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CARLIANE MARIA GUIMARÃES ALVES

EFICÁCIA DE ANTI-HELMÍNTICOS COMERCIAIS NO TRATAMENTO CONTRA MONOGENEA DAS BRÂNQUIAS DE *Colossoma macropomum* (SERRASALMIDAE)

MACAPÁ-AP
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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Ambientais (PPGCA) da Universidade Federal do Amapá, como requisito parcial para obtenção do título de Mestre em Ciências Ambientais.

Orientador: Dr. Marcos Tavares Dias.

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BANCA EXAMINADORA

Dr. Marcos Tavares Dias (Orientador)
Embrapa Amapá/ PPGBIO

Dra. Marcela Nunes Videira
Universidade do Estado do Amapá / UEAP

Dra. Eliane Tie Oba Yoshioka
Embrapa Amapá/PPGBIO

Dra. Jamile da Costa Araújo
Embrapa Amapá

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PREFÁCIO

Esta dissertação está dividida em dois capítulos, seguindo o formato alternativo proposto pelo Programa de Pós-Graduação em Ciências ambientais (PPGCA). Inicialmente, é feita uma introdução geral da dissertação, seguida pelo artigo 1 intitulado "**Albendazol, levamisol e ivermectin tem eficácia contra monogeneas de *Colossoma macropomum*, a Serrasalmidae da Amazônia"** que foi aceito para publicação no periódico Journal of Fish Diseases **(**Qualis A2**)**.

RESUMO

ALVES, C. M. G. Eficácia de anti-helmínticos comerciais no tratamento contra Monogenea das brânquias de *Colossoma macropomum* (Serrasalmidae). Dissertação de Mestrado, Programa de Pós-Graduação em Ciências ambientais, Universidade Federal do Amapá, Macapá, 2019.

Este estudo investigou os efeitos antiparasitários, *in vitro* e *in vivo*, de anti-helmínticos albendazol, ivermectina, levamisol, mebendazol e praziquantel para o controle de monogeneas das brânquias de *Colossoma macropomum* (Tambaqui). No ensaio *in vitro* foram testadas as eficácias do albendazol, ivermectina, levamisol, mebendazol e praziquantel contra monogeneas *Anacanthorus spathulatus*, *Notozothecium janauachensis*, *Mymarothecium boegeri* e *Linguadactyloides brinkmanni* em 4 diferentes concentrações e 3 repetições cada. Todas as concentrações de albendazol (500, 100, 1500 e 2000 mg/L), ivermectina (200, 250, 300 e 350 mg/L) e levamisol (50, 75, 100 e 125 mg/L) mostraram 100% de eficácia, enquanto a eficácia do mebendazol (125, 150, 175 e 200 mg/L) e praziquantel (5, 10, 15 e 20 mg/L) foi causada pelo diluente DMSO. Porém, nos controles expostos ao DMSO houve também mortalidade 100% dos parasitos, e nos controles com água do tanque de cultivo 100% de mortalidade dos monogeneas ocorreu somente após 6 horas de exposição. A partir dos resultados *in vitro* foram determinadas as concentrações e melhores resultados para uso nos banhos terapêuticos em tambaqui. Nos banhos terapêuticos, os peixes foram distribuídos em 4 tratamentos e expostos por 24 horas em concentrações de 500 mg/L albendazol, 200 mg/L de ivermectina ou 125 mg/L de levamisol. A concentração de 500 mg/L albendazol que não alteraram o comportamento dos peixes expostos e a mortalidade foi de 6,6%. Porém, 200 mg/L de ivermectina que causaram letargia e sinais de hipóxia em tambaqui após 1 hora e 100% de mortalidade em 2 horas de exposição, enquanto 125 mg/L de levamisol não causaram nenhuma mortalidade ou alteração comportamental. Após 24 horas dos banhos terapêuticos, 500 mg/L de albendazol teve eficácia de apenas 48,6% contra monogeneas e enquanto levamisol teve 88,2% de eficácia. Embora o albendazol, ivermectina, levamisol, mebendazol e praziquantel demonstraram atividade antiparasitária *in vitro* contra monogeneas de tambaqui, eficácia foi observada apenas para albendazol e levamisol, mas somente o levamisol pôde ser recomendado em banhos terapêuticos como estratégias para controle dos níveis de monogeneas nas brânquias de tambaqui em pisciculturas.

Palavras-chave: Anti-helmínticos, Brânquias, Monogeneas, Tambaqui, Tratamento.

ABSTRACT

ALVES, C. M. G. Efficacy of commercial anthelminthics in the treatment against Monogenea of the gills of *Colossoma macropomum* (Serrasalmidae). Master Dissertation – Postgraduate Program in Environmental Sciences, Federal University of Amapá, Macapá, 2019.

This study investigated the *in vitro* and *in vivo* anti-helminthic effects of albendazole, ivermectin, levamisole, mebendazole and praziquantel for the control of monogeneans of *Colosoma macropomum* gills (Tambaqui). In the *in vitro* assay, the efficacy of albendazole, ivermectin, levamisole, mebendazole and praziquantel in control of monogeneans *Anacanthorus spathulatus*, *Notozothecium janauachensis*, *Mymarothecium boegeri* and *Linguadactyloides brinkmanni* were tested using 4 concentrations and 3 replicates each. All concentrations of albendazole (500, 100, 1500 and 2000 mg / L), ivermectin (200, 250, 300 and 350 mg/L) and levamisole (50, 75, 100 and 125 mg/L) showed 100% efficacy, while the efficacy of mebendazole (125, 150, 175 and 200 mg/L) and praziquantel (5, 10, 15 and 20 mg/L) was caused by the DMSO diluent. However, in controls exposed to DMSO there was also 100% mortality of the parasites and in controls with water from the culture tank 100% mortality of the monogeneans occurred only after 6 h of exposure. From the *in vitro* results, the concentrations and best results for use in tambaqui therapeutic baths were determined. In the therapeutic baths, the fish were distributed in 4 treatments and exposed for 24 h at concentrations of 500 mg/L albendazole, 200 mg/L of ivermectin or 125 mg / L of levamisole. The concentration of 500 mg/L albendazole did not alter the behavior of exposed fish and mortality of fish was of 6.6%. However, 200 mg/L of ivermectin caused lethargy and signs of hypoxia in fish after 1 h and 100% mortality at 2 h of exposure, while levamisole 125 mg/L caused no mortality or behavioral change. After 24 h of the therapeutic baths, 500 mg/L albendazole had efficacy of only 48.6% against monogeneans and while levamisole had 88.2% efficacy. Although albendazole, ivermectin, levamisole, mebendazole and praziquantel demonstrated in vitro antiparasitic activity against monogeneans of the tambaqui, efficacy was observed only for albendazole and levamisole, but only levamisole is recommended in therapeutic baths as strategies to control the levels of monogeneans in the gills of tambaqui in fish farms.

Keywords: Anthelminthic, Gills, Monogeneans, Tambaqui, Treatment

SUMÁRIO

1 INTRODUÇÃO GERAL

A produção global da aquicultura de peixes atingiu quase 8 milhões de toneladas em 2016, representando cerca de US $ 232 bilhões (FAO, 2018). Entretanto, as populações naturais de peixes em todo o mundo diminuíram devido à sobrepesca de diferentes espécies sobre exploradas. Para atender à crescente demanda por pescados, a aquicultura foi proposta como uma solução, sendo observada na Agenda 2030 para o Desenvolvimento Sustentável como uma das metas a serem alcançadas na contribuição e condução da pesca e aquicultura, para a segurança alimentar e nutricional, visando garantir o desenvolvimento sustentável em termos econômicos, sociais e ambientais, no contexto do Código de Conduta da FAO para a Pesca Responsável (BASTOS GOMES et al., 2017; FAO, 2018).

Além disso, a aquicultura é uma prática agropecuária sustentável que pode contribuir para a conservação de espécies ameaçadas pela forte exploração de pesca. Assim, a aquicultura é uma atividade de rápido crescimento em todos os continentes, com taxa de cerca de 7% ao ano, representando mais da metade dos peixes utilizados no consumo do homem. Esta atividade é, portanto, uma importante fonte de alimento, nutrição, renda e meios de subsistência para centenas de milhões de pessoas ao redor do mundo. A piscicultura (classificada como um tipo de aquicultura) de água doce contribui com mais de dois terços da produção mundial da aquicultura (FAO, 2018).

O crescimento da piscicultura tem sido influenciado pelo aumento da demanda mundial por alimentos de origem proteica e por carnes brancas, bem como pelo alto valor de mercado de diferentes espécies de peixes de interesse zootécnico (TAVARES-DIAS, 2018). Estima-se que para 2025, a pesca e aquicultura deve registrar um crescimento de 104% na produção. O aumento na produção brasileira será o maior registrado seguido pelo México e Argentina (FAO, 2018). Tais aumentos no crescimento da piscicultura brasileira devem ser, principalmente, devido aos investimentos feitos no setor nos últimos anos.

O Brasil, possui condições excelentes para o desenvolvimento da piscicultura tais como clima, espécies com potencial zootécnico, mão de obra, mercado consumidor e outros fatores, com perspectivas de crescimento, além de grandes recursos hídricos, pois detém grande parte da água doce do planeta (KUBITZA et al., 2012). A grande extensão da bacia amazônica brasileira e a ampla diversidade ictiológica, com mais de 3.000 espécies (REIS et al., 2003; REIS et al., 2016), indicam que a piscicultura é o ramo da aquicultura que apresenta as maiores potencialidades de produção na região, tanto do ponto de vista da sustentabilidade ecológica

como nutricional e econômico (FIM, 1995). Porém, essa atividade agropecuária é bastante promissora em diversas regiões do país, com retorno financeiro.

Em 2016, a produção total de peixes de água doce no Brasil foi de aproximadamente 507 mil toneladas. A produção de *Oreochomis niloticus* (tilápia-do-nilo) foi cerca de 239 mil toneladas e de *Colossoma macropomum* (tambaqui) foi cerca de 137 mil toneladas. Essa produção de tilápia-do-nilo representou 47,1% da produção nacional e de tambaqui 27,0% (IBGE, 2016). A piscicultura é, portanto, de grande importância para o desenvolvimento socioeconômico do país, havendo uma grande capacidade para atender ao mercado local, regional, nacional e internacional.

Com a intensificação da piscicultura intensiva, os peixes podem se tornar mais susceptíveis às enfermidades em decorrência do estresse e má qualidade da água, que favorecem principalmente a proliferação de ectoparasitos e doenças infecciosas (MORAES; MARTINS, 2004). Dessa forma, com o aumento do cultivo de tambaqui o surgimento de doenças parasitárias é inevitável (MALTA et al., 2001; CHAGAS et al., 2016), principalmente devido as infecções por ectoparasitos monogeneas (CHAGAS et al., 2016; TAVARES-DIAS; MARTINS, 2017). Em peixes de água doce cultivados no Brasil, as perdas econômicas por doenças, relacionadas aos custos diretos e indiretos, são estimadas em US$ 84 milhões/ano (TAVARES-DIAS; MARTINS, 2017).

Nos sistemas de cultivo intensivo, para evitar tais perdas econômicas devido a ocorrência de doenças é de fundamental importância evitar alta densidade populacional, excesso de alimentação, falta de limpeza dos tanques, falta de monitoramento dos parâmetros aquáticos, dentre outros fatores LUQUE, 2004; MARTINS, 2004; TAVARES-DIAS; MARTINS, 2017). Portanto, além da necessidade de profilaxia no cultivo de tambaqui, deve-se buscar tratamentos com anti-helmínticos comerciais para eliminar monogeneas, visando reduzir as perdas econômicas na produção desse importante peixe amazônico.

1.1 *Colossoma macropomum* e *parasitos monogeneas*

Colossoma macropomum (Figura 1) é um Characiformes da família Serrasalmidae que habita lagos e áreas marginais alagadas associadas às calhas dos rios como Orinoco e Amazonas, que pode alcançar cerca de um metro de comprimento e atingir 30 kg de peso. É considerado o segundo maior peixe de escamas da bacia amazônica (GRAEF, 1995; FISCHER; MALTA, 2003; GOMES et al., 2010; SANTOS, 2013; CHAGAS et al., 2016). Este peixe onívoro alimenta-se principalmente de frutos e zooplâncton, apresentando hábitos diurnos e

migratórios, com presença de dentes molariformes e estômago bastante espesso (SOARES et al., 2011).

Espécie de peixe nativa mais cultivada no Brasil, principalmente nas regiões Norte e Nordeste, pois apresenta várias características zootécnicas favoráveis, para o cultivo, tais como fácil obtenção de alevinos, rápido crescimento e boa produtividade (SOARES et al., 2011; LOPERA-BARRERO et al., 2003; GODOI et al., 2012).

Figura 1 - Espécime de *Colossoma macropomum*.
Fonte: Marcos Tavares-Dias

Em tambaqui, entre os problemas com doenças parasitárias estão os monogeneas *Anacanthorus spathulatus*, *Mymarothecium boegeri*, *Notozothecium janauachensis* e *Linguadactyloides brinkmani* (TAVARES-DIAS et al., 2006; ARAÚJO; CHAGAS, 2006; COHEN; KOHN et al., 2009; MORAES et al., 2009; GODOI et al., 2012; SANTOS et al., 2013). Os monogeneas são ectoparasitos do grupo dos platelmintos caracterizados pela presença de um aparelho de fixação localizado geralmente na parte posterior do corpo (o haptor), que é uma estrutura formada por uma série de ganchos, barras e âncoras de número e tamanhos variáveis, que são introduzidos no corpo dos peixes hospedeiros (SCHMAHL; MEHLHORN, 1985; NOGA, 1996; THATCHER, 2006; PAVANELLI et al., 2008; ONAKA, 2009; EIRAS et al., 2006). Possuem a forma alongada, ovoidal ou circular e medem de 1 mm a 3 cm, são hermafroditas, de ciclo direto (monoxeno). Esses parasitos são encontrados principalmente nas brânquias e tegumentos dos peixes, mas podem ser encontrados também nas cavidades nasais e estômago dos hospedeiros (PAVANELLI et al., 2008; EIRAS et al., 2010).

Nos hospedeiros, os monogeneas podem provocar reações infecciosas quando em elevado parasitismo, principalmente em criações intensivas, onde existem altas aglomerações de indivíduos. Assim, monogenioses estão entre as mais importantes parasitoses da piscicultura. Nas brânquias, essas reações infecciosas podem variar com intensidade de parasitos e espécie do parasito, levando a hiperplasia celular, hipersecreção de muco, hemorragias e edemas nos filamentos branquiais, e ainda, favorecem infecções secundárias por bactérias (PAVANELLI et al., 2008; ONAKA, 2009). Como os monogeneas podem ser patogênicos, seu controle pode representar até 22% dos custos totais de produção dos peixes (FORWOOD et al., 2013).

Pelo fato da monogeniose causar mortalidade, além de medidas de profilaxia deve-se implementar o controle dos parasitos usando anti-helminticos comerciais como albendazol, ivermectina, levamisol, mebendazol e praziquantel, mas que precisam ser testados *in vitro* e *in vivo* para o uso contra monogeneas de tambaqui.

1.2 O uso de anti-helmínticos comerciais contra monogeneas de peixes

Na piscicultura, mesmo com o uso das Boas Práticas de Manejo (BPM) para prevenção de doenças, muitas vezes o uso de quimioterápicos é necessário no controle de mortalidade causada por parasitos ou para implementar programas sanitários durante pelo menos alguma fase do cultivo. Dentre os quimioterápicos anti-helmínticos usados em peixes de cultivo, destacam-se o albendazol, mebendazol (benzimidazóis), ivermectina (avermectina), levamisol (imidazotiazol) e praziquantel (pirazina-isoquinolínico), pois além de controlar parasitos podem também aumentar a resistência dos hospedeiros, em alguns casos (SCHMAHL, 1991; MARTINS et al., 2004; MARTINS et al., 2017). Assim, o controle das infecções parasitárias pode ser alcançado eliminando estágios de desenvolvimento nos hospedeiros, interrompendo assim o ciclo de vida dos parasitos (BADER et al., 2017). Porém, as concentrações desses diferentes fármacos devem ser seguras e eficazes, além de proporcionar uma baixa toxicidade aos peixes hospedeiros, além de serem seguras ao meio ambiente.

Existem duas formas de investigar a ação antiparasitária dos quimioterápicos; usando ensaios *in vitro*, que podem ser realizados em placa de Petri ou tubos de ensaios, enquanto os ensaios *in vivo* podem ser conduzidos usando banhos terapêuticos ou adição do fármaco na ração dos peixes (TAVARES-DIAS, 2018). Em peixes é frequentemente a utilização de anti-helmínticos na ração contra parasitos gastrointestinais (endoparasitos) e a inoculação intravenosa em reprodutores. Dar et al. (2017) relataram que a administração de anti-helmíntico

por via intraperitoneal, intramuscular e oral são métodos eficazes para tratamento de helmintos em peixes.

Usualmente, o que ocorre é a investigação prévia *in vitro* dos níveis de eficácia dos anti-helmínticos para posterior aplicação nos tratamentos *in vivo* (SITJA-BOBADILLA et al., 2006; CHAGAS et al., 2016; KHALIL et al., 2016). Os tratamentos devem levar em consideração a substância ativa de qualidade, concentração utilizada, o tempo de exposição e tipo de antihelmintico. A eficácia de um medicamento anti-helmíntico depende de uma concentração que seja tóxica para os parasitos por tempo suficiente para causar-lhes danos irreversíveis, mas sem toxicidade para os peixes. Assim, diferentes anti-helmínticos foram testados para controle *in vitro* e *in vivo* de monogeneas em espécies de peixes. Os resultados mostraram diferenças na eficácia relacionadas as concentrações dos diferentes produtos e tempo de exposição (Tabela 1 e 2). Porém, ainda não foram testados, *in vitro* e *in vivo*, alguns anti-helmínticos para controle de monogeneas de *C. macropomum*.

Tabela 1 - Eficácia *in vitro* de anti-helmínticos contra diferentes espécies de monogeneas em peixes.

Espécies de peixes	Espécies de parasitos	Anti-helmínticos	Concentrações	Eficácia (%)	Tempo de exposição	Referências
Oncorhynchus mykiss	*Gyrodactylus* sp.	Ivermectina	0,031 mg/L	100	60 minutos	SANTAMARINA et al. (1991)
Oncorhynchus mykiss	*Gyrodactylus* sp.	Ivermectina	0,031 mg/L	50	30 minutos	SANTAMARINA et al. (1991)
Takifugu rubripes	*Heterobothriumo kamotoi*	Levamisol	20 mg/L	100	3 horas	HIRAZAWA et al. (2000)
Takifugu rubripes	*Heterobothriumo kamotoi*	Levamisol	20 mg/L	66,7	2 horas	HIRAZAWA et al. (2000)
Oncorhynchus mykiss	*Gyrodactylus* sp.	Levamisol	100 mg/L	100	60 minutos	SANTAMARINA et al. (1991)
Oncorhynchus mykiss	*Gyrodactylus* sp.	Levamisol	100 mg/L	25	30 minutos	SANTAMARINA et al. (1991)
Oncorhynchus mykiss	*Gyrodactylus* sp.	Levamisol	50 mg/L	63	60 minutos	SANTAMARINA et al.(1991)
Carassius auratus	*Dactylogyrus vastator*	Mebendazol	0,02 mg/L	100	22 horas	ZHANG et al. (2014)
Carassius auratus	*Dactylogyrus vastator*	Mebendazol	0,04 mg/L	100	16 horas	ZHANG et al. (2014)
Takifugu rubripes	*Heterobothriumo kamotoi*	Praziquantel	20 mg/L	100	1 hora	HIRAZAWA et al. (2000)
Seriola quinqueradiata e *Seriola dumerili*	*Benedenia seriolae*	Praziquantel	0,5 mg/L	90	5 horas	HIRAZAWA et al. (2013)
Seriola quinqueradiata e *Seriola dumerili*	*Benedenia seriolae*	Praziquantel	1 mg/L	100	1 hora	HIRAZAWA et al. (2013)
Seriola quinqueradiata e *Seriola dumerili*	*Benedenia seriolae*	Praziquantel	2,5 mg/L	75	30 minutos	HIRAZAWA et al. (2013)
Seriola quinqueradiata e *Seriola dumerili*	*Benedenia seriolae*	Praziquantel	5 mg/L	100	1 hora	HIRAZAWA et al. (2013)
Seriola quinqueradiata e *Seriola dumerili*	*Neobenedenia girellae*	Praziquantel	0,5 mg/L	0	5 horas	HIRAZAWA et al. (2013)
Seriola quinqueradiata e *Seriola dumerili*	*Neobenedenia girellae*	Praziquantel	1mg/L	20	5 horas	HIRAZAWA et al. (2013)
Seriola quinqueradiata e *Seriola dumerili*	*Neobenedenia girellae*	Praziquantel	2,5 mg/L	100	4 horas	HIRAZAWA et al. (2013)
Seriola quinqueradiata e *Seriola dumerili*	*Neobenedenia girellae*	Praziquantel	5 mg/L	90	2 horas	HIRAZAWA et al. (2013)

Tabela 1 (Continuação) - Eficácia *in vitro* de anti-helmínticos contra diferentes espécies de monogenea em peixes

Espécies de peixes	Espécies de parasitos	Anti-helmínticos	Concentrações	Eficácia (%)	Tempo exposição	Referências
Sparus aurata	*Sparicotyle chrysophrii*	Praziquantel	25 mg/L	20	30 minutos	SITJÀ-BOBADILLA et al. (2006)
Sparus aurata	*Sparicotyle chrysophrii*	Praziquantel	50 mg/L	10	30 minutos	SITJÀ-BOBADILLA et al. (2006)
Sparus aurata	*Sparicotyle chrysophrii*	Praziquantel	100 mg/L	10	50 minutos	SITJÀ-BOBADILLA et al. (2006)
Carassius auratus	*Dactylogyrus vastator*	Praziquantel	10 mg/L	100	24 horas	ZHANG et al. (2014)
Carassius auratus	*Dactylogyrus vastator*	Praziquantel	20 mg/L	100	24 horas	ZHANG et al. (2014)
Oncorhynchus mykiss	*Gyrodactylus* sp.	Praziquantel	100 mg/L	100	60 minutos	SANTAMARINA et al. (1991)
Oncorhynchus mykiss	*Gyrodactylus* sp.	Praziquantel	30 mg/L	16,6	30 minutos	SANTAMARINA et al. (1991)
Sphoeroides annulatus	*Tagia ecuadori*	Praziquantel	2,5-20 mg/L	100	20 horas	MORALES-SERNA et al. (2018)
Sphoeroides annulatus	*Neobenedenia melleni*	Praziquantel	3 mg/L	87	12 horas	MORALES-SERNA et al. (2018)
Sphoeroides annulatus	*Neobenedenia melleni*	Praziquantel	10 mg/L	50	12 horas	MORALES-SERNA et al. (2018)
Sphoeroides annulatus	*Neobenedenia melleni*	Praziquantel	20 mg/L	22	12 horas	MORALES-SERNA et al. (2018)

Tabela 2 - Eficácia *in vivo* (banhos) de anti-helmínticos contra diferentes espécies de monogeneas em peixes.

Espécies de peixes	Espécies de parasitos	Anti-helmínticos	Concentrações	Eficácia (%)	Tempo exposição	Referências
Silurusglanis	*Urocleidus vistulensis*	Albendazol	1 a 500 mg/L	Baixa	4,5-26 horas	SZÉKELY e MOLNÁR (1990)
Piaractus mesopotamicus	*Anacanthorus penilabiatus*	Albendazol	200 mg/L	46,5	30 minutos	ONAKA et al. (2003)
Piaractus mesopotamicus	*Anacanthorus penilabiatus*	Albendazol	500 mg/L	32,7	30 minutos	ONAKA et al. (2003)
Anguilla anguilla	*Pseudodactylo gyrus* spp.	Albendazol	1 mg/L	67,3	24 horas	BUCHMANN e BJERREGAARD (1990)
Anguilla anguilla	*Pseudodactylo gyrus* spp.	Albendazol	10 mg/L	100	24 horas	BUCHMANN e BJERREGAARD (1990)
Anguilla anguilla	*Pseudodactylogyrus* spp.	Albendazol	100 mg/L	100	24 horas	BUCHMANN e BJERREGAARD (1990)
Anguilla anguilla	*Pseudodactylogyrus* spp.	Ivermectina	5 e 50 mg/L	0	24 horas	BUCHMANN e BJERREGAARD (1990)
Silurusglanis	*Urocleidus vistulensis*	Levamisol	20 a 50 mg/L	Baixa	10-18 minutos	SZÉKELY e MOLNÁR (1990)
Oncorhynchus mykiss	*Gyrodactylus* sp	Levamisol	100 mg/L	0	3 horas	SANTAMARINA et al. (1991)
Carassius auratus	*Dactylogyrus vastator*	Mebendazol	0,01 mg/L	10	48 horas	ZHANG et al. (2014)
Carassius auratus	*Dactylogyrus vastator*	Mebendazol	0,02 mg/L	25	48 horas	ZHANG et al. (2014)
Carassius auratus	*Dactylogyrus vastator*	Mebendazol	0,03 mg/L	46,9	48 horas	ZHANG et al. (2014)
Anguilla anguilla	*Pseudodactylogyrus* spp.	Mebendazol	1 mg/L	100	24 horas	BUCHMANN e BJERREGAARD (1990)
Carassuis auratus	*Gyrodactylus elegans*	Mebendazol	0,1 mg/L	100	24 horas	GOVEN e AMEND (1982)
Carassuis auratus	*Dactylogyrus vastator*	Mebendazol	0,1 e 4 mg/L	0	24 horas	GOVEN e AMEND (1982)
Pagrus pagrus	*Microcotyle* spp.	Mebendazol	400 mg/L	0	1 horas	KATHARIOS et al. (2006)
Piaractus mesopotamicus	*Anacanthorus penilabiatus*	Mebendazol	1 mg/L	58,7	24 horas	MARTINS et al. (2001)
Piaractus mesopotamicus	*Anacanthorus penilabiatus*	Mebendazol	10 mg/L	81,4	24 horas	MARTINS et al. (2001)
Piaractus mesopotamicus	*Anacanthorus penilabiatus*	Mebendazol	100 mg/L	77,9	24 horas	MARTINS et al. (2001)

Tabela 2 (Continuação) - Eficácia *in vivo* (banhos) de anti-helmínticos contra diferentes espécies de monogenea em peixes.

Espécies de peixes	**Espécies de parasitos**	**Anti-helmínticos**	**Concentrações**	**Eficácia (%)**	**Tempo exposição**	***Referências***
Silurus glanis	*Urocleidus vistulensis*	Mebendazol	10 mg/L	≅ 80	4 horas	SZEKELY e MOLNAR (1990)
Silurus glanis	*Urocleidus vistulensis*	Mebendazol	10 mg/L	≅ 80	26 horas	SZEKELY e MOLNAR (1990)
Silurus glanis	*Urocleidus vistulensis*	Mebendazol	100 mg/L	≅90	1,5 horas	SZEKELY e MOLNAR (1990)
Bidyanus bidianus	*Lipidotrema bidyana*	Praziquantel	10 mg/L		48 horas	FORWOOD et al. (2013)
Seriola lalandi	*Benedeniaseriolae*	Praziquantel	2,5 mg/L	99	24 horas	SHARP et al. (2004)
Seriola lalandi	*Benedeniaseriolae*	Praziquantel	2,5 mg/L	100	48 horas	SHARP et al. (2004)
Seriola lalandi	*Zeuxaptaseriolae*	Praziquantel	2,5 mg/L	100	24 horas	SHARP et al. (2004)
Seriola lalandi	*Zeuxaptaseriolae*	Praziquantel	2,5 mg/L	100	48 horas	SHARP et al. (2004)
Piaractus mesopotamicus	*Anacanthorus penilabiatus*	Praziquantel	500 mg/L	68,3	30 dias	ONAKA et al. (2003)
Carassius auratus	*Dactylogyrusvastator*	Praziquantel	10 mg/L	63	48 horas	ZHANG et al. (2014)
Carassius auratus	*Dactylogyrusvastator*	Praziquantel	15 mg/L	70	48 horas	ZHANG et al. (2014)
Carassius auratus	*Dactylogyrusvastator*	Praziquantel	20 mg/L	80	48 horas	ZHANG et al. (2014)
Oncorhynchus mykiss	*Gyrodactylus*sp.	Praziquantel	10 mg/L	97,7	3 horas	SANTAMARINA et al. (1991)
Silurus glanis	*Urocleidusvistulensis*	Praziquantel	10 mg/L	≅10	5 horas	SZEKELY e MOLNAR (1990)
Silurus glanis	*Urocleidusvistulensis*	Praziquantel	100 mg/L	≅ 60	18 minutos	SZEKELY e MOLNAR (1990)

Tem-se ainda uma preocupação sobre o destino final dessas drogas sintéticas no meio ambiente, uma vez que a dependência desses produtos químicos tornou-se maior com a intensificação da produção da aquicultura, alimentação formulada, manipulação e melhoramento da reprodução, promoção do crescimento, gestão da saúde, processamento e agregar valor ao produto final (DOUET et al., 2009). Portanto, os riscos desses produtos para o meio ambiental deve ser questionado.

1.2.1 Albendazol

Albendazol (metil [(5-propiltiol) –1 H – benzimidazol-2] carbamato) é um derivado imidazólico classificado como metil-carbamato, um fármaco que apresenta amplo espectro de ação com alta eficácia ovicida, larvicida e helminticida (CORRÊA, 1999; MORIWAKI et al., 2008; DAR et al., 2017; MARTINS et al., 2017). Foi desenvolvido em 1980 para uso contra helmintos (cestoides, *Strongyloides* sp., *Ascaris* sp. e *Trichuris* sp.) do homem (HORTON, 2003). Sua ação anti-helmíntica decorre da inibição da polimerização dos microtúbulos pela ligação à β-tubulina, prejudicando o aporte de nutrientes celulares nos helmintos (RANG et al., 2007; MARTINS et al., 2017). Esse anti-helmíntico tem sido usado contra diferentes helmintos do homem e animais, bem como contra diferentes espécies de nematoides (PRAVETTONI et al., 2012; ROMERO et al., 2014; OSMAN et al., 2016; KHALIL et al., 2016) e cestoides de peixes (ATHANASSOPOULOU et al., 2009; MARKOSKI et al., 2006). Contra monogenea de peixes esse fármaco tem mostrado eficácia de até 100%, quando usado em banhos terapêuticos (Tabela 2), mas não tem sido testado *in vitro*.

Em *Piaractus mesopotamicus*, após banhos terapêuticos com 50, 100, 200 e 500 mg de albendazol contra *Anacanthorus penilabiatus*, ONAKA et al. (2003) não observaram alterações histopatológicas nas brânquias, rim e fígado. Esses resultados demostram uma baixa toxicidade do albendazol, um anti-helmíntico que não tem sido usado contra monogeneas de tambaqui.

1.2.2 Ivermectina

Ivermectina, uma avermectina produzida da fermentação do actinomiceto de solo *Strepotomyces avermitilis* (CARRASCO et al., 1997; JOHNSON; HEINDEL, 2001; SCOTT et al., 2002), é uma droga antiparasitária de largo espectro, registrada em muitos países e administrada em diferentes animais (DINETTA, 1989). É efetiva para tratar infecções parasitárias na alimentação de mamíferos e também usada para tratar parasitos em espécies

aquáticas (COLLYMORE et al., 2014). Em peixes, esse anti-helmíntico tem sido usado contra helmintos nematoides (SAMUEL; GRAY, 1988; MANLEY; EMBIL, 1989).

Em peixes, a ivermectina tem sido pouco usada contra monogeneas (Tabela 1 e 2), pois apresenta toxicidade para os hospedeiros na dependência da concentração usada (SANTAMARINA et al, 1991). Em *Sparus aurata*, uma única injeção intraperitoneal de 100, 200, 400 ou 800 μg de ivermectina por kg/peixe resultou em alterações hematológicas sem causar alterações histopatológicas nos hospedeiros (KATHARIOS et al., 2002). Johnson et al. (1993) relataram que 5 a 200 mg de ivermectina por kg/ração causou diferenças na toxicidade para diferentes espécies de salmonídeos e levou a alterações sanguíneas, mas sem alterações histopatológicas no rim, fígado, baço e trato digestório. Porém, ivermectina não tem sido testada contra monogeneas de tambaqui.

Segundo Collier e Pinn (1998), a ivermectina é altamente fotolítica e dependendo de sua concentração pode ser tóxica para o hosdeiro. Além disso, é pouco solúvel em água e resistente à hidrólise e liga-se ao sedimento e não se degrada em condições anaeróbicas; assim, o risco para crustáceos é alto. A eliminação é lenta para *Salmo salar*, por exemplo, e composto não metabolizado pode ser encontrado nas fezes (77% do excretado é inalterado após um dia) (DOUET et al., 2009). Em estudos com *S. salar* foram relatados aumento da toxicidade após tratamento com ivermectina a temperaturas abaixo 10 °C (ATHANASSOPOULOU et al., 2002). A toxicidade aguda da ivermectina em mamíferos manifesta-se em efeitos no sistema nervoso central, e isso pode estar relacionado ao seu efeito sobre o GABA no cérebro de mamíferos, medula espinhal; e alguns sinais comuns de toxicidade aguda incluem ataxia, tremores e casos graves, coma e morte (CAMPBELL, 1993; MLADINEO et al., 2006; UCÁN-MARÍN et al., 2012; THIRIPURASUNDARI et al., 2014). Sinais de neurotoxicidade como letargia, perda de apetite e cor escura foram também observados com o uso excessivo de ivermectina (VARÓ et al., 2010).

1.2.3 Levamisol

Levamisol, fármaco na forma levógira do tetramisol, pertencente ao grupo dos imidotiazois, é um anti-helmíntico de amplo espectro utilizado no tratamento antiparasitário de diferentes espécies de interesse zootécnico (SCHALCH et al., 2009; MARTINS et al., 2017). Foi desenvolvido em 1971 para uso contra helmintos (*Ascaris* sp. e *Trichuris* sp.) do homem (HORTON, 2003). A ação do levamisol consiste na paralisia do parasito, devido ao bloqueio das sinapses musculares e concentra-se na estimulação dos gânglios parassimpáticos e

simpáticos dos hospedeiros (GUSTAFSSON et al., 1987; MARTINS et al., 2017). Este anti-helmíntico age como um nicotínico agonista, e pode ligar-se à acetilcolina nicotínica receptores (nAChR) de parasitos nematoides (ALVAREZ-PELLITERO et al., 2006). A toxicidade seletiva do levamisol parece basear-se nas propriedades únicas do nAChR do nematóide, que parecem farmacologicamente distintas daquelas do receptores homólogos em mamíferos. Além disso, pode causar variações na região anterior (pró-haptor) e posterior (opisto-haptor) de monogeneas após exposição (GUSTAFSSON et al., 1987; SCHMAHL; TARASCHRWSKI, 1987; FUJIMOTO et al., 2006).

Este fármaco também atua na estimulação de linfócitos, servindo como imunoestimulante (GUSTAFSSON et al., 1987; SCHMAHL; TARASCHRWSKI, 1987; FUJIMOTO et al., 2006). Segundo Renoux (1980), não só a recuperação de funções imunodeprimidas, mas também estimulação das funções imunológicas dos indivíduos podem ocorrer mamíferos que receberam levamisol. Assim, uma parte importante da informação sobre os mecanismos de ação do levamisol vem do seu uso em diferentes situações clínicas de alterações das funções imunológicas (LI et al., 2004; LI et al., 2006; ALVAREZ-PELLITERO et al., 2006; SADO et al., 2010; ZANON et al., 2014). Em alguns resultados do desafio bacteriano e atividade bactericida sugerem o aumento da fagocitose quando níveis mais elevados de levamisol foram usados, que têm um papel importante na prevenção de doenças infecciosas. A fagocitose por leucócitos fagocíticos é um processo de internalização, morte e digestão de microrganismos invasores, onde os fagócitos produzem oxigênio livre radicais durante a explosão respiratória, que é tóxico para as bactérias (BEDASSO, 2017)

Em peixes, esse anti-helmíntico tem sido usado para controle *in vivo* e *in vitro* de monogeneas (Tabelas 1 e 2), bem como no controle nematoides (UNTERGASSER, 1989) e cestoides (ATHANASSOPOULOU et al., 2009). Pahor et al. (2017), verificaram que 300 mg de levamisol por kg/dieta reduziu a infecção por nematoides *R. rondoni* em *P. mesopotamicus*, mas não teve eficácia contra monogeneas *A. penilabiatus* e causou alterações histológicas moderadas no fígado dos peixes. Para o controle de monogeneas, Kayis et al. (2009) citam que pode ser usado 50 mg/L de levamisol em banhos de 2 horas. Porém, em *P. mesopotamicus* dieta com 1 e 2 g de levamisol/kg de ração, durante 7 dias, resultou em baixa eficácia no controle de *A. penilabiatus*, pois aos 3, 7 e 15 dias após o tratamento a eficácia foi de 55, 20 e 22%, respectivamente. Essa baixa eficácia foi devido a solubilidade do produto em água, fazendo com que os peixes não ingerissem a quantidade suficiente do fármaco (SCHALCH et al., 2009). Além disso, peixes estressados podem também não estar se alimentando adequadamente da ração medicada, impedindo sua eficácia.

1.2.4 Mebendazol

Mebendazol (mentil [5-(benzoil)–H-benzimidazol-2] carbamato) foi desenvolvido em 1975 para uso contra helmintos tais como cestoides, *Strongyloides* sp., *Ascaris* sp. e *Trichuris* sp. do homem (HORTON, 2003). Este é um anti-helmíntico com a capacidade induzir alterações nos microtúbulos citoplasmáticos das células tegumentares intestinais dos parasitos afetando a sua captação de glicose e sua ação inclui a inibição da enzima fumarato-redutase mitocondrial, o desacoplamento da respiração e síntese de ATP, não ocorrendo a produção energética necessária para a respiração e reprodução dos parasitos (CORREA, 1999; MARTINS et al., 2017).

Este anti-helmíntico tem considerável eficácia contra monogeneas quando usado *in vitro* e na forma de banhos terapêuticos (Tabelas 1 e 2), bem como quando administrado na dieta dos peixes (KIM et al.,1998; KIM; CHOI, 1998). Taraschewski et al. (1988) relataram que o mebendazol pode ser usado também contra nematoides de peixes. Porém, para obter a efetividade com esse anti-helmíntico deve-se proceder a administração de doses terapêuticas adequadas, pois a utilização de doses sub-terapêuticas por períodos prolongados pode levar os parasitos a resistência do mebendazol (BUCHMANN et al., 1992), bem como de outros anti-helmínticos.

Araújo e Chagas (2006) adicionando 1 e 2 g de mebendazol/kg na dieta de *C. macropomum* para controle de monogeneas encontraram 100% de eficácia após 14 dias de alimentação. Chagas et al. (2016) usando 1 g de mebendazol por kg/ração para *C. macropomum*, após 14 dias de alimentação, encontraram uma eficácia de 89,2% contra *A. spathulatus*, *N. janauachensis*e, *M. boegeri*. Além disso, não houve alterações sanguíneas nos peixes tratados. Para o controle de monogeneas, Kayis et al. (2009) citam que pode ser usado 1 mg/L de mebendazol em banhos de 24 horas. Banhos terapêuticos com 100 mg/L de mebendazol, aplicados três vezes com intervalo de 2 dias cada, causou uma redução na mortalidade de *Arapaima gigas* parasitados por *Dawestrema* sp. (CARVERO et al., 2003). Como mebendazol tem baixa toxicidade para os peixes (KIM, 1998; MARTINS, 2001), este anti-helmíntico tem grande potencial para tratamentos contra parasitos; assim, precisa ser testado *in vitro* e banhos terapêuticos contra monogeneas de *C. macropomum*.

1.2.5 Praziquantel

Praziquantel é um derivado de pirazina-isoquinolínico que causa danos irreversíveis aos monogeneas, levando a alterações acentuadas na região de fixação do haptor, ou seja, ganchos e âncoras ficam distorcidos, além de intensa vacuolização no tegumento dos parasitos (SCHMAHL e TARASCHEWSKI, 1987). Em diferentes espécies de peixes, diversos estudos têm usado praziquantel no controle de monogeneas, devido sua alta eficácia (Tabelas 1 e 2). Além disso, esse fármaco tem sido usado também no controle espécies nematoides (FUJIMOTO et al., 2006), digeneas (SILVA et al., 2009; KAYIS et al. 2009; BADER et al., 2017) e cestoides (MARKOSKI et al., 2006). Porém, em banhos terapêuticos, a eficácia do praziquantel pode estar associada ao tempo de exposição ao produto e não à concentração utilizada, sendo indicadas concentrações menores por tempo prolongado (MARTINS et al., 2017). Além disso, Onaka et al. (2003) relataram que banhos terapêuticos com 50 a 500 mg/L de praziquantel resultou em toxicidade para *P. mesopotamicus*.

Hirazawa et al. (2013) observaram que a adição de 150 mg de praziquantel/kg na dieta de *Seriola quinqueradiata* e *Seriola dumerili*, para controlar monogeneas *Neobenedenia girellae* e *Benedenia seriolae*, foi mais eficiente contra *B. seriolae*. Porém, em *P. mesopotamicus* alimentados com ração contendo 1 g ou 2 g de praziquantel/kg de ração não houve eficácia contra *A. penilabiatus* (SCHALCH et al., 2009). Foi relatada eficácia de 79% contra *Lipidotrema bidyana* em *Bidyanus bidianus* de 75 mg praziquantel/kg de ração, usado durante 6 dias (FORWOOD et al., 2013).

As ações de toxicidade provocadas pelo praziquantel são alterações no perfil hematológico, incluindo anemia e que leva ao aumento da atividade da alanina aminotransferase e aspartato aminotransferase, níveis elevados de glicose e redução dos níveis de proteínas, sugerindo toxicidade para os peixes (ZUSKOVA et al., 2018). Portanto, precisam ser testados as concentrações albendazol, ivermectina, levamisol, mebendazol e praziquantel com eficácia *in vitro* e banhos terapêuticos para controle de monogeneas de *C. macropomum.*

2 PROBLEMAS

Entre os anti-helmínticos albendazol, ivermectina, levamisol, mebendazol e praziquantel, qual (is) o(s) mais eficiente(s), *in vitro* e *in vivo* (banhos) no controle de monogeneas das brânquias de *C. macropomum*? Qual a concentração mais eficaz de albendazol, ivermectina, levamisol, mebendazol e praziquantel contra monogeneas das brânquias de *C. macropomum*?

3 HIPÓTESES

Os anti-helmínticos levamisol (20 e 100 mg/L), mebendazol (0.02, 0.04 e 1 mg/L) praziquantel (1, 2.5, 10, 20 e 100 mg/L) são mais eficazes no tratamento *in vitro* e *in vivo* contra monogeneas das brânquias de *C. macropomum*, uma vez que mostram eficácia na eliminação desses parasitos de outras espécies peixes.

4 OBJETIVOS

4.1 Geral

Investigar a eficácia *in vitro* e *in vivo* do albendazol, ivermectina, levamisol, mebendazol e praziquantel contra monogeneas das brânquias de *C. macropomum*.

4.2 Específicos

- Avaliar concentrações *in vitro e in vivo* de albendazol, ivermectina levamisol, mebendazol e praziquantel que são eficazes contra monogeneas das brânquias de *C. macropomum*;
- Investigar o tempo e concentrações *in vivo* de albendazol, ivermectina levamisol, mebendazol e praziquantel com eficácia contra monogeneas das brânquias de *C. macropomum*;
- Em caso de comprovação de eficácia, indicar o melhor anti-helmíntico com sua concentração e estratégias para tratamento contra monogeneas de *C. macropomum*.

REFERÊNCIAS DA INTRODUÇÃO

ALVAREZ-PELLITERO, P.; SITJA-BOBADILLA, A.; BERMUDEZ, R.; QUIROGN, M. I. Levamisole activates several innate immune factors in *Scophthalmus maximus* (l.) (Teleostei). **International journal of immunopathology and pharmacology**, v. 19, n. 4, p. 727-738. 2006.

ARAUJO, L. D.; CHAGAS, E. C. Eficácia da administração oral de mebendazol no controle monogenoides parasitas de brânquias do tambaqui (*Colossoma macropomum*). Documentos 50. **Embrapa Amazônia oriental**, Manaus, p. 41- 46, 2006.

ATHANASSOPOULOU, F.; PAPPAS I. S.; BITCHAVA K. An overview of the treatments for parasitic diseas e in Mediterranean aquaculture. **Options Méditerranéennes,** v. 86, p. 65-83, 2009.

BADER, C.; CHELLADURAI, J. J.; STARLING , D. E.; JONES, D. E.; BREWER, M. T. Assessment of in vitro killing assays for detecting praziquantel induced death in *Posthodiplostomum minimum* metacercariae. **Experimental Parasitology**, v. 181, p. 70 -74, 2017.

BASTOS, GOMES, G.; JERRY, D.R.; MILLER, T.L.; HUTSON, K.S. Current status of parasitic ciliates *Chilodonella* spp. (Phyllopharyngea: Chilodonellidae) in freshwater fish aquaculture. **Journal of Fish Diseases**, v. 40, p. 703–715, 2017.

BEDASSO, G. T. A Study of Immune Response in Nile Tilapia (*Oreochromis niloticus*) Fed Levamisole Incorporated Diet. **Journal of Immunology and Infectious Diseases**, v. 4, p. 2394-6512, 2017.

BUCHMANN, K.; BJERREGAARD, J. Effect of lvermectin, pyrantel and morantel on the european eel and its monogenean parasites. **Bulletin of the European Association of Fish Pathologists**, v. 10, p. 146, 1990.

BUCHMANN, K.; ROESPSTORFF, A.; WALLER, P. J. Experimental selection of mebendazole-resistant gill monogeneans from the European eel, *Anguilla anguilla L*. **Journal of Fish Diseases**, v. 15, p. 393-400, 1992.

CARRASCO, M.S.D.; ESPUNY, A.; ESCUDERO, E.; CÁRCELES, Y. C. M. Farmacologia de los endectocidas: aplicaciones terapêuticas. **Anales Veterinária,** v. 13, p. 3-22, 1997.

CARVERO, B. A. S. Controle da taxa de mortalidade de pirarucu, Arapaima gigas, naturalmente parasitado por *Dawestrema* sp. (Monogenea: *Dactylogyridae*). In: URBINATI, E. C.; CYRINO, J. E. P. (Org.). XII Simpósio Brasileiro de Aquicultura. Jaboticabal: **Aquabio**, v. 2, p. 107-115, 2003.

CAMPBELL, W. C. Ivermectin, an antiparasitic agent. **Medicinal Research Reviews**, v. 13, n. 1, p. 61-79, 1993.

COLLYMORE, C.; WATRAL, V.; WHITE, J. R.; COLVIN, M. E.; RASMUSSEN, S.; TOLWANI, R. J.; KENT, M. L. Tolerance and efficacy of emamectin benzoate and ivermectin for the treatment of *Pseudocapillaria tomentosa* in laboratory zebrafish (*Danio rerio*). **Zebrafish**, v. 11, p.490-497, 2014.

COHEN, S. C. KOHN, A. On Dactylogyridae (Monogenea) of four species of characid fishes from Brasil. **Check List**, v. 5, p. 351 – 356, 2009.

CORREA, G. L. B. Antihelmínticos e sua importância no controle de zoonoses parasitárias. In: MAGALHAES, H. M. (Ed.). **Farmacologia veterinária**. Guaíba: Agropecuária, p. 17-39, 1999.

COLLIER, L.M.; PINN, E.H. An assessment of the acute impact of the sea lice treatment ivermectin on a benthic community. **Journal of Experimental Marine Biology and Ecology**, 230, 1, p. 131-147, 1998.

CHAGAS, E. C.; ARAÚJO, L. D.; MARTINS, M. L.; GOMES, L. C.; MALTA, J. C. O.; VARELLA, A. B.; JERÔNIMO, G. T. Mebendazole dietary supplementation controls Monogenoidea (Platyhelminthes: Dactylogyridae) and does not alter the physiology of the freshwater fish *Colossoma macropomum* (Cuvier, 1818). **Aquaculture**, v.464, p. 185–189, 2016.

DAR, S. A.; NAUTIYAL, V.; PHULIA, V.; GUPTA, S.; SARDAR, P.; SAHU, N. P. Bioavailability of albendazole and its metabolites in plasma of *Pangasianodon hypophthalmus* with High performance liquid Chromatography. **International Journal of Current Microbiology and Applied Sciences**, v.6, p. 2392-2400, 2017.

DINETTA, J. List of registrations. CAMPBELL, W.C (Ed.). Ivermectin and abamectin. New York: **Springer-Verlag**, p. 344-366, 1989.

DOUET, D. G.; BRIS, H.; Giraud, E. Environmental aspects of drug and chemical use in aquaculture: An overview. **Options Méditerranéennes,** p. 105-126, n. 86, 2009

EIRAS, J. C.; TAKEMOTO, R. M.; PAVANELLI, G. C. **Métodos de estudo e técnicas laboratoriais em parasitologia de peixes.** Ed. 2. Maringá: Eduem, p. 199, 2006.

EIRAS, J. C.; TAKEMOTO, R. M.; PAVANELLI, G. C. **Diversidade de parasitos de peixe de água doce**. Cicletec, Maringá. p.1 – 289, 2010.

FAO – Food and Agriculture Organization of the United Nations. (2018). **The State of World Fisheries and Aquaculture 2018**. Metting the sustainable development goals. Rome. 227 pp.

FISCHER, C.; MALTA, J.C.O.; VARELLA, A. M. B. A fauna de parasitas do tambaqui, *Colossoma macropomum* (Cuvier, 1818) (Characiformes: Characidae) do médio rio Solimões, Estado do Amazonas (AM) e do baixo rio Amazonas, Estado do Pará (PA), e seu potencial como indicador biológico. **Acta Amazonica,** v. 33, p. 651- 662, 2003.

FIM, J. D. I. Sistema integrado de cultivo entre animais e peixes. In: VAL, A. L.; HONCZARYK, A. Criando peixes na Amazônia. Manaus: **Instituto Nacional de Pesquisas da Amazônia**, p. 149, 1995.

FUJIMOTO, R. Y.; VENDRUSCOLO, L.; SCHALCH, S. H. S.; MORAES, F. R. Avaliação de três diferentes métodos para o controle de monogenéticos e *Capillaria* sp. (Nematoda:

Capillariidae), parasitos de acará-bandeira (*Pterophyllum scalare* Liechtenstein, 1823). **Boletim do Instituto de Pesca,** v. 32, p. 183 - 190, 2006.

FORWOOD, J. M.; HARRIS, J. O.; DEVENEY, M. R. Efficacy of bath and orally administered praziquantel and fenbendazole against *Lepidotrem abidyana* Murray, a monogenean parasite of silver of silver perch, *Bidyanus didyanus* (Mitchell). **Journal of Fish Diseases**, v. 36, p. 939-947, 2013.

GODOI, M. M. I. M.; ENGRACIA, V.; LIZAMA, M. L. A. P.; TAKEMOTO, R. M. Parasite-host relationship between the tambaqui (*Colossoma macropomum* Cuvier 1818) and ectoparasites, collected from fish farms in the city of Rolim de Moura, State of Rondônia, Western Amazon, Brazil. **Acta Amazonica,** v. 42, n. 4, p. 515 – 524, 2012.

GOMES, L.C.; SIMÕES, L.N.; ARAUJO-LIMA, C.A.R.M. Tambaqui (*Colossoma macropomum*). In: Baldisserotto, B., Gomes, L.C. (Eds.). **Espécies Nativas Para Piscicultura no Brasil** - Santa Maria, p. 175 – 204, 2010.

GOVEN, B.; AMEND, D. Mebendazole/trichlorfon combination: a new anthelmintic for removing monogenetic trematodes from fish. **Journal of Fish Biology**, v. 20, p. 373-378, 1982.

GUSTAFSSON, L. L.; BEERMAN, B.; ABDY, Y. A. **Handbook of drugs for tropical parasitic infections.** London: Ed. Taylor & Francis, p. 151, 1987.

GRAEF, E. W. As espécies de peixes com potenciais para a criação no Amazonas. In: Val AL, Honczaryk A**. Criação de Peixes na Amazônia.** Manaus: INPA, p. 29- 41, 1995.

HIRAZAWA, N.; OHTAKA, T.; HATA, K. Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*. **Aquaculture**, v. 188, p.1–13, 2000.

HIRAZAWA, N.; AKIYAMA, K.; UMEDA, N. Differences in sensitivity to the anthelmintic praziquantel by the skin-parasitic monogeneans *Benedenia seriolae* and *Neobenedenia girellae*. **Aquaculture**, v. 404-405, p. 59–64, 2013.

HORTON, J. Global anthelmintic chemotherapy programs: learning from history. **Trends in Parasitology**, v. 19, p. 405-408, 2003.

IBGE - Instituto Brasileiro de Geografia e Estatística. **Produção da Pecuária Municipal. Grandes Regiões e Unidades da Federação**. Rio de Janeiro: v. 41, 2016.

JOHNSON, S. C.; KENT, M. L.; WHITAKER, D. J.; MARGOLIS, L. Toxicity and pathological effects of orally administered ivermectin in Atlantic, chinook, and Coho salmon and steelhead trout. **Diseases of Aquatic Organisms,** v. 17, p. 107-112, 1993.

JOHNSON, K. A.; HEINDEL, J. A. Eficacy of manual removal and ivermectin gavage for control of *Salmincola californiensis* (Wilson) infestation of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. **Journal of Fish Diseases**, v. 24, p. 197-203, 2001.

KAYIS, S.; OZCELEP, T.; CAPKIN, E.; ALTINOK, I. Protozoan and metazoan parasites of cultured fish in turkey and their applied treatments. **Israeli Journal Aquaculture, Bamidged**, v. 61, n. 2, p. 93-102, 2009.

KATHARIOS**,** P**.;** ILIOPOULOU-GEORGUDAKI1, J.; KAPATA-ZOUMBOS, K.; SPIROPOULOS, S. Toxicity of intraperitoneally injected ivermectin in sea bream, *Sparus aurata***. Fish Physiology and Biochemistry**, v. 25, p. 99–108, 2002.

KATHARIOS, P.; PAPANDROULAKIS, N.; DIVANACH, P. Treatment of *Microcotyle* sp. (Monogenea) on the gills of cage-cultured red porgy, *Pagrus pagrus* following baths with formalin and mebendazole **Aquaculture**, v. 251,p. 167– 171, 2006.

KIM, K. H.; CHOI, E. S. Treatment of *Microcotyle sebastis* (Monogenea) on the gills of cultured rockfish (*Sebastes schelegeli*) with oral administration of mebendazole and bithionol. **Aquaculture,** v. 167, p. 115-121, 1998.

KIM, K. H.; PARK, S. I.; JEE, B. Y. Efficacy of oral administration of praziquantel and mebendazole against *Microcotyle sebastis* (Monogenea) infestation of cultured rockfish (*Sebastes schlegeli*). **Fish Pathology**, v. 33, p. 467-471, 1998.

KUBITZA, F.; CAMPOS, J. L.; ONO, E. A.; ISTCHUK, P. I.Panorama da piscicultura no Brasil particularidades regionais da piscicultura custo de produção e preço de venda e os gargalos que limitam a expansão dos cultivos. **Panorama Aquicultura**, v.22, n.134, p.14-23, 2012.

KHALIL, A. I.; OSMAN, G. Y.; MAGHRABI, O. A. M.; RADWAN, N. A.; MSALAM, M. A.In vitro Effect of LC_{90} of albendazole and *Allium sativum* water extract on the fine structure of *Capillaria* sp. (Capillaridae: Nematoda). **Journal of Bioscience and Applied Research**, v. 2, n. 2, p.125-135, 2016.

LI, P.; WANG, X.; GATLIN, D. M. Short communication excessive dietary levamisole suppresses growth performance of hybrid striped bass, *Morone chrysops* x*_M. saxatilis*, and elevated levamisole *In vitro* impairs macrophage function. **Aquaculture Research**, p. 1380-138, 2004.

LI, P.; Wang, X.; Gatlin, D. M. Evaluation of levamisole as a feed additive for growth and health management of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). **Aquaculture,** v. 251, n. 2-4, p. 201–209, 2006.

LOPERA-BARRERO, N. M. R. P.; RIBEIRO, J. A.; POVH, L. D. M.; VARGAS, A. R.; POVEDA, P.; DIGMAYER, M. As Principais Espécies Produzidas No Brasil, In: LOPERA-BARRERO, N. M. R. P.; RIBEIRO, J. A.; POVH, L. D. M.; VARELLA, M. B.A.; PEIRO, S. N.; MALTA, S. N. Monitoramento da parasitofauna de *Colossoma macropomum* (Curvier, 1818) (Osteichthyes: Characidae) cultivado em tanques rede em um lago de várzea na Amazônia. Aquabio. **XII Simpósio Brasileiro de Aquicultura**. Goiânia, p. 95-105, 2003.

LUQUE, J. L. Biologia, epidemiologia e controle de parasitos de peixes. In: XIII Congresso Brasileiro de Parasitologia Veterinária & I Simpósio Latino-Americano de Ricketisioses, Ouro Preto, MG, 2004. **Revista Brasileira de Parasitologia Veterinária**, v. 13, n. 1, p. 161, 2004.

MALTA, J.C.O.; GOMES, A.L.S.; ANDRADE, S.M.S.; VARELLA, A.M.B. Infestações maciças por acantocéfalos, *Neoechinorhynchus buttnerae* Golvan, 1956, (Eoacanthocephala: Neoechinorhynchidae) em tambaquis jovens, *Colossoma macropomum* (Cuvier, 1818) cultivados na Amazônia Central. **Acta Amazonica,** v. 31, n. 10, p. 133-143, 2001.

MANLEY, K. M.; EMBIL, J. A. *In vitro* effect of ivermectin on *Pseudoterranova decipiens* survival. **Journal of Helminthology,** v. 63, p. 72-74, 1989.

MARKOSKI, M. M.; TRINDADE, E. S.; CABRERA, G.; LASCHUK, A.; GALANTI, N.; ZAHA, A.; NADER, H. B.; FERREIRA, H. B. Praziquantel and albendazole damaging action on in vitro developing *Mesocestoides corti* (Platyhelminthes: Cestoda). **Parasitology International**, v.55, p. 51–61, 2006.

MARTINS, M. L. Cuidados básicos e alternativos no tratamento de enfermidades de peixes na aquicultura brasileira. In: RANZANI-PAIVA, M. J., TAKEMOTO, R. M., LIZAMA, M. A.P. **Sanidade de organismos aquáticos**. São Paulo: Editora Varela, p. 357- 370, 2004.

MARTINS, M. L.; ONAKA, E. M.; MORAES, F. R.; FUJIMOTO, R. Y. Mebendazole treatment againt *Anacanthorus penilabiatus* (monogenea, Dactylogyridae) gill parasite of cultivated *Piaractus mesopotamicus* (Osteichthyes, Characidae) in Brazil. Efficacy and Phematology. **Acta Parasitologica**, v. 46, p. 332-326, 2001.

MARTINS, M. L.; MOURINO, J. L. P.; CHAGAS, E. C. C.; SILVA, B. C.; FUJIMOTO, R. Y., PÁDUA, S. B. Ecotoparasitários na aquicultura. In: BALDISSEROTTO, B.; GOMES, L. C.; HEINZMANN, B. M.; CUNHA, M. A. **Farmacologia aplicada a aquicultura**. Santa Maria, RS: Ed. UFSM, 2017.

MORAES, F. R.; MARTINS, M. L. Condições predisponentes e principais enfermidades de teleósteos em piscicultura intensiva. In: CYRINO, J. E. P. et al. (ed). **Tópicos especiais em piscicultura de água doce tropical intensiva.** São Paulo: Tec Art,. Cap. 3, p. 343-386, 2004.

MORAIS, A. M.; VARELLA, A. M. B.; VILLACORTA-CORREA, M. A.; MALTA, J. C. O. A fauna de parasitos em juvenis de tambaqui *Colossoma macropomum* (Cuvier, 1818) (Characidae: Serrasalminae) criados em tanques-rede em lago de várzea da Amazônia central. **Biologia Geral e Experimental**, v. 9, p. 14-23, 2009.

MORALES-SERNAA, F. N.; CHAPA-LÓPEZA, M.; MARTÍNEZ-BROWNA, J. M.; IBARRA-CASTROA, L.; MEDINA-GUERREROA, R. M.; FAJER-ÁVILAA, E. J. Efficacy of praziquantel and a combination anthelmintic (Adecto®) in bath treatments against *Tagia ecuadori* and *Neobenedenia melleni* (Monogenea), parasites of bullseye puffer fish. **Aquaculture, v.** 492, p. 361–368, 2018.

MORIWAKI, C.; COSTA, G. L.; FERRACINI, C. N.; MORAES, F. F.; ZANIN, G. M.; PINEDA, E. A. G.; MATIOLIG. Enhancement of solubility of albendazole by complexation with β-cyclodextrin. **The Brazilian Journal of Chemical Engineering**, v. 25, n. 2, p. 255-267, 2008.

MLADINEO, I.; MARSIC-LUCIC, J.; BUZANCIC, M. Toxicity and gross pathology of ivermectin bath treatment in sea bream *Sparus aurata, L*. **Ecotoxicology and Environmental Safety**, p. 438–442, 2006.

NOGA, E. J. FishDisease. **Diagnosis and Treatment.** St. Louis: Mosby-Year Book, p.367, 1996.

ONAKA, E. M.; MARTINS, M. L.; MORAES, F. R. Eficácia do albendazol e praziquantel no controle de *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae), parasito de pacu *Piaractus mesopotamicus* (Osteichthyes: Characidae). I. banhos terapêuticos. **Boletim do Instituto de Pesca**, v. 29, p.101-107, 2003.

ONAKA, E. M. Principais parasitoses em peixes de água doce no Brasil. In: Tavares-Dias, M. **Manejo e Sanidade de peixes em cultivo**. Embrapa - Amapá, Macapá. p. 526 – 574, 2009.

OSMAN, G. Y.; KHALIL, A. I.; RADWAN, N. A.; OMAYMA, A. M.; EL-NABI, S. E.; MSALAM, A. M. A. In- vitro evaluation of antithelminthic activity of albendazole and three midicinal plant extracts on *Capillaria* sp. and its characterization with RAPD-PCR. **Research Journal of Pharmaceutical, Biological and Chemical Sciences**, v. 7, n. 4, p. 2965-2976, 2016.

PAHOR-FILHO, E.; JÚNIOR, J. P.; PILARSKI, F.; URBINATI, E. C. Levamisole reduces parasitic infection in juvenile pacu (Piaractus mesopotamicus). **Aquaculture**, v. 470, p. 123–128, 2017.

PAVANELLI, G. C.; EIRAS, J. C.; TAKEMOTO, R. M. **Doenças de peixes profilaxia, diagnóstico e tratamento**. Maringá: Editora da Universidade Estadual de Maringá, p. 99, 2008.

PRAVETTONI, V.; PRIMAVESI, L.; PIANTANIDA, M. *Anisakis* simplex: current knowledge. **European Annals of Allergy *and* Clinical Immunolog**, v. 44, n. 4, p. 150-156, 2012

RANG, H. P.; DALE, M. MAUREEN; RITTER, JAMES; FLOWER, *R. J.* Rang & Dale . **Farmacologia**. 6ª ed. Rio de janeiro: Elsevier, p. 829, 2007.

REIS, R. E.; ALBERT, J. S.; DI DARIO, F.; MINCARONES, M. M.; PETRY, P., ROCHA, L. A. Fish biodiversity and conservation in South America. **Journal of Fish Biology**, v. 89, p. 12–47, 2016.

REIS, R. E.; KULLANDER, S. O.; FERRARIS, C. J. **Check list of the freshwater fishes of South and Central America**. Edipucrs, Porto Alegre, Brasil. 2003.

ROMERO, C.; NAVARRO, C. I.; ANCHEZ, J. M.; VALERO, A. Peppermint (*Mentha piperita*) and albendazole against anisakiasis in an animal model. **Tropical Medicine & International Health**, v. 19, n. 12, p.1430–1436, 2014.

RENOUX, G. The general immunopharmacology oflevamisole. **Drugs** p. 19-89, 1980.

SADO, R. Y.; BICUDO, I. J. A. Dietary levamisole influenced hematological parameters of juvenile pacu, *Piaractus mesopotamicus* (Holmberg 1887). **Journal of the World Aquaculture Society.** v. 41, 2010.

SAMUEL, W. M.; GRAY, J. B. Efficacy of ivermectin against *Parelaphostrongylus Anderson* (Nematoda, Metastrongyloidea) in white-tailed deer *(Odocoileus virginianus).***Journal of Wildlife Diseases,**v. 24, p. 491-495, 1985.

SANTAMARINA, M. T.; TOJO, J.; UBEIRA, F. M.; QUINTEIRO, P.; SANMARTIN, M. L. Anthelmintic treatment against *Gyrodactylus* sp. infecting rainbow trout *Oncorhynchus mykiss*. **Diseases of Aquatic Orgamisms,** v. 10, p. 39-43, 1991.

SANTOS, E. F.; TAVARES-DIAS, M.; PINHEIRO, D. A.; NEVES, L. R.; MARINHO, R.G.B.; DIAS, M. K. R. Fauna parasitária de tambaqui *Colossoma macropomum* (Characidae) cultivado em tanque-rede no estado do Amapá, Amazônia oriental. **Acta Amazonica,** v. 43, p. 107-114, 2013.

SILVA, A. S.; PEDRON, F. A.; ZANETTE, R. A.; MONTEIRO, S. G.; NETO, J. R. Eficácia do praziquantel no controle ao parasito *Clinostomum complanatum* Rudolphi, 1918 (Digenea, Clinostomidae) em peixes da espécie *Rhamdia quelen* Quoy & Gaimard, 1824 (jundiá). **Pesquisa Agropecuária Gaúcha**, v.15, n.1, p.73-76, 2009.

SITJÀ-BOBADILLA, A.; FELIPE, M. C.; ALVAREZ-PELLITERO, P. *In vivo* and *in vitro* treatments against *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (*Sparus aurata* L.). **Aquaculture**, v. 261, p. 856–864, 2006.

SOARES, B. V.; NEVES, L. R.; OLIVEIRA, M. S. B.; CHAVES, F. C. M.; DIAS, M. K. R.; CHAGAS E. C.; TAVARES-DIAS, M. Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma macropomum* (tambaqui) and its physiological and histopathological effects. **Aquaculture**, v. 452, p. 107-114, 2017.

SOARES, M. G. M.; COSTA, E. L.; SIQUEIRA-SOUZA, F. K.; ANJOS, H. D. B; YAMAMOTO, K. C.; FREITAS, C. E. C. **Peixes de lagos do médio Rio Solimões**. 2ª ed. Instituto Piatam, Manaus, p. 1-160, 2011.

SCOTT, F. B.; MARTINS, I. V. F.; SOUZA, C.P.; CORREIA, T. R. Aspectos geraisdo controle da pulga *Ctenocephalides felis felis* em cães. **Hora Veterinária,** v. 125, p. 13 -18, 2002.

SCHMAHL, G.; MEHLHORN, H. Treatment of fish parasites: 1.Praziquantel effective against Monogenea (*Dactylogyrus vastor*, *Dactylogyrus extensus*, *Diplozoon paradoxum*. Zeitschrift für Parasitenkunde, v.71, p. 727-737, 1985.

SCHMAHL, G. The chemotherapy of monogeneans, which parasitize fish: a review. **Folia Parasitologica**, v. 38, p. 97-106, 1991.

SCHMAHL, G.; TARASCHEWSKI, H. Treatment of fish parasites: 2. Effects of praziquantel, niclosamide, levamizole-HCL, and metrifonate on monogeneas (*Gyrodactylus aculeate*, *Diplozoon paradoxum*). **Parasitology Research**, v. 73, n. 4, p. 341-351, 1987.

SCHALCH, S. H. C.; MORAES, F. R.; SOARES, V. E**.** Praziquantel, levamisol e diflubenzuron no controle de *Dolops carvalhoi* (Crustacea: Branchiura) e *Anacanthoruspenilabiatus* (Monogenea: Dactylogyridae) em *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae). **Revista Brasileira de Parasitologia Veterinária**, v. 18, n. 1, p. 53-59, 2009.

SHARP, N. J.; DIGGLES, B. K.; POORTENAAR, C. W.; WILLIS, T. J. Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi* lalandi in New Zealand. **Aquaculture**, v. 236, n.1, p. 67–83, 2004.

SZEKELY, C.; MOLNAR, K. Treatment of *Ancylodiscoides vistulensis* monogenean infestations of the European catfish (*Silurus glanis*). **Bulletin of the European Association of Fish Pathologists**, v. 10, p. 74, 1990.

TARASCHEWSKI, H.; RENNER, C.; MEHLHORN, H. Treatment of fish parasites. **Parasitology Research**, v. 74, p. 281-289, 1988.

TAVARES-DIAS, M.; LEMOS, J. R. G.; ANDRADE, S. M. S.; AQUINO-PEREIRA, S. L. Ocorrência de ectoparasitos em *Colossoma macropomum* Cuvier, 1818 (Characidae) cultivados em estação de pisciculturas na Amazônia central. **CIVA**; p. 726 -731, 2006.

TAVARES-DIAS, M.; MARTINS, M. L. An overall estimation of losses caused by diseases in the Brazilian fish farms. **Journal of Parasitic Diseases**, v. 41, n. 4, p. 913-918, 2017.

TAVARES-DIAS, M. Current knowledge on use of essential oils as alternative treatment against fish parasites. **Aquatic Living Resources,** v.31, 2018. https://doi.org/10.1051/ alr/2018001

THATCHER, V. E. **Amazon fish parasites**. 2ª edição. Sofia, Moscow: Pensoft Publishers, p.1-508, 2006.

THIRIPURASUNDARI, M.; SATHYA, K.; UMA, A.; SRINIVASAN, R. M.; RAJASEKAR, P. A comparative study on the toxicity of ivermectin in zebra fish and catla fish models. **Indo American Journal of Pharmaceutical Research**, p. 2231-6876, 2014.

UCÁN-MARÍN, F.; ERNST, W.; O'DOR, R. K.; SHERRY. J.; Effects of food borne ivermectin on juvenile Atlantic salmon (*Salmo salar* L.): survival, growth, behavior, and physiology. **Aquaculture**, p. 169–175, 2012.

UNTERGASSER, D. **Handbook of Fish Disease**. Plaza, Neptune City: TFH publications Inc. 1989, 160 p.

VARÓ, I.; RIGOS, G.; NAVARRO, J. C.; RAMO, J.; CALDUCH-GINER, J.; HERNÁNDEZ, A.; PERTUSA, J.; TORREBLANCA, A. Effect of ivermectin on the liver of gilthead sea bream *Sparus aurata*: A proteomic approach. **Chemosphere**, p. 570–577, 2010.

ZANON, R. B.; CEROZI, B. S.; CYRINO. J. E. P. Dietary Levamisole as Immunostimulant for Striped Surubim, *Pseudoplatystoma reticulatum.* **Journal of the World Aquaculture Society,** v. 45, n. 6, 2014.

ZHANG, X. P.; LI, W. X.; AI, T. S.; ZOU, H.; WU, S. G.; WANG, G. T. The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrusvastator* (Monogenea). **Aquaculture**, v. 420–421, p. 302-307, 2014.

ZUSKOVA, E., PIACKOVA, V., MACHOVA, J., CHUPANI, L., STEINBACH, C., STARA, A., VELISEK. Efficacy and toxicity of praziquantel in helminth-infected barbel (*Barbus barbus* L.). **Journal of Fish Diseases**, p. 643-649, 2018.

ARTIGO 1 - Albendazole, levamisole and ivermectin are effective against monogeneans of *Colossoma macropomum* (Pisces: Serrasalmidae)

Artigo aceito no periódico Journal of Fish Diseases

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

Running Head: Anthelminthics are effective against monogeneans of tambaqui

Carliane Maria Guimarães Alves[1], Joziele Neves Nogueira[2], Isaac Belo Barriga[2], Joilson Rodrigues dos Santos[3], Gracienhe Gomes Santos[3], Marcos Tavares-Dias[1,4*]

[1] Federal University of Amapá (UNIFAP), Postgraduate Program in Environmental Sciences (PPGCA), Macapá, AP, Brazil

[2] University of the State of Amapá (UEAP), Macapá, AP, Brazil

[3] Faculdade de Macapá (FAMA), Macapá, AP, Brazil

[4] Embrapa Amapá, Macapá, AP, Brazil

* Corresponding author: marcos.tavares@embrapa.br

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, 1500 and 2000 mg L^{-1}), ivermectin (200, 250, 300 and 350 mg L^{-1}) and levamisole (50, 75, 100 and 125 mg L^{-1}) were 100% effective against *Anacanthorus spatulatus*, *Notozothecium janauachensis*, *Mymarothecium boegeri* and *Linguadactyloides brinkmanni*, while mebendazole (125, 150, 175 and 200 mg L^{-1}) and praziquantel (5, 10, 15 and 20 mg L^{-1}) were ineffective. Fish mortality in 24 h therapeutic baths with 500 mg L^{-1} of albendazole was 6.6%, but the behavior of the animals remained unchanged, while 200 mg L^{-1} of ivermectin caused lethargy, signs of hypoxia and 100% mortality within 2 h, and 125 mg L^{-1} of levamisole caused no mortality. The efficacy of 500 mg/L of albendazole was 48.6% in the 24 h baths, while that of 125 mg L^{-1} 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^{-1} of levamisole to control and treat monogenean infestations on *C. macropomum* in fish farming.

Keywords: Anthelmintic, Infection, Fish, 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 poses a constant challenge, since these tasks are greatly complicated by the limited availability of licensed anthelmintic drugs (Zuskova et al., 2018; Morales-Serna et al., 2018) with varying degrees of effectiveness. Anthelmintic drugs such as albendazole, mebendazole, ivermectin, levamisole and praziquantel have been employed for the control and treatment against monogenean parasites that infest farmed fish (Santamarina et al., 1991; Kim & Choi, 1998; Hirazawa et al., 2000; Martins et al., 2001; Onaka et al., 2003; Sitjà-Bobadilla et al., 2006; Hirazawa et al., 2013; Zhang et al., 2014; Morales-Serna et al., 2018). Parasite infections can be controlled and treated by eliminating developmental stages in hosts, thus interrupting the life cycle of parasites (Bader et al., 2017). However, the concentrations of these different drugs must be not only safe and effective but also nontoxic 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 (Santamarina et al., 1991; Mladineo et al., 2006; Thiripurasundari et al., 2010; Varó et al., 2010), whereas clinical concentrations of albendazole, mebendazole, levamisole and praziquantel present a low toxicity (Kim & Choi, 1998; Martins et al., 2001; Onaka et al., 2003; Hirazawa et al., 2013). 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 seven 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 ± 0.1ºC, dissolved oxygen content was 5.6 ± 0.2 mg L^{-1}, pH was 5.3 ± 0.2, total ammonia was 0.5 ± 0.2 mg L^{-1}, alkalinity was 10.0 ± 0 mg L^{-1} and hardness was 10.0 ± 0 mg L^{-1}. 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^{-1}, 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^{-1} 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 h.

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^{-1})			
Albendazole	500	1000	1500	2000
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 h. The mean temperature in the tanks was 30.4 ± 0.1ºC, dissolved oxygen content was 5.5 ± 0.2 mg L^{-1}, pH was 5.3 ± 0.2, total ammonia was 0.5 ± 0.2 mg L^{-1}, alkalinity was 10.0 ± 0 mg L^{-1} and hardness was 10.0 ± 0 mg L^{-1}.

The therapeutic baths of 24 h consisted of four treatments (0 and 125 mg L^{-1} levamisole, 0 and 200 mg L^{-1} ivermectin and 0 and 500 mg L^{-1} albendazole) with three repetitions each, and

10 fish in each repetition, making a total of 30 fish per treatment. All treatment 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 h, the behavior 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 et al. (2006). After quantification of the parasites, the prevalence and mean abundance and mean intensity of infestation were calculated as described by Bush et al. (1997) and the efficacy of each treatment as described by Sommerville et al. (2016).

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 analyzed by the Kruskal-Wallis test, followed by Dunn's test for comparison among medians. The efficacy of albendazole and levamisol was compared using the test-t (Zar, 2010).

3. RESULTS

In vitro assays, all the albendazole concentrations caused immobilization 100% of the monogeneans of *C. macropomum* (*Anacanthorus spatulatus*, *Notozothecium janauachensis*, *Mymarothecium boegeri* and *Linguadactyloides brinkmanni*) after 2 h 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^{-1} caused total immobilization of the parasites in just 20 min of exposure. Mebendazole concentrations of 175 and 200 mg L^{-1} 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 h 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 was influenced by the solvent DMSO.

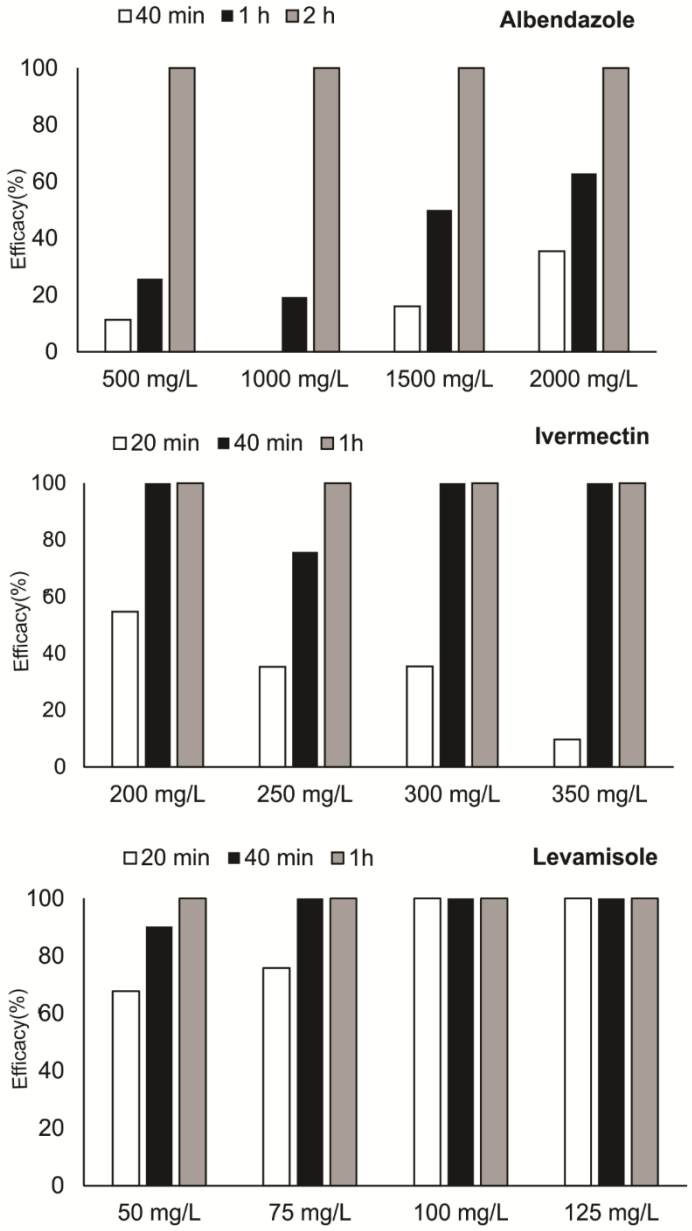

Figure 1. *In vitro* efficacy of anthelmintic drugs against monogeneans of *Colossoma macropomum* in different exposure times.

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^{-1} of albendazole was similar to that of the controls in water from the breeding tank (Table 3). The therapeutic baths containing 500 mg L^{-1} of albendazole showed 48.6% antiparasitic efficacy, while those containing 125 mg L^{-1} 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^{-1} of albendazole and 125 mg L^{-1} of levamisole.

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 h	Water of tank	20.7 ± 0.6	0
1 h	Water of tank	20.7 ± 0.6	0
3 h	Water of tank	20.7 ± 0.6	0
6 h	Water of tank	0	100
0 h	DMSO	20.0 ± 0	0
20 min	DMSO	9.0 ± 1.7	55.0
40 min	DMSO	4.0 ± 4.0	80.0
1 h	DMSO	0	100
0 h	500 mg L^{-1} of albendazole	26.0 ± 1.7	0
20 min	500 mg L^{-1} of albendazole	19.3 ± 1.1	5
40 min	500 mg L^{-1} of albendazole	18.3 ± 1.5	9.8
1 h	500 mg L^{-1} of albendazole	15.3 ± 4.7	24.6
2 h	500 mg L^{-1} of albendazole	0	100
0 h	1000 mg L^{-1} of albendazole	22.0 ± 1.0	0
20 min	1000 mg L^{-1} of albendazole	22.0 ± 1.0	0
40 min	1000 mg L^{-1} of albendazole	20.7 ± 1.1	6.1
1 h	1000 mg L^{-1} of albendazole	16.7 ± 3.0	24.2
2 h	1000 mg L^{-1} of albendazole	0	100
0 h	1500 mg L^{-1} of albendazole	20.0 ± 0	0
20 min	1500 mg L^{-1} of albendazole	20.0 ± 0	0
40 min	1500 mg L^{-1} of albendazole	17.3 ± 2.3	13.3
1 h	1500 mg L^{-1} of albendazole	10.3 ± 8.5	48.3
2 h	1500 mg L^{-1} of albendazole	0	100
0 h	2000 mg L^{-1} of albendazole	26.0 ± 1.7	0
20 min	2000 mg L^{-1} of albendazole	19.0 ± 5.3	27.0
40 min	2000 mg L^{-1} of albendazole	13.3 ± 8.5	48.7
1 h	2000 mg L^{-1} of albendazole	7.7 ± 7.1	70.5
2 h	2000 mg L^{-1} of albendazole	0	100

Table 2 (Continued). *In vitro* efficacy of the concentrations of anthelminthics against monogeneans of *Colossoma macropomum* in different exposure times.

Exposure time	Treatments	Live parasites	Mortality (%)
0 h	200 mg L^{-1} of ivermectin	21.6 ± 1.5	0
20 min	200 mg L^{-1} of ivermectin	9.3 ± 4.0	57.0
40 min	200 mg L^{-1} of ivermectin	0	100
0 h	250 mg L^{-1} of ivermectin	20.3 ± 0.6	0
20 min	250 mg L^{-1} of ivermectin	13.3 ± 4.9	34.4
40 min	250 mg L^{-1} of ivermectin	5.0 ± 5.0	75.4
1 h	250 mg L^{-1} of ivermectin	0	100
0 h	300 mg L^{-1} of ivermectin	21.6 ± 1.5	0
20 min	300 mg L^{-1} of ivermectin	11.6 ± 2.9	46.1
40 min	300 mg L^{-1} of ivermectin	0	100
0 h	350 mg L^{-1} of ivermectin	23.7 ± 1.1	0
20 min	350 mg L^{-1} of ivermectin	18.6 ± 3.2	46.1
40 min	350 mg L^{-1} of ivermectin	0	100
0 h	50 mg L^{-1} of levamisole	22.0 ± 2.0	0
20 min	50 mg L^{-1} of levamisole	6.7 ± 5.8	69.7
40 min	50 mg L^{-1} of levamisole	2.0 ± 2.0	90.9
1 h	50 mg L^{-1} of levamisole	0	100
0 h	75 mg L^{-1} of levamisole	19.3 ± 1.1	0
20 min	75 mg L^{-1} of levamisole	5.0 ± 5.0	74.1
40 min	75 mg L^{-1} of levamisole	0	100
0 h	100 mg L^{-1} of levamisole	19.3 ± 1.1	0
20 min	100 mg L^{-1} of levamisole	0	100
0 h	125 mg L^{-1} of levamisole	21.0 ± 1.0	0
20 min	125 mg L^{-1} of levamisole	0	100

Table 2 (Continued). *In vitro* efficacy of the concentrations of anthelminthics against monogeneans of *Colossoma macropomum* in different exposure times.

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

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^{-1} of levamisole (n =30		500 mg L^{-1} 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]

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

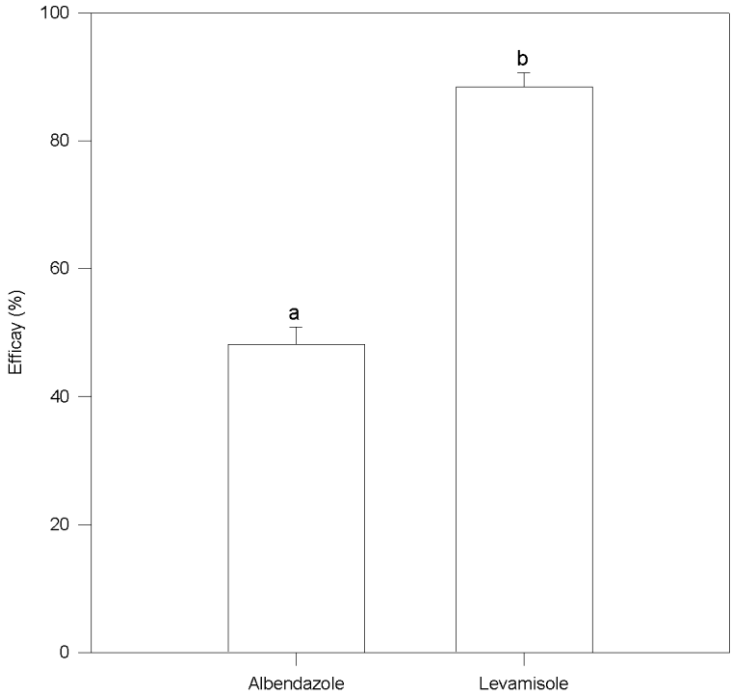

Figure 2. Therapeutic baths efficacy with 500 mg L^{-1} of albendazole and 125 mg L^{-1} levamisole in monogeneans of *Colossoma macropomum* after 24 h of treatment. Mean values followed by different letters indicate differences between treatments according to test-t ($p<0.05$).

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

4. DISCUSSION

All the concentrations of levamisole (50-125 mg L^{-1}), albendazole (500-2000 mg L^{-1}) 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^{-1} 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^{-1} 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, 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 is 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^{-1}) and praziquantel (5-20 mg L^{-1}) against monogeneans of *C. macropomum* to the solvent DMSO, given the low water solubility, and hence, low availability of both drugs (Swanepoel et al., 2003; Liu et al., 2018). 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^{-1}) against monogeneans *Neobenedenia melleni*. *In vitro* efficacy of 0.005 mg L^{-1} of mebendazole and 2.5 mg L^{-1} 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^{-1} of praziquantel against *Benedenia seriolae* and *Neobenedenia melleni* of *Seriola quinqueradiata* and *Seriola dumerili*. Given the *in vitro* efficacy of 2.5-20.0 mg L^{-1} praziquantel against *Tagia ecuadori* and *Neobenedenia 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 paralyzes 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 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^{-1} of ivermectin, 100% fish mortality occurred within 2 h of exposure, while 500 mg L^{-1} of albendazole caused a fish mortality rate of 6.6% within 24 h of exposure. *Piaractus mesopotamicus* has also reportedly exhibited good drug tolerance after 30 min of exposure to 50-500 mg L^{-1} of albendazole (Onaka et al., 2003). Therapeutic baths with 1.8 mg L^{-1} of ivermectin also caused 100% mortality of *Sparus aurata* after 96 h of exposure (Mladineo et al., 2006). Santamarina et al. (1991) reported that therapeutic baths with 0.031 mg L^{-1} 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^{-1}

of ivermectin, as did *Catla catla* exposed to 0.007 mg L^{-1} of this drug, due to neurotoxicity and hepatotoxicity (Thiripurasundari et al., 2010; 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^{-1} of albendazole were only 48.6% effective against monogeneans of *C. macropomum* after 24 h of exposure. In contrast, 125 mg L^{-1} 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^{-1} of albendazole were 46.5% affective against *Anacanthorus penilabiatus* of *P. mesopotamicus* (Onaka et al., 2003). In addition, 3h therapeutic baths with 100 mg L^{-1} of levamisole were effective against *Gyrodactylus* sp. of *O. mykiss* (Santamarina et al., 1991), and 10-18 min baths with 20-50 mg L^{-1} of levamisole were effective against *Urocleidus vistulensis* of *Silurus glanis* (Székely & Molnár, 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 were 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 500 mg L^{-1} 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 DISCLOSURES

This study was approved by the Ethics Committee on Animal Use of Embrapa Amapá (Protocol Nº 013/2018-CEUA/CPAFAP) and was conducted in accordance with the principles of the Brazilian College of Animal Experimentation (COBEA).

REFERENCES

Bader, C., Chelladurai, J. J., Starling, D. E., Jones, D. E., & Brewer, M. T. (2017). Assessment of *in vitro* killing assays for detecting praziquantel induced death in *Posthodiplostomum minimum* metacercariae. *Experimental Parasitology*, 181, 70-74.doi: 10.1016/j.exppara.2017.07.008

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. Revisited. *The Journal of Parasitology*, 83, 575-583.

Collymore, C., Watral, V., White, J. R., Colvin, M. E., Rasmussen, S., Tolwani, R. J., & Kent, M. L. (2014). Tolerance and efficacy of emamectin benzoate and ivermectin for the treatment of *Pseudocapillaria tomentosa* in laboratory Zebrafish (*Danio rerio*). *Zebrafish*, 11, 490–497.doi: 10.1089/zeb.2014.1021.

Eiras, J. C., Takemoto, R. M., & Pavanelli, G. C. (2006). *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes*. (2ª ed). Maringá, Eduem.

FAO – Food and Agriculture Organization of the United Nations. (2018). *The state of world fisheries and aquaculture 2018*. Meting the sustainable development goals. Rome.

Hashimoto, G.S.O., Ruiz-Neto, F.M., M.L., Achille, M., Chagas, E.C., Chaves, F.C.M., & Martins, M.L. (2016). Essential oils *of Lippia sidoides and Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia. *Aquaculture*, 450, 182–186.https://doi.org/10.1016/j.aquaculture.2015.07.029

Hirazawa, N., Ohtaka, T., & Hata, K. (2000). Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*. *Aquaculture*, 188, 1–13.

Hirazawa, N., Akiyama, K., & Umeda, N. (2013). Differences in sensitivity to the anthelmintic praziquantel by the skin-parasitic monogeneans *Benedenia seriolae* and *Neobenedenia girellae*. *Aquaculture*, 404-405, 59–64.https://doi.org/10.1016/j.aquaculture.2013.04.021

Kim, K. H., & Choi, E. S. (1998). Treatment of *Microcotyle sebastis* (Monogenea) on the gills of cultured rockfish (*Sebastes schlelegelii*) with oral administration of mebendazole and bithionol. *Aquaculture***,** 167, 115-121.https://doi.org/10.1016/S0044-8486(98)00300-7

Liu, Y., Wang, T., Ding, W., Dong, C., Wang, X., & Chen, J., LI, Y. (2018). Dissolution and oral bioavailability enhancement of praziquantel by solid dispersions. *Drug Delivery and Translational Research*, 8, 580–590.doi: 10.1007/s13346-018-0487-7.

Martins, M. L., Onaka, E. M., Moraes, F. R., & Fujimoto, R. Y. (2001). Mebendazole treatment againt *Anacanthorus penilabiatus* (monogenea, Dactylogyridae) gill parasite of cultivated *Piaractus mesopotamicus* (Osteichthyes, Characidae) in Brazil. Efficacy and Phematology. *Acta Parasitologica*, 46, 332-326.

Martins, M. L., Mourino, J. L. P., Chagas, E. C. C., Silva, B. C., Fujimoto, R. Y., & Pádua, S. B. (2017). Ectoparasitários na aquicultura. In: Baldisserotto, B., Gomes, L. C., Heinzmann, B. M., & Cunha, M. A. *Farmacologia aplicada a aquicultura* (pp. 127-181). Santa Maria: UFSM.

Morales-Serna, F. N., Chapa-López, M., Martínez-Brown, J. M., Ibarra-Castro, L., Medina-Guerrero, R. M., & Fajer-Ávila, E. J. (2018). Efficacy of praziquantel and a combination anthelmintic (Adecto®) in bath treatments against *Tagia ecuadori* and *Neobenedenia melleni* (Monogenea), parasites of bullseye puffer fish. *Aquaculture***,** 492, 361–368.https://doi.org/10.1016/j.aquaculture.2018.04.043

Mladineo, I., Marsic-lucic, J., Buzancic, M. (2006). Toxicity and gross pathology of ivermectin bath treatment in sea bream *Sparus aurata*, L. *Ecotoxicology and Environmental Safety***,** 63, 438–442.

Onaka, E. M., Martins, M. L., & Moraes, F. R. (2003). Eficácia do albendazol e praziquantel no controle de *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae), parasito de pacu *Piaractus mesopotamicus* (Osteichthyes: Characidae). I. banhos terapêuticos. *Boletim do Instituto de Pesca*, 29, 101-107.

Santamarina, M. T., Tojo, J., Ubeira, F. M., Quinteiro, P., & Sanmartin, M. L. (1991). Anthelmintic treatment against *Gyrodactylus* sp. infecting rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms*, 10, 39-43.

Sitja-Bobadilla, A., Felipe, M. C., & Alvarez-Pellitero, P. (2006). *In vivo* and *in vitro* treatments against *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (*Sparus aurata* L.). *Aquaculture*,261,856–864.https://doi.org/ 10.1016/j.aquaculture.2006.09.012

Soares, B.V., Neves, L.R., Oliveira, M.S.B., Chaves, F.C.M., Dias, M.K.R., Chagas E.C., & Tavares-Dias, M. (2017). Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma macropomum* (tambaqui) and its physiological and

histopathological effects. *Aquaculture*, 452, 107-114.https://doi.org/10.1016/ j.aquaculture.2015.10.029

Sommerville, C., Endris, R., Bell, T.A., Ogawa, K., Buchmann, K., & Sweeney, D. (2016).World association for the advancement of veterinary parasitology (WAAVP) guideline for testing the efficacy of ectoparasiticides for fish. *Veterinay Parasitology*, 219, 84–99. http://dx.doi.org/10.1016/j.vetpar.2015.11.003

Swanepoel, E., Liebenberg, W., Devarakonda, B., & Villiers, M. M. (2003). Developing a discriminating dissolution test for three mebendazole polymorphs based on solubility diferences. *Pharmazie*, 58, 117–121.

Szekely, C., & Molnar, K. (1990). Treatment of *Ancylodiscoides vistulensis* monogenean infestations of the European catfish (*Silurus glanis*). *Bulletin of the European Association of Fish Pathologists*, 10, 74-77.

Taraschewski, H., Renner, C., & Mehlhorn, H. (1988). Treatment of fish parasites. *Parasitology Research*, 74, 281-289.

Tavares-Dias, M., & Martins, M.L. (2017). An overall estimation of losses caused by diseases in the Brazilian fish farms. *Journal of Parasitic Diseases*, 41, 913-918.doi: 10.1007/s12639-017-0938-y

Thiripurasundari, M., Sathya, K., Uma. A., SRINIVASAN, M.R., RAJASEKAR, P. A. (2014). Comparative study on the toxicity of ivermectin in zebra fish and catla fish models. *Indo American Journal of Pharmaceutical Research*, 4, 2231-6876.

Varó, I., Rigos, G., Navarro, J.C.,Ramo, J. D., Calduch-Giner, J., Hernández, A., Pertusa, J., Torreblanca, A. (2010). Effect of ivermectin on the liver of gilthead sea bream *Sparus aurata*: a proteomic approach. *Chemosphere*, 80, 570–577.

Zar, J.H. (2010). *Biostatistical analysis* (5th ed). New Jersey, Prentice-Hall.

Zhang, X. P., Li, W. X., Ai. T. S., Zou H., Wu, S. G., & Wang, G. T. (2014). The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrus vastator* (Monogenea). *Aquaculture*, 420–421**,** 302–307.https://doi.org/10.1016/j.aquaculture.2013.09.022

Zuskova, E., Piackova, V., Machova, J., Chupani, L., Steinbach, C., Stara, A., & Velisek, J. (2018). Efficacy and toxicity of praziquantel in helminth-infected barbel (*Barbus barbus* L.). *Journal of Fish Diseases*, 41, 643-649. doi: 10.1111/jfd.12764. Epub2018 Jan 19.

CONCLUSÕES GERAIS

- Nos ensaios *in vitro* contra monogeneas de *C. macropomum*, todas as concentrações de levamisol (50-125 mg/L), albendazole (500-2000 mg/L) e ivermectina (200-350 mg/L) mostraram 100% de eficácia contra *A. spathulatus*, *N. janauachensis*, *M. boegeri* e *L. brinkmanni*.
- O praziquantel (5- 20 mg/L) e mebendazol (125-200 mg/L), utilizados *in vitro* tiveram eficácia devido ao solvente DMSO, pois ambos anti-helmínticos tem baixa solubilidade em água e baixa disponibilidade.
- Nos banhos terapêuticos em *C. macropomum* de 24 h, com 200 mg/L de ivermectina, ocorreu 100% de mortalidade dos peixes em até 2 h de exposição e 6.6% de mortalidade dos peixes expostos a 500 mg/L de albendazol. Portanto, este resultado indica que a ivermectina não é uma boa droga anti-helmíntica para controle e tratamento de peixes, devido a sua alta toxicidade, mesmo em baixas concentrações.
- Após 24 horas de banho terapêutico com 500 mg/L de albendazol houve eficácia de apenas 48,6% contra monogeneas de *C. macropomum*, enquanto 125 mg/L de levamisol apresentou 88,2% de eficácia. Portanto, 125 mg/L de levamisol pode recomendado para controlar monogeneas desse peixe na piscicultura. Sendo assim, mensurar a eficácia dos fármacos permite que estratégias adequadas de manejo sejam adotadas visando reduzir os custos com tratamentos ineficientes e mao de obra.

ANEXO

Prova gráfica do artigo publicado em Journal of Fish Diseases

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ORIGINAL ARTICLE

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

Carliane Maria Guimarães Alves[1] | Joziele Neves Nogueira[2] | Isaac Belo Barriga[2] | Joilson Rodrigues dos Santos[3] | Gracienhe Gomes Santos[3] | Marcos Tavares-Dias[1,4]

[1]Postgraduate Program in Environmental Sciences (PPGCA), Federal University of Amapá (UNIFAP), Macapá, Brazil

[2]University of the State of Amapá (UEAP), Macapá, Brazil

[3]Faculdade de Macapá (FAMA), Macapá, Brazil

[4]Embrapa Amapá, Macapá, Brazil

Correspondence
Marcos Tavares-Dias, Postgraduate Program in Environmental Sciences (PPGCA), Federal University of Amapá (UNIFAP), Macapá, Brazil.
Email: marcos.tavares@embrapa.br

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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

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-lucic, & 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).

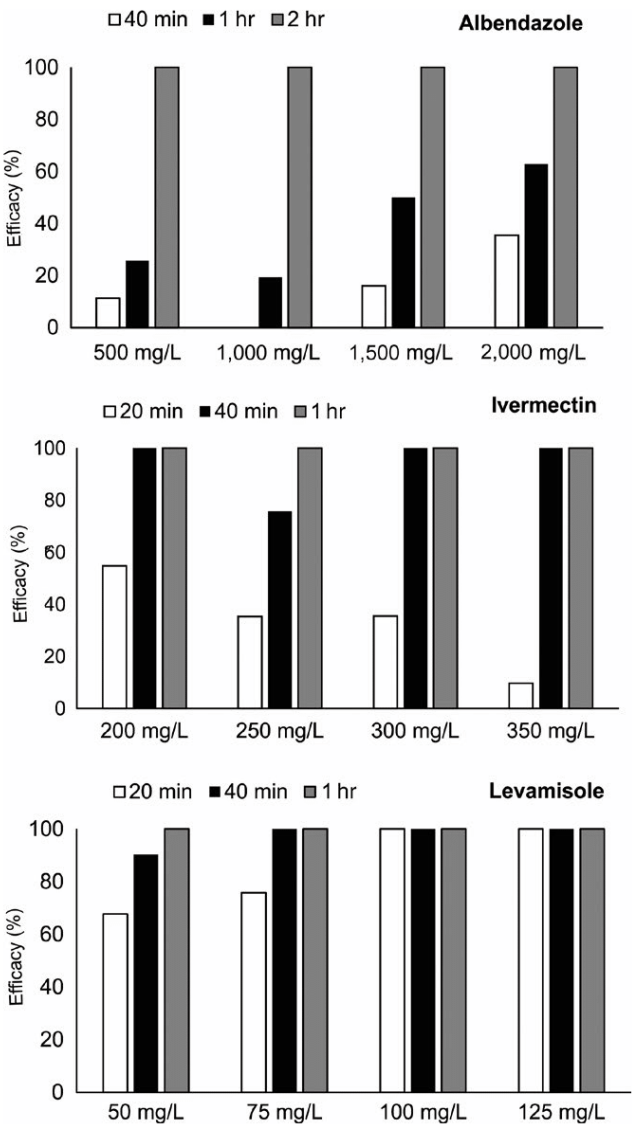

FIGURE 1 In vitro efficacy of anthelmintic drugs against monogeneans of *Colossoma macropomum* in different exposure times

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*

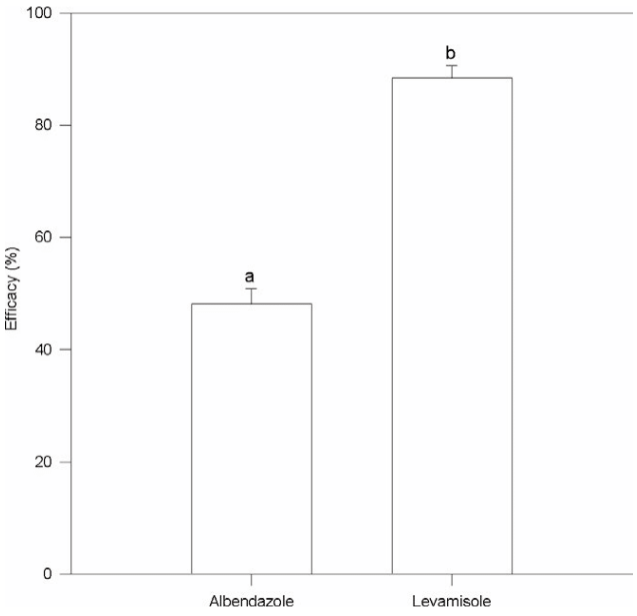

FIGURE 2 Therapeutic baths efficacy with 500 mg/L of albendazole and 125 mg/L levamisole in monogeneans of *Colossoma macropomum* after 24 hr of treatment. Mean values followed by different letters indicate differences between treatments according to *t* test ($p < 0.05$)

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).

ORCID

Marcos Tavares-Dias https://orcid.org/0000-0002-8376-1846

REFERENCES

Bader, C., Chelladurai, J. J., Starling, D. E., Jones, D. E., & Brewer, M. T. (2017). Assessment of in vitro killing assays for detecting praziquantel induced death in *Posthodiplostomum minimum* metacercariae. *Experimental Parasitology*, *181*, 70–74. https://doi.org/10.1016/j.exppara.2017.07.008

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, *83*(4), 575–583. https://doi.org/10.2307/3284227

Collymore, C., Watral, V., White, J. R., Colvin, M. E., Rasmussen, S., Tolwani, R. J., & Kent, M. L. (2014). Tolerance and efficacy of emamectin benzoate and ivermectin for the treatment of *Pseudocapillaria tomentosa* in laboratory Zebrafish (*Danio rerio*). *Zebrafish*, *11*, 490–497. https://doi.org/10.1089/zeb.2014.1021

Eiras, J. C., Takemoto, R. M., & Pavanelli, G. C. (2006). *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes* (2ª ed.). Maringá, Brazil: Eduem.

FAO – Food and Agriculture Organization of the United Nations (2018). *The state of world fisheries and aquaculture 2018. Meting the sustainable development goals*. Rome, Italy: FAO.

Hashimoto, G. S., Neto, F. M., Ruiz, M. L., Acchile, M., Chagas, E. C., Chaves, F. C. M., & Martins, M. L. (2016). Essential oils *of Lippia sidoides and Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia. *Aquaculture*, *450*, 182–186. https://doi.org/10.1016/j.aquaculture.2015.07.029

Hirazawa, N., Akiyama, K., & Umeda, N. (2013). Differences in sensitivity to the anthelmintic praziquantel by the skin-parasitic monogeneans *Benedenia seriolae* and *Neobenedenia girellae*. *Aquaculture*, *404-405*, 59–64. https://doi.org/10.1016/j.aquaculture.2013.04.021

Hirazawa, N., Ohtaka, T., & Hata, K. (2000). Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*. *Aquaculture*, *188*, 1–13. https://doi.org/10.1016/S0044-8486(00)00334-3

Kim, K. H., & Choi, E. S. (1998). Treatment of *Microcotyle sebastis* (Monogenea) on the gills of cultured rockfish (*Sebastes schlelegelii*)

with oral administration of mebendazole and bithionol. *Aquaculture*, *167*, 115–121. https://doi.org/10.1016/S0044-8486(98)00300-7

Liu, Y., Wang, T., Ding, W., Dong, C., Wang, X., Chen, J., & Li, Y. (2018). Dissolution and oral bioavailability enhancement of praziquantel by solid dispersions. *Drug Delivery and Translational Research*, *8*, 580–590. https://doi.org/10.1007/s13346-018-0487-7

Martins, M. L., Mourino, J. L. P., Chagas, E. C. C., Silva, B. C., Fujimoto, R. Y., & Pádua, S. B. (2017). Ectoparasitários na aquicultura. In B. Baldisserotto, L. C. Gomes, B. M. Heinzmann & M. A. Cunha (Eds.), *Farmacologia aplicada a aquicultura* (pp. 127–181). Santa Maria, CA: UFSM.

Martins, M. L., Onaka, E. M., Moraes, F. R., & Fujimoto, R. Y. (2001). Mebendazole treatment against *Anacanthorus penilabiatus* (monogenea, Dactylogyridae) gill parasite of cultivated *Piaractus mesopotamicus* (Osteichthyes, Characidae) in Brazil. efficacy and Phematology. *Acta Parasitologica*, *46*, 332–336.

Mladineo, I., Marsic-lucic, J., & Buzancic, M. (2006). Toxicity and gross pathology of ivermectin bath treatment in sea bream *Sparus aurata*, L. *Ecotoxicology and Environmental Safety*, *63*, 438–442. https://doi.org/10.1016/j.ecoenv.2005.02.015

Morales-Serna, F. N., Chapa-López, M., Martínez-Brown, J. M., Ibarra-Castro, L., Medina-Guerrero, R. M., & Fajer-Ávila, E. J. (2018). Efficacy of praziquantel and a combination anthelmintic (Adecto®) in bath treatments against *Tagia ecuadori* and *Neobenedenia melleni* (Monogenea), parasites of bullseye puffer fish. *Aquaculture*, *492*, 361–368. https://doi.org/10.1016/j.aquaculture.2018.04.043

Onaka, E. M., Martins, M. L., & Moraes, F. R. (2003). Eficácia do albendazol e praziquantel no controle de *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae), parasito de pacu *Piaractus mesopotamicus* (Osteichthyes: Characidae). I. banhos terapêuticos. *Boletim do Instituto De Pesca*, *29*, 101–107.

Santamarina, M. T., Tojo, J., Ubeira, F. M., Quinteiro, P., & Sanmartin, M. L. (1991). Anthelmintic treatment against *Gyrodactylus* sp. infecting rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms*, *10*, 39–43. https://doi.org/10.3354/dao010039

Sitjà-Bobadilla, A., Felipe, M. C., & Alvarez-Pellitero, P. (2006). In vivo and in vitro treatments against *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (*Sparus aurata* L.). *Aquaculture*, *261*(3), 856–864. https://doi.org/10.1016/j.aquaculture.2006.09.012

Soares, B. V., Neves, L. R., Oliveira, M. S. B., Chaves, F. C. M., Dias, M. K. R., Chagas, E. C., & Tavares-Dias, M. (2017). Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma macropomum* (tambaqui) and its physiological and histopathological effects. *Aquaculture*, *452*, 107–114. https://doi.org/10.1016/j.aquaculture.2015.10.029

Sommerville, C., Endris, R., Bell, T. A., Ogawa, K., Buchmann, K., & Sweeney, D. (2016). World association for the advancement of veterinary parasitology (WAAVP) guideline for testing the efficacy of ectoparasiticides for fish. *Veterinay Parasitology*, *219*, 84–99. https://doi.org/10.1016/j.vetpar.2015.11.003.

Swanepoel, E., Liebenberg, W., Devarakonda, B., & Villiers, M. M. (2003). Developing a discriminating dissolution test for three mebendazole polymorphs based on solubility diferences. *Die Pharmazie*, *58*, 117–121.

Szekely, C., & Molnar, K. (1990). Treatment of *Ancylodiscoides vistulensis* monogenean infestations of the European catfish (*Silurus glanis*). *Bulletin of the European Association of Fish Pathologists*, *10*, 74–77.

Taraschewski, H., Renner, C., & Mehlhorn, H. (1988). Treatment of fish parasites. *Parasitology Research*, *74*, 281–289. https://doi.org/10.1007/BF00539579

Tavares-Dias, M., & Martins, M. L. (2017). An overall estimation of losses caused by diseases in the Brazilian fish farms. *Journal of Parasitic Diseases*, *41*, 913–918. https://doi.org/10.1007/s12639-017-0938-y

Thiripurasundari, M., Sathya, K., Uma, A., Srinivasan, M. R., Rajasekar, P. A. (2014). Comparative study on the toxicity of ivermectin in zebra fish and catla fish models. *Indo American Journal of Pharmaceutical Research*, *4*, 2231–6876.

Varó, I., Rigos, G., Navarro, J. C., Ramo, J. D., Calduch-Giner, J., Hernández, A., … Torreblanca, A. (2010). Effect of ivermectin on the liver of gilthead sea bream *Sparus aurata*: A proteomic approach. *Chemosphere*, *80*, 570–577. https://doi.org/10.1016/j.chemosphere.2010.04.030

Zar, J. H. (2010). *Biostatistical analysis* (5th ed.). Englewood Cliffs, NJ: Prentice-Hall.

Zhang, X. P., Li, W. X., Ai, T. S., Zou, H., Wu, S. G., & Wang, G. T. (2014). The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrus vastator* (Monogenea). *Aquaculture*, *420-421*, 302–307. https://doi.org/10.1016/j.aquaculture.2013.09.022

Zuskova, E., Piackova, V., Machova, J., Chupani, L., Steinbach, C., Stara, A., & Velisek, J. (2018). Efficacy and toxicity of praziquantel in helminth-infected barbel (*Barbus barbus* L.). *Journal of Fish Diseases*, *41*, 643–649. https://doi.org/10.1111/jfd.12764

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