Efficacy of levamisole alone and in combination with mebendazole against *Gongylonema pulchrum* infection in rabbits

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**ABSTRACT.** *Gongylonema pulchrum* is an important parasite of captive primates. Twelve rabbits were infected with 30 third-stage larvae of *G. pulchrum*. At 4–7 months post-infection, animals were administered levamisole at a single dose of 12 mg/kg, levamisole at 8 mg/kg three times at 2-day intervals, levamisole at a single dose of 8 mg/kg after administration of mebendazole at 70 mg/kg for 3 days or 8 ml of distilled water for 3 days (control). Necropsy at 14 days after treatment revealed that single and multiple dosages of levamisole reduced nematode burdens by 68.4% and 89.5%, respectively. The combined regimen of mebendazole and levamisole exhibited high efficacy for treating *G. pulchrum* located widely within the upper digestive tract, with a reduction of 98.2%. These results suggest that this combined chemotherapy treatment may be effective against *G. pulchrum* infection, including buccal and lingual gongylonemiasis in primates.

**KEY WORDS:** combined treatment, *Gongylonema pulchrum*, levamisole, mebendazole

*Gongylonema pulchrum* (gullet worm) is a cosmopolitan parasite that occurs in the upper digestive tract of a variety of mammals, including domestic and wild ruminants, equids, swine, primates, squirrels, rabbits, bears, skunks, hedgehogs and humans [4]. In Japan, it is most commonly found in cattle, but it has also been reported in wild deer (*Cervus nippon*), wild macaques (*Macaca fuscata*) and captive primates (*Saimiri boliviensis*) [8, 13, 15–17]. The life cycle of *G. pulchrum* includes dung beetles and cockroaches as intermediate hosts.

While *G. pulchrum* is typically found in the esophageal mucosa of definitive hosts without any apparent pathogenicity, the nematode causes buccal and lingual gongyonemiasis associated with pathologic changes and clinical signs in primates, including humans. To date, numerous cases of *Gongylonema* spp. infection have been identified in captive primates at zoological parks [1, 13], and fatal cases of gongyonemiasis due to severe inflammation of the lips and tongue have been reported in Goeldi’s monkeys (*Callithrix goeldii*) and Common marmosets (*Callithrix jacchus*) [3, 5]. According to Brack [3], clinical signs of disease in the infected Common marmosets consisted of intensive itching and scratching of the edematous and hyperemic perioral tissues, with inflammation of the lips aggravated by the intense scratching. Duncan *et al.* [5] suggested that lingual gongyonemiasis associated with oral inflammation and irritation in Goeldi’s monkeys might have predisposed the animals to *Pasteurella septicemia*, which resulted in their death. In addition, in humans, infection by *G. pulchrum* is usually associated with local irritation of the buccal mucosa [4]. Nematode infection of the tongues of slaughtered pigs accompanied by mild and chronic inflammation of the lingual mucosa has also been reported [18].

Although anthelmintic treatment of *G. pulchrum* infection is not generally recommended in livestock, treatments using ivermectin, mebendazole and fenbendazole have been reported in callitrichid primates with gongylonemiasis [1, 3, 5]. In these studies on naturally infected callitrichids, a combined regimen of ivermectin and mebendazole was more effective for improving clinical signs than either monotherapy with each drug or combined chemotherapy with ivermectin and fenbendazole. In addition, clinical signs in human infections were resolved following treatment with levamisole and albendazole [2, 6]. However, because these reports lacked comprehensive data on the efficacy of anthelmintic treatment and the extent of the reduction in the number of nematodes after treatment, more detailed studies are necessary to assess the therapeutic efficacy of anthelmintics against gongylonemiasis. We previously demonstrated that levamisole is more effective against *G. pulchrum* infection than thiabendazole, mebendazole or ivermectin, by post-mortem examination of experimentally infected rabbits [11].

Experimental infection of rabbits with *G. pulchrum* is considered to be well suited for examining the therapeutic characteristics of buccal and lingual gongyonemiasis, because of the high susceptibility of rabbits to infection by this nematode and the location of the nematode burdens in the buccal mucosa and tongue of the animals [9, 10]. This paper presents additional information on the efficacy of levamisole monotherapy and a combined chemotherapy regimen of levamisole and mebendazole against *G. pulchrum* infection using rabbits.

The *G. pulchrum* used in this study was originally isolated from naturally infected dung beetles (*Aphodius rectus* and *Aphodius tasmanicus*) in Japan.
A. haroldianus) in Aomori Prefecture, Japan. The nematodes were maintained in our laboratory using cockroaches (Blattella germanica) as an intermediate host and rabbits as the definitive host. Using a stomach tube, 12 Japanese white rabbits (10-week-old males) were individually inoculated with 30 third-stage *G. pulchrum* larvae (L3) that were obtained from experimentally infected cockroaches. The prepatent period of *G. pulchrum* is 72–81 days in rabbits [10]. At 4–7 months post-infection, when the larvae were fully developed, the animals were divided into 4 groups containing 3 animals each. The following treatments were administered orally to each group: Group 1 was administered levamisole hydrochloride (powdered levamisole hydrochloride, Yuko Chemical Industries Co., Ltd., Nishinomiya, Japan) at a single dose of 12 mg/kg body weight; Group 2 was administered levamisole hydrochloride at a dose of 8 mg/kg three times at 2-day intervals; Group 3 was administered levamisole hydrochloride at a single dose of 8 mg/kg after administration of mebendazole (Mebendazole, Janssen Pharmaceutica, Beerse, Belgium) at 8 mg/kg after administration of mebendazole (Mebendazole administered levamisole hydrochloride at a single dose of 12 mg/kg body weight; Group 4 was administered levamisole hydrochloride at a single dose of 8 mg/kg × 1 + Levamisole 8 mg/kg × 1 + Distilled water 8 m/× 3 (Control) at a single dose of 8 mg/kg for 3 days; Group 4 received 8 ml of distilled water for 3 days (control). Each anthelmintic dose was suspended in 4 ml of distilled water, and the suspension was administered to the animals using a stomach tube under anesthesia with isoflurane. The animals were then given an additional 4 ml of distilled water to clear the inside of the stomach tube. All animals were euthanized using diethyl ether at 14 days post-treatment (PT). The upper digestive tracts (i.e. buccal mucosa, tongue, pharynx and esophagus) were removed from each animal and examined for nematodes. The tissues were then incubated in artificial gastric juice (0.5% pepsin and 0.5% HCl) for several hours at 37°C and examined for worms embedded in the mucosa using a dissecting microscope. The results were analyzed using Welch’s t-test to identify statistically significant differences among groups. Blood counts of the rabbits in groups 2–4 and clinical biochemistry profiles of the animals in groups 2 and 3 were performed at 0, 7 and 14 days PT, using an automatic cell counter (Nihon Kohden MEK-6358, Tokyo, Japan) and an automated analyzer (Olympus AU510, Tokyo, Japan), respectively. The biochemical examination included plasma concentrations of albumin, alanine aminotransferase and blood urea nitrogen. All experimental procedures were conducted according to the Guidelines for the Care and Use of Laboratory Animals of Kitasato University, and the experimental protocols were approved by the Animal Care and Use Committee of Kitasato University.

The number of nematodes recovered from the upper digestive tracts of rabbits after treatment with anthelmintics is shown in Table 1. The total nematode burden in each experimental group was significantly lower than in the control group (P<0.05). The nematode reduction rates at 14 days PT were highest for the combination of mebendazole and levamisole (98.2%), followed by levamisole at a dose of 8 mg/kg three times (89.5%) and at a single dose of 12 mg/kg (68.4%), respectively. The rate of nematode reduction in the combined chemotherapy was significantly higher than that obtained with a single dose of 12 mg/kg levamisole (P<0.05). Compared with the blood cell counts of group 4 (control) and the reference ranges for serum biochemistry values in normal rabbits [12], the blood tests revealed no side effects in drug-treated rabbits. In our previous study, necropsy of experimentally infected rabbits at 14 days PT revealed that monotherapy with levamisole at a single dose of 8 mg/kg, or mebendazole at 70 mg/kg for 3 days, reduced *G. pulchrum* burdens by 63.2% and 22.8%, respectively [11]. Compared with those results, no significant dose-dependent effect was observed when rabbits were treated with a higher single dose of levamisole (12 mg/kg) in this study. On the other hand, the efficacy of levamisole administered three times at a dose of 8 mg/kg was similar to that obtained using a single dose of 8 mg/kg. It therefore appears that a multiple dosage of levamisole has an additive effect in the treatment of *G. pulchrum*. Furthermore, the rate of nematode reduction obtained by combining mebendazole and levamisole was higher than that obtained with monotherapeutic treatment with each drug, suggesting that there is a synergistic effect between mebendazole and levamisole in the treatment of *G. pulchrum* infection. Indeed, a similar synergistic effect between these drugs has been reported in the treatment of *Trichuris muris* in mice [7].

While the number of nematodes recovered from the rabbit esophagi in groups 1 and 2 was markedly reduced compared to the number in the control group, some nematodes were found in the buccal mucosa, tongue and pharyngeal mucosa in both of these groups as well as in the control. These results indicate that multiple dosage of levamisole (group 2) may only be moderately effective for treating buccal and lingual gongylonemiasis in primates. On the other hand, except for the esophageal mucosa in group 3 (administered

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**Table 1. Recovery of Gongylonema pulchrum from the upper digestive tract of rabbits after treatment with levamisole and mebendazole**

| Groupa | Treatment | No. of worms recovered | Total | Reduction (%) |
|--------|-----------|------------------------|-------|---------------|
|        |           | Buccal mucosa | Tongue | Pharyngeal mucosa | Esophagus |       |
| 1      | Levamisole 12 mg/kg × 1 | 3 (2, 1, 0) | 2 (0, 0, 2) | 2 (0, 2, 0) | 11 (1, 4, 6) | 18 (3, 7, 8) | 68.4 |
| 2      | Levamisole 8 mg/kg × 3 | 0 (0, 0, 0) | 1 (1, 0, 0) | 1 (0, 0, 1) | 4 (0, 2, 2) | 6 (1, 2, 3) | 89.5 |
| 3      | Mebendazole 70 mg/kg × 3 + Levamisole 8 mg/kg × 1 | 0 (0, 0, 0) | 0 (0, 0, 0) | 0 (0, 0, 0) | 1 (0, 0, 1) | 1 (0, 0, 1) | 98.2 |
| 4      | Distilled water 8 ml × 3 (Control) | 2 (1, 1, 0) | 5 (1, 4, 0) | 4 (0, 3, 1) | 46 (14, 12, 20) | 57 (16, 20, 21) | – |

Groups of rabbits were inoculated with 30 L3 of *G. pulchrum* per animal before treatment at 4–7 months post-inoculation. a) Three rabbits per group.

b) Total number of worms and the number of worms from each animal are shown in parenthesis.
G. pulchrum

amined at 14 days PT. We therefore performed an additional
and no dead nematodes were found in any of the tissues ex-
the efficacy of mebendazole in stimulating migration of
Further studies are therefore necessary in order to clarify
the esophagus where they were then reduced by levamisole.
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Gnathostoma spinigerum

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G. pulchrum

per animal before treatment
fected with 100 L3 of
Rabbits were inoculated with 100 L3 of
G. pulchrum

in rabbits after levamisole treatment. Two rabbits were in-
-ble nematodes is shown in parenthesis.

Table 2. Recovery of Gongylonema pulchrum from the tissue and contents of the digestive tract of levamisole-treated rabbits at 12 and 48 hr after treatment

| Tissue of digestive tract      | No. of worms recovered | 12 hr post-treatment | 48 hr post-treatment |
|--------------------------------|------------------------|----------------------|----------------------|
| Buccal mucosa                  | na                     | 4                    |                      |
| Tongue                         | 1                      | 3                    |                      |
| Pharyngeal mucosa              | 1                      | 2                    |                      |
| Esophageal mucosa              | 5                      | 12                   |                      |
| Walls of stomach and intestine | 0                      | 0                    |                      |
| Contents of digestive tract    |                        |                      |                      |
| Esophagus                      | 3                      | 0                    |                      |
| Stomach                        | 13 (9)                 | 0                    |                      |
| Small intestine                | 2 (2)                  | 0                    |                      |
| Large intestine                | 19 (9)                 | 0                    |                      |
| Total                          | 44                     | 21                   |                      |

Rabbits were inoculated with 100 L3 of G. pulchrum per animal before treatment with levamisole (8 mg/kg) at 7 months post-inoculation. a) Not examined. b) Number of dead nematodes is shown in parenthesis.

mebendazole and levamisole), no nematodes were found in
the upper digestive tract, implying that mebendazole acted
to reduce the number of nematodes in the buccal mucosa,
tongue and pharyngeal mucosa. There is little information
on the efficacy of mebendazole treatment related to the dis-
tribution of nematode parasites within hosts. However, it has
been reported that albendazole, which is structurally similar
to mebendazole, stimulates the outward migration of Gna-
-thostoma spinigerum to the dermis in humans [14]. In the
same way, in this study, mebendazole may have caused the
migration of nematodes from the tongue and buccal cavity to
the esophagus where they were then reduced by levamisole.
Further studies are therefore necessary in order to clarify
the efficacy of mebendazole in stimulating migration of G. pulchrum.

The nematodes recovered from each group were all alive,
and no dead nematodes were found in any of the tissues ex-
amined at 14 days PT. We therefore performed an additional
examination to investigate the elimination of G. pulchrum
in rabbits after levamisole treatment. Two rabbits were in-
fected with 100 L3 of G. pulchrum per animal and treated
with levamisole at a single dose of 8 mg/kg at 7 months
after infection. The animals were sacrificed and examined
for worms at 12 and 48 hr PT as described above, and the
contents of the digestive tract were examined under a dis-
secting microscope.

As shown in Table 2, many nematodes were found in the
contents of the digestive tract, with a few nematodes recov-
ered from the tissues of the upper digestive tract at 12 hr PT.
However, by 48 hr PT, worm recovery was restricted to the
tissues of the upper digestive tract. Dead nematodes were
only found in the contents of the stomach, small intestine
and large intestine at 12 hr PT, indicating that the nematodes
in the gut mucosa migrate into the lumen of the upper di-
gestive tract immediately after levamisole treatment before
being eliminated in the feces within 48 hr PT. According to
Adkesson et al. [1], there is no reliable ante-mortem test for
evaluating drug treatment against gongylonemiasis in callit-
richid primates. Examining the feces for worms within 48 hr
after levamisole treatment is therefore considered useful for
evaluating the efficacy of drugs against G. pulchrum infec-
tion in these primates.

In conclusion, the combined chemotherapy regimen of
mebendazole and levamisole exhibited high efficacy for
treating G. pulchrum, which was located widely within the
upper digestive tract. These findings suggest that this com-
bined chemotherapy treatment may be effective against G. pulchrum infection, including buccal and lingual gongylo-
немiasis in primates. Indeed, it is considered that this drug
regimen is able to contribute to the prevention and control
of gongylonemiasis in captive primates, provided that co-
rophagous beetles and cockroaches are prevented from
entering the primate enclosures.

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