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Albendazole, levamisole and ivermectin are effective against monogeneans of *Colossoma macropomum* (Pisces: Serrasalmidae)

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Abstract

This study evaluated the efficacy of albendazole, ivermectin, levamisole, mebendazole and praziquantel on monogeneans of *Colossoma macropomum*, based on in vitro and in vivo assays. In vitro assays indicated that albendazole (500, 100, 1,500 and 2,000 mg/L), ivermectin (200, 250, 300 and 350 mg/L) and levamisole (50, 75, 100 and 125 mg/L) were 100% effective against *Anacanthorus spatulatus*, *Notozothecium janauachensis*, *Mymarothecium boegeri* and *Linguadactyloides brinkmanni*, while mebendazole (125, 150, 175 and 200 mg/L) and praziquantel (5, 10, 15 and 20 mg/L) were ineffective. Fish mortality in 24 hr therapeutic baths with 500 mg/L of albendazole was 6.6%, but the behaviour of the animals remained unchanged, while 200 mg/L of ivermectin caused lethargy, signs of hypoxia and 100% mortality within 2 hr, and 125 mg/L of levamisole caused no mortality. The efficacy of 500 mg/L of albendazole was 48.6% in the 24 hr baths, while that of 125 mg/L levamisole was 88.2%. Although ivermectin showed in vitro efficacy, the lowest concentration used in baths was highly toxic to fish. Therefore, we recommend the use of 125 mg/L of levamisole to control and treat monogenean infestations on *C. macropomum* in fish farming.

KEYWORDS
anthelmintic, fish, infection, parasites, treatment

1 | INTRODUCTION

Human societies face the enormous challenge of having to provide food and livelihoods to a global population that is estimated to exceed 9 billion people by the mid-21st century. In 2016, the global commercial production of fish, 88% of it destined for human consumption, reached an all-time high of 171 million tons (USD 362 billion) thanks to the relatively stable volume of fish captured by commercial fishing, reduced wastage and the steady growth of fish farming, which contributed 80 million tons of fish (USD 231.6 billion). This production resulted in a record high annual per capita consumption of 20.3 kg in 2016. The production of farmed fish in 2016 was of 54.1 million tons (FAO, 2018). However, all types of fish production are threatened by diseases caused by parasites such as monogeneans, resulting in significant economic losses (Tavares-Dias & Martins, 2017). Monogeneans are parasites with a direct and short life cycle, whose vertical transmission facilitates infection levels in intensive fish farming (Morales-Serna et al., 2018). Therefore, increasing interest has focused not only on understanding these diseases in fish but also on treating them correctly, using commercially available, low-cost and effective anthelmintic drugs.

In intensive fish farming, managing and controlling helminth infections pose a constant challenge, since these tasks are greatly complicated by the limited availability of licensed anthelmintic drugs (Morales-Serna et al., 2018; Zuskova et al., 2018) with varying degrees of effectiveness. Anthelmintic drugs

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such as albendazole, mebendazole, ivermectin, levamisole and praziquantel have been employed for the control and treatment against monogenean parasites that infest farmed fish (Hirazawa, Akiyama, & Umeda, 2013; Hirazawa, Ohtaka, & Hata, 2000; Kim & Choi, 1998; Martins, Onaka, Moraes, & Fujimoto, 2001; Morales-Serna et al., 2018; Onaka, Martins, & Moraes, 2003; Santamarina, Tojo, Ubeira, Quinteiro, & Sanmartin, 1991; Sitjà-Bobadilla, Felipe, & Alvarez-Pellitero, 2006; Zhang et al., 2014). Parasite infections can be controlled and treated by eliminating developmental stages in hosts, thus interrupting the life cycle of parasites (Bader, Chelladurai, Starling, Jones, & Brewer, 2017). However, the concentrations of these different drugs must be not only safe and effective but also non-toxic to fish. In addition, all forms of parasite control require in-depth knowledge about the environment and water quality parameters in fish farming.

Various studies have demonstrated ivermectin toxicity in fish (Mladineo, Marsic-Iucic, & Buzancic, 2006; Santamarina et al., 1991; Thiripurasundari, Sathya, Uma, Srinivasan, & Rajasekar, 2014; Varó et al., 2010), whereas clinical concentrations of albendazole, mebendazole, levamisole and praziquantel present a low toxicity (Hirazawa et al., 2013; Kim & Choi, 1998; Martins et al., 2001; Onaka et al., 2003). However, these anthelminthics have not been assayed to ascertain their effectiveness in controlling monogeneans of *Colossoma macropomum* (tambaqui), a fish of great economic importance to aquaculture from the Amazon. Thus, the purpose of this study was to investigate the in vitro and in vivo efficacy of albendazole, ivermectin, levamisole, mebendazole and praziquantel against monogeneans of *C. macropomum*.

2 | MATERIALS AND METHODS

2.1 | Fish and monogenean parasites

Two hundred *C. macropomum* fingerlings (±30 g) from a commercial fish farming in the municipality of Macapá, state of Amapá, were taken to Embrapa's fish Aquaculture and Fishery Laboratory in Macapá (Brazil). In the laboratory, the fish were acclimatized for 7 days in 500 L tanks kept at a constant water pressure and were fed twice daily with fish feed containing 32% crude protein. The mean temperature in the tanks was 30.6 ± 0.1°C, dissolved oxygen content was 5.6 ± 0.2 mg/L, pH was 5.3 ± 0.2, total ammonia was 0.5 ± 0.2 mg/L, alkalinity was 10.0 ± 0 mg/L, and hardness was 10.0 ± 0 mg/L. These water parameters were monitored using a multiparameter probe (Horiba Mod. U52, Japan).

These fish were used for all in vitro and in vivo assays. The monogeneans were obtained from naturally infested fish.

2.2 | Anthelmintic drugs

A solution of Albendathor 10® (Fabiani, Brazil) was used at a concentration of 10% albendazole. Ivomec Gold® solution at a concentration of 3.15% ivermectin was obtained from Merial, Brazil. Ripercol 150 F solution at a concentration of 18.8% levamisole was purchased from Zoetis, Brazil. Mebendasil® powder (100%) containing 5 g of mebendazole was supplied by Vansil, Brazil, and praziquantel powder (100%) was purchased from Shanxi Qianxiu Pharmaceutical Co. Ltd, China.

2.3 | In vitro assays of anthelminthics against monogeneans of *Colossoma macropomum*

Fifty *C. macropomum* fingerlings (12.9 ± 1.0 cm and 32.6 ± 9.3 g) were euthanized by medullary section and their parasitized gills removed in order to determine what duration of exposure to the four different concentrations of albendazole, ivermectin, levamisole, mebendazole and praziquantel would kill the monogeneans attached to the gills (Table 1). The various concentrations of albendazole, levamisole and ivermectin, diluted in water from the fish breeding tank to a volume of 5 m/L, were placed in Petri dishes (5.5 cm). Concentrations of mebendazole and praziquantel were diluted in 1.0 ml of dimethyl sulfoxide (DMSO), and then, water from the breeding tank was added to make up 5 ml in Petri dishes. Two control groups were prepared, one using 1.0 m/L of DMSO as solvent in water from the fish breeding tank and the other using only water from the breeding tanks.

Each branchial arch of *C. macropomum* naturally parasitized by monogeneans was placed separately in a Petri dish and immersed in the different concentrations of albendazole, ivermectin, levamisole, mebendazole and praziquantel (Table 1). Under a stereomicroscope, fields of view containing ±20 monogeneans were selected in each repetition, and after submerging the branchial arches in the different concentrations of anthelminthics, the parasites were observed under the microscope at 5-min intervals to count the number of live and dead monogeneans. The parasites were considered dead when they were detached from the gill tissue or when they were attached to the gill tissue but had completely lost their mobility (Soares et al., 2017). The efficacy of each treatment was estimated as proposed by Zhang et al. (2014). We recorded the time it took to kill 100% of the monogeneans and hypothesized that a treatment was effective if 100% parasite mortality was achieved within 2 hr.

Based on the in vitro results, the best concentrations were used in therapeutic baths against monogeneans of *C. macropomum*.

TABLE 1 Concentrations of the anthelmintic drugs used in the in vitro assays against monogeneans of *Colossoma macropomum*

Anthelmintic drugs	Concentrations (mg/L)			
Albendazole	500	1,000	1,500	2,000
Levamisole	50	75	100	125
Mebendazole	125	150	175	200
Ivermectin	200	250	300	350
Praziquantel	5	10	15	20

2.4 | In vivo assays of anthelminthics against monogeneans of *Colossoma macropomum*

One hundred and twenty *C. macropomum* fingerlings (length: 15.5 ± 1.1 cm, weight: 55.9 ± 12.0 g) naturally parasitized by monogeneans were randomly distributed in twelve 100 L^{-1} tanks, which were kept in a static water system under constant aeration for 24 hr. The mean temperature in the tanks was 30.4 ± 0.1°C, dissolved oxygen content was 5.5 ± 0.2 mg/L, pH was 5.3 ± 0.2, total ammonia was 0.5 ± 0.2 mg/L, alkalinity was 10.0 ± 0 mg/L, and hardness was 10.0 ± 0 mg/L.

The therapeutic baths of 24 hr consisted of four treatments (0 and 125 mg/L levamisole, 0 and 200 mg/L ivermectin and 0 and 500 mg/L albendazole) with three repetitions each, and 10 fish in each repetition, making a total of 30 fish per treatment. All treatments were performed in parallel with the control group. Since none of the in vitro concentrations of levamisole, ivermectin and albendazole proved to be dose-dependent, the lowest concentrations were chosen to avoid toxicity in fish. During the bath of 24 hr, the behaviour of the fish was observed and they were not fed.

After the therapeutic baths with levamisole, ivermectin and albendazole, the fish were euthanized by medullary section and their gills were excised, fixed in 5% formalin and examined under a stereomicroscope to identify and quantify the monogenean parasites. The parasites were prepared for identification as recommended by Eiras, Takemoto, and Pavanelli (2006). After quantification of the parasites, the prevalence and mean abundance and mean intensity of infestation were calculated as described by Bush, Lafferty, Lotz, and Shostak (1997) and the efficacy of each treatment as described by Sommerville et al. (2016).

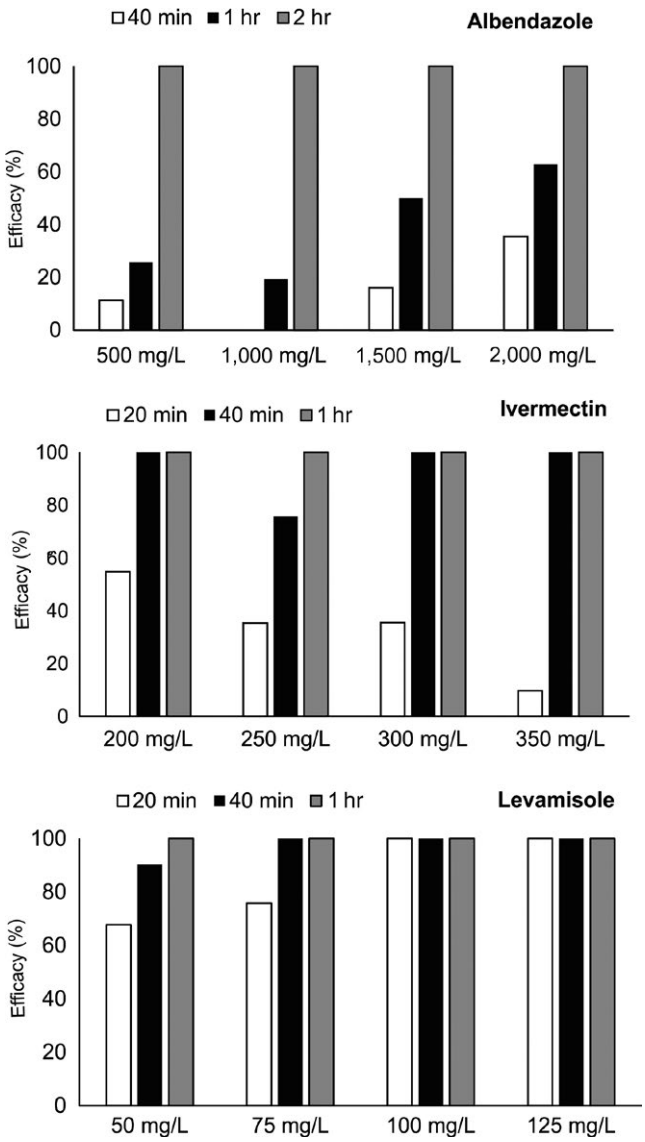

FIGURE 1 In vitro efficacy of anthelmintic drugs against monogeneans of *Colossoma macropomum* in different exposure times

2.5 | Statistical analyses

The abundance data of the bath treatments were evaluated based on the Shapiro–Wilk normality test and Bartlett's test of homoscedasticity. Because the intensity and abundance data were not normally distributed, they were analysed by the Kruskal–Wallis test, followed by Dunn's test for comparison among medians. The efficacy of albendazole and levamisole was compared using the *t* test (Zar, 2010).

3 | RESULTS

In vitro assays, all the albendazole concentrations caused immobilization 100% of the monogeneans of *C. macropomum* (*Anacanthorus*

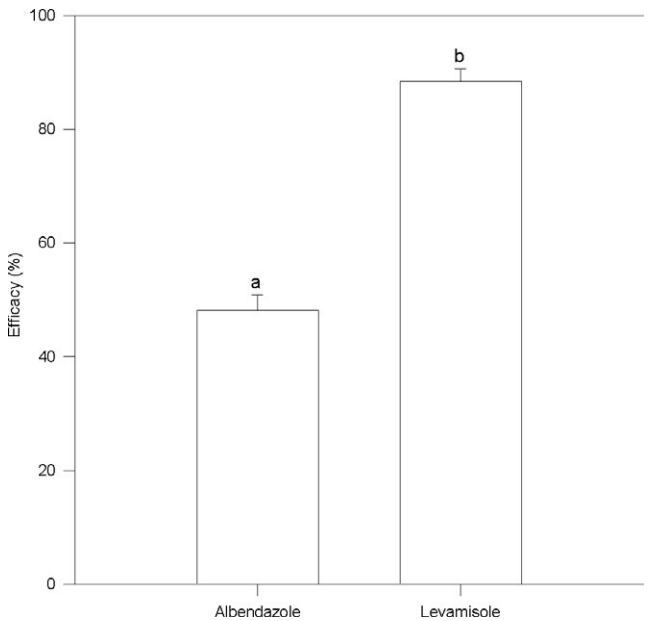

FIGURE 2 Therapeutic baths efficacy with 500 mg/L of albendazole and 125 mg/L levamisole in monogeneans of *Colossoma macropomum* after 24 hr of treatment. Mean values followed by different letters indicate differences between treatments according to *t* test ($p < 0.05$)

TABLE 2 In vitro efficacy of the concentrations of anthelminthics against monogeneans of *Colossoma macropomum* in different exposure times

Exposure time	Treatments	Live parasites	Mortality (%)
0 hr	Water of tank	20.7 ± 0.6	0
1 hr	Water of tank	20.7 ± 0.6	0
3 hr	Water of tank	20.7 ± 0.6	0
6 hr	Water of tank	0	100
0 hr	DMSO	20.0 ± 0	0
20 min	DMSO	9.0 ± 1.7	55.0
40 min	DMSO	4.0 ± 4.0	80.0
1 hr	DMSO	0	100
0 hr	500 mg/L of albendazole	26.0 ± 1.7	0
20 min	500 mg/L of albendazole	19.3 ± 1.1	5
40 min	500 mg/L of albendazole	18.3 ± 1.5	9.8
1 hr	500 mg/L of albendazole	15.3 ± 4.7	24.6
2 hr	500 mg/L of albendazole	0	100
0 hr	1,000 mg/L of albendazole	22.0 ± 1.0	0
20 min	1,000 mg/L of albendazole	22.0 ± 1.0	0
40 min	1,000 mg/L of albendazole	20.7 ± 1.1	6.1
1 hr	1,000 mg/L of albendazole	16.7 ± 3.0	24.2
2 hr	1,000 mg/L of albendazole	0	100
0 hr	1,500 mg/L of albendazole	20.0 ± 0	0
20 min	1,500 mg/L of albendazole	20.0 ± 0	0
40 min	1,500 mg/L of albendazole	17.3 ± 2.3	13.3
1 hr	1,500 mg/L of albendazole	10.3 ± 8.5	48.3
2 hr	1,500 mg/L of albendazole	0	100
0 hr	2,000 mg/L of albendazole	26.0 ± 1.7	0
20 min	2,000 mg/L of albendazole	19.0 ± 5.3	27.0
40 min	2,000 mg/L of albendazole	13.3 ± 8.5	48.7
1 hr	2,000 mg/L of albendazole	7.7 ± 7.1	70.5
2 hr	2,000 mg/L of albendazole	0	100
0 hr	200 mg/L of ivermectin	21.6 ± 1.5	0
20 min	200 mg/L of ivermectin	9.3 ± 4.0	57.0
40 min	200 mg/L of ivermectin	0	100
0 hr	250 mg/L of ivermectin	20.3 ± 0.6	0
20 min	250 mg/L of ivermectin	13.3 ± 4.9	34.4
40 min	250 mg/L of ivermectin	5.0 ± 5.0	75.4
1 hr	250 mg/L of ivermectin	0	100
0 hr	300 mg/L of ivermectin	21.6 ± 1.5	0
20 min	300 mg/L of ivermectin	11.6 ± 2.9	46.1
40 min	300 mg/L of ivermectin	0	100
0 hr	350 mg/L of ivermectin	23.7 ± 1.1	0
20 min	350 mg/L of ivermectin	18.6 ± 3.2	46.1
40 min	350 mg/L of ivermectin	0	100
0 hr	50 mg/L of levamisole	22.0 ± 2.0	0
20 min	50 mg/L of levamisole	6.7 ± 5.8	69.7
40 min	50 mg/L of levamisole	2.0 ± 2.0	90.9

(Continues)

TABLE 2 (Continued)

Exposure time	Treatments	Live parasites	Mortality (%)
1 hr	50 mg/L of levamisole	0	100
0 hr	75 mg/L of levamisole	19.3 ± 1.1	0
20 min	75 mg/L of levamisole	5.0 ± 5.0	74.1
40 min	75 mg/L of levamisole	0	100
0 hr	100 mg/L of levamisole	19.3 ± 1.1	0
20 min	100 mg/L of levamisole	0	100
0 hr	125 mg/L of levamisole	21.0 ± 1.0	0
20 min	125 mg/L of levamisole	0	100
0 hr	125 mg/L of mebendazole	22.3 ± 2.5	0
20 min	125 mg/L of mebendazole	22.3 ± 2.5	0
40 min	125 mg/L of mebendazole	9.7 ± 12.4	56.7
1 hr	125 mg/L of mebendazole	6.3 ± 11.0	71.6
2 hr	125 mg/L of mebendazole	0	100
0 hr	150 mg/L of mebendazole	20.0 ± 0	0
20 min	150 mg/L of mebendazole	19.3 ± 1.1	3.3
40 min	150 mg/L of mebendazole	10.3 ± 4.9	48.3
1 hr	150 mg/L of mebendazole	1.6 ± 2.9	91.6
2 hr	150 mg/L of mebendazole	0	100
0 hr	175 mg/L of mebendazole	21.0 ± 1.7	0
20 min	175 mg/L of mebendazole	7.0 ± 1.7	66.6
40 min	175 mg/L of mebendazole	2.6 ± 4.6	87.3
1 hr	175 mg/L of mebendazole	0	100
0 hr	200 mg/L of mebendazole	20.6 ± 1.1	0
20 min	200 mg/L of mebendazole	9.3 ± 1.1	54.8
40 min	200 mg/L of mebendazole	3.3 ± 3.0	83.9
1 hr	200 mg/L of mebendazole	0	100
0 hr	5 mg/L of praziquantel	23.0 ± 2.6	0
20 min	5 mg/L of praziquantel	23.0 ± 2.6	0
40 min	5 mg/L of praziquantel	23.0 ± 2.6	0
1 hr	5 mg/L of praziquantel	23.0 ± 2.6	0
2 hr	5 mg/L of praziquantel	23.0 ± 2.6	0
3 hr	5 mg/L of praziquantel	0	100
0 hr	10 mg/L of praziquantel	20.3 ± 0.6	0
20 min	10 mg/L of praziquantel	19.6 ± 1.5	3.3
40 min	10 mg/L of praziquantel	15.0 ± 2.0	26.2
1 hr	10 mg/L of praziquantel	8.0 ± 3.5	60.6
2 hr	10 mg/L of praziquantel	0	100
0 hr	15 mg/L of praziquantel	20.0 ± 0.0	0
20 min	15 mg/L of praziquantel	19.6 ± 0.6	1.7
40 min	15 mg/L of praziquantel	14.0 ± 3.6	30.0
1 hr	15 mg/L of praziquantel	9.3 ± 2.1	53.3
2 hr	15 mg/L of praziquantel	0	100
0 hr	20 mg/L of praziquantel	21.3 ± 1.5	0
20 min	20 mg/L of praziquantel	19.6 ± 2.9	7.8
40 min	20 mg/L of praziquantel	14.7 ± 2.5	31.2
1 hr	20 mg/L of praziquantel	0	100

spatulatus, *Notozothecium janauachensis*, *Mymarothecium boegeri* and *Linguadactyloides brinkmanni*) after 2 hr of exposure, while concentrations of 200, 300 and 350 mg/L of ivermectin caused immobilization of the parasites after 40 min of exposure. Levamisole concentrations of 100 and 125 mg/L caused total immobilization of the parasites in just 20 min of exposure. Mebendazole concentrations of 175 and 200 mg/L and all the praziquantel concentrations also caused immobilization 100% of the parasites. Moreover, the parasites in the control groups exposed to DMSO also suffered immobilization 100%. In the controls treated with water from the breeding tank, the monogeneans showed immobilization 100% only after 6 hr of exposure (Table 2). Thus, only albendazole, ivermectin and levamisole showed in vitro efficacy against monogeneans of *C. macropomum* (Figure 1), since mebendazole and praziquantel efficacy were influenced by the solvent DMSO.

Anacanthorus spatulatus, *N. janauachensis*, *M. boegeri* and *L. brinkmanni* were also identified on gills of *C. macropomum* used in the therapeutic baths. A high prevalence of monogeneans was observed in the fish of therapeutic baths with levamisole, albendazole and controls. The prevalence of *A. spatulatus* in baths with 500 mg/L of albendazole was similar to that of the controls in water from the breeding tank (Table 3). The therapeutic baths containing 500 mg/L of albendazole showed 48.6% antiparasitic efficacy, while those containing 125 mg/L of levamisole showed 88.2% efficacy (Figure 2). Moreover, the intensity of monogeneans in control treatments was higher ($H = 60.5$, $p = 0.0001$) than in treatment with 500 mg/L of albendazole and 125 mg/L of levamisole.

Colossoma macropomum exhibited lethargy and signs of hypoxia after 1 hr of therapeutic baths with 200 mg/L of ivermectin, culminating in 100% fish mortality within 2 hr of exposure. However, therapeutic baths containing 500 mg/L of albendazole caused a mortality of 6.6% within 24 hr, but did not change the behaviour of exposed fish, while 125 mg/L of levamisole caused no fish mortality or behavioural changes.

4 | DISCUSSION

All the concentrations of levamisole (50–125 mg/L), albendazole (500–2,000 mg/L) and ivermectin (200–350 mg/L) in the in vitro assays were 100% effective against *A. spatulatus*, *N. janauachensis*, *M. boegeri* and *L. brinkmanni* of *C. macropomum*. Similarly, 100 mg/L of levamisole and 0.031 mg/L of ivermectin were also 100% effective in vitro against *Gyrodactylus* sp. of *Oncorhynchus mykiss* (Santamarina et al., 1991). Hirazawa et al. (2000) also reported 100% efficacy of 20 mg/L of levamisole against *Heterobothrium okamotoi* of *Takifugu rubripes*. However, albendazole had been not tested in vitro against monogeneans, at the present moment. Exposure to levamisole induces changes in the haptor of monogeneans (Taraschewski, Renner, & Mehlhorn, 1988), causing these parasites to detach from the gills of fish. The anthelmintic effect of albendazole is that it inhibits microtubule polymerization by binding to β-tubulin, impairing the supply of cellular nutrients to helminths (Martins et al., 2017). The mode of action of avermectins such as ivermectin is paralysis of helminths (Collymore et al., 2014).

Managing and controlling monogenean infestations are a constant challenge in fish farming, given the limited availability of effective anthelmintic drugs (Morales-Serna et al., 2018). We attribute the in vitro efficacy of mebendazole (125–200 mg/L) and praziquantel (5–20 mg/L) against monogeneans of *C. macropomum* to the solvent DMSO, given the low water solubility, and hence, low availability of both drugs (Liu et al., 2018; Swanepoel, Liebenberg, Devarakonda, & Villiers, 2003). Moreover, has been reported in vitro efficacy of DMSO against the monogeneans *Cichlidogyrus tilapiae*, *Cichlidogyrus thurstonae*, *Cichlidogyrus halli* and *Scutogyrus longicornis* of *Oreochromis niloticus* (Hashimoto et al., 2016). Morales-Serna et al. (2018) also suggest effects of alcohol used as solvent for praziquantel (2.5–20.0 mg/L) against monogeneans *Neobenedenia melleni*. In vitro efficacy of 0.005 mg/L of mebendazole and 2.5 mg/L of praziquantel against *Dactylogyrus vastator* of *Carassius auratus* has also been reported (Zhang et al., 2014). Hirazawa et al. (2013) observed 80%–100% in vitro efficacy of 0.5 mg/L of praziquantel against *Benedenia seriolae* and *N. melleni* of *Seriola quinqueradiata* and *Seriola dumerili*. Given the in vitro efficacy of 2.5–20.0 mg/L praziquantel against *Tagia ecuadori* and *N. melleni*, thus was suggested that a low concentration of this drug can be used in long-term therapeutic baths (Morales-Serna et al., 2018). Although praziquantel paralyses monogeneans, it does not always kill them (Hirazawa et al., 2013; Morales-Serna et al., 2018). In view of the growing interest in controlling and treating monogenean infestations using

TABLE 3 Prevalence (*P*) and mean abundance (MA) of monogeneans on gills of *Colossoma macropomum* exposed to anthelmintic drugs

Treatments	Controls ($n = 30$)		125 mg/L of levamisole ($n = 30$		500 mg/L of albendazole ($n = 30$)	
Parasite species	*P* (%)	MA ± *SD*	*P* (%)	MA ± *SD*	P (%)	MA ± *SD*
Anacanthorus spatulatus	100	47.3 ± 26.4[a]	96.7	7.0 ± 6.3[b]	100	39.6 ± 30.6[a]
Mymarothecium boegeri	96.7	14.5 ± 13.2[a]	16.7	0.8 ± 2.5[b]	33.3	2.5 ± 5.1[b]
Notozothecium janauachensis	100	46.9 ± 55.5[a]	73.3	5.1 ± 6.6[b]	90.0	14.2 ± 13.1[b]
Linguadactyloides brinkmanni	10.0	0.8 ± 2.5[a]	3.3	0.03 ± 0.2[a]	3.3	0.03 ± 0.2[a]

Note. Mean values followed by different letters on the same line indicate differences between treatments according to Dunn's test ($p < 0.05$).

low-cost, effective and commercially available anthelmintic drugs, it is therefore highly desirable for such drugs to be used directly in the water of fish breeding tanks.

Safe anthelmintic drugs that can be administered in therapeutic baths are suitable for use in fish farming. However, in the therapeutic baths of *C. macropomum* with 200 mg/L of ivermectin, 100% fish mortality occurred within 2 hr of exposure, while 500 mg/L of albendazole caused a fish mortality rate of 6.6% within 24 hr of exposure. *Piaractus mesopotamicus* has also reportedly exhibited good drug tolerance after 30 min of exposure to 50–500 mg/L of albendazole (Onaka et al., 2003). Therapeutic baths with 1.8 mg/L of ivermectin also caused 100% mortality of *Sparus aurata* after 96 hr of exposure (Mladineo et al., 2006). Santamarina et al. (1991) reported that therapeutic baths with 0.031 mg/L of ivermectin caused a high mortality rate among *O. mykiss* after just a few minutes of exposure. *Danio rerio* also reportedly suffered 100% mortality after exposure to 0.007 and 0.009 mg/L of ivermectin, as did *Catla catla* exposed to 0.007 mg/L of this drug, due to neurotoxicity and hepatotoxicity (Thiripurasundari et al., 2014; Varó et al., 2010). These reported results indicate that ivermectin is not a good anthelmintic drug for control and treatment, given its high toxicity to fish even at low concentrations.

In intensive fish farming, even when good management practices are employed to prevent monogenean infestations, treatments are often necessary to control mortality rates or to implement sanitation programs during at least some stage of fish breeding. Therefore, knowledge about suitable strategies to control and treat against these parasitic diseases is essential. Therapeutic baths with 500 mg/L of albendazole were only 48.6% effective against monogeneans of *C. macropomum* after 24 hr of exposure. In contrast, 125 mg/L of levamisole was 88.2% effective against monogeneans because it blocked neuromuscular junctions and stimulated parasympathetic and sympathetic ganglia in the hosts (Martins et al., 2017), causing the parasites to detach from the host gills. Thirty minute therapeutic baths with 500 mg/L of albendazole were 32.7% effective, while 200 mg/L of albendazole was 46.5% affective against *Anacanthorus penilabiatus* of *P. mesopotamicus* (Onaka et al., 2003). In addition, 3 h therapeutic baths with 100 mg/L of levamisole were effective against *Gyrodactylus* sp. of *O. mykiss* (Santamarina et al., 1991), and 10–18 min baths with 20–50 mg/L of levamisole were effective against *Urocleidus vistulensis* of *Silurus glanis* (Szekely & Molnar, 1990). Therefore, albendazole efficacy depends on the concentration and exposure time of the hosts to the drug.

In conclusion, although the treatment in vitro with albendazole, ivermectin and levamisole was 100% effective against monogeneans of *C. macropomum*, only levamisole showed good efficacy in therapeutic baths, since albendazole showed low efficacy and exposure to ivermectin was highly toxic to fish. Therefore, we recommend the use of long duration antiparasitic therapeutic baths with 125 mg/L of levamisole to control monogeneans of *C. macropomum* in fish farming.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

This study was approved by the Ethics Committee on Animal Use of Embrapa Amapá (Protocol N° 013/2018-CEUA/CPAFAP) and was conducted in accordance with the principles of the Brazilian College of Animal Experimentation (COBEA).

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