomy, morphometry and micrphotography. [13] The generic diagnosis of collected roundworms was based on *Systema Helminthum* vol. III “The Nematodes of Vertebrates”. [14] The liver tissue of infected wild rats was fixed in picroforma or Bouin’s fluid for histopathological investigation and processed for paraffin microtomy after standardized laboratory protocol. [15] The microphotographs were captured by MOTIC image analyzer using Nikon trinocular computerized micro-photographic unit and Biovis image plus software. All the morphometric measurements were given in micrometer (µm) as range followed by mean ± standard error in parentheses. [16] The nemic infection prevalence (IP%), female to male ratio (FMR) and pattern of infection distribution (Poisson distribution) were calculated by advanced biostatistical tools. [17-19]

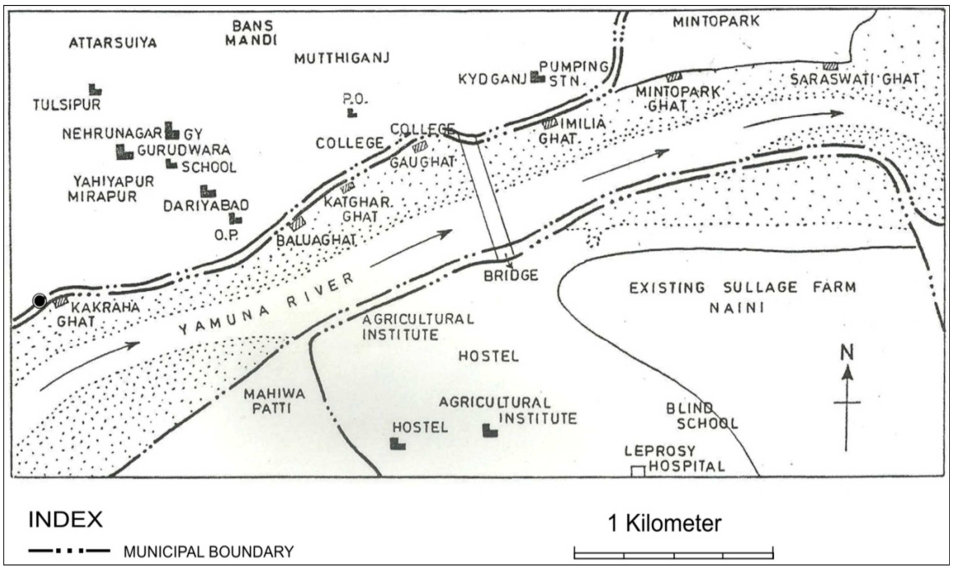

Figure 1. *Wild rats trapping sites of urban localities from the Gangetic geographical areas of Allahabad, Uttar Pradesh, India for enoplid round worms investigation.*

3. Results

3.1. Morphotaxonomy

The roundworms isolated from the intestine of wild rats, *R. rattus* (Mammalia: Rodentia) were characterized as *Trichuris* spp. (Nematoda: Enoplea) by typical whip like body, cuticle with fine striae, narrow cephalic end, indistinct oral papillae and dorsally curved caudal end with subterminal anus (Figure 2). The male worms comprised slender, cuticularized, distally sharp and pointed spicule. However, females had non-protrusible muscular vulva and long mono-opisthodelphic uterus after key to the Nematoda.

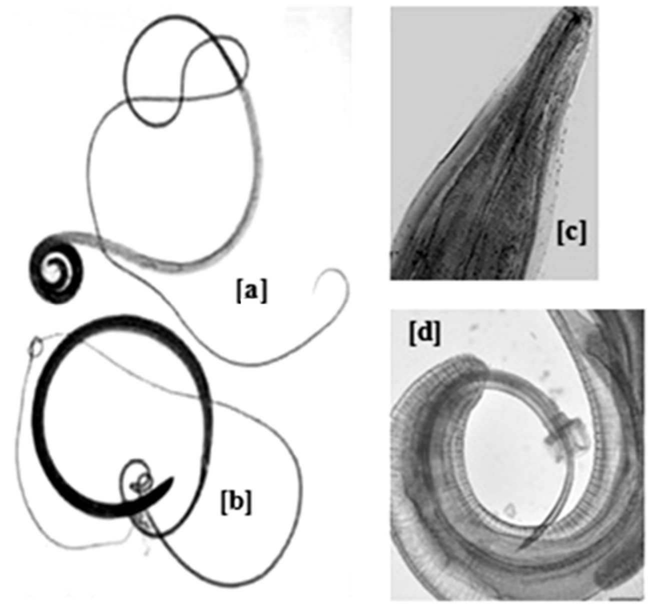

Figure 2. *Microphotographs of adult Trichuris spp.: a. male (wm); b. female (wm); c. anterior end (magnified); d. posterior end male (magnified). Not to scale bar.*

3.1.1. Male

Body size 61013–66229 (64044±1520) × 198–792 (429±45). The narrow cephalic end was indistinct with fairly small head and oral papillae (Figure 2a). The body was clearly divisible in two parts which refers to the shape of the worm like a whip 38318–41278 (40554±1018) × 198–360 (279±21) with wider handles at the posterior end 21205–24387 (23490±1139) × 306–792 (564±52). The narrower anterior part covered about 63.222% while the posterior wider region about 36.678% of the total body length. The male individual had slender well cuticularized, distally sharp and pointed spicule with 1123–1325 (1224±109) × 27–72 (49±8) size (Figure 2d). The length ratio of spicule *vs* total body length was about 1:52.324. The tail was dorsally curved and 85–94 (90±6) µm long.

3.1.2. Female

Worms with 75128–79367 (77976±2032) × 126–1118 (601±85) body size with whip like narrow anterior part 51426–56201 (53802±1202) × 126–306 (212±20) and broader handle shaped posterior region 22917–25694 (24174±1321) × 306–1118 (829±82) (Figure 2b). The head was comparatively smaller in size 9–18 (13±4) × 18–36 (27±5) (Figure 2c). The narrower anterior part in female made about 68.998% and posterior wider part about 31.002% of the total body length. The female worms had non-protrusible post equatorial muscular vulva at a distance of 73.499% of the body length from anterior end with long mono-opisthodelphic uterus about 19098 µm. The tail was comparatively shorter and 81–90 (85±5) µm in length.

3.1.3. Taxonomic Summary

Type host: *Rattus rattus* (Mammalia: Rodentia)

Type locality: Urban localities of Allahabad, Uttar Pradesh, India

Habitat: Intestine and liver

Type specimen: Holotype (male) and Paratype (female) deposited with Department of Zoology, University of Allahabad, Allahabad, Uttar Pradesh, India.

Etymology: The common term whip worm of the recovered nemic fauna named after its distinct shape.

3.2. Histopathology and Distribution

The nemic infection in liver was diagnosed by the presence of numerous creamish-white patches and cysts all over the surface. The intensive histopathological analyses indicated that the adult worms reached to the liver from the intestine by direct penetration of gut wall and migrated to the destination, liver. The mature female worms after copulation started egg laying and laid their eggs in the intracellular and intercellular hepatic spaces or cavities (Figure 3). The cross section of infected part of liver tissues comprised larger necrotic cavities that were filled with scattered eggs. The eggs were lemon or barrel-shaped, with two polar plugs and about 27–90 (65±9) × 9–45 (29±6) in size and diagnosed as eggs of *Trichuris* spp. after key to the Nematoda.

During the investigation it was recorded that about 68.3% of the host population was found to be infected by *Trichuris* spp. Out of the total infected male hosts, 50.0% were infected by the male nematodes and 33.3% by female nematodes. However, the rate of infection (infection prevalence%) and mean worm burden was recorded higher in female hosts and about 72.3–81.5% and 3–6 respectively. The findings were also provided evidence of seasonality on the female to male ratio (FMR) of roundworms in infected hosts and highest FMR was calculated during spring (7.38) than winter (5.11). The month wise agreement of nemic prevalence and mean intensity in host population was analyzed during the period of investigation to assess the distribution pattern of infection. It was statistically found to be significant on Poisson series ($p<0.50$), thereby indicating that nemic population were found to be over-dispersed in hosts *R. rattus*.

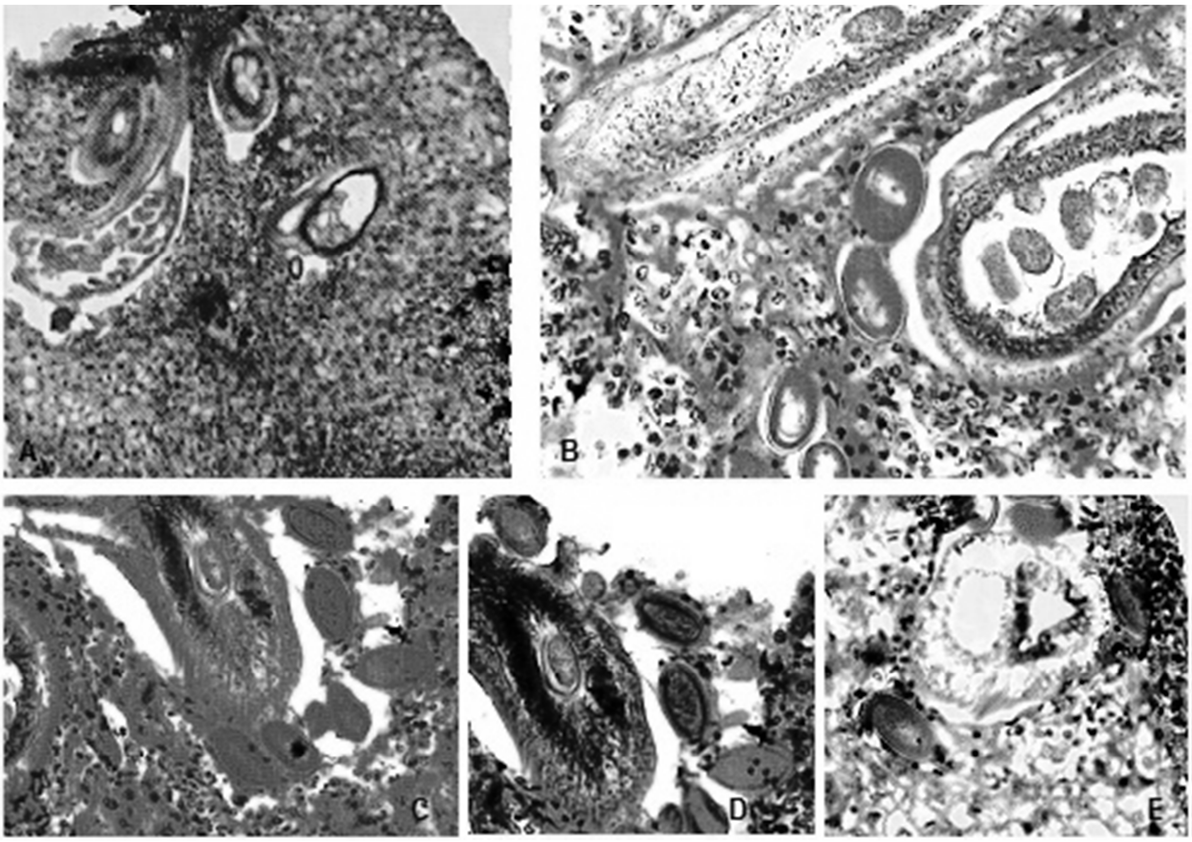

Figure 3. *Histological sections of rat liver with lemon shaped bipolar eggs of Trichuris spp. (in situ). Not to scale bar.*

4. Discussion

In India the work related to the survey and taxonomy of nematodes of domestic and wild animals was started in the early twentieth century and from the time to time the parasites specimens collected in India found its way into the hands of specialists such as Cobbold in England, Railliet in France, Parona in Italy and Linstow in Germany. Accuracy of identification is, therefore, fundamental to our understanding and communication of the ecological role of any organism. Parasites are one group of pathogens that thrive under such environmental conditions, and they continue unabated to exploit the behavioral patterns of their hosts to further transmission among animals and to humans. [11] About billions of people round the world are found to be suffered by trichriasis due to the infection of a soil transmitted helminthes (STH) or geohelminthes *Trichuris trichiura*, being particularly ubiquitous where hygiene and sanitation are pitiable. [20] The perisinusoidal spaces and spaces between reticulo-endothelial and hepatic cells increased in the infected liver than the non-infected host tissue corroborated the findings of the earlier workers. [21, 22] The adult worms were recovered from the intestine and migratory mature female after copulation laid lemon or barrel-shaped bipolar eggs in the intracellular and intercellular hepatic lumens was noticed in cross section of liver. The findings of hisological microscopic examination during present investigation was supported by the colonoscopic finding of a case report conducted in Korea showed a long slender whitish *T. trichiura* worm in the caecum, and histology and microscopic observation reflected section of uterine tubules with barrel-shaped bipolar eggs dispersed in the lumen with moderate eosinophilic infiltration. [23]

The nemic prevalence among selected model population was comparatively higher in the present investigation (68.3%) than the yesteryears scholars (46.2%). [24] The morphometric analysis was conducted and it was found that the worms were comparatively longer than the whip worms recovered from the human beings and pigs, length ratio of spicule *vs* body and narrow anterior *vs* wide posterior region did not differed significantly and corroborated with the findings of Meekums et al. [10] The ten years consecutive investigation indicated that the larger the rats, greater the infection as published earlier. [25] The reported study also described younger rats to be more resistant than the older ones to infections by *Trichuris* spp. The correlation of feeding habits with size of the host played a major role in heavier infection due to greater intake of resources, potentially capable of exposing these hosts which played a major role to more infectious stages of parasites through incidental ingestion. [26, 27] The sex biased nemic prevalence i.e. the parasite of one sex preferred to harbor the host of same sex was reflected in the present study corroborated to the finding of Upadhyay worked out in yesteryears. [11]

5. Conclusion

The nemic investigation in rodents of subhumid area of eastern Uttar Pradesh, India concluded that the adult worms were inhabited in intestine but female may migrated to liver after copulation to utilized hepatic spaces as microhabitat for egg laying. These worms caused a neglected tropical disease, when come in contact of human beings *via* rodents as paratenic or intermediate host. The genera of the worms diagnosed as *Trichuris* spp. by archetypal whip-shaped body, cuticle with fine striae, narrow cephalic end, indistinct oral papillae and dorsally curved tail with subterminal anus. The male comprised slender, cuticularized and arcuate spicule, while females had non-protrusible muscular vulva and long monodelphic uterus. As the population ecology concerned, the advanced numerical paraphernalia showed that about 68.3% of the hosts were found to be infected with seasonality based female to male nemic ratio. The nemic prevalence based on Poisson series in rodents were over dispersed ($p<0.50$). The present study also described that the older hosts were more sensitive to infection than younger one related to feeding habits, greater intake of resources and potentially more exposed to extrinsic factors. The *Trichuris* spp. infection in rodents might suppose to be contributing in active transmission of worms to human beings; hence play a significant role in zoonoses, trichuriasis or trichocephaliasis or trichocephalosis, as a major problem in the area where the wild animals have such nemic infection. Therefore, the present investigation was taken in consideration to work out taxomic, histopathological and distribution aspects of *Trichuris* spp. in the urban localities for health, mankind and eco-societal welfare.

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Conflicts of Interest

The author declares no conflict of interest because did not receive financial support from any government and non-government agency or organization to conduct this research work. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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