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Original Article

Morphological and molecular identification of *Hymenolepis* spp. in *Rattus rattus* and children with diarrhea from Upper Egypt

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Abstract
Introduction: Hymenolepiasis remains among the most common parasitic zoonoses in developing countries. Little information is available about hymenolepiasis in children in Upper Egypt and rodents' contribution to maintaining the disease's epidemiology.
Methodology: A cross-sectional study was carried out to investigate the occurrence of *Hymenolepis* spp. in *Rattus rattus* and children in Asyut Governorate, Egypt. Rodents (*n* = 100) were randomly trapped from various localities in Asyut Governorate, and stool samples from 120 children were collected from the same localities. Laboratory examination of the collected samples involved investigation of the small intestine of *R. rattus* for adult worm detection by morphological examination, followed by examination of stool samples of children using direct smear, formol-ether sedimentation technique, and Sheather's sugar flotation technique. Confirmation of *Hymenolepis* spp. positive samples were performed using polymerase chain reaction targeting the internal transcribed spacer 1 (ITS-1) and restriction fragment length polymorphism (PCR-RFLP).
Results: This study revealed the occurrence of *Hymenolepis* spp. in 45% of the examined *R. rattus*, comprising 43% positivity for *H. diminuta* and 2% for mixed infection by *H. nana* and *H. diminuta*. *Hymenolepis nana* was detected in 28.3% of the examined children. PCR–RFLP confirmed these findings, showing 100% sensitivity. Collectively, these findings reveal the potential contribution of *R. rattus* as an important reservoir for *Hymenolepis* infection in Upper Egypt.
Conclusions: This study concluded that personal education, periodical deworming of children, rodent control, and hygienic measures should be implemented by governmental and nongovernmental organizations to reduce the incidence of infection.

Key words: *Hymenolepis nana*; *Hymenolepis diminuta*; *Rattus rattus*; children; PCR–RFLP; Egypt.

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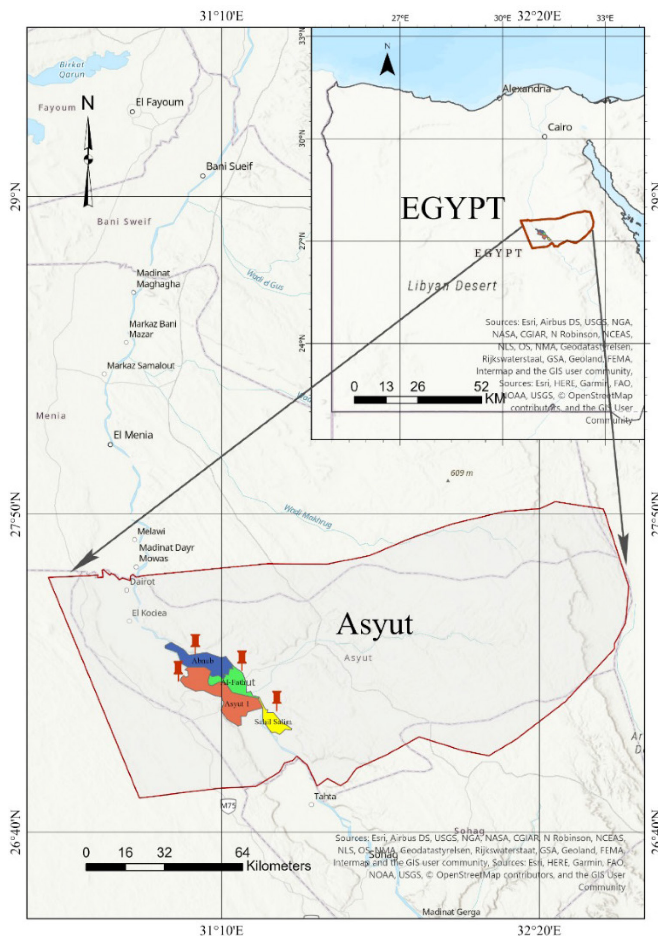
Introduction
Zoonotic parasites are among the main causes of morbidity and mortality worldwide [1]. Several recent studies reported that 30% of the total population worldwide is infected with zoonotic intestinal parasites, among others [2,3]. Children living under conditions of poor sanitation, inadequate health education, and a low socioeconomic level are considered the main susceptible group. Among others, hymenolepiasis is considered a parasitic zoonotic disease with a global distribution [4,5]. The disease is caused by *Hymenolepis* spp., comprising two main species including *Hymenolepis nana* and *Hymenolepis diminuta*. Collectively, these are the most common zoonotic cestodes distributed worldwide, but both parasite species are endemic in Asia, eastern and southern Europe, central and southern America, and Africa [6].

Regarding its epidemiological profile, *H. nana* (dwarf tapeworm) mostly infects humans who act as

definitive hosts. Meanwhile, *H. diminuta (*rat tapeworm) mainly infects rodents and infrequently humans. Taken into consideration, coprophilic arthropods, including fleas, Lepidoptera, and coleopteran, are also implicated as intermediate hosts for the transmission of *H. diminuta*. Among other species, *R. rattus* is widely distributed in Egypt especially in Upper Egypt. The distribution of *R. rattus* (house rat) in houses of cities or villages is very irregular depending on the kind of shelter and food supply, and they usually do not inhabit places where brown rats (*Rattus norvegicus*) are present. Transmission of the infection to human mainly occurs through the fecal-oral route [7] by ingestion of contaminated food/water with feces of infected cases. Transmission of the infection rarely occurs through accidental ingestion of rat fleas infected with cysticercoids [8].

It should be stressed that the different developmental stages (egg, cysticercoid, and adult worm) of *H. nana* might occur in the definitive host without requiring an intermediate host to complete its life cycle. However, it should be noted that intermediate hosts might be implicated in the transmission of the infection [9]. In contrast, *H. diminuta* are maintained in rodents and their transmission to humans rarely occurs through accidental ingestion of rat fleas infected with cysticercoids [8]. Regarding its clinical impact, hymenolepiasis is usually asymptomatic; however, heavy infections may cause gastrointestinal disturbance, including abdominal pain, diarrhea, nausea, vomiting, and loss of appetite [10].

Figure 1. Map of sampling sites and different localities.



The diagnosis of *Hymenolepis* infection in rodents occurs through macroscopic examination of the small intestines, followed by the preparation of the adult *Hymenolepis* spp. for examination [11]. In humans, microscopic examination of the stool is considered the main diagnostic method to detect *Hymenolepis* eggs using several techniques, but no single stool examination may be conclusive. Several molecular approaches have been adopted to diagnose and differentiate parasites that are morphologically identical but genetically different, including protein and DNA-based techniques such as polymerase chain reaction (PCR) [12]. PCR is considered an ideal technique for the molecular detection of the minute quantities of DNA extracted from *Hymenolepis* spp. eggs [13]. Reviewing the available literature, many surveys revealed the prevalence of *Hymenolepis* spp., mainly *H. nana*, worldwide. However, very limited information is available about the occurrence of *Hymenolepis* infection in Upper Egypt, and the real contribution of *R. rattus* in the transmission of the infection to humans. This study was designed to provide preliminary data on the occurrence of *Hymenolepis* spp. in *Rattus rattus* and children in Upper Egypt.

Methodology

Sample collection

This study was conducted in several localities from the Asyut Governorate (Egypt). One hundred commensal house rats (*R. rattus*) were randomly trapped in four locations including rural areas (Abnoub *n* = 32; Sahel Sleem *n* = 29) and urban areas (Asyut city *n* = 18; Elfath *n* = 21) from December 2015 to May 2016. The map of sampling sites is shown in Figure 1. Four rat traps were baited with moist bait and placed along rodent runways at every location. Rats were identified according to their morphological features. All captured rodents were identified to be *R. rattus* and they were black with a lighter color on the belly and varied in weight between 70 and 300 g. The head and body length varied between 16 and 22 cm, while the tail

length reached ≥ 19 cm (tail length equal to the length of the head and body) [14].

One hundred twenty children with diarrhea were randomly included in the study from different localities in Asyut Governorate and examined at the Children's Hospital, Assiut University Hospital, Egypt. Stool samples were collected from the investigated children. A complete case history was obtained from each patient or guardian, including the duration of illness, frequency, and consistency of stools, and any other complaints.

Detection of Hymenolepis spp. from R. rattus

The captured rats were euthanized using an overdose of Thiopentone as described in a previous study [15]. Later, a midventral incision was made, and the intestines were removed and placed in 0.9% saline solution and incised longitudinally. The intestinal contents were then examined for adult *Hymenolepis* spp. with the naked eye and using coprological techniques under a hand lens and a light microscope, respectively, for the identification of eggs, proglottids, and adult worms [16]. The intestinal contents of the rats were evacuated into labeled 15 mL Falcon tubes and mixed with distilled water and left to sediment. The supernatant fluid was decanted and the sediment was washed several times with distilled water and examined for minute worms under a binocular microscope. The worms were preserved in glycerin alcohol [95 parts ethyl alcohol (70%) and 5 parts glycerin] in screw-capped vials [17]. An examination of the small intestine of *R. rattus* for adult worm detection and identification was performed as described in [11]. The detected worms were then preserved in a solution containing sodium acetate-acetic acid-formalin (SAF) until further molecular examination [18,19].

Detection of Hymenolepis spp. in children

The collection of stool samples and parasitological examination was performed according to the technique described in Pietrzak-Johnston *et al.* [18]. Stool samples were examined using direct smear and confirmed by formol-ether sedimentation and Sheather's sugar flotation techniques. For the direct smear method, briefly, each stool sample was strained through two layers of gauze inside a funnel into a glass beaker and then centrifuged at 272 × g for 5 minutes. A drop of saline was placed on the center of a slide and a drop of 1% Lugol's iodine (50%) on the second slide. A drop from the sediment of the centrifuged stool was added to each slide, then a cover glass was placed on each slide, and examined using a binocular microscope (Olympus, CX 21, Japan) [19].

For formol-ether sedimentation technique, each stool sample (2 mL) was centrifuged at 272 × g for 5 minutes. The supernatant was discarded and 5 mL formalin (10%) was then added and mixed well; then, ether (3 mL) was added, covered firmly with a rubber stopper, shaken vigorously for 1 minute, and centrifuged at 272 × g for 5 minutes. Four separate layers were obtained, including a top layer of ether, formalin, a plug of fecal debris, and a bottom layer of sediment containing parasites. The plug of debris was loosened from the sides of the tube using a stick and the top three layers were decanted. The sediment was slurred and examined by direct smear as described in [20]. For Sheather's sugar flotation technique, intestinal contents (2 mL) were added to a centrifuge tube containing 8 mL of the sugar solution (500 g sucrose and 6.5 g phenol crystals were dissolved in 320 mL distilled water) and centrifuged at 1500 rpm for 5 minutes. A wire loop was used to touch the surface film and five drops of the film were placed on a slide, covered with a cover glass, and examined using bright-field microscopy. Stool samples infected with *Hymenolepis* spp. eggs were preserved in SAF solution [21].

Molecular examination of Hymenolepis spp. positive samples

DNA was purified from *H. nana* and *H. diminuta* tissues using the QIAmp tissue purification kit (Qiagen, Hilden, Germany), according to manufacturer's protocol. DNA was then eluted in 300 µL Tris–EDTA (TE), and 1 µL of the diluted extract was used as a template in the PCR mixture [22]. In addition, DNA was purified from infected human stool samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol.

A PCR amplifying the *ITS-1* gene was performed to identify the conserved regions in *H. nana* and *H. diminuta* using a set of primers designated from these regions [21]. DNA templates of *H. nana* (extracted from eggs of human stool and tissues from adult worms in *R. rattus*) and *H. diminuta* were amplified in 67-mM Tris–HCl (pH 8.8), 2-mM $MgCl_2$, 16.6-mM $(NH_4)_2SO_4$, 0.5-unit Tth plus polymerase, 200 mM of each dNTP and 12.5 pmoles of each primer F3 (5′ GCGGAAGGATCATTACACGTTC 3′) and R3 (5′ GCTCGACTCTTCATCGATCCACG 3′). PCR thermal conditions were set as follows: one cycle of 94 °C for 2 minutes, 63 °C for 2 minutes, 72 °C for 1 minute, which was followed by 50 cycles of 94 °C for

20 seconds, 63 °C for 20 seconds, 72 °C for 45 seconds, and a final step of 72 °C for 7 minutes [21].

PCR products (3 µL) were digested overnight with 10 units of restriction enzyme (*Msp I*), 2 µL digestion buffer, and sterile ultra-pure H_2O to a final volume of 20 µL. The predicted restriction fragments for *H. nana* are 223 and 423 bp [21,23]. Meanwhile, *H. diminuta PCR* product remains undigested and represented by one band of 748 bp [21,23].

Statistical analysis

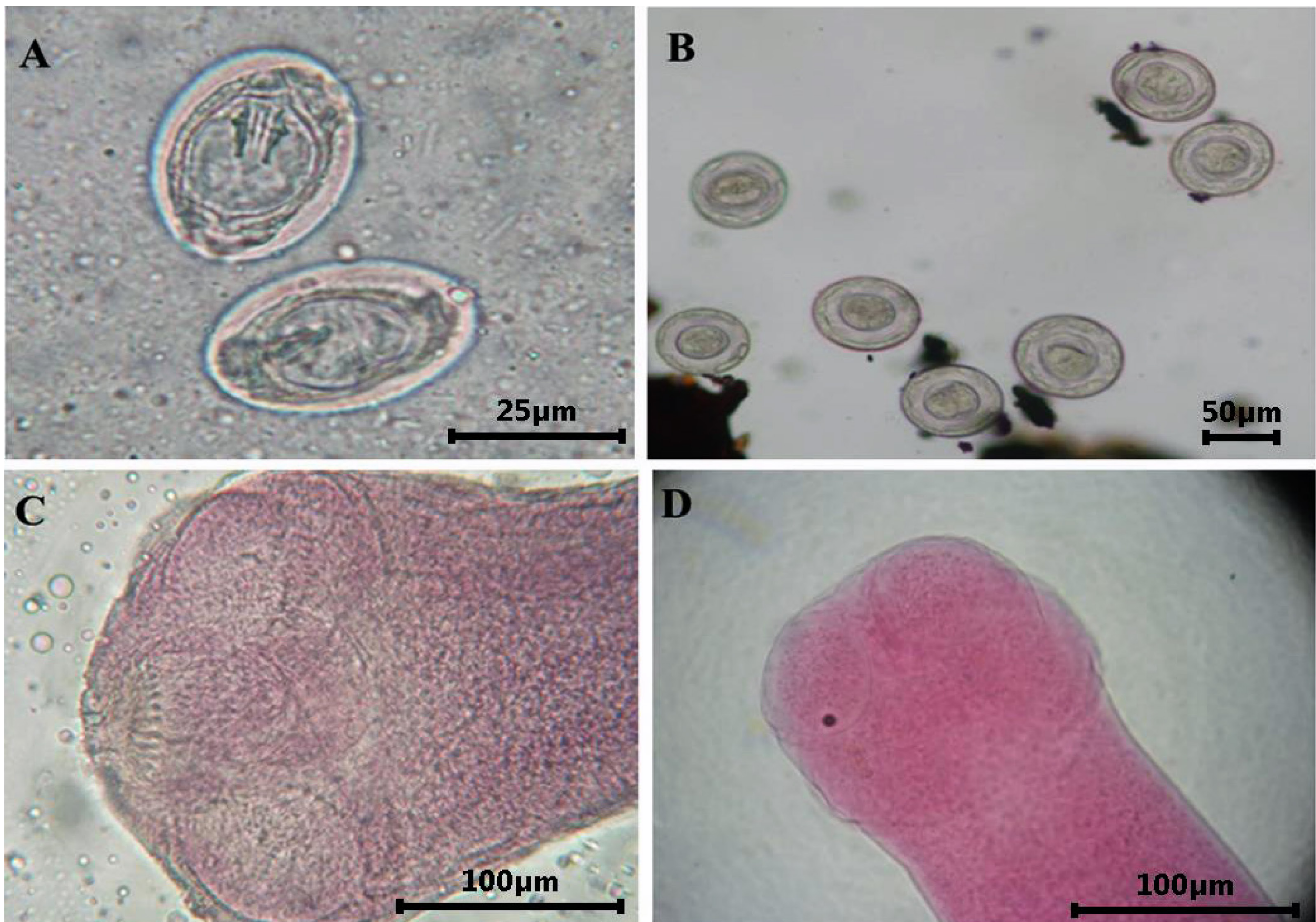
Number and percentage were used for the description of categorical variables. Meanwhile, continuous variables were expressed by the mean and standard deviation (mean ± SD). The Chi-square test was used to compare categorical variables and T-test was used for comparison of continuous variables. The Pearson correlation coefficient was used to assess the association between continuous variables. The *p* value of ≤ 0.05, ≤ 0.001, and > 0.05 were considered statistically significant, highly significant, and statistically not significant, respectively. Data were collected and then tabulated and the statistical analysis was conducted using SPSS 20.0 software. Fisher's exact test was examined to test differences in the prevalence of *Hymenolepis* spp. infection among *R. rattus individuals* in different localities, a Bonferroni correction was applied as a *post hoc* test to adjust the p-values for multiple group comparisons. A $p < 0.05$ is statistically considered significant.

Results

Morphological characteristics of identified Hymenolepis spp.

This study identified two *Hymenolepis* spp, *H. diminuta,* and *H. nana,* based on their morphological characteristics. Figure 2 shows the identified species of the parasite based on microscopic examination. As shown in Figure 2A, *H. nana* eggs appeared slightly oval with a thin shell and measured ~40–60 µm × 30–

Figure 2. A. *H. nana* eggs with the oncosphere bearing three pairs of hooklets surrounded by a membrane with two polar thickenings; **B.** *H. diminuta* eggs measure 70–85 µm × 60–80 µm and have no polar filaments; **C.** the scolex of *H. nana* with four muscular suckers and a rostellum; **D.** *H. diminuta* scolex with four muscular suckers without rostellum.



50 µm. *H. nana* eggs contain the oncosphere, which bears three pairs of hooklets surrounded by a membrane with two polar thickenings. Meanwhile, *H. diminuta* eggs measured ~70–85 µm × 60–80 µm and had no polar filaments (Figure 2B). Concerning suckers and rostellum, *H. nana* had four muscular suckers and a rostellum (Figure 2C), while *H. diminuta* had no rostellum (Figure 2D).

Prevalence of Hymenolepis spp. among examined R. rattus

In this study, the total prevalence of *Hymenolepis* spp. infection was 45% among examined *R. rattus*, with 33.3% (20/60) for males and 62.5% (25/40) for females. Table 1 shows that two species of *Hymenolepis* (*H. diminuta* and *H. nana)* were detected in the infected rats. The total prevalence of *H. diminuta* infection was 43%; the highest prevalence was found in *R. rattus* collected from the Abnoub locality 93.7% (30/32), and mixed infection with *H. diminuta* and *H. nana* was 6.25% (2/32) in the same locality (p = 0.05). Meanwhile, lower prevalence [(37.9%) (11/29)] of *H. diminuta* infection was detected in Sahel Sleem and there was no infection identified in Asyut City. Significant variation was found in the prevalence of *H.diminuta* infection in Sahel-Sleem, Elfath, and Asyut City versus Abnoub. The difference in *H. diminuta* infection rate between Sahel-Sleem and Asyut City was significant ($p < 0.05$). The comparison of *Hymenolepis* spp. infections in *Rattus rattus* across various localities are presented in Supplementary Table 1.

Prevalence of Hymenolepis spp. among examined children

As shown in Table 2, the prevalence of *Hymenolepis* spp. infection in the examined children was 28.3% (34/120) and *H. nana* was the only species reported. *H. diminuta* infection was not detected among the examined children in this work. In accordance with the individual variable factors under study (Table 2), the prevalence of *H. nana* infection was higher in male (32.8%) than female children (23.7%), but the difference was not significant (p = 0.650). Regarding age, the highest prevalence (36.3%) of *H. nana* infection was detected among the age group of 10–16 years, followed by a prevalence rate of 28.5% among children in the age group of 5–10 years, and the lowest prevalence (14.2%) was in the age group of 3–5 years. However, there were no statistically significant differences between the age groups ($p > 0.05$).

Table 2. Demographic characteristics of the children.

Characteristics	Total Examined	Number of infected children (%)
Gender		
Male	61	20 (32.8%)
Female	59	14 (23.7%)
Age groups		
3 - >5 Years	14	2 (14.2%)
5 ->10 Years	84	24 (28.5%)
10 - 16 Years	22	8 (36.3%)
Stool sample Positive to *H. nana*	120	34 (28.3%)

Molecular examination of Hymenolepis spp. positive samples

The parasitological examination in this study detected two *Hymenolepis* spp. from humans and animals, as shown in Table 3, including *H. nana* (n = 36) and *H. diminuta* (n = 45). The molecular testing was carried out to corroborate the *Hymenolepis* spp. positive samples identified morphologically. The positive samples to the ITS-1 gene of *H. nana* exhibited two bands of 223 and 423 bp in length, as revealed by PCR-restriction fragment length polymorphism (RFLP). *Hymenolepis diminuta* PCR products, on the other hand, remained undigested and were represented by a single band of 748 bp.

Discussion

This study provides interesting information and represents a comparative study of hymenolepiasis in humans and animals in Upper Egypt and the potential role of *R. rattus* in maintaining the zoonotic foci of the disease through the combined use of morphological and molecular methods. The potential risk factors that could be associated with the infection were also studied. Although the detection of different *Hymenolepis* spp. infections in rats and humans depend mainly on the

Table 1. Prevalence of *Hymenolepis* spp. infection among *R. rattus* in different localities.

Locality	n	*H. diminuta*		*H. nana and H. diminuta (Mixed infection)*	
		Infected (%)	Non-infected	Infected (%)	Non-infected
Sahel-Sleem	29	11 (37.9)^{b}	18	0^{b}	29
Elfath	21	2 (9.5)^{bc}	19	0^{b}	21
Abnoub	32	30 (93.7)^{a}	2	2 (6.25)^{a}	30
Asyut city	18	0^{c}	18	0^{b}	18
Total	100	43	57	2	98
Fisher's Exact Test		< 0.0001		< 0.0001	

Superscript letters (a, b, c, and d) mean within the same column carrying different superscripts are significantly different at $p < 0.05$ based on Bonferroni-Adjusted p value.

Table 3. Morphological and molecular identification *Hymenolepis* spp.

Characteristics	Number	*Hymenolepis* species (%)		
		H. nana	*H. diminuta*	*H. nana and H. diminuta* (Mixed infection)
Morphological identification				
Rodents	100	0.0	43 (43%)	2 (2%)
Children	120	34 (28.3%)	0.0	0.0
Molecular identification				
Rodents	45	0.0	43 (95.6%)	2 (4.4 %)
Children	34	34 (100%)	0.00	0.0

differences in the morphological characters of adult worms and eggs, molecular tools are considered alternative methods to a) identify *Hymenolepis* at species level, b) confirm the morphological identification, and c) differentiate the *Hymenolepis* spp. adults/eggs [24,25]. Molecular tools have revolutionized how closely related parasite species can be characterized [24-26]. PCR–RFLP represents a useful and inexpensive molecular tool used for confirmation of the parasite, especially in heavy and mixed infections [8]. Similarly, a previous study identified *Hymenolepis microstoma* among 11 individuals using PCR–RFLP [23]. Taken together, this study reveals the relatively high occurrence of *Hymenolepis* spp. among *R. rattus* and children which warrants conducting stricter control and preventive measures to reduce the risk of infection.

This study identified two *Hymenolepis* spp., *H. nana and H. diminuta*, based on the morphological characteristics of the parasites, which is consistent with several previous studies [5,27]. In this work, the total prevalence of *Hymenolepis* spp. infection in *R. rattus* was 45% (43% positivity for *H. diminuta* and 2% for mixed infection by *H. nana* and *H. diminuta*). *H. diminuta* infection rates varied in the examined localities. Significant variation was found in the prevalence of *H. diminuta* infection in Sahel-Sleem, Elfath, and Asyut City versus Abnoub. Moreover, there was a significant variation in *H. diminuta* infection rates between Sahel-Sleem and Asyut city. Reviewing the available literature, various prevalence rates were reported either at the national (Egypt) or international levels. In this respect, our present results revealed a higher occurrence rate than a previous study in Aswan province and Asyut Governorate, Egypt, whereas 8.70% and 11.67% of the examined rats were found infected by *H. diminuta*, respectively [28,29]. Furthermore, lower prevalence rates of 36.8% and 40.6% for *H. diminuta* were reported in rodents from the Nile Delta and Suez Canal zone (Egypt), respectively [30,31]. Another study carried out on fecal samples of rodents in Beni-Suef province (Egypt) revealed infection rates of 20% and 12.6% for *H. diminuta* and *H. nana*, respectively [32]. Furthermore, Abd-el-Wahed *et al.* [33] reported a prevalence rate of 3.8% for *H. diminuta* among wild rats from the Qalyobia Governorate, Egypt. Meanwhile, at the international level, lower prevalence rates of *Hymenolepis* spp. infection (1.3%–26.3%) have been reported in several studies [7]. This study recorded a mixed infection in *R. rattus* by both *Hymenolepis* spp.; however, the occurrence of *H. nana* in rats was low [2%] and it was detected only in Abnoub locality. Reviewing the available literature, higher prevalence rates of 10.3% and 19.6% were reported in previous studies in Kuala Lumpur and Sudan, respectively [7,34]. It seems that the differences in the reported infection rates in different areas might be closely associated with the degree of endemicity of *Hymenolepis* spp. infection in the investigated areas. The variation in the prevalence rates of hymenolepiasis could also be attributed mainly to the level of hygienic practices, level of sanitation and socioeconomic status [35]. Moreover, climatic and ecological conditions, habitat characteristics, abundance of rodents, and host vulnerability to infection might influence the prevalence of hymenolepiasis [35].

Furthermore, this present work reported a higher prevalence in female than in male rats. The present results are inconsistent with a previous study that showed a higher prevalence rate in males (20%) than in females (16.2%), but the difference was not significant [36]. Clearly, the variation of prevalence reported among males and females in several studies indicates that acquiring *Hymenolepis* infection is unlikely to be gender-linked, and the infection occurs when contact occurs with infected rodents or intermediate hosts.

In accordance with the findings of *Hymenolepis* spp. infection among children under study, *H. nana* was the only detected species with a prevalence rate of 28.3% (34/120). *Hymenolepis nana* is considered one of the most common zoonotic parasites in humans, estimated to cause infections in 75 million people globally [37]. In accordance with its occurrence at the national level (Egypt), various prevalence rates (3.9%–49.6%) have been reported in several previous studies

[38-39]. As in many developing countries, Egypt suffers from hymenolepiasis with variable detection rates. According to a previous study on 2,292 farmers from Menoufia Governorate, *H. nana* eggs were detected in 3% of the examined stool samples [40]. In another study at a hospital in the Dakahlia Governorate, *H. nana* eggs were detected in 3.9% of the examined stool samples [41]. A study on human stool in Beni-Suef province (Egypt) revealed that the prevalence rates of *H. nana* and *H. diminuta* were 12.5% and 0.18%, respectively, by direct fecal smears and concentration methods [15]. These studies reported lower prevalence rates of the parasite in Egypt than our present work. However, another study reported a higher prevalence rate of 49.6% [39]. Moreover, many surveys revealed the prevalence of *Hymenolepis* spp., mainly *H. nana*, infection worldwide. A previous study in Mexico reported that the prevalence rate of *H. nana* among children was 25% [42]. Based on the literature, *H. diminuta* infection in humans is very rare [43], which is consistent with our present findings. According to a MEDLINE search, since 1965, 48 cases have been recorded in the United States [43]. The World Health Organization reported six human cases infected with *H. diminuta*, from India, Italy, Jamaica, Spain, the US, and Yugoslavia, during the period from 1989 to 1999 [45], in addition to another case from India [45]. At the national level, a lower prevalence of *H. diminuta* (1.4%) was determined in Dakahlia Governorate, Egypt [17]. In contrast, *H. diminuta* could not be detected in children in this study, which is consistent with several studies. Given its rarity, *H. diminuta* human infection is rather uncommon and a possible explanation could be that humans rarely ingest intermediate hosts containing its cysticercoid larvae [46].

Regarding the variable factors, this study reports a higher number of infected males, by *H. nana*, (20/61) than females (14/59). However, there was no statistically significant difference in the prevalence rate of *H. nana* infection among males/females ($p > 0.05$). Similarly, the prevalence of *H. nana* infection was higher in males (16.5%) than in females (10.4%) in Ethiopia [47]. Another study carried out in Sudan observed higher prevalence rates of *H. nana* among males, and the association was statistically significant ($p < 0.001$) [48]. In contrast, a survey in Burkina Faso recorded no differences in the prevalence rates of *H.* among female and male children ($p = 0.963$) [49]. It is concluded that infection with *Hymenolepis* spp. is not associated with gender, but it varies between sexes in different studies. Regarding the age of children, this study revealed no statistical difference in the prevalence of *H. nana* among the various age groups. A similar age-related pattern was described in another study, which indicated that the prevalence of *H. nana* infection among children in the age group of > 10 years was higher than the infection among the younger age groups [47]. In contrast, the present results disagree with another study, which found that children below the age of 10 years had a higher infection rate (50%) of *H. nana* than the older age groups [50]. Factors that might influence this association include sample size, climatic and ecological factors, the level of sanitary measures, and socioeconomic level. These findings could be explained by the fact that, at this age, children do not strictly follow hygienic measures, including washing their hands after using the toilet.

Conclusions

Using a variety of morphological and molecular identification approaches, this study offers intriguing information regarding the occurrence of *H. diminuta* and *H. nana* in rodents and children from Upper Egypt. *Hymenolepis nana* infection, for example, endangers children's health due to its capacity to complete its life cycle without an intermediate host. To minimize infection, governmental and nonprofit organizations should implement personal education, periodical deworming of children, rodent control, and environmental hygiene measures. Further research into hymenolepiasis in rodents and people on a bigger scale in Egypt, as well as the primary circulating genotypes of the parasites in the country, might be beneficial in combating this disease.

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Authors' Contributions

A.S.M.S., M.Z.A., D.A.Y., A.A.M.A, and. designed the idea of the conception, performed the methodology, formal analysis, data curation and supervision besides revision of the manuscript. A.S.M.S, A.A.M.A., M.F.E-K., M.S.L, and E.K.E., participated in the methodology, formal analysis, data curation and contributed their scientific advice. M.Z.A., A.S.M.S., and E.K.E drafted the manuscript, and prepared the manuscript for publication and revision. The manuscript was read by all authors who agreed to the published version.

Institutional Review Board Statement

Animal procedures in this study were conducted following the rules and standards of animal research of Assiut

University, Egypt. The procedures were ethically approved by the Scientific Research Committee and Ethics Board of Faculty of Medicine, Assiut University, Asyut, Egypt (IRB no: 17101287). Helsinki Declarations in relation to ethical standards with human participants were also followed.

Informed Consent Statement

The study was performed in full accordance with the principle of Good Clinical Practice (GCP) and according to the guidelines of Helsinki Declaration and an informed consent was also obtained from all participants. Written informed consent to participate in this study was provided by the participants' legal guardian.

Data Availability Statement

The data that support the findings of this study is contained within the article.

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Annex – Supplementary Items

Supplementary Table 1. Comparison of *Hymenolepis* spp. infection among *R. rattus* in different localities.

	Unadjusted *p* value	**Bonferroni-Adjusted *p* value**	**Significant**
Comparison of localities for *H. dimuniuta*			
Sahel-Sleem vs. Elfath	0.1074	0.6443	No
Sahel-Sleem vs. Abnoub	0.00000000000000142	0.00000000000000853	Yes
Sahel-Sleem vs. Asyut city	0.00000000000000000000000000000349	0.00000000000000000000000000000209	Yes
Elfath vs. Abnoub	0.00000000000439	0.00000000002634	Yes
Elfath vs. Asyut city	0.0138	0.0826	No
Abnoub vs. Asyut city	0.00000000000000000119	0.00000000000000000712	Yes
Comparison of localities for mixed infection			
Sahel-Sleem vs. Elfath	1		No
Sahel-Sleem vs. Abnoub	0.00000000000000263	0.0000000000000158	Yes
Sahel-Sleem vs. Asyut city	1	……	No
Elfath vs. Abnoub	0.00000000000486	0.0000000000292	Yes
Elfath vs. Asyut city	1	….	No
Abnoub vs. Asyut city	0.00000000000000258	0.0000000000000155	Yes