ACTA VETERINARIA INDONESIANA
P-ISSN 2337-3202, E-ISSN 2337-4373

Research

Vol. 13, No. 2: 106–111, Juli 2025

In Vitro Evaluation of Levamisole Efficacy Toward Gastrointestinal Worms of Domestic Chicken in Yogyakarta

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Received: 5 February 2025, Approved: 1 June 2025

ABSTRACT

Levamisole is a broad-spectrum anthelmintic from the imidazothiazole derivative group for mammals and poultry. This study aims to evaluate the anthelmintic effectiveness of levamisole against gastrointestinal worms in domestic chickens in Yogyakarta. The findings of this study could potentially lead to the development of more effective strategies for controlling helminthiasis in poultry, thereby improving the health and productivity of domestic chickens. This study is an *in vitro* laboratory experiment that uses *Ascaridia galli* (AG) and *Raillietina* spp. (RL), in a petri dish with ten treatment groups (n=3). The groups consisted of two negative controls, 0.9% sodium chloride AG and RL (G1, G2), two groups of AG and RL in Carboxymethylcellulose Sodium (G3, G4), and levamisole treatment groups with doses of 0.02, 0.1, and 0.2% for three groups of AG (G5-G7) and three groups of RL (G8-G10), respectively. The worm's mortality time was observed for 24 hours, while descriptive analyses were conducted between treatments with the scoring method. The 0.2% levamisole solution showed anthelmintic efficacy toward AG and RL of a domestic chicken, which in RL has the fastest time to die, 30 minutes of mortality time.

Key Words: *Ascaridia galli*, domestic chicken, levamisole, mortality time, *Raillietina* spp.

ABSTRAK

Levamisol merupakan obat cacing berspektrum luas golongan imidazotiasol untuk mamalia dan unggas. Tujuan dari penelitian ini adalah untuk mengevaluasi tingkat efektivitas obat terhadap cacing saluran pencernaan ayam kampung di wilayah Yogyakarta. Hasil studi diharapkan mampu mengevaluasi efektivitas obat sehingga dapat mengembangkan strategi pengobatan cacing yang tepat, serta meningkatkan kesehatan dan produktivitas ayam kampung menjadi lebih baik. Penelitian ini merupakan kajian in vitro laboratorium menggunakan cacing hidup Ascaridia galli (AG) dan Raillietina spp. (RL) dalam cawan petri dengan 10 perlakuan (n=3). Dua kelompok kontrol negatif berisi AG (G1) dan RL (G2) yang direndam dalam larutan natrium klorida 0,9%. Dua kelompok AG dan RL dalam larutan Natrium Karboksimetil selulosa (G3, G4), tiga kelompok AG dalam larutan levamisole 0,02; 0,1; dan 0,2% (G5, G6, G7) dan tiga kelompok RL dalam larutan Levamisol 0,02; 0,1; dan 0,2% (G8, G9, G10). Waktu kematian cacing diamati selama 24 jam, dan dilakukan analisis deskriptif terhadap hasil pengamatan dengan metode skoring. Hasil penelitian menunjukkan levamisole konsentrasi 0,2% memberikan efektivitas terbaik terhadap AG dan RL. Kematian tercepat dialami oleh RL dengan waktu kematian 30 menit.

Kata kunci: Ascaridia galli, ayam kampung, levamisol, Raillietina spp., waktu kematian

INTRODUCTION

Helminthiasis is a disease caused by infection with endoparasites, such as nematodes, cestodes, and trematodes. Free-range chickens are more vulnerable than purebred chickens because they have not been affected by genetic engineering to grow meat or eggs. Based on research by Moenek & Oematan (2017), the frequency of the presence of endoparasites in free-range chickens in the Kupang region was found to be *Ascaridia galli* (88.58%) and *Raillietina* spp (5.71%). As reported by Siddiqui et al. (2023), *Raillietina* spp. found to be 72% infected the indigenous chickens in Bangladesh and caused a reduction of eggs in windowless poultry houses in Japan (Oshima et al., 2024). In Australia, helminth infection in cage-free laying chickens survey found worm prevalence, a high proportion of respondents reported the detection of *Ascaridia galli* (77%), followed by tapeworms (69%) (Shifaw et al., 2023). Sharma et al. (2019) and Dao et al. (2019) reported that *Ascaridia galli* infection in chicken depressed the immune system function, which can increase susceptibility to secondary infections. The cause of this high prevalence rate in free-range chickens is probably due to the rearing system, unclean feed problems, and environmental factors.

The loss of domestic chicken production because of helminths needs to be solved by finding a new strategy for using effective anthelminthic for gastrointestinal worms. Levamisole is widely used to paralyze gastrointestinal nematode worms by penetrating the worm cuticle layer (Balqis et al., 2016). A pharmacokinetic and therapeutic study of levamisole for *Ascaridia galli* infection in ducks resulted in no significant dose for infected or healthy with a single dose of treatment (Tabari et al.,2022). Research conducted by Ritu et al. (2024) reported the effectiveness of levamisole on *Ascaridia galli* infection in endogenous chicken in Bangladesh. The immunostimulant and growth-promoting effect of levamisole was studied (El-Sawi et al. (2022; Valpotic et al., 2021) by making levamisole as a supplement through feed or water.

The effectiveness of levamisole in cestode class, especially *Raillietina* spp., still needs to be studied, especially in domestic chicken in Indonesia. As described earlier, these two species of worms are often found in domestic and free-range chicken in African, Asian, and Australian regions. The study aims to know the efficacy of levamisole on *Ascaridia galli* and *Raillietina spp*. obtained from domestic chickens' gastrointestinal tract, especially in the Yogyakarta, Indonesia. The mortality time of worms will be counted for 24 hours after contact with the anthelmintic solutions in laboratory conditions and then analyzed to find the anthelmintic effectiveness.

MATERIALS AND METHODS

Study Period and Location

The research was conducted in the Yogyakarta area from February to May 2024. The in vitro experiment was conducted at the Pharmacology Laboratory of the Faculty of Veterinary Medicine, Universitas Gadjah Mada. The research did not use live animals, so no ethical clearance was required. The live worm samples were taken from the intestinal waste from chicken slaughterhouses.

Material and Equipment

The materials and equipment used in the research were Levamisole LMS 200® (PT. SANBE FARMA, Bandung), the intestinal tract of domestic chicken from Pasar Terban Yogyakarta, carboxymethylcellulose sodium (CMC-Sodium), 0.9 % of sodium chloride (Merck, Germany), stirrer, volume flask, petri dish, object glass, deck glass, blade, camera Optilab® (Advance Lite), and microscope AmScope B120C 40X-2500X to worm identification.

The Sampling and Worm Identification

The intestinal samples were taken from ten infected chickens slaughtered at Pasar Terban, Yogyakarta City, which had one or more of the following clinical signs, such as weakness, diarrhea, thin, dull fur, and lack of appetite. The intestinal samples were taken to the pharmacology laboratory, the Faculty of Veterinary Medicine, Universitas Gadjah Mada, in a clean and solid container. The intestines were opened to find the *Ascaridia galli* (AG) and *Raillietina* spp. (RL). Microscopic identification was conducted to confirm the morphology. The AG was identified as circular-gill-shaped, elongated, and white-cream; meanwhile, RL was segmented, various in length, with or without scolex, and pale white color. The chosen samples were motile segments or live worms that had been washed and soaked in 0.9% sodium chloride.

The Treatments

Before conducting in vitro treatments, the levamisole was dissolved in 0.2 % CMC-sodium as a standard solution and then diluted into lower concentrations of treatments (0.1 % and 0.02 %). Meanwhile, we used the 0.9 % sodium chloride as the normal physiologic solution. A total of 30 worms were divided into ten groups containing three worms each

(n=3), with two replications of treatments. The order of the treatment follows Table 1 and is provided in Figure 1. The observations of the worm mortality have been made for 24 hours with a one-hour interval at the room temperature.

Data Analysis

The mortality time is analyzed descriptively with Microsoft Excel 2007. The effectiveness of the anthelmintic solution will be seen through its ability to cause the death of worms in a shorter time. The rate at which the worm moves until it dies is calculated using a scoring method (Table 2).

RESULTS

Mortality time

The results of the worm mortality time after 24 observations are described in Table 3. Based on the results, RL is more sensitive to levamisole than AG. It took at least double the time of AG to die in the most concentrated solution (G7 compared to G10). The higher the levamisole concentration (G5-G7 for AG; G8-G10 for RL), the faster the mortality time seems.

Morphological change

Figure 2 shows the rupture proglottid of RL after soaking in 0.2% levamisole solution under scanning of a microscope. The body content was floating out of the proglottid. The abnormal form of the proglottid is caused by the leakage of the tegument.

Worm scoring for moving

As described in Table 3, AG has longer survival in CMC-sodium solution than RL (G3, G4). However, they have the same score in sodium chloride (G1, G2), which means they have the same survival. The efficacy of 0.1 and 0.2% levamisole solutions toward AG have the same movement score; meanwhile, in RL, the 0.2% levamisole solution has better efficacy, which causes zero movement (died) more quickly. The 0.2% levamisole solution has an anthelmintic effect, which can kill AG and RL *in vitro* in less than 60 and 30 minutes, respectively.

Table 1 The scores and description of the worm's movement until dead

Score	description
0	worms do not move anymore, even if it is disturbed by a pin (dead)
1	Worms do not move but move when touched with a pin
2	Only part of the worm's body moves
3	The worm's entire body is still actively moving

Figure 1 Treating levamisole solutions against Ascaridia galli (G5, G6, G7) and Raillietina spp. (G8, G9, G10). The worms were also soaked in solutions of 0,9% sodium chloride (G1, G2) and 0.2% CMC-sodium (G3, G4) as negative controls. The observations were taken for 24 hours at the laboratory room

Table 2 The results of worms' mortality time after soaking in control (G1-G4) and levamisole (G5-G10) solutions for 24 hours.

Group of treatment	Mortality time (hour, minute)
G1	24 hs
G2	24 hs
G3	3 hs
G4	1 h
G5	1 h 30 min
G6	1 h 15 min
G7	1 h 15 min
G8	45 min
G9	45 min
G10	30 min

Table 3. The result of worm movement scoring in treatment groups

Time of observation (hour, minute)	G1, G2		G3, G4		G5	G6	G7	G8	G9	G10
	AG	RL	AG	RL	AG				RL	
0	3	3	3	3	3	3	3	3	3	3
15 min	3	3	3	3	3	3	3	3	3	3
30 min	3	3	3	3	2	2	2	1	1	0
45 min	3	3	3	3	2	2	2	0	0	0
1 h	3	3	3	0	2	2	2	0	0	0
1 h, 15 min	3	3	1	0	2	0	0	0	0	0
1 h, 30 min	3	3	1	0	0	0	0	0	0	0
1 h, 45 min	3	3	1	0	0	0	0	0	0	0
2 hs	3	3	1	0	0	0	0	0	0	0
3 hs	3	3	0	0	0	0	0	0	0	0
6 hs	3	3	0	0	0	0	0	0	0	0
12 hs	3	3	0	0	0	0	0	0	0	0
24 hs	0	0	0	0	0	0	0	0	0	0

DISCUSION

A study of levamisole by *ex vivo* incubation using the Reversed Phase-High Performance Liquid Chromatography method by Ayaz *et al*. (2018) found that the drug exposure time to cestodes only takes 5 seconds to 30 minutes. A similar result showed in this in vitro that RL (G10) had 30 minutes of mortality time.

The levamisole molecule from the imidazothiazole group has a high affinity for lipids and proteins in the bodies of cestodes and nematodes (Solomon & Hayes, 2017). The mechanism of action of levamisole on RL, which infects chickens, has not been explained much. However, it is generally explained that drug absorption occurs by trans-tegumental diffusion through the cestode proglottid parenchyma either partially or completely. The drug absorption mechanism through the cestode's outer structure is based on the level of lipophilicity so that the drug will reach therapeutic concentrations at the target parasite receptors (Ayaz et al., 2018). Anthelmintic activity in nematodes is done by penetrating the worm cuticle. The antagonistic action of levamisole against acetylcholine is by constant depolarization of cells, such as muscle cells, resulting in spastic nematode paralysis. Levamisole will work directly on the cholinergic effects of the acetylcholine receptor (nicotinic acetylcholine receptor, nAChR), a transmembrane protein with five subunits (Balqis et al., 2016). According to Bilandz et al. (2018), the overuse of anthelmintics, including levamisole in chicken, led to residue problems in products, and Collins et al. (2015) mention the emergence of resistance of anthelmintic benzimidazole class in turkeys. Table 2 shows that the longer mortality time of AG than RL in levamisole solutions needs to be further investigated concerning the incidence of resistance.

Figure 2 shows the rupture proglottid of RL after soaking in 0.2% levamisole solution under scanning of

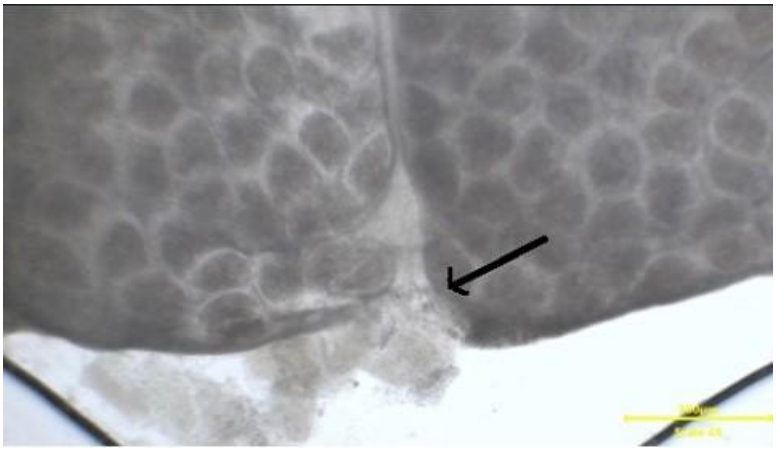

Figure 2 The rupture proglottid of RL in the 0.2% levamisole solution (arrow).

a microscope. Many studies reported levamisole as a deworming agent in poultry for nematodes (Feyera et al., 2021; Soudkolaei et al., 2021), but it is rarely reported in cestodes. Cestode infection in farm animals does not cause much economic loss (Dever et al., 2015), however, heavy worm infestation will certainly affect the animal's growth. Studies of active compounds from plants, herbal, and feed supplements have continuously reported the potential anthelmintic effect for cestodes, including *Raillietina* species (El-Bahy & Bazh, 2015; Lathanpuii & Lalchhandama, 2019; Yazwinsky et al., 2020).

An interesting result from this study is the worm's time mortality in the CMC-sodium negative control group, which was significantly different from the sodium chloride control group. In the previous preliminary study, CMC-sodium was a better solvent for levamisole than water or 0.9 of sodium chloride. The CMC-sodium solution functions as a suspension agent, slowing down settling and increasing the viscosity of the preparation (Eryani et al., 2022). Increased viscosity can disrupt the osmotic balance of the worms and cause damage to the worm structure. As described in Table 3, AG has longer survival in CMC-sodium solution than RL (G3, G4). However, they have the same score in sodium chloride (G1, G2), which means they have the same survival. Further investigation is needed to find the anthelmintic

Eflect of CMC-Sodium, Both In Vitro and In Vivo Studies.

Deworming strategies for domestic or free-range chicken are important to maintain the egg product and carcass quality. A decrease in the level of anthelmintic efficacy can occur due to the wrong choice of drug type or because resistance to certain drugs has occurred. A study conducted by Feyera et al. (2021) showed that the choice of anthelmintic is related to the efficacy of the deworming program in chickens. The study revealed that levamisole, fenbendazole, and flubendazole are still effective in nematodes (*A. galli*., *H. gallinarum*, and *Capillaria* spp.) and some cestodes. The lower effectiveness of piperazine at a certain stage of the nematode was caused by the drug's lack of efficacy instead of a resistance mechanism.

The efficacy of 0.1 and 0.2% levamisole solutions toward AG have the same movement score; meanwhile, in RL, the 0.2% levamisole solution has better efficacy, which causes zero movement (died) more quickly. The 0.2% levamisole solution has an anthelmintic effect, which can kill AG and RL *in vitro* in less than 60 and 30 minutes, respectively.

Based on the result, it was concluded that the levamisole solution showed anthelmintic efficacy toward *Ascaridia galli* and *Raillietina* spp. of domestic chicken. The activity of levamisole is more potent in *Raillietina* spp., which has the fastest of 30 minutes of mortality time.

ACKNOWLEDGEMENT

The authors thank the Faculty of Veterinary Medicine, Universitas Gadjah Mada, for the Competitive Research Funding year 2023 so that this research can be carried out.

"The author declares that there is no conflict of interest with the parties involved in this research."

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