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# The role of beetles in expanding of spread of parasitic infection with *Hymenolepis nana*

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## ABSTRACT

*Hymenolepis nana*, commonly known as the dwarf tapeworm, is a significant zoonotic parasite with a cosmopolitan distribution. This study aimed to investigate the prevalence of *H. nana* infection in human populations and the role of stored-product beetles, specifically *Tribolium confusum*, as intermediate hosts within food substrates like flour and rice. A total of 75 human stool samples were collected and examined using the Petri dish method and Ziehl-Neelsen staining. Simultaneously, 200 food samples (100 flour and 100 rice) were analyzed for beetle infestation. Statistical analysis was performed using the Chi-Square test at a probability level of ($p \leq 0.05$). The overall infection rate of *H. nana* was 24% (18/75 cases). While females showed a slightly higher infection rate (25.5%) compared to males (21.8%), the difference was not statistically significant. However, age was a critical factor; children under 10 years exhibited the highest prevalence (40%), which decreased significantly with age, reaching 17.6% in individuals over 40. Infection was strongly associated with gastrointestinal distress ($p \leq 0.05$), with abdominal pain (33.3%) and diarrhea (30%) being the most common symptoms. Notably, 8% of positive cases were asymptomatic. A highly significant difference was found in beetle infestation between food types. Flour recorded a dominant infestation rate of 70% and a prevalence of 93.88% (2,071 beetles), whereas rice showed only a 5% infestation rate and 6.12% prevalence (135 beetles).

**Keywords:** ***Hymenolepis nana, Tribolium confusum*, Food, Flour, Prevalence.**

## Introduction

*Hymenolepis nana* generally known as the dwarf tapeworm, is one of the most Common tapeworms of Humans, in which the parasite can cause hymenolepiasis. This Zoonotic tapeworm has a cosmopolitan Distribution with socio-economic and medical Significance which may occur in many countries, worldwide (Cheng et al., 2016). Hymenolepiasis is the most common intestinal tapeworm Infection of humans with an estimated 50 to 75 million infections worldwide, caused by *Hymenolepis nana* which is commonly known as dwarf Tapeworm, this tapeworm is Endemic in Asia, Africa and southern and eastern Europe (CDC., 2014, Mirdha and Samantrai, 2002). A person becomes diseased by coincidence eating eggs of tapeworm Parasite, This can occur by eating nutrients or liquid contaminated with stool by Touching mouth by contaminated limbs, or by eating contaminated earth, Individuals be able to also become diseased if they by coincidence eat an Infected insect such as Arthropod for example small or large beetle that has caught into the nutrition, adult parasite ( *H.n.*) are so very minor compared to further tapeworms belong to class Cestoda and may be up to fifty-fourth mm long, adult parasite are made up of from various small Strobilia, when the dwarf tapeworm parasite develops within the human digestive system, Specifically in the small intestine, its parts disintegrate and are transmitted to the stool this

parasite Can live for 21- 42 days. After Ingestion of eggs by an insect intermediate host such as beetles or fleas, the oncosphere Is released in the intestine, penetrates the intestinal wall, and develops into a Cysticercoid larva within the body cavity of the insect. Infection of the definitive host occurs when the infected insect containing the cysticercoid is ingested, allowing the parasite to mature into an adult tapeworm in the intestine (CDC., 2023).

## Materials and Methods

The Petri dish method was used for preparing flour and rice samples. 10 grams of Each flour or rice sample were weighed using a clean laboratory balance and placed in a Sterile Petri dish for subsequent examination, as in previous studies (Marangi et al., 2003; Roberts, Janovy and Nadler, 2013). The samples were visually examined for the presence Of insects or other contaminants, and any detected insects were separated manually using fine forceps or a small sieve, following standard entomological procedures, as in Previous studies (Sokoloff, 1974).

**Samples collection:** Stool, flour, and rice samples were systematically collected from different areas within the study region for the detection of *Hymenolepis nana*. Fresh stool specimens were obtained from individuals presenting with abdominal pain. Each participant was provided with a sterile, tightly sealed plastic container and instructed to collect an adequate amount of fresh stool. All samples were carefully coded and labeled with relevant demographic information, including age, gender, and date of collection, to ensure accurate documentation and traceability. The collected specimens were transported promptly to the laboratory in cooled containers and examined as soon as possible to preserve sample integrity and minimize the risk of contamination.

In parallel, flour and rice samples were collected from different households within the study area using clean plastic bags. Each sample was appropriately labeled with the location and date of collection. The flour and rice samples were transported to the laboratory for detailed examination of possible insect contamination, particularly the presence of flour beetles such as *Tribolium confusum*, which may serve as a potential intermediate host in the transmission cycle. The insects were manually separated using a sieve and prepared for microscopic examination in accordance with the study protocol.

**Stool sample preparation (Petri dish method):** Ziehl-Nelsen stain, produced by Ismailia Company for laboratory medical supplies in Egypt, was used according to the steps shown below:

1- Prepare a slide by taking a stool sample (2 mg) and distributing it on the glass slide and leaving it to dry and fix it using heat.
2- Dip the slide in carbol fuchsin solution for 5 minutes.
3- Wash the slide with distilled water.
4- Dip the slide in sulfuric acid H2SO4 at a concentration of 20% for one minute.
5- Wash with distilled water to remove excess sulfuric acid.
6- Dip the slide in methylene blue for one minute.
7- The slide is washed with distilled water to remove the excess dye and placed in the Slide holder until it dries and then examined Isolation and diagnosis of parasites: Examination using a dissecting microscope and a NOVEX-HOLLAND compound Microscope (40x and 100x), samples examined directly. For the purpose of diagnosing Parasites based on by Peter et al., (2003), the percentage of parasitic Infestation was calculated.

## Results and Discussion

**The percentage of infection *Hymenolepis nana* with according to sex:** A total of 75 samples were examined, of which 18 were positive, giving an overall prevalence rate of 24%. The infection rate among females (25.5%) was slightly higher than that among Males (21.8%) (Table1). Statistical analysis using the Chi-Square ($X^2$) test indicated that These differences are not statistically significant at the probability level of $p \leq 0.05$. This Suggests that the slight numerical increase observed in females does not represent a biological susceptibility, but rather a coincidental or minor variation in sample Distribution within the studied population This variation may be explained by differences in environmental exposure, hygiene practices, and the nature of daily activities that may Increase the risk of transmission. These findings are consistent with the study conducted By Alasadiy on the prevalence of intestinal parasites, which reported slight differences Between males and females in infection rates, with females sometimes showing higher Prevalence due to behavioral and environmental factors (Alasadiy, 2020). Similarly, Migue et al. (2016) reported no statistically significant differences between genders in the prevalence of *H.n.* infection, attributing this to similar living conditions and Comparable exposure to sources of infection (Wadhah et al., 2022).

**Table (1): The Percentage of infection *Hymenolepis nana* with according to sex.**

| Sex | Total examined | Positive cases | Negative cases | Infection rate % | Statistical Results |
|---|---|---|---|---|---|
| Male | 32 | 7 | 25 | 21.8% | Chi-Square ($x^2$) ~0.165 |
| Female | 43 | 11 | 32 | 25.5% | Degrees of Freedom (df): 1 |
| Total | 75 | 18 | 57 | 24% | P-value: p≤0.05<br>Not statistically significant (ns) |

In addition, Wali et al. (2018) indicated that gender-related differences are often limited, and that environmental and hygienic factors play a more significant role in determining prevalence than gender Itself (Wali et al., 2018).

**Distribution of patients infected with *H.n.* according to age groups:** The table shows that the highest infection rate of *H.n*. is concentrated in the age group under 10 years at 40%. This percentage then begins to decrease gradually as age increases, reaching 22.2% in the (10-20 years) group and 20% in the (21-40 years) group, until it records its lowest levels among people over 40 years old at 17.6% (Table 2). By examining the total sample of 75 individuals, only 18 positive cases were found, making the overall average infection rate for this study 24%. This clearly indicates that young children are the group most vulnerable to infection compared to adults and the elderly. These results are in high agreement with several local studies conducted across various Iraqi provinces, which consistently identify Hymenolepiasis as a major public health concern, particularly among pediatric populations. Our findings align with Al-Saeed and Issa (2006) in Dohuk, who observed the prevalence was more pronounced in male subjects compared to females. Among the positive cases, a subset of 70 out of 1261 samples exhibited mixed parasitic infections (either double or triple co-infections), where Hymenolepis nana co-existed alongside *Entamoeba histolytica, Iodamoeba buetschlii* and *Blastocystis hominis*, *G. lamblia*.

Furthermore, the 24% overall prevalence mirrors trends reported by Al-Sultany and Al-Morshidy (2023) in the holy city of Karbala. The proliferation of intestinal parasitic infections was heavily influenced by several epidemiological parameters, notably the patients' age and gender, alongside the specific timeframe of the study. The peak infection rate in the youngest age group (40%) can be attributed to poor personal hygiene habits and the lack of acquired immunity in children, which is a common conclusion in Iraqi epidemiological literature, According to a prior investigation conducted by Husam and Jawad (2019) in Babylon Province, the overall infection rate of H. nana was notably high at 11.8%. Conversely, a significant decline in clinical manifestations was observed among patients older than 15 years, with the infection rate dropping to a mere 0.33%. In alignment with Khder (2024) in Erbil, the researcher proposes that despite the noticeable reduction in intestinal parasite contraction rates, this persistent health issue still requires the maintenance of active prevention and awareness campaigns within the local community. The persistence of a 24% infection rate in this study underscores the necessity of integrated control measures that target both direct transmission and indirect routes involving stored-product beetles to mitigate the spread of the parasite in the Iraqi Environment. The statistical non-significance observed in this study supports the conclusions of Wadhah et al. (2022), suggesting that in areas with shared environmental contamination (such as water and soil), the entire community remains at risk regardless age.

**Table (2): Distribution of patients infected with *H.n.* according to age groups.**

| Age groups | Total examined | Positive | Infection rate % | Statistical Results |
|---|---|---|---|---|
| <10 | 15 | 6 | 40% | Chi-Square ($x^2$) ~3.41 |
| 10-20 | 18 | 4 | 22.2% | Degrees of Freedom (df): 1 |
| 21-40 | 25 | 5 | 20% | P-value: p≤0.05 |
| >40 | 17 | 3 | 17.6% | Not statistically significant (ns) |
| Total | 75 | 18 | 24% | |

**Distribution of patients infected with *H.n.* according to clinical symptoms**. Illustrates The relationship between *Hymenolepis nana* infection and clinical symptoms. The Highest infection rate was recorded among patients suffering from abdominal pain (33.3%), followed by diarrhea (30%) (Table3). The lower a small percentage of Infected individuals (8%) showed no symptoms, indicating that asymptomatic Infection can occur. Statistical evaluation using the Chi-Square (X2) indicates that the distribution of symptoms is statistically significant p≤0.05 This suggests that *H. Nana.* infection is not random in its clinical presentation but is strongly associated with gastrointestinal distress.

**Table (3): Distribution of patients infected with *H. N.* according to clinical symptoms.**

| Clinical symptoms | Total cases | Positive | Infection rate % | Statistical Results |
|---|---|---|---|---|
| Abdominal pain | 30 | 10 | 33.3% | Chi-Square ($x^2$) 7.82 |
| Diarrhea | 20 | 6 | 30% | Degrees of Freedom (df): 1 |
| No symptoms | 25 | 2 | 8% | P-value: p≤0.05 |
| Total | 75 | 18 | 24% | statistically significant ($s^*$) |

**Comparison of Beetle Prevalence and Infestation Rates Between Flour and Rice:** The results demonstrate a highly significant difference (p≤0.05) in the prevalence and infestation rates of beetles between flour and rice, with flour emerging as the vastly superior environment for population growth. Out of the 200 total samples, flour recorded a dominant infestation rate of 70% and a prevalence of 93.88% (2071 beetles), whereas rice showed a significantly lower infestation rate of 5% and a prevalence of only 6.12% (135 beetles). This statistical disparity is biologically justified by the fact that these beetles are secondary pests, lacking the strong mandibles required to penetrate hard, intact rice grains; conversely, the powdered nature of flour provides an accessible, high-surface-area food source for both adults and larvae (Hagstrum and Subramanyam, 2006). Furthermore, the fine texture of flour serves as an ideal substrate for oviposition, offering protection and camouflage for eggs that the large, unstable interstitial spaces of rice grains cannot provide (Sokoloff, 1974). Additionally, because flour absorbs atmospheric moisture more efficiently due to its small particle size, it creates a high-humidity microenvironment that significantly accelerates fertility rates and the overall life cycle, leading to the observed population explosion in flour compared to rice (Rees, 2004) (Table 4).

**Table (4): Comparison of Beetle Prevalence and Infestation Rates Between Flour and Rice.**

| Food source | Total samples examined | Number of infested samples | Percentage of infestation % | Average number of beetles Per 10 g | Total number of beetles | Percentage prevalence (%) | Statistical results |
|---|---|---|---|---|---|---|---|
| Flour | 100 | 70 | 70 | 29.58 | 2071 | 93.88 | Chi-Square ($x^2$) 9.06 |
| Rice | 100 | 5 | 5 | 27 | 135 | 6.12 | Degrees of Freedom (df):1 |
| Total | 200 | 75 | 75 | | 2206 | 100 | P-value: p≤0.05 statistically significant ($s^{**}$) |

## Conclusions

1. Age-Driven Epidemiology: A significant inverse correlation exists between Age and infection rates, with a peak prevalence of 40% among children under 10. This identifies underdeveloped immunity and suboptimal hygiene as the Primary drivers of Hymenolepiasis.
2. Gender-Neutral Exposure: Biological sex is not a determinant of Susceptibility, as evidenced by comparable rates in both genders. Infection Risk is instead governed by shared environmental conditions and uniform Exposure to contamination sources.
3. Substrate-Host Dynamics: Flour acts as a high-risk reservoir for Intermediate hosts, exhibiting an exceptional infestation rate of 3.88%. Its Physical properties create an ideal micro environment for beetle proliferation, thereby facilitating the indirect transmission cycle.
4. Clinical Correlation and Latent Risks: While infection is strongly associated with acute gastrointestinal symptoms, the presence of asymptomatic carriers (8%) identifies a "silent reservoir" that sustains the parasite's

epidemiological Persistence within the population.

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