The Effectiveness of Two Anthelmintics, Vermox® (mebendazole) and Povan® (pyrvinium pamoate), on Thelastomatid Nematodes (Nematoda: Oxyuroidea) of the Cockroach, Gromphadorhina portentosa¹

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ABSTRACT. The anthelmintics Vermox (mebendazole) and Povan (pyrvinium pamoate) were tested for their effectiveness against thelastomatid nematode, Leidyinema portentosae and Hammerschmidtia diesingi, infections in the cockroach, Gromphadorhina portentosa. Vermox was tested in doses ranging from 0.2 mg to 5 mg and Povan was tested in doses ranging from 0.1 mg to 12.5 mg. Vermox was found much more effective in curing the thelastomatid infection of cockroaches than Povan. Although Povan was able to reduce markedly the infection rate of the adult nematodes, it was significantly less effective on the thelastomatid juveniles and on the adult H. diesingi. Host sex had no noticeable impact on the sensitivity of either nematode to either anthelmintic at all dose levels tested. The results obtained with the G. portentosa system were in agreement with studies in other host-parasite systems.

INTRODUCTION

Gromphadorhina portentosa is a large malagasy cockroach that is usually infected with two thelastomatid nematodes, Leidyinema portentosae and Hammerschmidtia diesingi (Nematoda: Oxyuroidea). These two nematodes live in the hindgut of their hosts and feed on gut contents, but usually do not harm their host (Jarry 1964, Welch 1965, Holoman 1980). The high occurrence of infection by these nematodes makes it quite difficult to obtain nematode-free G. portentosa. However, in order to perform certain experiments, “clean” hosts are required. One possible way to solve this problem is to use an effective anthelmintic that is powerful enough to cure the nematode infection without harming the host.

Vermox (mebendazole) and Povan (pyrvinium pamoate) are two of the most widely used anthelmintics against the pinworm, Enterobius vermicularis (Rochette 1985). Because E. vermicularis is in the same superfAMILY Oxyuroidea as the thelastomatids (Chitwood 1974), this close taxonomical relationship allowed us to assume the potential for Vermox and Povan to be effective against L. portentosae and H. diesingi. Thus, experiments were designed to test the effectiveness of these two anthelmintics against infections by the thelastomatid nematodes of G. portentosa.

MATERIALS AND METHODS

Cockroaches, G. portentosa, were supplied by the Insect Culturing Laboratory at The Ohio State University. Males weighing between 5-8 g and females weighing between 6-9 g were used in the present study. All male and female cockroaches were selected to be thelastomatid nematode-egg positive by light microscopic examination of fecal material before the test. Nine groups of animals were used with 10 male and 10 female cockroaches per group. One group served as pretest control, one served as a 0 mg dosage control, and seven served to test the various doses of two anthelmintic compounds. Pretest control animals were dissected at the beginning of the experiment to determine initial parasite load. Each cockroach in the experiment was isolated in a plastic cup having an 11 cm diameter opening and a depth of 7.5 cm. Milk-Bone dog biscuits (Nabisco Inc., East Hanover, NJ 07936) were used as food, and water was supplied from a cotton wick in a small vial filled with tap water. The plastic cups and the dog biscuits were preheated at 75°C for at least 12 hours to eliminate all possible nematode egg contamination. The food, water, and the plastic cups were changed every three days.

Two anthelmintics were tested in this study. Vermox (mebendazole; Janssen Pharmaceutica Inc., Piscataway, NJ 08854) was administered in doses of 0.2 mg, 1.0 mg, or 5.0 mg per animal, and Povan (pyrvinium pamoate; Warner-Lambert Inc., Morris Plains, NJ 07950) was given at 0.1 mg, 0.5 mg, 2.5 mg, or 12.5 mg per animal. All animals were fasted for 24 hours before receiving the dose. Each dose was mixed with 160 mg of crushed fresh apple for each cockroach. The mixture was placed on a 22 mm x 22 mm cover slip and given to the cockroach. Each cockroach in the 0 mg dosage control group was given 160 mg of crushed fresh apple without the anthelmintics. Most of the cockroaches consumed all of the crushed fresh apple within 30 minutes regardless of presence of dosage of anthelmintic. All of the cockroaches finished feeding within two hours. Normal food and water were given immediately thereafter and for the remainder of the 16-day test period. Every three days fecal material from each cockroach was examined by light microscopy for the presence of nematode eggs. At the end of the period, all the cockroaches were dissected, and the contents of the intestine were inspected using a microscope and the species, sex, and age (adult or juvenile) of the helminths found were recorded. Since the early stage juveniles of the two nematodes are morphologically very similar to each other, it is difficult to distinguish them accurately with light microscopy. Therefore, the juveniles of the two nematodes were not recorded separately in this

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Data were expressed as percent of host infected and as number of parasites per infected host. The data were analyzed statistically using the BMDP Logistic Regression Analyses (Dixon 1983) or Fisher's Exact Test. All comparisons with P values equal to or less than 0.05 were considered to be significant.

RESULTS

Before the experiment, 80% of male cockroaches in the pretest control group contained male *L. portentosae* and 30% contained female nematodes of this species; 60% of female cockroaches contained male *L. portentosae* and 70% contained female nematodes of the same species (Figs. 1A, 2A). Male *H. diesingi* infected 100% of male cockroaches and 80% of female hosts in the pretest control group; female parasites of the same species were present in 90% of male hosts and 80% of female hosts (Figs. 1B, 2B). These percentages were not modified (P>0.1) 16 days after administering crushed apple without anthelmintic drug (0 dosage; Figs. 1, 2). There were no significant differences in the infection rates of the nematodes between the host sexes (P>0.05). The juveniles of the two nematodes were present in about 80% of male or female hosts and the eggs were found in 100% of the hosts (Table 1).

The rate of eradication of *L. portentosae* and *H. diesingi* in the three Vermox groups was excellent when compared to the control infection (P<0.01) (Fig. 1). At a dose of 1 mg or higher, no nematodes were found in the hosts. Although an 11-22% infection rate was recorded with the 0.2 mg dose, there was no significant difference (P>0.05) in terms of the rate of infection between the three doses. The sex of the host had little, if any, impact on the sensitivity of the nematodes to Vermox at any dosage used for the test groups (P>0.1).

The only group in which infection of juvenile nematodes remained after the treatment with Vermox was the female hosts with 0.2 mg dosage; juveniles were present in 22.2% cockroaches. However, neither sex, nor developmental stage (juvenile or adult), of the two nematodes resulted in differences in sensitivity to the anthelmintic (P>0.1). Nematode eggs were present randomly in the hosts after the treatment with Vermox. Presence was not correlated to the dosages tested. With 0.2 mg dosage, the eggs were present in 11.1% male hosts and 55.6% female hosts. With 1 mg dosage, the eggs were present in 20% male hosts and 22.2% female hosts. With 5 mg dosage, the eggs were present in 0% male hosts and 55.6% female hosts.

At a dose of 0.1 mg or higher, Povan was able to cure infections of adult *L. portentosae* as effectively as Vermox (P>0.1) (Fig. 2). At a dose of 0.1 mg, a 10-22% infection of *L. portentosae* was found. This, nevertheless, was not significantly different from the cure rates at the higher doses (P>0.05). Both sexes of *L. portentosae* were equally sensitive to Povan (P>0.1). However, adult *H. diesingi* was more resistant to Povan than adult *L. portentosae* (P<0.01). As with Vermox, the sex of the host had little impact on the sensitivity of the nematodes to Povan, at any dosage used for the test groups (P>0.1).

It was found that the juvenile stages of *L. portentosae* and *H. diesingi* were not sensitive to Povan, as all doses tested were ineffective in reducing the infection rate (P>0.05). With 0.1 mg Povan, 40% male hosts and 33.3% female hosts were infected with juveniles. With 0.5 mg dosage, 60% male hosts and 66.7% female hosts were infected. Thirty percent of male hosts and 40% female hosts were infected after treatment with 2.5 mg. With 12.5 mg dosage, 90% male hosts and 62.5% female hosts were infected. After Powan treatment, nematode eggs were present in 10% male hosts and 22.2% female hosts with 0.1 mg dosage; in 20% male hosts and 33-3% female hosts with 0.5 mg dosage; in 10% male hosts and 0% female hosts with 2.5 mg dosage; and, in 0% male hosts and 0% female hosts with 12.5 mg dosage.

The final infection rates of adult *H. diesingi* after treatment with Povan were influenced by the sex of the nematode (Fig. 2B). Male *H. diesingi* were less sensitive to varying doses of Povan than females regardless of dose.

### Table 1

Average number of *L. portentosae* and *H. diesingi* nematode parasites in untreated (Pretest; 0 mg dosage) G. portentosa*.

<table>
<thead>
<tr>
<th>Test</th>
<th>Host sex</th>
<th>Host #</th>
<th>Both sp.</th>
<th><em>L. portentosae</em></th>
<th><em>H. diesingi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Juvenile</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Pretest</td>
<td>♂️</td>
<td>10</td>
<td>12.8±2.9</td>
<td>8.7±2.8</td>
<td>3.4±2.5</td>
</tr>
<tr>
<td></td>
<td>♀️</td>
<td>10</td>
<td>14.9±6.1</td>
<td>8.6±3.6</td>
<td>7.1±4.6</td>
</tr>
<tr>
<td>0 mg</td>
<td>♂️</td>
<td>10</td>
<td>11.4±2.9</td>
<td>8.5±2.7</td>
<td>3.4±2.5</td>
</tr>
<tr>
<td></td>
<td>♀️</td>
<td>10</td>
<td>14.1±5.8</td>
<td>8.6±3.6</td>
<td>7.1±4.6</td>
</tr>
</tbody>
</table>

* Mean ± SEM (SEM = standard error of mean).
(P>0.05). Female \textit{H. diesingi}, on the other hand, were more sensitive to higher doses. With doses of 0.5 mg or higher, Povan effectively cured the infections of female \textit{H. diesingi} when compared to the control group (P<0.001) and was significantly better than the 0.1 mg dose (P<0.05). However, 0.1 mg of Povan still reduced the infection rate of female \textit{H. diesingi} noticeably as compared to that of the control group (P<0.05). The infection rates of female \textit{H. diesingi} after treatment with Povan did not differ between the doses 0.5, 2.5, and 12.5 mg (P>0.1).

**DISCUSSION**

There have been several investigations to test anthelmintics in cockroaches (Owens 1956, de Sylva 1960, Pawlik 1966, Holoman 1980). However, the experimental methods used in these previous studies have some drawbacks. For example, in some cases, the anthelmintics were mixed in water and the cockroaches used in the tests were expected to drink the anthelmintic solution. Unfortunately, most of the anthelmintics are insoluble or only partially soluble in water so that the final concentration of the anthelmintic solution could be far from that of the experimental design. Even if the anthelmintics completely dissolved in water, it is unlikely that all the cockroaches completely consumed the test anthelmintic solution, which could result in misleading conclusions. In some studies, the range of weight of the test cockroaches was not controlled. The individual weight of adult cockroaches, in the case of \textit{G. portentosa}, can vary from 3 g to more than 13 g. Such a large variation in weight will influence the final results of the test, even though each individual cockroach is given the same dose. These pitfalls were considered and eliminated in the design of the present study.

The chemical name of Vermox is mebendazole, a benzimidazole derivative. Reports show that mebendazole has the broadest spectrum of activity among all anthelmintics, and is actively effective against juvenile and adult stages of both nematodes and cestodes in large experimental animals such as mice, rats, cats, dogs, sheep, cattle, and human hosts (Rochette 1985).

In the present study, mebendazole was tested in an insect-nematode system and the results are in agreement with the previous studies (Rochette 1985). The efficacy of Vermox in curing the infection of thelastomatid nematodes in \textit{G. portentosa} is excellent. It was able not only to effectively remove both adult \textit{L. portentosae} and \textit{H. diesingi}, but also their juveniles, from the hosts at doses of 0.2 mg or higher.

The exact mechanism of anthelmintic activity of mebendazole has not been established fully. However, several studies have shown that mebendazole appears to inhibit the assembly of microtubules (Köhler and Bachmann 1980, 1981), to inhibit the uptake of glucose and other low molecular weight nutrients (Rahman and Bryant 1977), and to diminish ATP synthesis and/or turnover of adenine nucleotides (Behm and Bryant 1979). The present study demonstrated that Vermox effectively eliminates parasitic nematodes in the absence of harmful effects to the host. Thus, the mechanism of action may be through nematode-specific metabolic pathways.

The chemical name of Povan is pyrvinium pamoate, which is a cyanine dye. The cyanine dyes are able to inhibit oxygen uptake in nematodes having aerobic metabolic components (Bueding 1949). They are also effective against anaerobic adult nematodes because they inhibit glucose transport (Bueding et al. 1960, Davis 1973, Botero 1978). The ability of pyrvinium pamoate to cure some nematode infections, such as \textit{E. vermicularis}, has been well documented (Biguet et al. 1952, Poyer and Baronikoff 1962, Mathies 1973, Alekseeva and Pucenko 1980). Nevertheless, it has also been reported to have a narrow nematocidal activity and to be less active against immature worms (Meira et al. 1961, Wagner 1963, Tanaka et al. 1965, Vilela et al. 1964). The present study of Povan in the treatment of thelastomatid nematodes in \textit{G. portentosa} demonstrated the narrow nematocide effect, as Povan treated the two closely related thelastomatids differently. Povan is very effective against both sexes of adult \textit{L. portentosae}, but it is significantly less effective against adult female \textit{H. diesingi}, even less effective against the adult male \textit{H. diesingi}, and totally ineffective against the
juveniles. Nevertheless, Povan has also proved to be safe for host insects, since the cockroaches given the highest dose in the present study, equivalent to 1700 mg/kg, behaved the same as the control group.

The results of this experiment provide some ideas for selecting anthelmintics to treat nematode infections in cockroaches. The highly selective functions of Povan suggest that it may not be a good choice if there is more than one nematode species present. Its ineffectiveness against juveniles indicates that it may not be an appropriate choice if a complete cure is required. In contrast, Vermox could be highly recommended in such cases, and is a particularly good choice to generate nematode-free *G. portentosa*.

In the present study, it was observed that the percentage of nematode eggs present in the fecal material during the test did not accurately reflect the actual infection rate of the nematode in the host. In some hosts where no nematodes were found when assessed at the conclusion of the test, nematode eggs were sometimes present in their fecal material. It appears that the rate at which eggs are expelled with feces is slower than the rate at which the nematodes are removed from the host by the anthelmintics. Presumably this results from the small size of the eggs. This means that using the percentage of nematode eggs in feces as the only measurement of effectiveness of an anthelmintic may lead to false estimations unless given a sufficient time period for observation.

In this test, crushed fresh apple was used as a vehicle to administer the anthelmintics. The experiment showed that crushed fresh apple is a good choice for administering anthelmintics because it attracts the cockroaches well and does not appear to influence nematode infections as indicated by almost identical infection rates between the two control groups. In addition, the comparison of the two control groups demonstrated that the 16-day time period of the experiment had no influence on the infection rates. Thus, it can be said that the differences between the test groups and the control group were due to the anthelmintics.

From this study we have found that the large size of *G. portentosa*, the stable relationship between the host and its parasites, and the ease of maintaining this host-parasite system in a laboratory, make it a favorable model system for tests and experiments. One particular attraction is that *L. portentosae* and *H. diesingi* are phylogenetically close to the human pinworm *E. vermicularis*. Welch (1965) suggested that cockroaches have approximately the same rate of food passage as a human. Furthermore, the results of the anthelmintic sensitivity test performed using *G. portentosa* are strikingly similar to the results using many large experimental animals and humans. This suggests the potential usefulness of this relatively inexpensive model system for screening anthelmintics against *E. vermicularis*.

**LITERATURE CITED**


