INVESTIGATION OF HELMINTH PARASITIC INFECTION OF LABORATORY ANIMALS (RATS & MICE) WITH SPECIAL REFERENCE TO CONTROL OF HYMENOLEPIS NANA AS A ZOONOTIC PARASITE

ABSTRACT:
A total of one hundred and twenty rats and 200 mice were investigated during the present study to identify the helminth parasites of laboratory animals purchased from Abu rawash area, Giza, Egypt. The collected obtained helminthes were *Hymenolepis* (*H.* nana, *H. diminuta*, *Syphacia* (*S.* muris), and cyst of *Strobilocercus fasciolaris* (*Cysticercus fasciolaris*) in rats and *H. nana*, *S. obvilata*, *Aspiculuris* (*A.* tetraptera and *Strobilocercus* (*S.* fasciolaris) (*Cysticercus*) in mice. The common worm infection in rat was *H. diminuta* (25%) while in mice it was *S. obvilata* (55%). Infection of cats by the obtained *Strobilocercus* cysts gave adult worms of *Taenia* (*T.* taeniaeformis) after 55 days post-infection. Treatment of *H. nana* infected rats with single dose of Zanide (15 mg oxyclozanide and 7.5 mg levamizole per kg body weight) completely eradicated *H. nana* infection after one week post treatment. However, treatment of the infected rats with single dose of alzental (albendazole, 32 mg/kg body weight) was not efficient in *H. nana* worm elimination, but led only to an increase in *H. nana* egg output then decrease in number.

Key words:
Helminth Parasitic Infection, *Hymenolepis nana*.

INTRODUCTION:
Efforts have been made worldwide in order to detect, identify and study ecto- and endo-parasites of laboratory animals, aiming to achievement the proper procedures regarding the control or eradication of parasitism, considering the important role of these animals in scientific research. Besides the high mortality in young animals caused by some infections, parasites can complicate research by inducing physiological and immunological alterations in the hosts, increasing or diminishing host susceptibility to experimental stress, inducing tissue damages, stimulating abnormal tissue growth, competing with the host for nutrients, decreasing the volume of host's blood and body fluids and by mechanical interference (Hsu, 1980).

*Hymenolepis* species are tapeworms occurring throughout the world in temperate to tropical conditions of poor sanitation. Over 400 species are found in higher vertebrates, while the definitive hosts are rodents (Little and Ambrose, 2000; Bahadir, 2002). *H. nana* (*Vampirolepis nana*) is a zoonotic parasite and is common in children and institutionalized groups (Rausch, 1993; Alvez et al., 2003). Albendazole is widely used as antihelminthic drug for *H. nana* (Prasad et al., 1985; Jagota, 1986; Amato et al., 1990). Oxyclozanide was used in control of nematodes in sheep and treatment of infected animals. It led to disappearance of parasite eggs from feces after 7 and 14 days (Tinar et al., 1997).

*Taeniaeformis* is a cestode of family Taeniidae and it occurs as adult tapeworms in the small intestine of carnivores as definite hosts. Rodents (intermediate hosts) become infected when ingested eggs of this worm where they develop as *Strobilocercus fasciolaris* or *Cysticercus fasciolaris* (Tucek et al., 1973; Hsu, 1979; Ismail et al., 1983; Hasslinger et al., 1988; Huh et al., 1993, Sohn and Chai, 2005; Mahesh Kumar et al., 2006). Once a cat ingests the...
2.1. Experimental infection of cats:

Strobilocercus, the posterior portion of the larva is digested away and then the anterior portion begins to develop into adult worm (Hutchison, 1959). In 1981, Williams and Shearer, stated that the patent infection by *T. taeniaeformis* develop between 32 to 80 days after strobilocerci are ingested by a cat. The larval stages of *T. taeniaeformis* (*Cysticercus fasciolaris* or *Strobilocercus fasciolaris*) were found in the liver and peritoneum of muskrat especially in adult rats (Borgsteede et al., 2003). These larvae were fed to domestic dogs and the adult *T. taeniaeformis* were obtained (Rossin et al., 2004).

*H. nana*, *H. diminuta*, *S. muris*, *S. obvilata* and *Strobilocercus fasciolaris* are common parasites of rodents (Soo et al., 1968; Ito and Itagaki, 2003). Pinworms (*S. muris*, *S. obvilata* and *A. tetraperta*) are the most common contaminants of rat and mice (Nakagawa et al., 1984; Coghlan et al., 1993; Pritchett and Johnson, 2002).

Many research endeavors and practical student teaching in many of Egyptian universities are carried on experimental animals especially rats and mice purchases from Abo rawash area, Giza, Egypt. These animals bred and housed under unknown conditions. Therefore this study aimed to investigate the helminthic parasites prevalence in these laboratory animals, as well as as evaluation of the most effective drugs in controlling the zoonotic worm *H. nana*.

1. Animals:

A total of 120 rats and 200 mice were collected between April 2005 and May 2007 from different scientific departments in the faculties of Veterinary Medicine and Science, Beni-Sueif University, Beni-Sueif Governorate, Egypt. These animals were brought to the faculties from Abo-rawash area, Giza, Egypt and were killed by ether inhalation in the laboratory. They were dissected and gastrointestinal tracts, livers, lungs, kidneys, gonads, body cavities and subcutaneous tissues were examined for helminth infections using stereomicroscope.

2. Parasites:

Adult and larval stages of different helminthes were collected. Cestodes were pressed between two slides, fixed in 10% formalin and stained with acetocarmine technique (Pritchard and Kruse, 1982). Nematodes were fixed in 70% glycerol alcohol and mounting in glycerol jelly. Taxonomic identification of helminthes was based on Yamaguti (1958).

2.1. Experimental infection of cats:

Faeces of 4 cats (*Felis catus*) were examined to ensure that they were free from parasitic helminth infection especially *Taenia* species. Three cats were fed on the parts of livers of either mice or rats that contained *T. taeniaeformis* cysts. Each cat was infected by 5 cysts. The fourth was lifted as control without infection to ensure the fed cyst was the only source of the infection. To prove the presence of the adult worms, faeces of cats were examined weekly after 5 weeks post-infection for the detection of *T. taeniaeformis* eggs by using flotation technique (Soulsby, 1982). Cats were sacrificed at 55 days post–infection, their intestines were examined and adult worms were collected and identified.

3. Treatment design of *H. nana*.

3.1. Experimental infection of rat by *H. nana*.

All rats were coprofecoally examined and the infected ones were reared in the laboratory as donors for *H. nana*. These animals were scarified and the gravid segments of *H. nana* were collected in 0.9 % saline and used for experimental infection. Thirty parasite free rats were used for experimental infection. Each rat was infected orally by 10 gravid segments (average 100 eggs per animal) using stomach tube according to Maki and Yanagisawa (1987). After 10 days post infection, faecal examination was carried out to observe the appearance of *H. nana* eggs.

3.2. Treatment groups:

*H. nana* experimentally infected rats were divided into 3 groups 10 rats each.

- **Group A**: Infected rats were treated with Zanide (Med Mac, Cairo, Egypt) which contain oxyclozanide 30 mg and levamizole 15 mg for each one ml. Rats were received orally a single dose of 15 mg oxyclozanide and 7.5 mg levamizole per kg body weight.

- **Group B**: Infected rats were treated one time with albendazole (EPICO, Cairo, Egypt). Rats were received orally 32 mg albendazole / kg body weight by using stomach tube.

- **Group C**: 10 animals of *H. nana* infected rats were kept as control untreated.

All of the treated animals were observed during the next week after oral administration of the drugs to check any expelled proglottids in their faeces as well as count of eggs per gram (g) faeces.

3.3. Egg count:

The count of eggs per gram faeces (e. p. g.) was calculated for each animal before and after treatment by Mac Master technique according to Soulsby (1982) with some modifications. Briefly, 1 g of fresh faeces was collected from each animal and soaked in 10 ml water until it was sufficiently soft. Ten ml saturated salt solution was added to
each sample. After rough shaking 200 µl was withdrawn and run into the counting chambers. 3 samples were counted for each animal and the mean eggs per g faeces were calculated.

Total egg count / g = total No. of eggs counted in chambers X 100

Efficacy of drug = (a – b/ a) X 100

a = No. of eggs before treatment
b = No. of eggs post treatment.

RESULTS:

1. Identification of adult helminthes and larvae:

The helminth parasites detected in this study were identified as cestodes and nematodes according to Yamaguti (1958). About 120 rats and 200 mice were sacrificed and examined for helminthic infection. The prevalence of infection in rats was 45.83%, while in mice was 60% (Table 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>Examined No.</th>
<th>Infected No.</th>
<th>Prevalence of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>120</td>
<td>55</td>
<td>45.83</td>
</tr>
<tr>
<td>Mice</td>
<td>200</td>
<td>120</td>
<td>60</td>
</tr>
</tbody>
</table>

Four species of helminthes parasites were found and isolated from rats (Table 2).

<table>
<thead>
<tr>
<th>Helminthes</th>
<th>Exam. No.</th>
<th>Inf. No.</th>
<th>Prevalence of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hymenolepis nana</td>
<td>120</td>
<td>15</td>
<td>12.5</td>
</tr>
<tr>
<td>H. diminuta</td>
<td>120</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Strobilocercus fasciolaris</td>
<td>120</td>
<td>5</td>
<td>4.17</td>
</tr>
<tr>
<td>Syphacia muris</td>
<td>120</td>
<td>25</td>
<td>20.83</td>
</tr>
</tbody>
</table>

The prevalence of *H. nana* (Fig. 1a) in examined rats was 12.5%, *H. diminuta* (Fig. 1b) was 25%, *Strobilocercus fasciolaris* (Fig. 3b) was 4.17% and *S. muris* (Fig. 2i) was 20.83%. 2.5% of infected rats were found to harbor a mixed infection of the two Hymenolepis species. The highest mixed infection in rats was by *H. diminuta* with *S. muris* (12.5%), while infection by *H. nana* with *S. muris* was 4.17%. There was no mixed infection in rats that were infected with *Strobilocercus fasciolaris* (Table 3). No rat was found to harbor more than two different species of helminthes.

Only four species of helminthes parasites were found in examined mice (Table 4). The prevalence of *H. nana* infection in mice was 35%, *S. obvilata* (Fig. 2ii) was 55%, *A. tetraptera* (Fig. 2iii) was 25% and *Strobilocercus fasciolaris* was 10%. Infection by *H. nana* and *S. obvilata* was the highest mixed infection (25%) in mice, while only 3% of infected mice were found to harbor *H. nana* with *Strobilocercus fasciolaris* (Table 5). In mice, there was no mixed infection between *Strobilocercus fasciolaris* and *S. obvilata*. There was no mouse found to be infected with the 3 different parasites at the same time.
and gravid segment had several lateral branches (Figs 3a-i, 3a-ii & 3a-iii).

Fig. 3 a- Taenia taeniaeformis i- Scolex, ii- Mature segment, iii- Gravid segment. b- Strobilocercus fasciolaris (Scolex).

**Fig. 4. Efficacy of zanide and alzental on H. nana egg output.**

Table 3. Mixed infection by helminthes in rats.

<table>
<thead>
<tr>
<th>Helminthes</th>
<th>Exam. No.</th>
<th>Inf. No.</th>
<th>Prevalence of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. nana &amp; H. diminuta</td>
<td>120</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>H. diminuta &amp; S. muris</td>
<td>120</td>
<td>15</td>
<td>12.5</td>
</tr>
<tr>
<td>Strobilocercus fasciolaris &amp; others</td>
<td>120</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H. nana &amp; S. fasciolaris</td>
<td>200</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4. The helminth parasites in the examined mice.

<table>
<thead>
<tr>
<th>Helminthes</th>
<th>Exam. No.</th>
<th>Inf. No.</th>
<th>Prevalence of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hymenolepis nana</td>
<td>200</td>
<td>75</td>
<td>38</td>
</tr>
<tr>
<td>Syphacia obvilata</td>
<td>200</td>
<td>105</td>
<td>55</td>
</tr>
<tr>
<td>Aspiculuris tetraptera</td>
<td>200</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Strobilocercus fasciolaris</td>
<td>200</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 5. Mixed infection by helminthes in mice.

<table>
<thead>
<tr>
<th>Helminthes</th>
<th>Exam. No.</th>
<th>Inf. No.</th>
<th>Prevalence of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. nana &amp; S. obvilata</td>
<td>200</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>H. nana &amp; A. tetraperta</td>
<td>200</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>S. obvilata &amp; A. tetraperta</td>
<td>200</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Nematode &amp; S. fasciolaris</td>
<td>200</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>H. nana &amp; S. fasciolaris</td>
<td>200</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

2. Efficacy of both zanide and albendazole against H. nana:

No eggs were found in H. nana infected rats treated with a single dose of Zanide (15 mg oxyclozanide and 7.5 mg levamizole per kg body weight) one week post treatment. As shown in figure 4 the eggs / g faeces of infected treated animals began to decrease after 24 hours and completely disappeared at 7 days post treatment with Zanide.

On the other hand, infected rats treated with a single dose of alzental (32 mg / kg body weight) showed an increase in eggs / g faeces from the 1st until the 3rd day post treatment. Then the eggs / g faeces decreased but still detected in the faeces until the 7th day post infection (Fig. 1).

Parasites infect laboratory rats and mice worldwide at high prevalence and usually highly contagious infection proceeds without symptoms unless heavy infection occurs (Flynn, 1973; Tanaka, et al., 1974; Baker, 1998). However, these infections have several adverse effects on biological researches, specially altering immune responses, blood parameters, nutritional and radiation studies making some experimental results invalid. Thus, animals infected with parasites are not suitable for cancer and disease research or helminthes biology experiments (Zubaid and Majeed, 1981; Baird, 1982; Ito, 1982). Moreover, experimental animals infected by zoonotic diseases can transmit diseases to human. Some parasites frequently found in mouse and rat colonies, are potentially zoonotic agents and the health hazards for students, technicians and researchers (Fox et al., 1984). Moreover, experimental results from research performed with living laboratory animals may be affected by infectious agents (Clough, 1982; Pakes et al., 1984). Therefore, the present study aimed to investigate the prevalence of the helminthic infections in rats and mice purchased from Abo rawash area, Giza, Egypt and usually use in research work and practical student teaching in many universities of Egypt.

The data of the present study showed that 45.83% of the rats and 60% of the mice purchased from Abo-rawash area, Giza, Egypt were infected with helminthes. Rats were infected with H. nana (12.5%), H. diminuta (25%), Strobilocercus fasciolaris
(4.17%) and S. muris (20.83%), while mice were infected with H. nana (35%), S. obvlilata (55%) and Strobilocercus fasciolaris (10%). Rats and mice become infected due to bad hygienic measures of breeding and housing of the animals. The results of the present survey were not different from those obtained in the past from Egypt and other countries (Tanaka et al., 1974; Casebolt et al., 1988; Abdella, et al., 2006). The data of the present study are in agreement with the study of Gilioili, et al. (2000) who stated that the prevalence of H. nana is 53.3%, of S. obvlilata is 86.6% in mice and of S. muris is 80.0%, of H. nana is 40.0% in rats.

The data of the present study revealed that 20.84% of the examined rats were found to harbour a mixed infection. 2.5% of rats were found infected by H. nana with H. diminuta. Also, there were a mixed infection between S. muris and H. diminuta (12.5%), and S. muris and H. nana (4.17%) in rats. These results indicate that infection by one of Hymenolepis species not prevent colonization by the other species. So, infection by Hymenolepis species not induces a protective immunity against the infection by the other helminthes in rats. However, rats infected with Strobilocercus fasciolaris showed no mixed infection. The possible explanation for this results not clear and needs a further investigation.

On the other hand, about 38% of examined mice showed a mixed infection by H. nana with S. obvlilata (25%), H. nana with A. tetraptera (10%), and H. nana with Strobilocercus fasciolaris (3%). So, infection of mice by H. nana not prevents infection by other species. This finding indicates that H. nana infection not stimulate a protective immunity against the infection by the other helminthes species. There was no mixed infection in mice between Strobilocercus fasciolaris and S. obvlilata. The main possible explanation for this result unclear and needs a further immunological investigations.

In both rats and mice there was no infection by three different species of helminthes in the same animal. The more likely explanation for this result is that, each of either rat or mouse can not tolerate infection by more than two different helminthes parasite at the same time, and the infection by more than two parasites species may lead to the death of the animal.

The resulted adult cestode from the experimental infection identified as Taenia taeniaformis based on its typical taenoid hooks and structure of mature segments and branches of gravid segments. The adult worm was similar to that obtained by Williams and Shearer (1981) and Rossin et al. (2004). Also Mahesh Kumar et al. (2006) confirmed that the obtained tapeworm cysts from liver of Wistar rats in India and the tapeworm larva was confirmed as Strobilocercus fasciolaris by PCR linked mitochondrial DNA sequencing.

The two most problematic for humans are H. nana (Vampyrolepis nana) and H. diminuta. H. nana is mainly a parasite of humans, but found more commonly in rats and mice, and has been widely used as a model system for the study of cestode tapeworm biology (Ito and Itagaki, 2003). Effective treatment of such parasite can protect human from hazards of infection. The data of the present study showed that using of Zanide in a single dose (15 mg oxyclozanide and 7.5 mg levamizole per kg body weight) for control of H. nana in rats is effective. However, treatment with alzental in a single dose (32 mg / kg body weight) was not effective in a complete eradication of H. nana from rats. There results are in agreement with the finding of Coghlan et al. (1993) and Zenner (1998).

Accordingly, we recommended to investigate all the experimental animals specially rats and mice for helminthic infections before using in any research endeavor or practical student work, especially if these animals bred and maintained in low hygienic area such as Abo rawash area.

REFERENCES:


استكشاف الديدان الطفيلية في حيوانات التجارب (الجرذان والفئران) ومحاولة لعلاج هيمينوليس نانا كطفيل مشترك

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قسم علم الحيوان- كلية العلوم- جامعة بني سويف

تم فحص 130 جرذ و 300 فأر لمعرفة الديدان المتطفلة في حيوانات التجارب (الجرذان والفئران) المشتراة من منطقة أبواسحا بالعجوزة، ووجد في الجرذان ديدان هيمينوليس نانا و هيمينوليس دايمينونا و سيشيفيا مورس و حويصلات أسترويلوسيركس فاشيولاريس. و في الفئران هيمينوليس نانا و سيشيفيا أوبيلانا و أسترويلوسيركس تيرانزا و حويصلات أسترويلوسيركس فاشيولاريس في الفئران، و كانت جرذان هيمينوليس دايمينونا (60%) الأكثر انتشارا في الجرذان بينما جرذان سيشيفيا أوبيلانا (85%) الأكثر في الفئران. و تم عد عدد القطط بحويصلات أسترويلوسيركس فاشيولاريس للحصول على الأطوار

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