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Microscopic detection study of *Hymenolepis nana* in children in Babylon province, Iraq

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Abstract
This study was conducted to investigate the prevalence of *Hymenolepis nana* at Babylon Governorate for during the period from December. 2023 till April 2024, examined about 75 fecal samples of house rats male (47) and female (28). In addition, 75 stool samples of humans (children) in different areas (Al- Qasim, Al- Musayyib, Al- Hilla, Al- Kifl, Al- Hamza Al- Gharbi, Al- Shomali) and study the effects of sex, areas with ages in humans. the infection rates of *Hymenolepis* parasite in humans by using Microscopic, the study revealed in humans on the infection rates by traditional methods (Direct microscope, formalin ethyl acetate and flotation method). In humans *H. nana* was showed an infection rate 9.3% (7/75), the high infection rate was 10% (2/20) in Al- Hilla and it was 5.9% (1/17) in Al-Qasime with significant difference., 10% (1/10) Al Musayyib,12,5% (1/8) Al Kifl, 10% (1/10) Al-Hamza Al Gharbi, 10% (1/10) Al Shomali) .The results were showed the parasite *H. nana* of the infected humans in this study tack three gropes 1-5 years about 16% (4/25), 6-10 years. 32(2/6.2%) and 11-15 years 18(1/5.6%). 75 stool samples of humans (children) in different areas (Al Qasim, Al Musayyib, Al Hilla, Al Kifl, Al Hamza Al Gharbi, Al Shomali) and study the effects of sex, areas with ages in humans on the infection rates by traditional methods (Direct microscope, formalin ethyl acetate and flotation method). In the male 6.4(3/47) and in female 7.1(2/28).

Keywords: *Hymenolepis nana*, humans, formalin ethyl acetate, flotation method

Introduction
Hymenolepis nana is the predominant tapeworm found in both humans and rodents globally, prevalent in tropical and subtropical regions, especially in rural areas with poor or insufficient sanitation. Hymenolepiasis is a type of helminthiasis infection in humans, which is considered a neglected tropical disease. The illness is common in dry, hot, and low-resource areas [2]. It is a frequent parasitic disease in children and is seen as an opportunistic illness with a potentially deadly impact on immunocompromised individuals due to the spread of cysticercoids. It shows a high rate of infection in populations residing in tropical and subtropical countries with low hygiene levels and poverty [4]. *Hymenolepis nana*, also called dwarf tapeworm is a small cestode parasite named as Vampirolepis nana, Hymenolepis fraternal, and *Taenia nana* [5]. It is present worldwide and is a prevalent cestode parasite in the phylum Platyhelminths. It infects various domestic and wild animals, as well as humans, particularly children, in temperate regions, with a higher incidence among institutionalized individuals [6]. Minchin showed that fleas are capable of acting as intermediate hosts between humans [7]. *Hymenolepis* nana is the sole tapeworm that can finish its life cycle without the need for an intermediary host (direct life cycle); typically, tapeworms need one or two intermediary hosts and *H. nana* can also go through an intermediary host (indirect life cycle) [8]. Similarly, H. diminuta does not always rely on arthropods like flour beetles from the Tenebrionidae family to complete its life cycle [9]. Grassi noted in 1887 that transmission between rats did not need an intermediary host. Later in 1921, Saeki showed that *H. nana* could be transmitted directly between people without the need for an intermediate host [10].
In their study, Nicholl and Minchin showed that fleas can act as middle hosts between humans in the direct cycle [11]. Rodents harbor human pathogens, and if mice in a confined space are infected with the same parasites, it is mostly associated with the life cycle of *H. nana* and coprophagia, which is a typical feeding behavior of rodents that helps them retrieve unused nutrients from their first digestion. Infection is transmitted through the ingestion of eggs from contaminated hands via the fecal-oral route, often through food and water [12].

This worm is capable of infecting itself and finishing its life cycle independently without requiring a different host. Yet, some fleas and beetles have the ability to act as middle hosts, where the infectious phase grows inside them prior to being consumed by the final host. People can become infected by touching directly, consuming eggs, or sometimes by unknowingly ingesting a beetle or flea carrying the larvae. In addition, the easy transmission of the parasite among rodents sharing an enclosure, due to close physical contact between pet rodents and humans, increases the risk of *H. nana* infection in pets. This poses a transmission threat, especially to children who may not always wash their hands thoroughly after handling animals [13].

Materials and Methods

1. Human samples (stool)

During the month of December. A surveillance study was conducted in Babylon from 2023 to April 2024. The study included 75 individuals ranging from one to fifteen years old, with each person providing a single stool sample that was examined directly. Samples of feces were examined to detect parasitic structures (head, body parts, and reproductive cells), while details regarding where and the sex of individuals were recorded. The stool sample needs to be gathered in a tidy, empty screw-top container [14]. Firstly, a microscopic examination is conducted to identify eggs using a microscope.

2. Microscopic examination :- (Human)

1. Direct smear

For the direct smear method, around 2 mg of rodent feces and human stool samples were placed on glass slides using glass rods and combined with saline. A small amount of each water sample was placed onto a slide, while 200 grams of each green salad sample were manipulated by a food handler and stored in a plastic bag until juice was released, from which a small amount was added to the glass slide. Next, the slides were covered with a coverslip and examined using a microscope at a 40x magnification for identification as stated in [15].

2. Flotation method

The flotation technique was used to analyze fecal samples from both house rats and humans. Fifty grams of poop were put in a 250 mL glass container and covered with 0.95% salt water solution until reaching a final volume of 200 mL. The blend was completely mixed using a glass rod and allowed to settle for 5 minutes before being filtered to remove large particles. Sediment along with other solid particles were eliminated. The blend was filtered through a fine mesh sieve and then moved into a pre-labeled 15 mL conical tube, which was subsequently spun at 2000 g for 3 minutes. A positive meniscus was formed in each tube by adding an extra 0.95% sodium chloride solution, and then a coverslip was placed on top. Following a 5-minute period, the coverslip was taken off and positioned on a slide with a pre-assigned number for inspection using a light microscope set at 40× magnification [16]. The presence of the polar filament of dark brown-colored eggs confirmed the diagnosis of *H. nana* infection [17].

Results

Microscopic Result

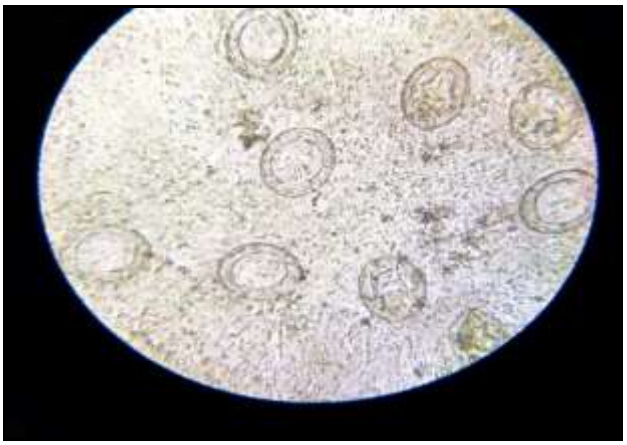

Fig 1: Egg of *Hymenolepis nana* under the microscopic (X40), in stool of human show overall in shape *H. nann* under the microscope.

Table 1: Infection in rate of *Hymenolepis* nana in humans according to sex in Microscopic

Gender	No. of samples examined	Positive samples	
Male	44	No. of positive	Percentage of total (%)
		5	11.4
Female	31	2	6.5
Total	75	7	9.3
X^2	0.518549		
P value	0.471461NS		

NS: no significant differences at ($p \leq 0.05$).

Table 2: Infection rate of *Hymenolepis nana* in human according to age groups (Microscopic)

	No. of the exam. samples	Positive samples	
		No.	% of total
1-5	25	4	16
6-10	32	2	6.2
11-15	18	1	5.6
Total	43	26	9.3
X^2	1.976103		
P value	0.372301NS		

NS: no significant differences at ($p \leq 0.05$).

Table 3: Infection of *Hymenolepis* nana in`````````````` humans according to areas of study by (Microscopic)

Areas	No. of the exam. Samples	Positive samples	
		No.	% of total
Al Musayyib	10	1	10
Al Hilla	20	2	10
Al Kifl	8	1	12.5
Al Hamza Al Gharbi	10	1	10
Al Qasim	17	1	5.9
Al Shomali	10	1	10
Total	75	7	9.3
X^2	0.360310		
P value	0.996353 NS		

NS: no significant differences at ($p \leq 0.05$).

Discussion

Infection rate of *Hymenolepis nana* in human according to area

Seventy-five samples of stool were gathered, with 9.3% (7/75) showing signs of *H. nana* infection in laboratory tests. The highest infection rate was 10% (2/10) in Al Hilla, and equal rates of 10% (1/10) were found in both Al Musayyib and Al Hamza Al Gharbi. In addition, rates of 5.9% (1/17) in Al-Qasim, 12.5% (1/8) in Al Kifl, and 10% (1/10) in Al Shomali were reported.

Hymenolepis nana is the most frequently identified tapeworm in humans all over the world. It is common among kids in tropical and subtropical regions, especially in rural impoverished areas with insufficient or missing sanitation [18]. The rate of *H. nana* can also be influenced by asymptomatic individuals in the community, as they are the main source of infection by continuously excreting eggs in their stool. Evidence suggests that rats living near humans in urban environments, benefiting from resources like food and shelter, can spread numerous diseases. Human activities like clearing forests are creating new habitats for commensal rodents. 62 The findings of this study align with other research that found a low infection rate of *H. nana* in humans, noting a 3.0% infection rate in Abha, South Western, Saudi Arabia. And who documented a 1.8% infection rate of *H. nana* among children based on 634 stool samples in Baghdad Al-Rasafa.

Infection rate of *Hymenolepis* nana in humans according to sex

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Infection rate of *Hymenolepis* nana in human according to age group

A sum of 75 patients diagnosed with hemenolepis nana, consisting of 44 males and 31 females. 16% of infections occurred in children aged 1-5, while 6.2% occurred in children aged 6-10. Infections in the 11-15 age group accounted for 5.6%, with no significant difference between these age groups. Other age groups, such as 6-10 and 11-15 years old, also experienced infections. In people of all ages, infection can happen. Yet, school-aged children are at the highest risk of Hymenolepiasis due to their increased likelihood of being exposed to human feces. It is rare among adults. The study found that the highest infection rates of 10% and 6.2% were in the 1-15 age group due to possible poor personal hygiene and self-dependence in using the bathroom. The findings of this study align with previous research done by [27], which reported a 25% prevalence of *H. nana* infection among children aged 6-10 years in rural Mexico. It was also noted that the highest infection rate of 2.97% was recorded among children aged 4-6 years. Additionally, it was revealed that the patients were categorized based on age ranges of 1-5, 6-10, 11-15, and 16-20 years. Patients under 10 years old had a higher proportion (50%), while the older age groups had the lowest percentages. Therefore, according to the study, those aged 1-10 years had a higher infection rate (30.0%) compared to other age groups [29]. The data shows that the majority of infections occurred in age groups 6 to 9-12 years, with rates of 93.8% and 88.2% respectively. The lowest rate of infection was seen in children under one year old at 27.1%. Additionally, 11.4% of infections were in the age group >7 years, and 8.8% were in the age group ≥7 years. Percentage of positive *H. nana* cases ranged from 1-5% at 7.14%, 6-10% at 7.40%, 11-15% at 0.00%, 16-20% at 0.00%, and ≥ 20% at 0.00%. Overall, the total percentage was 3. Chi-Square (χ 2) value was 3.41 which was not significant. The findings of this study contradicted those of [31] who found the highest infection rate among individuals under 30 years old. The current study found that *H. nana* infection is prevalent in children aged 1-5 and 6-10, possibly because they do not wash their hands after using the toilet or wash vegetables before eating, which are significant risk factors. Primary causes for this are the lack of parental awareness and economic circumstances.

Conclusion

1. *Hymenolepis* infection is higher in males than females
2. The higher infection rate of *Hymenolepis nana* is found in in Al-Hilla
3. Human *H. nana* infection is recorded in the age group between 1-5 years.
4. Morphologyical examination by microscope can be used as a primitive diagnosis of *Hymenolepis* spp. but molecular techniques is needed for definitive identification of the species.

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