Prevalence of *Hymenolepis nana* Infection in Aswan Governorate and Associated Risk Factors Assessment

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**Abstract**

*Hymenolepis nana* infections are among the most important global socioeconomic and health problems worldwide notably in developing countries with hot temperature and dry climates. This study aimed to estimate the linkage between the prevalence of *H. nana* infection in humans at variant ages and its presence in different sources. A cross sectional study was achieved in the period between 2019 and 2021 during which 100, 50, 50, and 175 samples of rodent feces, water, green salad, and human stool were gathered, respectively and investigated for *H. nana* eggs. For checking the diagnostic efficacy of direct smear and flotation techniques compared to formalin-ethyl acetate concentration technique, rodent and human fecal samples were examined by the three methods. It has been found that one (1.0%), two (2.0%), and five (5.0%) out of 100 rodent fecal samples contained *H. nana* eggs, respectively. In Parallel, 2 (1.14%), 5 (2.86%), and 7 (4.0%) of 175 human fecal samples were positive, respectively by the three methods emphasizing that formalin-ethyl acetate concentration technique is the most sensitive. On the other hand, samples of water and green salad were examined by direct smear method only yielding 6.0% (3/50) contaminated vegetable samples while tape water samples weren’t contaminated by *H. nana* eggs. Interestingly, the prevalence of hymenolepiasis was significant (p<0.05 at 95%CI) with washing of hands, washing of vegetables/fruits, fingernail trimming, and water supply. Therefore, an efficient program to manage *H. nana* infection should emphasize educating the hygienic practices and improving sources of drinking water.

**Keywords:** FECT, Human, *Hymenolepis nana*, Risk factors, Rodent

DOI: 10.21608/svu.2023.191180.1256  Received: February 1, 2023  Accepted: May 5, 2023  Published: June 18, 2023

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**Citation:** Badry et al., Prevalence of *Hymenolepis nana* Infection in Aswan Governorate and Associated Risk Factors Assessment. SVU-IJVS 2023, 6(2): 55-69.

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**Competing interest:** The authors have declared that no competing interest exists.
Introduction

*Hymenolepis nana* is a miniature tapeworm, and the most widespread human cestode in the world (Magalhaes, 2013) especially in warm climates (Cabada et al., 2016). Various prevalence rates were detected in different regions, including Africa (1.8-2.9%), Europe (0.5-5%), Asia (0.2-28.4%), and Americans (0.9-23%) (Houmsou et al., 2010; Soriano et al., 2011; Mohd et al., 2012).

Rodents are known as reservoirs or carriers of different zoonotic pathogens threatening the public health by dissemination of various diseases via food or water contamination including *H. nana*. The role of rodents as main reservoir hosts especially brown rats in transmission of the parasite has been recorded (Yang et al. 2017).

Recently, the consumption of raw vegetables contributes to a large epidemic role in the transmission of parasitic foodborne diseases resulting in health hazards when consumed without proper washing or cooking (Khan and Ujjan, 2022). This may be attributed to changes in agronomic and processing practices, increased consumption of raw or minimally processed fruits and vegetables, and increased number of immunocompromised consumers. As well as contaminated drinking water is one of the largest risks to public health. Approximately 1 to 2 billion people worldwide lack access to clean drinking water, and 30,000 people die every week from contaminated water (WHO, 2018). In wastewater, pathogens do not survive for a long time when exposed to high temperature, while worm’s eggs have a high resistance that can exceed several years in nature. The presence of *H. nana* eggs in sewage sludge and wastewaters increases the risk of human infestation either directly or indirectly through the reuse of sludge for agricultural purposes (WHO, 2006).

*Hymenolepis nana* also can be transmitted directly from person to person and has the ability to complete its entire life cycle in a single host (Magalhaes, 2013). Worldwide, more than 175 million cases of *H. nana* infection have been reported (Cabada et al., 2016) and the infection was particularly common in children living in developing countries related to various factors such as poor sanitation, crowding, low education and low socioeconomic status.

The inter-human transmission of the parasite can be occurred mainly by the fecal-oral route without an intermediate host; eggs are infectious when shed and can reinfest the host leading to long-lasting infections. *Hymenolepiasis* is often asymptomatic but when the level of infection rises, it causes diarrhea, abdominal pain, headache, dizziness, loss of appetite, anal itching, and weakness (Cabada et al., 2016; Panti-May et al. 2020). Seriously, the infection can ultimately cause severe diseases, and even life-threatening conditions in immunosuppressed and malnourished children (Muehlenbachs et al., 2015; Amer et al., 2018).

Detection of eggs in the host feces plays an important role in the diagnosis of *H. nana* infection (Nkouawa et al., 2016; Jirků et al., 2018; Řežábková et al., 2019). Microscopic examination remains the foundation stone of parasitological diagnosis, as it provides an epidemiological assessment of the parasite burden and due to lack of specificity of techniques based on immunology especially for parasites localized in the intestine (Momčilović et al., 2019). It mainly depends on the identification of morphological features of
the parasite in different biological samples which requires a high level of experience. Although flotation technique yields a clean preparation and somewhat sensitive, some helminth eggs don’t concentrate well so, formalin-ethyl acetate concentration technique is recommended due to its rapidity and suitability for fresh or preserved stool, as well as it overcomes the poor results caused by extreme amounts of fats and fatty acids during flotation technique (El-Nadi et al., 2019).

However, many studies have been conducted on the prevalence of different intestinal parasitic infections in Egypt, a few of them have included the occurrence of *H. nana* infection as one of the neglected zoonotic parasitic diseases. So, in this study we described the prevalence of *H. nana* infection in rodents as reservoir of infection, water and vegetables as essential sources, and in outpatients in addition to detecting the associated risk factors.

**Materials and methods**

**Study area**

This study was carried out in Aswan Governorate, which is located in the south of Egypt (Upper Egypt) at a latitude of 24° 5' 20.18” N, 32° 53’ 59.39” E toward 680 km south of Cairo. A descriptive cross-sectional study was conducted on 175 outpatients attending El Fouad laboratories, which located in different areas in the Governorate, at ages ranged from 2 to 25 years suffering from abdominal pain, anorexia, and some of them suffering diarrhea. In addition, 100 rodent fecal samples, 50 green salad, and 50 tape water samples were collected from various localities in Aswan Governorate during the period from December 2019 to October 2021. Demographic and lifestyle were obtained through a survey questionnaire which included sex, age, residence, washing hands before eating and after defecation, washing of vegetables and fruits before consumption, trimming of fingernails periodically, and water supply sources.

**Samples collection and laboratory processing**

Fresh fecal samples were collected from rodent (*Rattus rattus rattus*) in different localities of Aswan Governorate where human stool samples were taken. Green salad samples were purchased randomly from different food shops and street vendors. At the same time, samples of drinking water were collected from rural and urban areas in the Governorate. All samples were put in sterile, covered, and labeled plastic bags and transferred to the laboratory, to be investigated.

**Macroscopic examination**

Few grams of each fresh fecal sample of rodent and human stool were inspected macroscopic for the presence of worms or their segments using a necked eye. This method is suitable only for fecal samples investigation.

**Microscopic examination**

**Direct smear method**

Approximately 2 mg of each rodent feces and human stool sample were put on glass slides by using glass rods and mixed with saline. Few drops of each water sample were directly placed on a slide, while 200 gm of each green salad sample were handled mechanically by a food handler and was kept in a plastic bag until juice was produced from which few drops were put on the glass slide. Then the slides were coverslipped and observed under a microscope at a magnification of 40x for identification according to Ukaga et al.(2002).
Flotation technique

Rodent and human fecal samples were assessed using the flotation technique. Fifty grams of fecal matter were placed in 250 mL glass beaker and topped with 0.95% sodium chloride solution to a final volume of 200 mL. The mixture was thoroughly homogenized with a glass stick and was left to decant for 5 min before being filtered to eliminate big particles. Sediment and other solids were removed. The mixture was poured through a fine-mesh strainer and then transferred to a pre-numbered 15 mL conical tube, which was then centrifuged at 2000 g for 3 min. An additional 0.95% sodium chloride solution was pipetted into each tube to create a positive meniscus onto which a coverslip was placed. After 5 min, the coverslip was removed and placed on a pre-numbered slide for examination under a light microscope at 40× magnification (Dryden et al., 2005). The diagnosis of *H. nana* infection was confirmed by the finding of the polar filament of dark brown-colored eggs (Dovč et al., 2017).

Formalin-ethyl acetate concentration technique

Four grams of each fecal sample was emulsified in 10 mL of formalin 10% in a centrifuge tube 15 mL then was strained by gauze placed over a funnel into 15 mL centrifuge tube and the tube was completed with formalin to bring the volume to 15 mL, then centrifuged at 500g for 10 minutes. The supernatant was discarded then 10 mL formalin was added and mixed well with a wooden applicator stick. This process was done two times to wash the stool sample. Then the sediment was suspended in 4mL of ethyl acetate and the mixture was sealed with a rubber bung and shaken vigorously in an inverted position for 30 seconds, then the stopper was removed carefully. It was spun at 500g for 10 min, and then separated into three parts. The first from the bottom was the sediment followed by a layer of formalin in the middle and at the top was coarse fecal debris and ethyl acetate. The layers above the sediment were carefully aspirated and discarded using the pipette. The side of the tube was cleaned using a cotton swab to remove ethyl acetate and debris. The sediment mixed well with the remaining fluid, and 1 to 2 drops of formalin were added in case of dryness then picked one drop on a clean glass slide, cover slipped and examined under the microscope for the presence of eggs (Ukaga et al., 2002).

Statistical analysis

Data were statistically analyzed using SPSS version 22. The Fisher's Exact Test / Monte Carlo test was performed to predicate the association between the independent and dependent variables at 5% significant level followed by Eta Squared test and ANOVA test to display a one-way analysis-of-variance. Eta and Eta-squared (measures of association) were calculated for each independent variable. Furthermore, the McNemar test was applied for a nonparametric test for two related dichotomous variables using the chi-square distribution followed by Cohen's kappa to measure the agreement between the evaluations. Finally, diagnostic accuracy of used procedure was calculated to give discriminates between certain two conditions of interest, this discriminative ability can be quantified by the measures of sensitivity, specificity, positive and negative predicative values (PPV, NPV), likelihood ratio, diagnostic efficiency, and discrimination ability. Receiver operating characteristic (ROC) curve values were calculated using area under the curve (AUC) as a diagnostic...
accuracy test to validate the prediction of *H. nana*; at level of 95% was considered statistically significant.

**Ethical approval**

The ethical committee of South Valley University, Qena, Egypt (No.72/27.9.2022), approved the protocol of the current study. In addition, Oral consent was obtained from each participant.

**Results**

The present study showed only 4.0% (15/375) of the examined samples were infected with *Hymenolepis nana* eggs. The percentage of each sample infected was given in Table 1 as 5.0% of rodent feces, 6.0% of green salad, and 4.0% of human stool while couldn't be detected in tape water samples. Statistically, there were no significant differences between the source of infection and hymenolepiasis (*P* = 0.747).

Through checking the diagnostic efficacy of direct smear and flotation technique methods compared to formalin-ethyl acetate concentration technique (a gold standard [reference method]), out of 100 rodent fecal samples, 1(1.0%), 2(2.0%), and 5(5.0%), respectively were hosted *H. nana*, while out of 175 human fecal samples 2 (1.14%), 5 (2.86%), and 7 (4.0%) were positive using the three techniques, respectively (Table 2, Fig. 1).

**Table 1. Prevalence of Hymenolepis nana in the examined samples**

<table>
<thead>
<tr>
<th>Sources of samples</th>
<th>No. of examined Samples</th>
<th>Infected cases No. (%)</th>
<th>Within- samples Fisher’s Exact Test</th>
<th>Monte Carlo Sig. (2-sided) all samples</th>
<th>Monte Carlo Sig. (2-sided) all sub-samples</th>
<th>Eta Squared test</th>
<th>ANOVA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent fecal samples</td>
<td>100</td>
<td>5 (5.0)</td>
<td>NA</td>
<td>Value =0.563; <em>P</em> = 0.747</td>
<td>Value =3.079; <em>P</em> = 0.539b</td>
<td>0.00</td>
<td><em>F</em> = 0.10; <em>P</em> = 1.000</td>
</tr>
<tr>
<td>Tape water</td>
<td>50</td>
<td>0 (0.0)</td>
<td><em>P</em> = 0.242</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green salad</td>
<td>50</td>
<td>3 (6.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human stool or diarrhea samples</td>
<td>175</td>
<td>7 (4.0)</td>
<td><em>P</em> = 1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>375</td>
<td>15 (4.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Comparative approach to different diagnostic techniques for Hymenolepis nana eggs detection in the examined fecal samples**

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>No. of examined samples</th>
<th>Direct smear</th>
<th>Flotation technique</th>
<th>Formalin-ethyl acetate concentration technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. infected (%)</td>
<td>No. infected (%)</td>
<td>No. infected (%)</td>
<td>No. infected (%)</td>
</tr>
<tr>
<td>Rodent fecal sample</td>
<td>100</td>
<td>1 (1.0)</td>
<td>2 (2.0)</td>
<td>5 (5.0)</td>
</tr>
<tr>
<td>Human stool or diarrhea sample</td>
<td>175</td>
<td>2 (1.14)</td>
<td>5 (2.86)</td>
<td>7 (4.0)</td>
</tr>
</tbody>
</table>
Compatibly, the sensitivity of the diagnostic accuracy of direct smear and flotation technique were 20% and 40%, respectively compared to formalin-ethyl acetate concentration technique with 100% specificity for rodent fecal samples with the area under the curve 0.40 (0.109-0.691) and 0.30 (0.006-0.594), respectively (Table 3). Harmoniously, the sensitivity was 0% and 20%, respectively with 99.16% specificity for human stool or diarrhea samples with the area under the curve of 0.504 (0.247-0.761) and 0.404 (0.115-0.693), respectively as illustrated in Table 4.

Table 3. Sensitivity and specificity of Direct smear and Flotation as diagnostic screening techniques for *H. nana* compared to the Formalin-ethyl acetate concentration technique as the gold standard in rodent fecal samples

<table>
<thead>
<tr>
<th>Screening tests</th>
<th>Diagnostic efficiency %</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
<th>PPV % (95% CI)</th>
<th>NPV % (95% CI)</th>
<th>PLR (95% CI)</th>
<th>NLR (95% CI)</th>
<th>DA %</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct smear</td>
<td>96%</td>
<td>20 (1.05-70.12)</td>
<td>100 (95.15-100)</td>
<td>100 (5.46-100)</td>
<td>95.95 (89.38-98.69)</td>
<td>Infinity</td>
<td>0.8 (0.51-1.24)</td>
<td>95.95</td>
<td>0.40 (0.109-0.691)</td>
</tr>
<tr>
<td>Flotation technique</td>
<td>97%</td>
<td>40 (7.25-82.95)</td>
<td>100 (95.15-100)</td>
<td>100 (19.78-100)</td>
<td>96.93 (90.67-99.20)</td>
<td>Infinity</td>
<td>0.6 (0.29-1.22)</td>
<td>96.93</td>
<td>0.30 (0.006-0.594)</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value; PLR, likelihood ratio for positive results; NPL, likelihood ratio for negative results; DA, discrimination ability (PPV+ NPV-100)100%; AUC area under the curve.
Table 4. Sensitivity and specificity of Direct smear and Flotation as diagnostic screening techniques for *H. nana* compared to the Formalin-ethyl acetate concentration technique as the gold standard in human fecal samples

<table>
<thead>
<tr>
<th>Screening tests</th>
<th>Diagnostic efficiency %</th>
<th>Sensitivity % (95%CI)</th>
<th>Specificity % (95%CI)</th>
<th>PPV % (95% CI)</th>
<th>NPV % (95% CI)</th>
<th>PLR (95% CI)</th>
<th>NLR (95% CI)</th>
<th>DA %</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct smear</td>
<td>95.2%</td>
<td>0 (0– 53.70)</td>
<td>99.16 (94.76- 99.95)</td>
<td>0 (0- 94.53)</td>
<td>95.96 (90.37- 98.50)</td>
<td>NaN</td>
<td>1.008 (1.0082 - 1.0085)</td>
<td>4.04</td>
<td>0.504 (0.247 - 0.761)</td>
</tr>
<tr>
<td>Flotation technique</td>
<td>96%</td>
<td>20 (1.05- 70.12)</td>
<td>99.16 (94.99.95)</td>
<td>50 (2.66- 97.33)</td>
<td>96.74 (91.38- 98.95)</td>
<td>24 (1.74- 330.80)</td>
<td>0.80 (0.52- 1.25)</td>
<td>46.7</td>
<td>0.404 (0.115 - 0.693)</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value; PLR, likelihood ratio for positive results; NPL, likelihood ratio for negative results; DA, discrimination ability (PPV+ NPV-100)/100%; AUC area under the curve.

The Demographic characteristics and risk factors associated with *H. nana* infection in humans were reported in Table 5. During the study, 175 outpatients participated through collecting fecal samples during the period from December 2019 to October 2021, they aged 2-25 years with 78 males and 97 females. There was no significant difference perceived in the sex-wise analysis although a higher prevalence of *hymenolepis* infection was distinguished in males (6.41%) than in females (2.06%). Taken together, the age-wise analysis exposed the highest infection rate among 2-7 years age group (6.67%) followed by group of 20-25 years (3.85%) and 14-19 age group (3.45%) in compared to ages 8-13 years where the infection couldn’t be detected with no significant relationship (*P*=0.558b). Among the participants, 95 were from rural areas who showed a higher rate of *H. nana* infection (4.21%) than 80 participants from urban areas (3.75%).

On the contrary, statistically significant differences were noticed between the following risk factors and *Hymenolepis* infection, washing of hands, washing of vegetables/fruits, finger nail trimming, and water supply (*P* =.007*, 0.000*, 0.043*, 0.032*, respectively).

Table 5. Prevalence of *H. nana* infection with associated risk factors in humans under investigation

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of examined cases</th>
<th>No. of positive cases (%)</th>
<th>Fisher’s Exact Test/ Monte Carlo test</th>
<th>Risk factors Odd ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>78</td>
<td>5 (6.41)</td>
<td><em>P</em>=0.244</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>97</td>
<td>2 (2.06)</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>2-7</td>
<td>60</td>
<td>4 (6.67)</td>
<td><em>Value =2.196</em></td>
</tr>
<tr>
<td></td>
<td>8-13</td>
<td>34</td>
<td>0 (0.0)</td>
<td><em>P= 0.558b</em></td>
</tr>
<tr>
<td></td>
<td>14-19</td>
<td>29</td>
<td>1 (3.45)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-25</td>
<td>52</td>
<td>2 (3.85)</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>Rural</td>
<td>95</td>
<td>4 (4.21)</td>
<td><em>P= 1.000</em></td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>80</td>
<td>3 (3.75)</td>
<td></td>
</tr>
</tbody>
</table>
Washing of hands

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>115</th>
<th>1 (0.87)</th>
<th>P=0.007*</th>
<th>0.079 (0.009-0.672)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>60</td>
<td>6 (10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Washing of vegetables/fruits

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>133</th>
<th>0 (0.0)</th>
<th>P= 0.000*</th>
<th>1.2 (1.048-1.374)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>42</td>
<td>7 (16.67)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Finger nails trimming

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>100</th>
<th>1 (1.0)</th>
<th>P=0.043*</th>
<th>0.116 (0.014-0.987)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>75</td>
<td>6 (8.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Water supply

<table>
<thead>
<tr>
<th></th>
<th>Public network</th>
<th>120</th>
<th>2 (1.64)</th>
<th>P=0.032*</th>
<th>0.169 (0.032-0.903)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cistern trucks</td>
<td>55</td>
<td>5 (9.09)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to the calculated area under curve at level of 95% CI in Table 6, Fig. 2, washing of vegetables/fruits gets attention as the most significant risk factor followed by washing of hands, fingernail trimming and water supply with AUC values of 0.896, 0.768, 0.723, 0.708, respectively.

Table 6. The significance of the associated risk factors with H. nana infection in humans according to Area Under Curve (AUC)

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area</th>
<th>Std. Error a</th>
<th>Asymptotic Sig. b</th>
<th>Asymptotic 95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>.360</td>
<td>.103</td>
<td>.210</td>
<td>.159</td>
</tr>
<tr>
<td>Age (year)</td>
<td>.427</td>
<td>.123</td>
<td>.513</td>
<td>.185</td>
</tr>
<tr>
<td>Residence</td>
<td>.485</td>
<td>.111</td>
<td>.894</td>
<td>.267</td>
</tr>
<tr>
<td>Washing of hands</td>
<td>.768</td>
<td>.082</td>
<td>.016</td>
<td>.607</td>
</tr>
<tr>
<td>Washing of vegetables/fruits</td>
<td>.896</td>
<td>.030</td>
<td>.000</td>
<td>.837</td>
</tr>
<tr>
<td>Fingernails trimming</td>
<td>.723</td>
<td>.085</td>
<td>.046</td>
<td>.556</td>
</tr>
<tr>
<td>Water supply</td>
<td>.708</td>
<td>.101</td>
<td>.062</td>
<td>.510</td>
</tr>
</tbody>
</table>

The test result variable(s): sex, age (year), residence, washing of hands, washing of vegetables/fruits, finger nails trimming, water supply has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Fig. 2 Receiver operating characteristic
Discussion

Intestinal parasitic infections are regarded as one of the major socioeconomic and public health problems that negatively affect the health of millions of people globally. These infections can cause anemia, growth retardation in children besides affecting physical and mental health conditions (Alharazi et al., 2020). *Hymenolepis nana* is the most common cestode in humans adversely affecting the host nutritional status (Ahmed et al., 2021).

This study was conducted in Aswan Governorate in a wide age group and different sample types to detect the prevalence rate of hymenolepiasis through different techniques. Rodents showed 5.0% prevalence rate of *H. nana* in their feces by using formalin-ethyl acetate concentration technique (the gold standard method). Hymenolepiasis, according to Thompson (2015), is one of a series of rodent-borne parasitic diseases. Lower incidences were recorded by Franssen et al. (2016) as 3.3% in farms and 4.1% in rural environments in the Netherlands, and Companioni-Ibañez et al. (2016) as 2.56% in Cuba. Contrariwise other authors recorded higher prevalence rates of *H. nana* in rodent as Azzam et al. (2016) who recorded infection rate of 16.67% in Egypt, Simões et al. (2016) as 8.8% in Brazil, Yang et al. (2017) as 21.8% in Taiwan, Fitte et al. (2018) as 8.2% in Argentina, and Coello-Peralta et al. (2021) as 20.99% in Ecuador. These variations can be attributed to the environmental conditions; countries with high humidity and temperature, and low rainfall which showed increased prevalence rate of *H. nana*.

The occurrence of *H. nana* in humans is usually achieved by fecal-oral route, through the egg ingestion with infected materials either water or vegetables. Due to their capacity for resistance, *H. nana* eggs have been recorded in different environmental samples, especially surface water and sewage (Ribas et al., 2017). Our study couldn’t detect any *H. nana* eggs in the examined water samples. In line with this result, Periago et al. (2018) discovered that water didn’t considered as a risk factor in *Hymenolepis* infection in humans. In contrast, Al-Tameemi et al. (2019) found that *H. nana* eggs were the most prevalent at a rate of 2.78 eggs/L and Sugianto et al. (2019) who discovered that water source from a river was the main risk factor of parasitic infection including *H. nana*.

The consumption of raw or not thoroughly washed vegetables, significantly contributes to the epidemiology of parasitic foodborne illnesses. In this study, green salad investigation revealed 6.0% prevalence rate. Compared with previous studies, our contamination rate was higher than those recorded by Said (2012) as 2.6% in Alexandria, Egypt, Ebrahimzadeh et al. (2013) as 2.4% in Libya, Eraky et al. (2014) as 2.4% in Benha, Egypt. Also, El Bakri et al. (2020) detected *H. nana* in 3.0% of the examined fresh vegetables. Contrarily higher incidences were recorded by Al-Megrm (2010) as 14.5% in Riyadh, Saudi Arabia. Contamination of vegetables may occur in a variety of ways, but mainly associated with the water used for irrigation that the use of sewage contaminated water for irrigation is a common practice in developing countries including Egypt or contamination may occur directly through domestic animals.

The prevalence of *H. nana* in 175 outpatients aged 2 to 25 years was 4% of the examined stool or diarrhea samples. Different occurrence rates were recorded in other Egyptian governments; 3.9% among
patients in Dakahlia, 3% among rural participants in Menoufia, 5.8% among patients in Assiut, and 7% of random fecal samples in Sohag while across the world, the prevalence of *H. nana* was recorded as 8.3% in Brazil and 7.8% in Mexico (Ahmed et al., 2021).

Lower *H. nana* infection rates were recorded by Al-Daoody et al. (2017) and Geneidy (2019) as 4.04% and 1.0%, respectively. On the other hand, Barreto et al. (2017) could detect *H. nana* in a higher rate as 7.61% of children fecal samples aged from 2-15 years. This variation could be attributed to the different climatic circumstances, environmental hygiene, socioeconomic level, and alterations in host susceptibility to parasitic diseases.

Our study showed a higher prevalence of infection in males (6.41%) than in females (2.06%) without statistically significant difference ($P=0.244$). Symmetrically, Bagayan et al. (2015) declared that *H. nana* infection was not significantly associated with gender in a study conducted in Burkina Faso. In agreement with our results, Barreto et al. (2017), and Mahmoudvand et al. (2020) showed that males had a higher prevalence of the infections than females (7.1, 4.8% and 12.2, 7.1%, respectively). In our country culture, males are more likely to spend time outdoors away from their home; therefore, hygienic habits would be less optimal compared to females which may explain the results obtained. Contrarily, Al-Daoody et al. (2017) recorded a percentage of infection in females as 6.59% which was higher than that in males (3.33%).

The study was performed on four age groups, statistically there is insignificant association between *H. nana* prevalence and age however the results declared that 2-7 years age group was the most susceptible to the infection (6.67%) followed by 20-25 years age group (3.85%) and finally that of 14-19 years (3.45%) while couldn’t be detected in those of 8-13 years. Our findings approved the results recorded by several authors as Barreto et al. (2017) who found that prevalence of *H. nana* decreased by age and Ahmed et al. (2021) who found a significant association between *H. nana* prevalence and the age group with the highest among 5-7 years age group. This finding may be due to poor hygiene in younger ages which makes it easier for *H. nana* to spread through the eating of food contaminated with feces that contain *H. nana* eggs. Besides, its expansion is facilitated by the presence of an infected individual in a crowded area.

It is worthy noted that there was no statistically significant difference in the prevalence of hymenolepiasis between rural and urban residents however the rate of infection was 4.21% among people living in rural regions versus 3.75% of those living in urban regions. This result was supported by Mahmoudvand et al. (2020) and Ahmed et al. (2021) who found that living in rural areas was substantially related with the incidence of intestinal helminthes as well as *H. nana*. Though, disagreed results were obtained by Barreto et al. (2017) in Peru who found that the prevalence of *H. nana* in rural areas was lower than in peri-urban areas (6.4% and 8.6%, respectively). This might be due to the difference in the exposure to sources of infection which might occur through eating fast food prepared by the infected food handlers in both areas. Also, poor conditions in rural areas are suitable breeding sites for rodents.
As regards life patterns and health management behavior risk factors, the present work approved a significant higher prevalence of infection among patients with poor hand washing before eating and after defecation (10%) \( (P=0.007; \text{OR}=0.079(0.009-0.672)) \), and among those who didn’t wash vegetables and fruits before consumption (16.67%) \( (P=0.000; \text{OR}=1.2(1.048-1.374)) \). Also, a linear association between fingernails trimming, water supply and hymenolepiasis were found. For instance, it was reported that people who didn’t practice fingernails trimming were more likely to be infected (8%) than others \( (P=0.043; \text{OR}=0.116(0.014-0.987)) \). A number of studies have addressed the correlation between unsafe drinking water contaminated with soil or feces and hymenolepiasis which acts as a carrier of infectious H. nana eggs and this was evident in our study that patients who lived in areas where the water was delivered through trucks that filled onsite cisterns acquisized the highest rate of infection (9.09%) unlike those received water through public networks (1.64%) with significant association \( (P=0.032; \text{OR}=0.169(0.032-0.903)) \). The same results were demonstrated and supported by Abd-El-Mageed et al. (2010) in Assiut, Zenu, et al. (2019) in Ethiopia, Mahmoudvand et al. (2020) in Iran, and Ahmed et al. (2021) in Sohag who found that poor personal hygiene before eating, untrimmed fingernails and consuming raw or unwashed vegetables/fruits were significantly associated with parasitic infections.

**Conclusion**

Our results clearly show that there is a strong inverse association between H. nana infection in human and economic situation, personal hygiene, and basic sanitary services in the home besides the presence of animal reservoirs, particularly rodents. Therefore, the basic rules of food and personal hygiene should always be followed by customers through washing vegetables before eating and washing hands before meals. In addition to that, public health and veterinary officers must implement prevention and monitoring programs to combat the sources and reservoirs of hymenolepiasis.

**Conflicts of Interest**

The authors declare that they have no competing interests.

**References**


Al-Megrn WAI (2010). Prevalence intestinal parasites in leafy


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Zenu S, Alemayehu E, Woldemichael K (2019). Prevalence of intestinal...