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Investigation of Zoonotic Cestode (Hymenolepididae: Cyclophyllidea) from Rodents in Suburban Hyderabad: Prevalence and Public Health Risk

Maree Rajper[1*] and Nadir Ali Birmani[1]

[1]Department of Zoology, University of Sindh, Jamshoro, Pakistan

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***Corresponding Author:**
Maree Rajper
Department of Zoology, University of Sindh, Jamshoro, Pakistan
rajparmarvi@gmail.com

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ABSTRACT

The Rats and mice are well-known vectors of ecto and endo parasites and have zoonotic and veterinary importance. **Objectives:** To study two Hymenolepididae species in sub-urban rodents: Rattus rattus and Mus musculus and to analyse the elements contributing to their occurrence in the environment and causing sanitary risks and to evaluate the prevalence, mean, and abundance. **Methods:** 40 samples were collected including 21 rats and 19 mice captured from sub-urban areas of Hyderabad. Prevalence means and abundance was recorded with the help of Quantitative Parasitology-version 3.0. Morphological characters were studied using line diagrams and photographs of this cestode. Identification of cestode was done with key books and recent research papers. **Results:** Morphological analysis of hosts i-e Rattus rattus and Mus. musculus revealed that two cestode species (Hymenolepis diminuta, H. nana) were found in the sub-urban localities of the Hyderabad district. These localities are mostly under development, lack freshwater facilities, and have sanitary risks. This cestode is found in the small intestine. Morphometric studies were conducted on both species: H. nana and H. diminuta. Statistical value: Prevalence, mean, abundance, and mean intensity were calculated, also observed host species correlation with sex, localities, and season. **Conclusions:** It was concluded that cestode parasites have public health importance. Studies provide valuable data to local and provincial organizations and also help in the diagnosis of zoonotic diseases. This study also provides references to minimize the rodent population, especially in suburban areas where the sewage system is poor and zoonotic diseases are common.

INTRODUCTION

Helminthiases was neglected in about 20% of people affected in Latin- America, and throughout the world, more than 3800 people were infected [1]. These helminths infections are more prevalent in rural and overpopulated areas that are poorly constructed, and sanitation issues that cause environmental contamination. [2]. Previously, this helminthiases disease was cosmopolitan, especially in an anthropogenic environment. This disease was infested worldwide and was affected by cestode genera H. diminuta (Rudolphi, 1819) and H. nana (Von Siebold, 1852). These cestode species life cycles involve definitive hosts i-e humans, rats, and mice, and intermediate hosts and arthropods. The frequent host species around humans are rats, mice, and arthropods. The disease is more prevalent in young than adults and is mostly in marshy areas [3-5]. The rat and mice are definitive for H. diminuta (Rudolphi, 1819) species. For parasitosis, humans by complete life cycle transmitted from the intermediate host an arthropod [6]. Whereas, H. nana (Von Siebold, 1852) type host is humans, although it infested other mammals. Previously, around the world, this species infected more than 20 million by direct contact [7, 8]. Furthermore, epidemiologically seen higher infection in children of trashed and overpopulated areas [6]. The climate of Hyderabad is dry with little rainfall. In day time observed hot up to 40°C meanwhile, nights are pretty cold and airy [9]. In Pakistan, Hyderabad is the fifth largest city and in Sindh, it stands as the 2nd city of Province. It is estimated in the 2017 census of Pakistan that more than 13 million homes were included in Municipal Corporations and cantonments. Slum houses were not documented. After the 2022 flood, most affected were shifted to Hyderabad [10]. Therefore, the

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Hyderabad district seems favorable for urban rodents, the most frequent species were black rats (Rattus rattus Linnaeus, 1758) and house mice (Mus musculus Linnaeus, 1758). These hosts were bearing parasitic infections. In cestode mostly H. diminuta (Rudolphi, 1819) and H. nana (Von Siebold, 1852) parasitosis in definite hosts i-e hosts [11-14]. These two species are cosmopolitan, and distributed in trashed areas. No studies have been conducted on the family of Murids in Pakistan. Only a few authors have been given intentions at different times. [15-17]. A study on zoonotic and non-zoonotic parasites in Rattus rattus in the Sawat district of Pakistan has been conducted. Eight species were noticed, including H. diminuta and two other species of two species from Hymenolepis genus [18]. In another part of the country of Pakistan, Malakand has been studied for intestinal parasites in school children. About eight species were found including H. nana in stool samples of children [19]. However, the purpose of this study is to analyse zoonotic infection of Hymenolepididae species present in overpopulated and poor sanity areas of district Hyderabad. This study aims to evaluate the major reason for the Hymenolepididae species in these sub-urban hosts i-e House mice and black rats.

METHODS

Hyderabad district is located on the east bank of the Indus River, at 25.367 °N latitude and 68.367 °E longitude. It consists of four localities of approximately consist on 5 lac households and 2 and half lac populated districts. This study was conducted on four localities: Qasimabad, Latifabad, Hyderabad Sadar, and Hyderabad City. All studies conducted on sub-urban sides mainly focus on a higher level of trash areas, where houses have low hygiene and are overcrowded. Moreover, target slum areas that are poorly developed. These areas lack fresh water facilities and are forced to use higher containment water. The sample was collected from August 2021 to 2023 December. The Captured collected were divided into two periods: Summer-spring with an average temperature is 40°C high and low of 23°C and winter-autumn with an average temperature higher than 29°C and a low of 15°C. Rat and Mice were collected in a cage and brought to the parasitology lab of the Zoology Department of the University of Sindh Jamshoro. Hosts were dissected, visceral organs detached and placed separately in Patri Plates. The organs were perturbed in the body cavity and teased in normal saline. The visual and AmScope New Dual Lit 6W LED Trinocular Stereo Zoom microscope observation for the presence of Cestode in the small intestine of hosts. The cestodes were collected and stored in 70% alcohol. Specimens were stained with borax carmine, dehydrated with an alcohol series, and made permanent slides by using Canada balsam. Photomicrographs were taken with an OMAX 40X-2500X Trinocular Compound Microscope with a 10MP camera. Line drawings were drawn using a microscope Olympus ch 20 drawing tube. Measurements were in millimetres [20]. Identification by key books and research papers. Statistical value: Prevalence, mean, abundance and mean intensity were calculated and also observed host species correlation with sex, localities and seasons [21]. To analyse prevalence, mean, abundance and mean intensity by using the Fisher test and difference of proportions, while Bootstrap test 97.5% confidence limit by using Quantitative Parasitology 3.0 software [22].

RESULTS

A total of 40 samples were captured and analysed for the current study: 21 R. rattus and 19 Mus musculus. The two Hymenolepididae species were found in the sub-urban localities of Hyderabad district. These localities are mostly under development, lack freshwater facilities and have sanitary risks. This cestode is found in the small intestine. Morphometric studies were conducted on both species: H. nana and H. diminuta. H. nana has been identified based on 375 samples. These cestode were white creamy. The body of the cestode is thin and long. Body consist on scolex, immature proglottids, and mature proglottids. In this species scolex with armed rostellum has crown shaped 20-22 hooks. Multistrobia broader length than body length. Usually, immature proglottids were short in size. Mature proglottids contain male as well as female reproductive organs. In the segment male reproductive organ has three globular testes, one is polar and two are antipodal. There is a seminal vesicle, one on the outer, and one on the inner side, that opens to cirrus and is surrounded by a cirrus pouch. The female organ ovary is usually bi-lobed. However, in the present specimen, it was not prominent. Ootype was connected with the vitelline duct from the vitelline glands. The gravid segments contain several eggs, each egg has six hooks, the oncosphere is covered with a thin layer called hyaline, and a hard outer layer called filaments (Figure 1).

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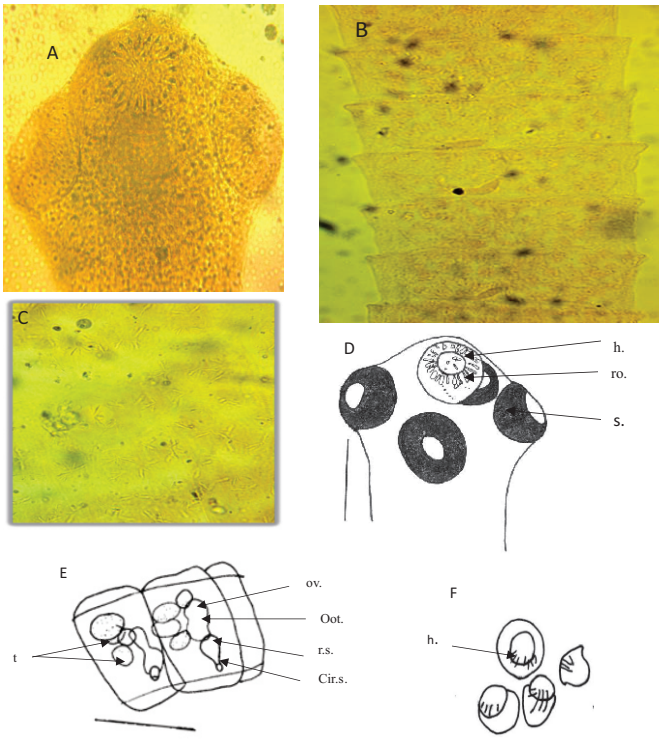

Figure 1: Hymenolepis Nana (Dwarf Tapeworm) A, B, and C Photographs on Omax 40 x2500 Trinocular Compound Microscope with a 10MP camera. D, E and F Line Drawing on Olympus ch.20.(D)Scolex,

h=Hooks, ro=rostellum, s=suckers. (E) Mature segment t=Testis, cir.s.=Cirrus Sac/Pouch, oot. =Öotype, ov. =Ovary, and r. s.=Receptaculum Seminis.(F)Eggs with Hooks. Scale bar 0.1mm.

H. diminuta was identified based on 327 specimens of cestode. Strobila was well- developed,reaching its maximum length. The body is consisting of colex, immature proglottid, and mature proglottid. Scolex was broader, with a non- recognizable neck. Scolex was flattened slightly towards the dorsal and ventral surface. Suckers were round or oval without armed, and located anterolateral. Rostellar pouch was present. Neck was wider, slightly smaller than segment. Mature proglottids were diagonal with quadri-lateral single pairs of parallel sides. Testes were round spherical, small almost equal in size, one was polar and the other two were antipodal. Cirrus sac was bipartite, well developed, one elongated, short and the other part was overly ventral x-axis canal. The genital atrium was situated at lateral side of proglottids, however, it was not fully developed. Ovary was broad, irregularly lobed, and situated at the middle field of the proglottids vitellarium posterior to the ovary, barely lobed. The gravid segment was an elongated fully developed uterus. The uterus contains numerous small eggs. Egg were round, and oncosphere had embryonic hooks(Figure 2).

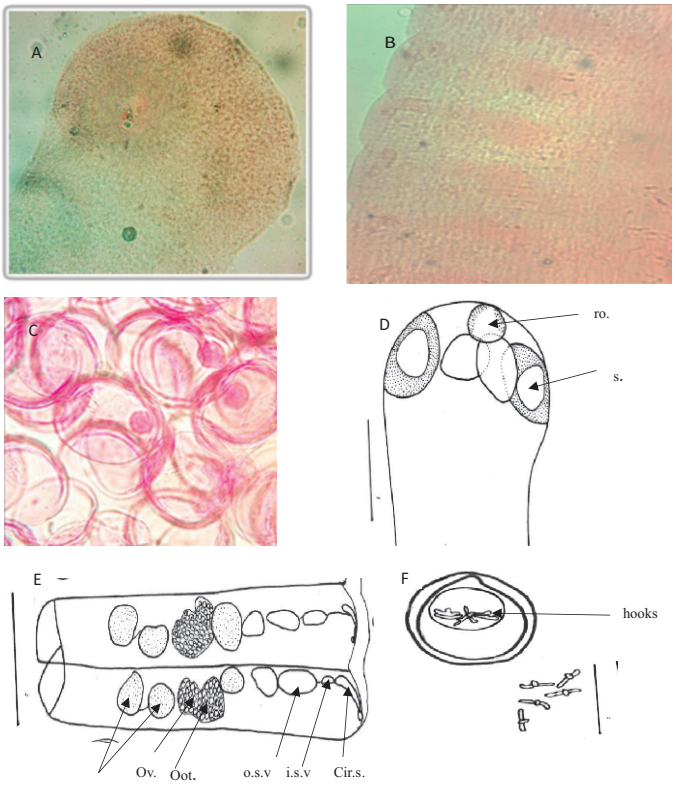

Figure 2: *Hymenolepis diminuta* A, B, and C Photographs on Omax 40 x2500 Trinocular Compound Microscope with a 10MP Camera. D, E and F Line Drawing on Olympus ch.20.(D)Scolex,

Table 1: Biometric Data of *H. nana* and *H. diminuta*

Body Parts	*H. nana*	*H. diminuta*
Scolex	0.0638X0.083	0.236X0.111
Suckers	0.055X0.066	0.097X0.08
Rostellum	0.083X0.611	0.055X0.027
Hooks	0.0166	-
Immature Segment	0.111	0.222
Mature Segment	0.152	2
Testes	0.0277	0.122
Ovary	0.055	0.244
Gravid Segment	0.116	1.777
Eggs	0.027	0.333X0.311
Eggs Hooks	0.0083	0.07

Statistical analysis of rodents revealed that the maximum prevalence of Hymenolepididae species was more frequent in Rattus rattus than in Mus musculus. The maximum mean intensity and mean abundance of H. diminuta was more in Mus musculus, and that of H. nana was more in Rattus rattus. The allocated localities, maximum prevalence, mean intensity and abundance in Qasimabad, followed by Saddar remaining two are minimum prevalent. Moreover, in season prevalence, mean intensity and abundance are maximum in Summer- spring than winter –autumn. However, no significant differences were seen in survey areas, divided years, and host species (Table 2).

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Table 2: Prevalence, Mean Intensity, and Abundance

Host	Statistical Analysis	*H. nana*	*H. diminuta*
Rattus Rattus Linnaeus, (1758) n=21	P(%)	(18) 85.7	(18) 85.7
	Mean Intensity	(255) 14.16	(194) 11
	Mean Abundance	(255) 12.14	(194) 9.8
Mus Musculus Linnaeus, (1758) n=19	P(%)	(15) 78.9%	(15) 78.9%
	Mean Intensity	(120) 8	(233) 15.53
	Mean Abundance	(120) 6.31	(233) 12.26
Areas			
Qasimabad n=10	P(%)	(8) 80	(8) 80
	Mean Intensity	(36) 4.50	(72) 9.50
	Mean Abundance	(36) 3.60	(72) 7.60
Latifabad n=9	P(%)	(5) 55.6	(5) 55.6
	Mean Intensity	(15) 3.00	(15) 6.00
	Mean Abundance	(15) 1.67	(15) 3.33
Sadar n=6	P(%)	(4) 66	(4) 66
	Mean Intensity	(20) 5.00	(30) 7.50
	Mean Abundance	(20) 3.33	(30) 5.00
Hyderabad City n=5	P(%)	(3) 60	(3) 60
	Mean Intensity	(16) 5.33	(16) 5.33
	Mean Abundance	(16) 3.20	(16) 3.20
Season			
Winter –Autumn n=20	P(%)	(8) 40	(8) 40
	Mean Intensity	(36) 4.50	(51) 5.67
	Mean Abundance	(36) 1.80	(51) 2.55
Summer –Spring n=20	P(%)	(16) 80	(16) 80
	Mean Intensity	(158) 9.50	(158) 9.50
	Mean Abundance	(158) 7.60	(158) 7.60

DISCUSSION

The objective of the current research is to study intestinal cestode species in rats and mice. Therefore, we evaluated the prevalence, mean and abundance in urban environments to reach the relevance of rats and mice zoonotic parasites for public health. Rats and mice captured can be separated by location type and season. Most rats and mice were captured from Qasimabad: location type summer and spring seasons. In statistical analysis of the current study, H. nana and H. diminuta species were more prevalent in Rattus rattus than Mus musculus, and significantly higher in Qasimabad than any other location type. Previous studies have reported that H. nana and H. diminuta are zoonotic parasites from zoonotic humans. These cestodes are transmitted to humans by intermediated hosts. Whereas, in humans, it is asymptomatic and causes headache, weakness, stomach ache and sometimes diarrhoea [23]. Veterinary-relevant species H. diminuta, and H. nana have been reported previously in the Netherlands in three areas: Farms, rural and suburban environments. The prevalence of H. diminuta was more in brown rats in suburban areas [24]. In contrast, our study showed that both species were frequently seen in sub-urban rats and mice. It may be due to different hygiene conditions of environments. Endo-parasites are related to human health in rodents: Rattus rattus, Rattus norvegicus, and Mastomys natalensis. A study on the effect of parasites in various aspects such as location, seasons, age of hosts, and gender of hosts was done previously and reported the prevalence of eight species including H. nana (0.8%) and H. diminuta (17.2%) in the metropolitan area of South Africa [25]. These findings are concordant with our findings. Current data revealed that H. nana and H. diminuta are more prevalent in Rattus rattus than in Mus musculus. However, identifying typical cestode of rodents is tricky, especially hymenolepid species because H. nana and H. diminuta act as cryptic species. Furthermore, morphological identification by combining molecular and phylogenetic analysis has been done previously in Egypt, where they found prevalence of H. nana (57%) and H. diminuta (35%) in Rattus rattus in different locations of Egypt Aswan Governorate. Mainain aim of the study was to shed light on zoonotic cestodes for the better health of humans [26]. Rodents-borne cestode especially H. nana has been reported in Aswan School children. These rodents lead to parasitic risk for humans and have zoonotic importance [27-29]. Similarly, in Pakistan, urban localities of Lahore have been studied for H. diminuta in three different host species: Rattus rattus, Rattus norvegicus, and Mus musculus to study the prevalence of H. diminuta in seasons and months. The maximum prevalence of H. diminuta was seen in rat species than in mice [30]. Our study poses that the current findings are important for dynamics, health risk and socio-environmental factors, concerning cestode infection in humans. Taking into account the prevalence of cestode parasites could lessen the burden of zoonotic diseases. Furthermore, measures should be taken to improve the sewage system for better health prospects.

CONCLUSIONS

It was concluded that there were considerable differences between host species and cestode species. Rattus rattus (85%) was infected than Mus musculus (78%). Location-wise, the most prevalent area was Qasmabad. Both cestode species were prevalent in the summer and spring seasons. The rats and mice have been living with humans, therefore, it is an initiative to minimize the population of rodents.

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Authors Contribution

Conceptualization: MR
Methodology: MR, NAB
Formal analysis: MR
Writing review and editing: NAB

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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