

## SENSITIVITY TO IVERMECTIN AND PYRANTEL OF *ANCYLOSTOMA CEYLANICUM* AND *NECATOR AMERICANUS*

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**Abstract**—BEHNKE J.M., ROSE R. and GARSIDE P. 1993. Sensitivity to ivermectin and pyrantel of *Ancylostoma ceylanicum* and *Necator americanus*. *International Journal for Parasitology* 23: 945-952. Experiments were carried out in the hamster to compare the relative susceptibility of *Necator americanus* and *Ancylostoma ceylanicum* to treatment with ivermectin. *A. ceylanicum* was found to be 300 times more sensitive to the anthelmintic with a 50% effective dose (ED<sub>50</sub>) of the order of 10-15 µg kg<sup>-1</sup> body weight whilst that for *N. americanus* approximated to 3-5 mg kg<sup>-1</sup>. Furthermore, whereas complete clearance of *A. ceylanicum* was observed with a dose of 100 µg kg<sup>-1</sup>, *N. americanus* was not totally removed after treatment with 25 mg kg<sup>-1</sup>, the highest dose tested. Both parasites proved equally sensitive to pyrantel with an ED<sub>50</sub> of 1-12 mg kg<sup>-1</sup> for *A. ceylanicum* and 5-25 mg kg<sup>-1</sup> for *N. americanus*. Treatment with pyrantel at 100 mg kg<sup>-1</sup> completely eliminated worms of both species and doses of 25-50 mg kg<sup>-1</sup> were > 90% effective. In addition to worm burdens, changes in host weight and PCV were also recorded and it was shown that both parameters could be used to evaluate the success/failure of treatment.

**INDEX KEY WORDS:** *Necator americanus*; *Ancylostoma ceylanicum*; hamster; ivermectin; pyrantel.

### INTRODUCTION

THE introduction of ivermectin, in 1981 as an antiparasitic drug for helminths and arthropods has had a marked impact on the treatment of animal diseases and recently, following successful trials in humans, a licence has been granted for treatment of patients with onchocerciasis (Campbell, 1985; Green, Brown & Taylor, 1989). Many of the common gastrointestinal nematodes are highly susceptible to ivermectin, at concentrations measured in µg kg<sup>-1</sup> body weight, considerably smaller than the mg kg<sup>-1</sup> concentrations required for effective antiparasite activity of other anthelmintics. However, some parasites have proved to be exceptionally resistant to ivermectin, (*Heligmosomoides polygyrus*, *Trichuris trichiura* and hookworms; Wahid, Behnke & Conway, 1989; Whitworth, Morgan, Maude, McNicholas & Taylor, 1991).

Several field studies have shown that ivermectin, at the doses suitable for treatment of humans, has little

impact on hookworm infection (Whitworth *et al.*, 1991; Richard-Lenoble, Kombila, Rupp, Pappayliou, Gaxotte, Nguiri & Aziz, 1988). However, these observations contrast with the results of studies in animals which have indicated that *Ancylostoma* spp. are susceptible (Egerton, Eary & Suhayda, 1985). Field data is mostly based on *Necator americanus*, a human hookworm which has no close relative among laboratory-maintained animal parasites and which itself cannot be easily maintained in dogs, the hosts generally used for chemotherapeutic studies of hookworm infection (Behnke, 1990). The implied difference in relative susceptibility to ivermectin of hookworm species from the genera *Ancylostoma* and *Necator* therefore requires urgent evaluation. In this paper we describe experiments in which we compared the relative susceptibility to ivermectin of *Ancylostoma ceylanicum* and *N. americanus* in hamsters, a host in which both species develop to patent infection. Our results establish that there is indeed a profound difference in susceptibility to ivermectin.

### MATERIALS AND METHODS

*Parasites and hosts.* Infective larvae of *N. americanus* were obtained in 1983 from Dr Rajasekariah of CIBA-GEIGY

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TABLE 1.—EXPERIMENTAL DESIGN

Experiment	No. of hamsters/ group	Parasite*	No. of larvae administered	Anthelmintic†	Day of infection when treated	Interval to autopsy (days)
1	3	<i>A. ceylanicum</i>	50	Ivermectin	18	3
2	3	<i>A. ceylanicum</i>	100	Ivermectin	21	3
3	4–5	<i>A. ceylanicum</i>	70	Ivermectin	22	6
4	3	<i>N. americanus</i>	108	Ivermectin	35	3
5	3	<i>N. americanus</i>	144	Ivermectin	35	3
6	4–5	<i>N. americanus</i>	100	Ivermectin	28	8
7	3	<i>A. ceylanicum</i>	100	Pyrantel	21	3
8	4–6	<i>A. ceylanicum</i>	50	Pyrantel	21	7
9	4–5	<i>A. ceylanicum</i>	66	Pyrantel	22	6
10	3	<i>N. americanus</i>	108	Pyrantel	35	3
11	3	<i>N. americanus</i>	160	Pyrantel	35	3
12	3	<i>N. americanus</i>	70	Pyrantel	30	7

\**Ancylostoma ceylanicum* larvae were administered orally to mature hamsters. *Necator americanus* larvae were given percutaneously to neonatal (2–3-day-old) hamsters.

†Ivermectin was administered subcutaneously. Pyrantel was given orally.

Hindustan Ltd., Bombay, India and the parasite has been maintained since by regular passage through hamsters as described originally by Sen (1972) and Behnke, Wells & Brown (1986). It is important to note here that *N. americanus* will only mature to patency following exposure of neonatal (1–3-day-old) hamsters to infection (Rajasekariah, Deb, Dhage & Bose, 1985). Experiments with *N. americanus* were therefore limited by the need for synchronized mating of female hamsters and by resulting litter sizes. *A. ceylanicum* was also obtained from Dr Rajasekariah and was passaged through adult hamsters using techniques which have been described by Garside & Behnke (1989). All the animals used in this work were syngenic DSN hamsters originally purchased from Intersimian Ltd, Oxford, U.K., but now maintained, under conventional conditions with access to food and water *ad libitum*, as a closed breeding colony in the Department of Life Science at Nottingham University. Both sexes were used but all the animals in any one experiment were of the same sex. Experimental groups were set up in separate cages at least 1–2 weeks before infection. In some experiments all individuals were weighed a week before infection, on the day of infection and treatment and on the day of autopsy. Fifty  $\mu$ l blood samples were obtained in heparinized capillary tubes, under trilene anaesthesia for measurement of the packed cell volume (PCV) at times stated.

**Anthelmintic treatment.** Ivermectin was available as a commercial preparation, Ivomec which contains 1% w/v of the anthelmintic (Merck Sharp & Dohme AGVET). The required concentrations of ivermectin were obtained by appropriate dilution of this preparation with sterile distilled water. The resulting fine suspension was injected subcutaneously in volumes of 0.05–0.4 ml within minutes of preparation. Pyrantel embonate (Strongid-P paste, Pfizer) was administered orally to hamsters after appropriate dilution of the stock with sterile distilled water.

**Statistical analysis of results.** The results are presented as group mean values (MWR)  $\pm$  standard error (S.E.M.). Non-

parametric statistical procedures were used to analyse the data sets, because small sample sizes precluded the assumption of normal distribution (Sokal & Rohlf, 1969). When more than 2 groups required comparison at a single time point the Kruskal–Wallis statistic *H* was calculated to determine whether there was a significant treatment effect. If significant, specific groups were compared to the control group (or as stated) by the Mann–Whitney *U*-test. Correlations between variables were tested by the Spearman Rank Order Correlation Test and the statistic *r<sub>s</sub>* is given, as appropriate. Probabilities were calculated from statistics tables and are presented as follows: \*  $P = 0.05$ ; \*\*  $0.05 > P \geq 0.02$ ; \*\*\*  $0.02 > P \geq 0.01$ ; \*\*\*\*  $0.01 > P \geq 0.001$ ; \*\*\*\*\*  $P < 0.001$ .

## RESULTS

### *Effect of ivermectin on A. ceylanicum*

In a preliminary experiment (Experiment 1) 5 groups of hamsters, each of 3 animals, were treated either with 20, 5, 1.25 or 0.31 mg kg<sup>-1</sup> body weight of ivermectin subcutaneously 18 days after exposure to 50 L3. One group was left untreated. When killed 3 days after treatment worms were recovered only from the control untreated animals (20.3  $\pm$  4.6).

Two further experiments were then carried out to establish the cut-off point for drug efficacy and to obtain data for 50% effective dose (ED<sub>50</sub>) and complete clearance of worms. The experimental design of this (and all other experiments) is given in Table 1 and the results are illustrated in Fig. 1(A). *A. ceylanicum* proved to be sensitive to ivermectin with a significant reduction in worm burden being achieved with doses of 10–100  $\mu$ g kg<sup>-1</sup> body weight. However, complete clearance of worms required 100–500  $\mu$ g kg<sup>-1</sup> body weight.

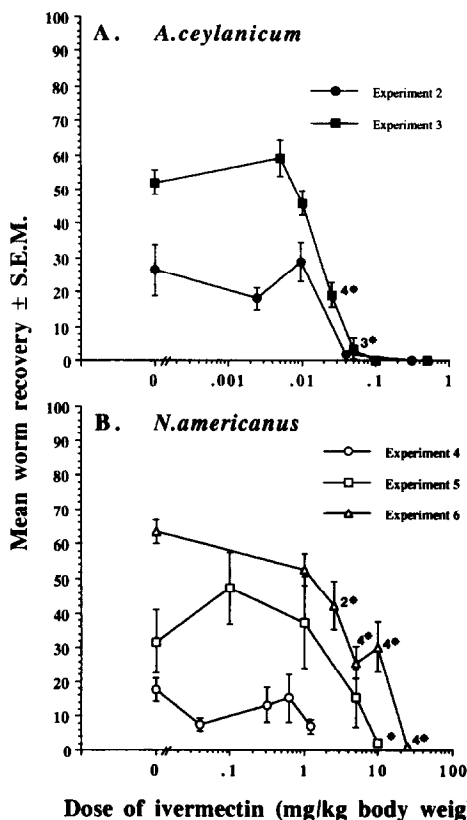


FIG. 1. *Ancylostoma ceylanicum* and *Necator americanus* adult worm burdens in hamsters treated with various doses of ivermectin. For details of the experimental designs see Table 1. Statistical analysis: Groups were compared to the untreated control group in each case. \*  $P = 0.05$ ; 2\*  $0.05 > P \geq 0.025$ ; 3\*  $0.025 > P \geq 0.01$ ; 4\*  $0.01 > P \geq 0.001$ .

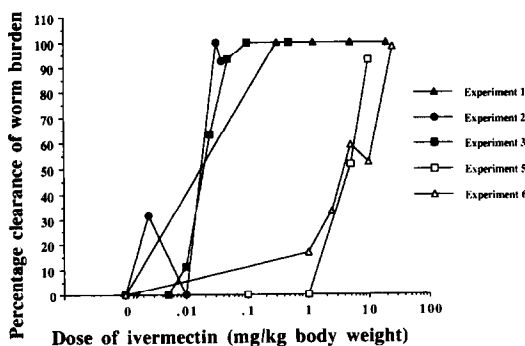


FIG. 2. Comparison of the efficacy of ivermectin against *Ancylostoma ceylanicum* and *Necator americanus*. Solid symbols represent experiments with *A. ceylanicum* and open symbols *N. americanus*.

**Effect of ivermectin on *N. americanus***

Three experiments were carried out to determine the efficacy of ivermectin against *N. americanus* (Experi-

ments 4–6). The doses investigated are given in Fig. 1(B). In Experiment 4 no significant reduction in worm burden was obtained when doses ranging from 39  $\mu\text{g}$  to 1.25  $\text{mg kg}^{-1}$  were examined. In Experiment 5, 5  $\text{mg kg}^{-1}$  resulted in a 51.6% reduction in worm burden but because of small group size and variation in worm establishment this was not a significant reduction. However, 10  $\text{mg kg}^{-1}$ , gave 92.6% clearance of worms ( $P = 0.05$ ). In Experiment 6, a dose of 5  $\text{mg kg}^{-1}$  gave 59.4% protection ( $P = 0.008$ ) and a significant effect was also detected with 2.5  $\text{mg kg}^{-1}$  (33.4% reduction,  $P < 0.032$ ) but complete clearance of worms was not achieved even with a dose of 25  $\text{mg kg}^{-1}$  (98.4% reduction in worm burden). The results therefore show that *N. americanus* is not affected by doses lower than 2.5  $\text{mg kg}^{-1}$  and that complete clearance of worms requires doses in excess of 25  $\text{mg kg}^{-1}$ .

**Comparison of the effect of ivermectin on *A. ceylanicum* and *N. americanus***

Figure 2 summarizes the data from 5 experiments. Experiment 4 was omitted because none of the doses examined were effective against *N. americanus*. It can be seen that  $\text{ED}_{50}$  for *A. ceylanicum* is of the order of 10–15  $\mu\text{g kg}^{-1}$  whilst that of *N. americanus* approximates to 3–5  $\text{mg kg}^{-1}$ . On the basis of these results *A. ceylanicum* is approximately 300 times more sensitive to ivermectin than *N. americanus*. Likewise, whereas complete clearance of *A. ceylanicum* was observed with a dose of 100  $\mu\text{g kg}^{-1}$ , *N. americanus* was not totally removed after treatment with 25  $\text{mg kg}^{-1}$ , the highest dose tested.

**Comparison of the effect of pyrantel on *A. ceylanicum* and *N. americanus***

The difference in sensitivity to ivermectin between these 2 hookworm species prompted us to compare sensitivity to pyrantel, an anthelmintic known to have comparable efficacy against worms of both genera. We were particularly concerned that our results with ivermectin might reflect an artifact of the system rather than an intrinsic difference in drug sensitivity. Therefore, 6 further experiments were carried out in which hamsters were infected with either *A. ceylanicum* or *N. americanus* and were treated with various doses of pyrantel.

The results of 5 experiments are summarized in Figs. 3 and 4. Experiment 10, which is not illustrated, comprised 5 groups of 3 hamsters each exposed to 164 L3 of *N. americanus*. Four of the groups were dosed 35 days p.i. with either 1, 2.5, 5.0 or 10  $\text{mg kg}^{-1}$  of pyrantel. The recovery of worms from the control group on this occasion was more variable than normal ( $49.3 \pm 25.4$ ) and there was no evidence of an effect at 5  $\text{mg kg}^{-1}$  ( $57.0 \pm 23.52$ ) but the group treated with 10

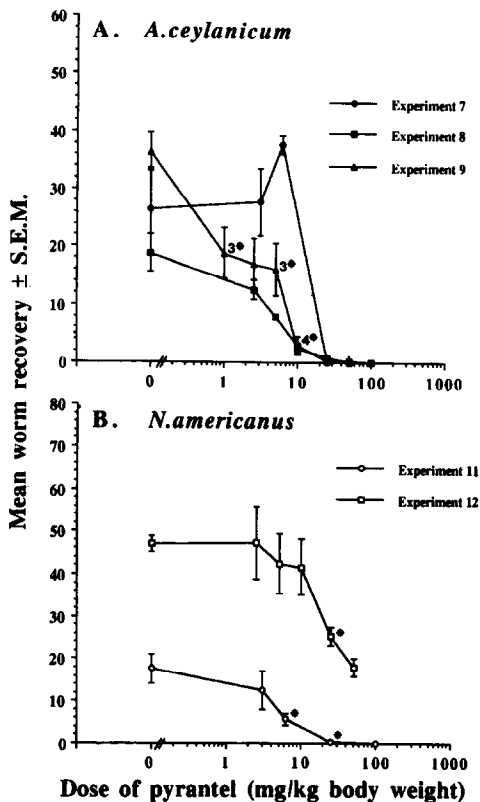


FIG. 3. *Ancylostoma ceylanicum* and *Necator americanus* adult worm burdens in hamsters treated with various doses of pyrantel. For details of the experimental designs see Table 1. Statistical analysis: Groups were compared to the untreated control group in each case. \*  $P = 0.05$ ; 3\*  $0.025 > P \geq 0.01$ ; 4\*  $0.01 > P \geq 0.001$ .

mg kg<sup>-1</sup> had a 50% reduction relative to the control group ( $24.67 \pm 11.3$ ). This result was broadly confirmed in Experiment 11 in which 6.1 mg kg<sup>-1</sup> gave 67.9% protection (Fig. 3B). However, in Experiment 12 *N. americanus* appeared to be less sensitive with 10 mg kg<sup>-1</sup> giving only a 11.3% reduction relative to controls and 25 mg kg<sup>-1</sup> 46.1% (Fig. 3B). The animals in Experiment 12 harboured heavier worm burdens than those in Experiment 11 and this may have been a factor affecting overall drug efficacy.

The sensitivity of *A. ceylanicum* to pyrantel was similar to that of *N. americanus* (Fig. 3A). Again, there was some variation between the experiments but LD<sub>50</sub> approximated to 1–12 mg kg<sup>-1</sup> for *A. ceylanicum* and 5–25 mg kg<sup>-1</sup> for *N. americanus* (Fig. 4). The lowest dose of pyrantel giving complete elimination of *A. ceylanicum* was 25 mg kg<sup>-1</sup> in Experiment 7, but the same dose left a few worms in Experiments 8 and 9 (98.9 and 97.8% protection, respectively). Fifty mg kg<sup>-1</sup> completely cleared worms in Experiment 9 but

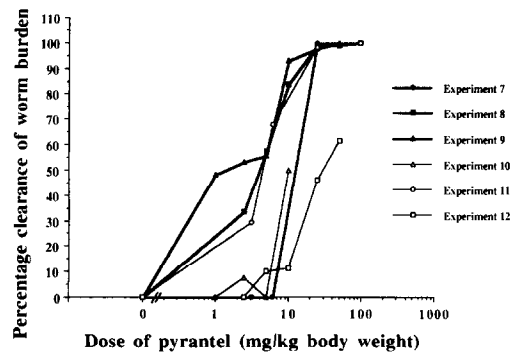


FIG. 4. Comparison of the efficacy of pyrantel against *Ancylostoma ceylanicum* and *Necator americanus*. Solid symbols represent experiments with *A. ceylanicum* and open symbols *N. americanus*.

left some adult worms in Experiment 8 (98.9% protection). Therefore, the minimum dose which guaranteed complete removal of all adult worms was established as 100 mg kg<sup>-1</sup>. This pattern was similar for *N. americanus* with 25 mg kg<sup>-1</sup> giving 98.3% protection in Experiment 11 but only 46.1% in Experiment 12. We tested 100 mg kg<sup>-1</sup> on one occasion against *N. americanus* and the result was 100% clearance of all worms (Fig. 3B, Experiment 11).

#### Comparison of the effect of both anthelmintics on changes in weight and PCV of hamsters infected with *A. ceylanicum* and *N. americanus*

In addition to recording changes in worm burden following treatment we also monitored changes in PCV (Fig. 5) and body weight (not illustrated) in some experiments. Normal PCV values in adult hamsters average about 52% with a range from 50 to 55% (Fig. 5C). In Experiments 3 and 9, in which adult hamsters were infected with *A. ceylanicum*, PCV dropped uniformly to just below 30% (Experiment 3) or just above 30% (Experiment 9) in all groups by 7 or 6 days p.i., respectively. The difference in magnitude of the reduction of the PCVs was a reflection of the heavier mean worm burden in Experiment 3 (Fig. 1A, MWR =  $52 \pm 7.1$ ) relative to that of Experiment 9 (Fig. 3A, MWR =  $36.4 \pm 3.3$ ). Following treatment with either ivermectin or pyrantel at doses which did not reduce the worm burden, PCV remained depressed. However, all groups in which treatment resulted in worm loss, had improved PCV with restoration closest to normal levels in those groups which showed more than 90% reduction in worm burdens. Worm loss was closely paralleled by increase in PCV (Experiment 3,  $r_s = 0.82$ ,  $P = 0.024$ ; Experiment 9,  $r_s = 0.922$ ,  $P = 0.001$ ) over the period between treatment and autopsy for worm counts.

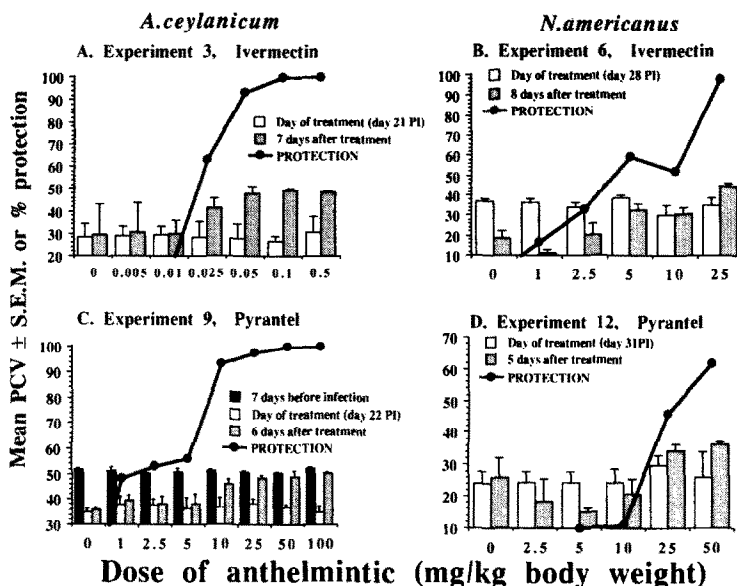


FIG. 5. Relationship between changes in PCV in the period between treatment and autopsy, and protection (% of worm burden lost) as a result of treatment.

The data for changes in weight were less clear-cut, but nevertheless support similar conclusions to those linking worm counts and changes in PCV. In Experiment 3 the mean weight of the untreated control group was less on the day of autopsy than on the day of treatment ( $97.4 \pm 23.7$ ,  $104.4 \pm 16.4$ , respectively) and similar losses of weight were detected in animals given 5 or  $10 \mu\text{g kg}^{-1}$  of ivermectin in which there was no loss of worms following treatment. All the groups treated with ivermectin at more than  $25 \mu\text{g kg}^{-1}$  had higher means, indicating that there was some weight gain (e.g. hamsters given  $100 \mu\text{g kg}^{-1}$  weighed  $104.5 \pm 6.4$  g on the day of treatment and  $110.2 \pm 5.1$  g, 7 days later at autopsy), but there were no significant differences between the groups. In Experiment 9, where the number of worms establishing was lower than in Experiment 3, all groups (except animals treated with  $5 \text{ mg kg}^{-1}$  which lost 2 gm) continued to increase in weight between the day of treatment with pyrantel and autopsy. The mean weight gain in groups given pyrantel at  $10 \text{ mg kg}^{-1}$  or more ranged from 6.1 to 11.2 g. In contrast those groups receiving less than  $10 \text{ mg kg}^{-1}$  showed a maximum mean weight gain of 4.25 g. Thus, there was some indication that animals in which the worm burden was effectively reduced gained weight faster than those in which the parasites persisted.

In experiments with *N. americanus* the interval between infection and autopsy was longer because *N. americanus* moult to the preadult stage 21–23 days p.i. and do not become patent until after 4 weeks of infection. The effect of *N. americanus* on the PCV was

more severe in Experiments 6 and 12 than that of *A. ceylanicum* in Experiments 3 and 9 and, as can be seen from Figs. 5(B) and (D), PCVs continued to decline in animals which were not treated and in those given doses of anthelmintic which failed to make a significant impact on the worm burdens. In Experiment 6 hamsters given more than  $5 \text{ mg kg}^{-1}$  of ivermectin (Fig. 5B) either stabilized their PCV or showed an increase over the interval between treatment and autopsy. There was a significant relationship between the change in PCV and loss of worms following treatment with anthelmintic ( $r_s = 0.886$ ,  $P = 0.019$ ). However, in Experiment 12 where a shorter interval separated treatment and assessment of PCV (5 days) this relationship was not significant ( $r_s = 0.667$ ).

Changes in weight in Experiments 6 and 12 closely paralleled anthelmintic efficacy. Thus in Experiment 6 hamsters given  $2.5 \text{ mg kg}^{-1}$  of ivermectin or less, lost weight between treatment and autopsy, while those given more gained weight. There was an exception in the group given  $10 \text{ mg kg}^{-1}$ , but this group also showed a smaller reduction in worms than animals given only  $5 \text{ mg kg}^{-1}$ . Overall there was a perfect correlation between the change in weight and loss of worms following treatment ( $r_s = 1$ ,  $P < 0.001$ ). In Experiment 12, only hamsters given  $2.5 \text{ mg kg}^{-1}$  of pyrantel or untreated lost weight between treatment and autopsy (e.g. the mean weight of untreated controls was  $42.8 \pm 2.7$  g on the day of treatment and  $42.4 \pm 6.8$  g at autopsy). All groups given  $5 \text{ mg kg}^{-1}$ , or more, gained weight (e.g. mean weight of hamsters given  $50 \text{ mg kg}^{-1}$  was  $45.3 \pm 13.8$  and  $57.4 \pm 16.9$  at

treatment and autopsy, respectively). Again there was a significant relationship between the mean change in weight over the period from treatment to autopsy and loss of worms ( $r_s = 0.928$ ,  $P = 0.008$ ).

### DISCUSSION

The results reported in this paper provide the first direct comparison of hookworm species from the two important genera *Ancylostoma* and *Necator* for their sensitivity to ivermectin. *Ancylostoma* is 300 times more sensitive than *Necator* to treatment with this anthelmintic. The relative insensitivity of *N.americanus* to ivermectin in the hamster model has been reported previously by Rajasekariah, Deb, Dhage & Bose (1989) with a  $ED_{50}$  of  $8.32 \text{ mg kg}^{-1}$  (marginally higher than our estimate of  $3\text{--}5 \text{ mg kg}^{-1}$ ) and the minimum dose required for complete clearance of worms calculated as  $18 \text{ mg kg}^{-1}$  (whereas we were unable to demonstrate total loss of worms with doses less than  $25 \text{ mg kg}^{-1}$ ).

This marked difference in sensitivity to ivermectin between hookworm genera has potential significance for the epidemiology of human hookworm infections in regions where *A.duodenale* and *N.americanus* are sympatric and where ivermectin is being used for the control of filariasis. Indeed there is already evidence from such studies in Africa that hookworm infections, which are predominantly *N.americanus*, are unaffected by ivermectin at the doses which are employed to treat communities with onchocerciasis ( $150 \mu\text{g kg}^{-1}$ , Whitworth *et al.*, 1991) or loiasis ( $5\text{--}200 \mu\text{g kg}^{-1}$ , Richard-Lenoble *et al.*, 1988).

To our knowledge there is no clear-cut data on the effectiveness of ivermectin in patients with *A.duodenale* but in a study of patients harbouring single or mixed species infections Naquira, Jimenez, Guerra, Bernal, Nalin, Neu & Aziz (1989) reported that doses ranging from  $50$  to  $2 \times 200 \mu\text{g kg}^{-1}$  did not significantly affect prevalence of either species. At the highest dose level tested the cure rate was higher in patients harbouring *A.duodenale* alone compared with those harbouring *N.americanus* (67 and 38%, respectively), perhaps indicating differential sensitivity but the sample sizes were too small to establish this point. However, there is considerable evidence from studies in animals that *A.caninum* in dogs (Egerton *et al.*, 1985) and *A.tubaeformae* in cats (Fukase, In, Chinone, Akihama & Itagaki, 1991) are readily affected by doses of ivermectin comparable to those which we employed. Wang, Huang, Zhang, Yen & Wen (1989) reported complete clearance of *A.caninum* from dogs after treatment with only  $10 \mu\text{g kg}^{-1}$  body weight, a dose corresponding in our study to  $ED_{50}$  for *A.ceylanicum*. In hamsters complete clearance of *A.ceylanicum* necessitated a minimum dose of  $100 \mu\text{g kg}^{-1}$ .

However, it is well established that the metabolic activity of small animals is higher than that of larger animals and there is a negative correlation between body size and  $ED_{50}$  as well as  $ED_{100}$ . Thus, the 10-fold difference in the results reported here and those of Wang *et al.* (1989) is consistent with this interpretation, but we can only speculate until comparative experiments employing both *Ancylostoma* species in dogs are completed. The canine system could also be used to examine *A.duodenale* which can be maintained in dogs (Leiby, El Naggar & Schad, 1987).

We were concerned that the difference between species in sensitivity to ivermectin may be attributable to some artifactual consequence of the experimental system which we employed (see Behnke, 1990), since both species of hookworm used in this study were adapted to passage through hamsters and were not tested in their natural definitive hosts. However, our results with pyrantel were reassuring since we concluded that *A.ceylanicum* was marginally, if at all, more sensitive to pyrantel than *N.americanus*, and this is consistent with the reported comparable efficacy of pyrantel against both human hookworms (Ghadirian & Sanati, 1972; Rossignol, 1990). The standard treatment for humans is  $10 \text{ mg kg}^{-1}$  of pyrantel base for 3 days (Migasena & Gilles, 1991) which, taking relative body size into consideration, is also close to our values for over 90% efficacy in hamsters. Furthermore, our  $ED_{50}$  value for *N.americanus* ( $5\text{--}25 \text{ mg kg}^{-1}$ ) was similar to that of  $23.3 \text{ mg kg}^{-1}$  given by Rajasekariah *et al.* (1989).

However, the most interesting conclusion from this study is that *N.americanus* is extremely resistant to ivermectin, more so than *Heligmosomoides polygyrus* which is reputed for its insensitivity to this anthelmintic (Wahid *et al.* 1989). The intriguing question which follows is why 2 organisms, representing 2 genera closely related to each other, should show such marked disparity in sensitivity to ivermectin, despite comparable sensitivities to other commonly employed anthelmintics. It may also be pertinent that hookworms of the genera *Bunostomum* and *Gaigeria* (see Yazwinski, 1988; Benz, Roncalli & Gross, 1989) which are more closely related to *Necator* than *Ancylostoma* are sensitive to ivermectin at doses of  $< 200 \mu\text{g kg}^{-1}$ . One possibility may be that *in vivo*, subcutaneously injected ivermectin has less access to *N.americanus* than to worms of other hookworm genera and in this context *A.duodenale* generally penetrate deeper into the gut mucosa than *N.americanus* (see Bonne, 1942; Zimmerman, 1946). Furthermore, *A.duodenale* is known to cause more profound blood loss than *N.americanus* in humans (Roche & Layrisse, 1966) but in our system *A.ceylanicum* is comparably the more

pathogenic (Behnke, 1991). However, we feel that this is an unlikely explanation for the relative insensitivity of *N. americanus* to ivermectin because other parasites such as *Ascaris lumbricoides* are extremely sensitive to ivermectin in orally treated patients whereas *N. americanus* is not. Furthermore, *Uncinaria stenocephala* and *A. braziliense* (see Egerton *et al.*, 1985; Henriques, Martin & Sievers, 1985; Campbell, 1989) which cause only minimal blood loss and are regarded as mucosal surface browsers, are sensitive to subcutaneously administered ivermectin at doses of  $< 50 \mu\text{g kg}^{-1}$ .

Although it was originally widely believed that the principal target of ivermectin was GABA mediated transmission of signals to longitudinal muscles (Campbell, 1985) the exact mode of action is now disputed. The physiological effect of ivermectin is an increase in permeability of cell membranes to chloride ions (Turner & Schaeffer, 1989) and it has recently been proposed that a novel  $\text{Cl}^-$  channel may be the receptor (reviewed Geary, Klein, Vanover, Bowman & Thompson, 1992). In *Caenorhabditis elegans* ivermectin mediates its effect by binding to a specific high affinity binding site. The differential sensitivity observed in our experiments may thus result from some fundamental difference in the binding sites or chloride channels between worms from the genera *Ancylostoma* and *Necator*; but the differential susceptibility of dogs to ivermectin has been clearly demonstrated not to be a result of chloride channel binding (Schaeffer, Rohrer, Cully & Arena, 1992) and hence there may be other explanations. Since there is evidence that ivermectin passes through the nematode cuticle (Ho, Geary, Barsuhn, Sims & Thompson, 1992), our observations may suggest fundamental differences in the organization of the cuticle, perhaps at the molecular level. Alternatively differences between the genera in permeability to ivermectin at the gut level following oral ingestion by the parasites or through cuticular pores associated with excretory activity or sensory structures such as cuticular papillae, amphids and phasmids may result in less ivermectin having access to relevant nerve junctions in worms of the genus *Necator* compared with *Ancylostoma*. Pharmacokinetic differences between the genera may be responsible for different concentrations of ivermectin reaching target sites. Such differences have been proposed to account for resistance of insects to commercial insecticides (Ahn, Funabi & Motoyama, 1992). One way to establish whether ivermectin has comparable or differential access to the nervous systems of *Ancylostoma* and *Necator* would be to employ fluorescent labelled ivermectin to quantify drug concentrations at nerve junctions following uniform exposure *in vitro* and/or *in vivo*

(Martin, Kusel, Robertson, Minta & Haugland, 1992), an approach which we are currently investigating.

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