

Comparative Efficacy of Ivermectin and Levamisole for Reduction of Migrating and Encapsulated Larvae of *Baylisascaris transfuga* in Mice

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Abstract: The comparative efficacy of 2 anthelmintics (ivermectin and levamisole) against *Baylisascaris transfuga* migrating and encapsulated larvae was studied in mice. A total of 60 BALB/c mice inoculated each with about 1,000 embryonated *B. transfuga* eggs were equally divided into 6 groups (A-F) randomly. Mice of groups A and B were treated with ivermectin and levamisole, respectively, on day 3 post-infection (PI). Mice of groups A-C were killed on day 13 PI. Similarly, groups D and E were treated with ivermectin and levamisole, respectively, on day 14 PI, and all mice of groups D-F were treated on day 24 PI. The groups C and F were controls. Microexamination was conducted to count the larvae recovering from each mouse. The percentages of reduction in the number of migrating larvae recovered from group A (ivermectin) and B (levamisole) were 88.3% and 81.1%, respectively. In addition, the reduction in encapsulated larvae counts achieved by ivermectin (group D) and levamisole (group E) was 75.0% and 49.2%, respectively. The results suggested that, to a certain extent, both anthelmintics appeared to be more effective against migrating larvae than encapsulated larvae. However, in the incipient stage of infection, ivermectin may be more competent than levamisole as a larvicidal drug for *B. transfuga*.

Key words: *Baylisascaris transfuga*, ivermectin, levamisole, migrating larvae, encapsulated larvae, granuloma

INTRODUCTION

As a potential pathogen of zoonosis, *Baylisascaris transfuga* is a ubiquitous gastrointestinal nematode of almost all species of bears, including giant panda *Ailuropoda melanoleuca*, Malayan sun bears *Helarctos malayanus*, sloth bears *Melursus ursinus*, American black bears *Ursus americanus*, brown bears *Ursus arctos*, polar bears *Ursus maritimus*, Asiatic black bears *Selenarctos thibetanus*, and Andean bears *Tremarctos ornatus*. Within as few as 2 weeks, *B. transfuga* eggs, which are evacuated along with feces of bears, can not only mature to infective larvae, but also remain viable in zoo environment for up to 5 years. Under laboratory conditions, *B. transfuga* eggs retain their infectivity for at least 15 months. Herbaceous plant, soil, and water sources contaminated by *B. transfuga* eggs are the major infec-

tive agent of this zoonosis. *B. transfuga* can impact natural host survival and reproduction directly through pathologic effects and indirectly by reducing host condition. Furthermore, it can give rise to lethal larva migrans syndromes in occasional hosts, such as alternative livestock, free ranging domestic fowls and humans [1].

Due to its widespread transmission among bear populations, it is more possible for people who get in touch with bears and their feces frequently, such as zookeepers and animal trainers, to be infected. In certain countries, some cases indicated that bears were implicated in human visceral larva migrans by *B. transfuga* [2]. According to previous studies on larval migration of *Baylisascaris* in mice, following oral infection with infective eggs, most of the larvae hatched in the gastrointestinal tract and remained in the intestinal wall, while others migrated through the liver and lungs to reach other organs, including the brain, and mostly the musculature [3,4]. Hence, infective larvae can not complete the whole growth cycle in the incidental host, but can invade into central nervous system (CNS) and implicate in irreversible nervous larvae migrans (NLM). Otherwise, Papini [5] experimentally inoculated

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chickens with *B. transfuga* infected eggs, and considered that the larvae may be able to penetrate the liver, lungs, carcass, and brain of infected chickens. However, a great number of larvae accumulated in the liver [5]. In most cases, within 2 weeks, *Baylisascaris* larvae can be encapsulated by granulation tissues, so that they tend to settle and/or trapped in muscles or other tissues. Becoming encapsulated, larvae can survive in tissues of mice for several months, even after freezing and putrefaction [3]. In experimentally infected monkeys and rats, *Baylisascaris procyonis* larval granulomas, often visible grossly, were abundant in many organs and tissues, including the liver, lungs, heart, intestinal wall, skeletal muscles, brain, and eyes [4].

Several anthelmintics have been proved to be effective against adult *Baylisascaris* in definitive hosts, including raccoons, skunks, dogs, and bears. Bauer and Gey [6] treated raccoons infected with *B. procyonis* with ivermectin (1 mg/kg) orally, found out that 24 adult raccoon roundworms were expelled in the feces on day 2 post-treatment [6]. No worms were observed in corpses on day 7 post-treatment. In addition, another 5 anthelmintics, including pyrantel embonate, moxidectin, albendazole, fenbendazole, and flubendazole, were considered useful against *B. procyonis*, based on their studies. However, due to the lack of early diagnosis, rare anthelmintics have been proved to be effective enough to control *Baylisascaris* larva migrans [7,8].

Ivermectin, a member of the macrocyclic lactone endectocides, is a broad-spectrum anthelmintic. It has been successfully used to control a wide range of gastrointestinal helminths in livestock [9,10]. Levamisole also is used in helminth control programmes with good results and has been frequently used in clinical application.

However, due to the fact that once larvae have entered CNS, significant neurological damage quickly occurs, for the most part, irreversible. Unfortunately, this is the time when infection is still asymptomatic and likely to be unrecognized [4,7]. Papini et al. [8] treated mice infected with 3,000 *B. transfuga* eggs with a large dose of ivermectin (2 mg/kg) subcutaneously, and supported that, to a certain extent, ivermectin was useful in limiting the nervous migratory activity of larvae, based on the highest percentage reduction of larvae in brain (66.7%). Nevertheless, as far as we know, few have been reported on the comparative efficacy of levamisole and ivermectin against *B. transfuga* larvae, and whether ivermectin could produce more marked effects on their migratory activity in other organs of mice. Therefore, the objective of the present trial was to com-

pare the efficacy of ivermectin and levamisole against *B. transfuga* migrating larvae and encapsulated larvae in mice.

MATERIALS AND METHODS

Baylisascaris transfuga eggs and animals

In order to compare the efficacy of ivermectin and levamisole for control of *B. transfuga* larvae in mice and whether ivermectin offered advantages to limit *B. transfuga* larval migration in various organs (not only the brain), *Baylisascaris transfuga* adult females were recovered from a captive polar bear living in the Chengdu Zoological Garden, Chengdu, China. Eggs were obtained and cultured as described by Papini and Casarosa [1].

Sixty BALB/c mice of both sexes, aged 4 weeks and weighing 20-25 g each, were obtained from Laboratory Animal Center of Sichuan University (Chengdu, China), and randomly assigned into 6 groups (A-F). Each group contained 10 mice, males and females. Institutional Ethical and Animal Care guidelines were adhered to during the sampling exercise, and all procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

During the experimental period, the different sexes were kept separately, and maintained on commercial diet and water discretionally. Ivermectin and levamisole used in this study were brought from Qian Kun Inc. (Chengdu, China).

Innoculation and treatment

As described by Papini and Casarosa [1], each mouse of all groups was infected orally with about 1,000 infective eggs. Mice were examined daily for clinical signs.

Mice were treated as follows:

Group A: ivermectin, by subcutaneous (SC) injection with 0.3 mg/kg for 1 time, on day 3 post-infection (PI).

Group B: levamisole, by intramuscular (IM) injection with 8 mg/kg for 1 time, on day 3 PI.

Group C: untreated controls (Control 1).

Group D: ivermectin, by SC injection with 0.3 mg/kg for 1 time, on day 14 PI.

Group E: levamisole, by IM injection with 8 mg/kg for 1 time, on day 14 PI.

Group F: untreated controls (Control 2).

Larvae collection and procession

The mice of groups A-C were killed by ether anesthesia on

day 13 PI, and processed respectively. For each carcass, the small and large intestines were removed, opened longitudinally, and rinsed vigorously in 3 changes of normal saline. As described by Cho et al. [11], the intestinal wall, liver, lungs, and skeletal muscles, including bones, were separately minced with scissors and digested in artificial gastric juice (0.5% 1:10,000 pepsin, 0.7% hydrochloric acid) for 2 hr at 37°C along with vigorous agitation. *B. transfuga* larvae were recovered by Baermann's apparatus. Sediments were examined for larvae in a grid-marked glass by stereoscopic microscope (Olympus Inc., Tokyo, Japan). The brain was directly examined under a stereoscopic microscope after squashing between 2 large glass slides [11]. Larvae recovered from brain, skeletal muscle, and internal organs (including liver, lungs, and intestinal wall) of each mouse were observed and counted using microscopic examinations. Counting apparatus were produced by Fujihira Inc. (Tokyo, Japan). Identically, mice of groups E-F were examined in the same way on day 24 PI, and the number of larvae recovered from them was also recorded. The percentage of efficacy of the treatments was calculated from the count reduction of recovered larvae, using the arithmetic mean of larvae count with the following formula:

$$\text{Efficacy (\%)} = 100 \times (\text{mean larvae recovered in controls} - \text{mean larvae recovered in treated mice}) / \text{mean larvae recovered in controls}$$

It is worth mentioning that larvae recovered from groups A-C and groups E-F represent migrating larvae and encapsulated larvae, respectively.

Data processing and statistical analysis

The number of larvae recovered was expressed as means \pm SD for each experimental group. Comparisons between experimental groups were performed by Duncan's multiple range using the statistical pack SPSS version 13.0 for Windows. The level of significance for analyses was set at $P \leq 0.05$ and ≤ 0.01 .

RESULTS

Clinical symptoms

None of groups A and B mice displayed any clinical symptoms during the examination. Comparatively, clinical signs of verminous pneumonitis were obvious in 7 mice of group C, within day 4 PI. Mice of group C ($n = 4$) showed mild clinical symptoms of nervous larvae migrans (NLM), which is caused by larvae migrating into the host brain, such as spinning

around the longitudinal axis, continuous circling or lateral recumbency, on day 10 PI.

Group D ($n = 3$), group E ($n = 7$), and group F ($n = 5$) mice showed respiratory symptoms during the early stage of infection (days 0-4 PI). None of groups D and E mice appeared to have patent neurological symptoms, such as gait difficulty and circulatory movements until day 12 PI. It seemed that 6 mice with clinical NLM, which were treated with ivermectin in group D, failed to improve, but continued to deteriorate. Two out of 5 mice with neurological symptoms in group E took a favorable turn on day 17 PI, after having been treated with levamisole.

Necropsy examination

During necropsy examination of groups A-C, several pinpointed grayish nodules and irregular petechial and ecchymotic hemorrhages were evident on the surfaces of the liver and lungs of group C mice. There existed no significant pathological changes on the mice of groups A and B.

On the other hand, investigating the skeletal muscle and organs of groups D-F, some encapsulating reactions were found to involve the intestinal wall and subcutaneous tissues of the neck, back, and legs, as well as the diaphragm. Obvious hemorrhagic foci and congestion were found on the surface of the brains of groups D-F.

Number of larvae recovered and the efficacy of treatment

Mean numbers of larvae recovered from groups A-F are shown in Fig. 1. On one hand, comparing the mean total numbers of larvae recovered from 6 groups, respectively, it was easy to find out that there was no statistical difference between groups A and B, but the recovery in some groups was significantly reduced ($P < 0.01$) compared with that in group C. Similarly, larval recovery in group D or E was strikingly reduced ($P < 0.01$) from that in group F. However, the mean total number of larvae recovered from group D was remarkably lower than that from group E ($P < 0.01$). In addition, there existed remarkable differences ($P < 0.01$) in total larval recovery, between groups A and D, as well as between group B and E.

On the other hand, in different tissues and organs except the brain, it was also apparent that the larval recovery in treatment groups was significantly reduced from that in control groups ($P < 0.01$). Moreover, larval recovery from skeletal muscles in groups A and D, groups B and E, and groups D and E was notably different between each other. In contrast, there ex-

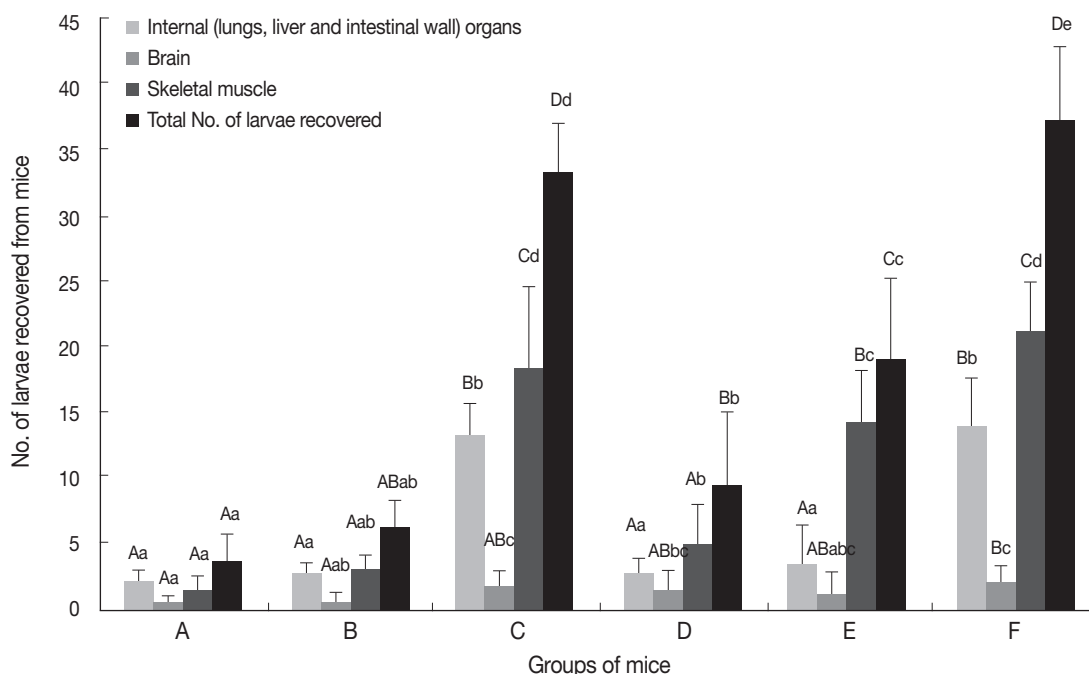


Fig. 1. The number of larvae recovered from different tissues of the 6 groups of mice inoculated orally with approximately 1,000 *Baylisascaris transfuga* infective eggs, and examined on the day post-infection (PI). Values were expressed as the mean value \pm SD for each group of 10 mice. Above the columns with the same color, values with the same letter mean no significant differences ($P > 0.05$), values with different small letter mean significant differences ($P < 0.05$), and values with different capital letter mean highly significant differences ($P < 0.01$).

isted no significant differences between the 4 treatment groups in internal organs recovery. As for the larvae count in the brain, the difference between groups A and D was marked ($P < 0.05$). However, the lack of difference in the brain recovery among groups D-F provided food for thought.

The efficacy of the anthelmintics was estimated based on the % larvae reduction. As illustrated in Table 1, the total percentage reduction in larvae counts in mice following treatment with ivermectin in group A and levamisole in group B was 88.3% and 81.1%, respectively. Similarly, it was 75.0% in group D (ivermectin) and 49.2% in group E (levamisole), respectively.

Examined the matter from other angles, there was something valuable to be noticed. By comparison of the data of 2 ivermectin groups, which were treated on different days PI, total percentage reduction in group A (88.3%) was higher than that in group D (75.0%). The same phenomenon existed in levamisole groups, as levamisole showed greatly higher efficacy in group B (81.1%) than in group E (49.2%).

On the other hand, for both group A (ivermectin-treating on day 3 PI) and group B (levamisole-treating on day 3 PI), in different organs, there were no significant differences in reduc-

Table 1. Reduction in the number of larvae recovered in different organs of treatment-group mice on days 13 and 24 PI

Treatment groups	Percentage (%) reduction in the number of larvae recovered from different organs			Total reduction (%)
	Internal organs (lungs, liver and intestinal wall)	Brain	Skeletal muscle	
A (ivermectin, on day 13 PI)	84.1	76.5	92.4	88.3
B (levamisole, on day 13 PI)	79.6	70.6	83.2	81.1
D (ivermectin, on day 24 PI)	79.9	23.8	76.9	75.0
E (levamisole, on day 24 PI)	74.1	42.9	33.5	49.2

tion percentage. In contrast, treatments conducted on day 14 PI manifested the difference. Ivermectin administered in group D showed much lower larval reduction percentage in the brain (23.8%) than in other organs (79.9% in the intestinal organs and 76.9% in the skeletal muscle). For levamisole used in group E, reduction percentages in the brain and skeletal muscle (24.9% and 33.5%, respectively) were much lower than that in intestinal organs (79.9%). It was remarkable that, in the brain, efficacy of treatments on day 14 PI (group D:

23.8%; group E: 42.9%) was much lower than those on day 3 PI (group A: 76.5%; group B: 70.6%).

DISCUSSION

The goal of this study was to compare the efficacy of ivermectin and levamisole for control of *B. transfuga* larvae in mice and whether ivermectin offered advantages to limit *B. transfuga* larval migrans in various organs, not only the brain.

The data demonstrated that ivermectin and levamisole were both useful in control of *B. transfuga* larvae in mice but at different rates. In addition, both of the 2 drugs were found to be more efficacious for migrating larvae than for encapsulated larvae. In the mass, ivermectin was superior to levamisole with regard to decreasing both free and encapsulated larvae. However, ivermectin had little effect on *B. transfuga* larvae invading into the brain. This result coincided with that obtained by Kazacos [4]. Comfortably, our study showed that ivermectin was capable of reducing the larvae in other organs, including liver, lungs, intestinal wall, and muscle. Levamisole, on the other hand, showed steady qualities on control of visceral larva migrans. However, it seemed to be failed to deal with larval granulomas in the skeletal muscle and brain.

As is known to all, administration of ivermectin via subcutaneous injection can achieve the greatest bioavailability. Due to its high lipophilic nature, ivermectin is extensively distributed in the organism. Moreover, its extremely low water solubility and its precipitation in subcutaneous tissues favours slow absorption from the injection site, leading to a long duration of activity [12]. In relative terms, administered by intramuscular injection, levamisole can distribute broadly as well, and be absorbed quickly, but fails to prolong presence in the blood stream.

Ivermectin acts by binding to a glutamate-gated chloride channel receptor in nematode and arthropod nerve cells, and also increases the release of γ -aminobutyric acid (GABA) from synaptosomes of the nervous system and blocks the transmission of nervous stimuli to muscles resulting in flaccid paralysis of affected parasites, followed by their death or expulsion [13,14].

On the other hand, levamisole is agonists at nicotinic acetylcholine receptors of nematode muscles and cause spastic paralysis. However, it seems that levamisole can not kill larvae in tissues immediately, as well as adult worms. In normal times, after administration with levamisole, gastrointestinal nema-

todes expelled with feces are still alive. Furthermore, as an immunoregulatory agent, levamisole simulates the thymic hormone thymopoietin, which probably targets at the stimulation of phagocytosis and regulatory T cells to restore homeostasis in a perturbed immune system [15]. So, there is every reason to believe that the efficacy of levamisole against larvae in the tissues, to a great extent, depends on the immunocompetence of the hosts. Comparatively speaking, the mechanism of action of ivermectin may be more direct and effective. All the features of pharmacokinetic and mode of action which we mentioned above should successfully explain the difference between the 2 drugs in the efficacy against *B. transfuga* larvae.

All previous treatment attempts of which the authors are aware indicate that ivermectin is unsuccessful in NLM treatment, because in mammals ivermectin apparently is excluded from the CNS by the blood-brain barrier (BBB) [4,16]. The present study proved the viewpoint again, as the efficacy of ivermectin in the brain of groups A and D were only 76.5% and 23.8%, respectively. However, the significant difference between the 2 sets of data meant that it could markedly prevent motile larvae from invading into the brain in the early stage of infection. The efficacy of levamisole in the brain of group E mice (42.9%), even though in a low level, was much higher than that of ivermectin in group D (23.8%). This phenomenon conformed to the distribution of unbound levamisole in the anesthetized rat brains, as Lin and Tsai described [17]. It is indicated that levamisole should cross BBB more easily.

About 2 weeks later, larvae in tissues became encapsulated in well-circumscribed eosinophilic granulomas [4]. Yet, from the formation of granulomas in mouse tissues in response to trapped *Schistosoma japonicum* eggs, we may deduce that only within a few days PI, neutrophils should begin to infiltrate around the *B. transfuga* larvae, and inflammatory cells begin to infiltrate around and inside the lesions [18]. In the meantime, collagenous fibers are steadily generated by fibroblasts around the larvae. Finally, once the threat from larvae has been reduced or eliminated, the infiltrating population is reduced and the formation of scar tissues is induced, accompanied by the atrophy of micrangium around the granuloma. Maturation of granuloma ends in fibrosis. Drugs may hardly reach into the granuloma due to the atrophy of micrangium. In addition, tight and firm fibrosis which serves to wall off the granuloma contents [19], may block the infiltration of drugs. These may be the reason why the efficacy of both ivermectin and levami-

sole against encapsulated larvae was much lower than that against migrating larvae.

In our experiment, we conducted culture of eggs, inoculation, larvae collection and procession, and microscopic examination for counting larvae completely as previously described. However, the recovery in the controls were less than 4%. Sprent [20] administered 5,000 *B. transfuga* eggs to each of white mice and recovered around 7.8% of the larvae on day 14 PI. Papini et al. [8] orally infected chicken with about 5,000 embryonated eggs of *B. transfuga*, and the recovery was 4.9% on day 15 PI. Cho et al. [11] inoculated 1,000 *B. transfuga* eggs into gerbils orally, and the recovery on day 14 PI was above 12.5%. The differences among the larval recovery rates, on the one hand, may be attributed to susceptibility differences for various intermediate hosts, even for different strains of mice. Sugane and Oshima [21] orally infected 8 inbred strains of mice with 500 embryonated *Toxocara canis* eggs each. Different inbred strains of mice showed different larval recovery rates ranging from 9% to 24%. Therefore, it is assumed that different strains of mice may be susceptible to *B. transfuga* in varying degrees. Certainly, further investigations would be needed to verify this hypothesis. On the other hand, it may be ascribed to different infectivity of eggs, which was correlated with length of time in cultures. Papini and Casarosa [22] demonstrated that *B. transfuga* eggs reached infectivity after 2 weeks in cultures, peaked after 4 weeks, and then waned over the next 18 months [22]. The eggs used in this experiment had been cultured for about 7 weeks before inoculated, and may be not the most infective. However, this did not affect the reliability of the results, because the same eggs were administered to treatment and control groups. The reduction % of larvae was still accurate and reliable.

From what has discussed above, we may reasonably arrive at the conclusion that, in the absence of a completely effective larvicide, an early chemotherapy of using ivermectin or levamisole is feasible and useful in limiting the migratory activity of *B. transfuga* larvae, especially for migrating larvae. In addition, ivermectin is superior to levamisole, in the mass, with regard to decreasing both free and encapsulated larvae in mice.

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