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## Tilapia, high socio-economic cichlid fish, as host of trematode parasites with zoonotic potential

La tilapia, pez cíclido de alto valor socio-económico, como hospedador de parásitos trematodos con potencial zoonótico



Acosta-Pérez Johan<sup>1</sup> ID, De-la-Rosa-Arana Jorge<sup>2</sup> ID, Vega-Sánchez Vicente<sup>1</sup> ID, Reyes-Rodríguez Nydia<sup>1</sup> ID, Zepeda-Velázquez Andrea<sup>1</sup> ID, Gómez-De-Anda Fabian<sup>1</sup> ID\*

<sup>1</sup>Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Área Académica de Medicina Veterinaria y Zootecnia, México. <sup>2</sup>Microbiología en Salud Humana. Facultad de Estudios Superiores Cuautitlán. Universidad Nacional Autónoma de México. Avenida 1 de mayo S/N, Campo Uno, Cuautitlán Izcalli, CP 54743, Estado de México, México. \* Corresponding Author: Gómez-De-Anda Fabián, Rancho Universitario Av. Universidad km 1, A.P. 32 CP.43600. 01771717 2000 ext. 2454, Ex-Hda. de Aquetzalpa, Hidalgo, México. josvi10itz@gmail.com, delarosa.jl@gmail.com, vicente\_vega11156@uaeh.edu.mx, nydia\_reyes@uaeh.edu.mx, andrea\_zepeda@uaeh.edu.mx, fabian\_gomez9891@uaeh.edu.mx

### Abstract

The production and consumption of tilapia (Cichlidae) is very popular in the world, so the safety of the meat produced must be ensured given the potential risk of zoonotic transmission of parasites; For this reason, the objective of this work was to carry out a bibliographic review on the prevalence, distribution and hosts that intervene in the life cycle of trematode helminths that can be transmitted zoonotically in the consumption of tilapia. The bibliographic review was carried out with six specialized search engines. 1,044 articles were analyzed, of which 113 included epidemiological data. Tilapia was reported as the intermediate host of 15 species of trematodes that affect humans, 6 parasites were reported at the genus level and 2 parasites at the family level. The described flukes belong to the families Heterophyidae and Opisthorchiidae. Reported prevalences range from 1% in infections by *Haplorchis pumilio* and *Centrocestus formosanus* to 93.64% in multiparasitic infections by *Haplorchis yokogawi*, *Pygidiopsis genata* and *Phagicola ascolonga*. Although the biodiversity of documented helminths in tilapia is abundant, the available information is still insufficient, situating tilapia as a potential transmitter of these helminths in humans.

**Keywords:** tilapia, trematodes, zoonotic parasites, aquaculture, fisheries, host.

### Resumen

La producción y consumo de tilapias (Cichlidae) es muy popular en el mundo, por lo que debe asegurarse la inocuidad de la carne producida ante el riesgo potencial de la transmisión zoonótica de parásitos; por ello, el objetivo de este trabajo fue realizar una revisión bibliográfica sobre la prevalencia, distribución y hospederos que intervienen en el ciclo de vida de los helmintos trematodos que pueden transmitirse de manera zoonótica en el consumo de la tilapia. La revisión bibliográfica se llevó a cabo con seis motores de búsqueda especializada. Se analizaron 1,044 artículos, de los cuales 113 incluían datos epidemiológicos. La tilapia fue reportada como el hospedador intermediario de 15 especies de trematodos que afectan al



humano, 6 parásitos se reportaron a nivel de género y 2 parásitos a nivel de familia. Los trematodos descritos pertenecen a las familias Heterophyidae y Opisthorchiidae. Las prevalencias reportadas oscilan de 1% en infecciones por *Haplorchis pumilio* y *Centrocestus formosanus* hasta 93.64% en infecciones multiparasitarias de *Haplorchis yokogawi*, *Pygidiopsis genata* y *Phagicola ascolonga*. Aunque la biodiversidad de helmintos documentados en la tilapia es abundante, aún es insuficiente la información disponible, situando a la tilapia como un transmisor potencial de estos helmintos en el humano.

**Palabras clave:** tilapia, trematodos, parásitos zoonóticos, acuacultura, pesca, hospederos.

## INTRODUCTION

There are many papers that describe the nutritional and economic importance of worldwide fishing and aquaculture ([Adugna et al., 2020](#); [Chibwana et al., 2020](#); [Okoye et al., 2014](#)). This activities annually produce more than 179 million tons of fish ([FAO, 2021](#)) and one of the most produced, is the fish named “tilapia”, a term that is used to refer to cichlid fishes of the genera, *Oreochromis*, *Sarotherodon* and *Tilapia*. In Mexico, during the last 10 years, an increase in tilapia production has been reported, with an average annual growth of 3.1% ([Huerta-Mata & Valenzuela-Oyadener, 2019](#); [SIAP, 2022](#)). Due to the expansion of fishing and aquaculture, requirements of food safety strategies are mandatory, *v. gr.*, the presence of potential pathogen microorganism, as the parasites, compromises the safety of fishery products ([Ananda-Raja & Jithendran, 2015](#); [Williams et al., 2020<sup>a</sup>](#)).

Among the helminths, the most widely studied for their zoonotic transmission from fish meat are the trematodes, colloquially named “flukes”, from the families *Heterophyidae* (intestinal flukes), *Echinostomatidae* (intestinal flukes), and *Opisthorchiidae* (liver flukes), which are represented by the species *Clonorchis sinensis*, *Opisthorchis viverrini*, *O. felineus*, *Metagonimus yokogawai* and *Heterophyes* spp. ([Lima dos Santos & Howgate, 2011](#); [Wiriya et al., 2013](#)). The tapeworms *Diphyllobothrium latum* and *D. pacifica* as well as the nematodes *Capillaria philippinensis*, *Gnathostoma hispidum*, *G. spinigerum*, *G. doloresi*, *G. nipponicum*, *Pseudoterranova decipiens*, *Contracaecum osculatum* and *Anisakis simplex* are also studied. As example of medical and economical importance, there are annually more than 1,000 clinical cases of anisakidosis in Japan transmitted by the ingest of more than 100 species of fishes that are eaten without cooking ([Bao et al., 2019](#)). Gnathostomosis is another helminthic disease reported frequently in tourists ([Bravo and Gontijo, 2018](#)). Although more than 1,000 cases of gnathostomosis had been recorded in Mexico between 1970 and 1999 ([Lamothe-Argumedo, 1999](#)), there are not enough systematic studies of prevalence and distribution of helminths with zoonotic potential that parasitized fish of economic importance ([Carrique-Mas & Bryant, 2013](#); [Pritt, 2015](#)). However, it is known that in in Asia, 18 million people are infected by trematodes ([Mahmoud et al., 2016](#); [Wiriya et al., 2013](#)) and in the world there are 500 million people at risk of infection ([Chi et al., 2008](#)). In this way, considering the importance of helminths



and the commercial level of tilapia (Gulelat *et al.*, 2013; Mahmoud *et al.*, 2016; Soler Jiménez *et al.*, 2016; Watterson *et al.*, 2012); the objective of this work was to carry out a literature review about the prevalence and distribution of trematode helminths that can be transmitted as zoonosis during the tilapia production chain.

## MATERIAL AND METHODS

### Bibliographic research

The review was carried out with search engines based on the use of the keywords: zoonotic parasite, trematode, foodborne parasites, *Oreochromis* and tilapia. The variants that make references to the zoonotic parasite disease were centrocestiosis (*Centrocestus*), clonorchiosis (*Clonorchis*), opisthorchiosis (*Opisthorchis*), heterophiosis (*Heterophyes*) and haplorchiosis (*Haplorchis*). Taking in account these words, the inclusion as a whole, was structured as shown, “(*Centrocestus* or centrocestiosis) and (*Oreochromis* or tilapia)” for each organism / parasitic disease. Six specialized bibliography search engines were used (ScienceDirect, PubMed, Primo, CONRICyT, LILAES and AJOL), a total of 1,044 search results were obtained to which exclusion criteria were applied, eliminating zoonotic parasitic diseases in other taxa of parasites and from fish that were not within the scope of this review. Replicas were eliminated and studies were defined as those that included basic epidemiological data, prevalence and distribution. The purification delimited 80 bibliographic sources for the qualitative analysis and 33 bibliographic sources referring to prevalence that was part of the information analyzed in this review, thus, a total of 113 papers were analyzed. Subsequently, the information was captured in tables to data presentation in geographic distribution map, using the free access computer program RSstudio (Boston, MA, USES).

## RESULTS AND DISCUSSION

### General life cycle of Trematodes

Flukes are helminths taxonomically located within the group of flatworms. In general, flatworms meet three characteristics: they are acellomate, protostomate and triblastic (Negrete & Damborenea, 2017). Trematodes include a number of parasitic species of animals that accidentally reach humans. Most trematodes have complex life cycles involving multiple hosts (Fang *et al.*, 2018). The general life cycle of trematodes begins with the monoecious adult worm that is established in the viscera of the digestive system of a vertebrate. The adult worms lay eggs that use the host's faeces as a vehicle for dispersal. The eggs are dispersed in the water and from there, the first larva emerges, which is called miracidium and it is mobile. The miracidium stage looks for the first



intermediate host (gastropod mollusk), in host develops into a sporocyst and this, in turn, into redias that reproduce asexually, finally mollusk expels cercariae stage. Cercariae swim and disperse in the aquatic environment until they find the second intermediate host, which can be a fish (Lima dos Santos & Howgate, 2011; Zhao-Rong *et al.*, 2005). In the skeletal muscle of the second intermediate host, the cercariae become metacercariae, which are surrounded by a chitin wall that allows them to resist climatic changes or simply the passage through the digestive tract of the definitive host that in a natural way it is a vertebrate animal with ichthyophagous habits (Mutengu *et al.*, 2018). In the definitive host, the metacercariae are released from the capsule and establish themselves in some of the viscera of the digestive system, to become adult worm stage (Galaktionov & Dobrovolskij 2003; Burton *et al.*, 2019). Foodborne zoonosis are infections that affect humans and are acquired through the ingestion of food of vertebrate animal origin (Carrique-Mas & Bryant, 2013). This type of food can transmit the infection to humans because it comes from animals that are part of the life cycle of parasites; for example, from raw fish meat. In particular, fish flukes have life cycles with multiple hosts (Fang *et al.*, 2018), including a definitive single host for the adult worm stage and one or more intermediate hosts, which harbor the different stages of parasite development (Chibwana *et al.*, 2020; Hung *et al.*, 2015). Gastropod mollusks are primary intermediate hosts (Chi *et al.*, 2008), while fish are secondary intermediate hosts and have a transcendental role in the transmission of the parasite through the trophic chain (Koinari *et al.*, 2013).

### Cercariae in tilapia environments

Given the importance of gastropods mollusks in the life cycle of trematodes, removing snails from ponds for tilapia production is recommended (Kang *et al.*, 2013). In the aquaculture and fishing of has been identified some species of gastropods as primary host of trematodes. Table 1 shows the prevalence of cercariae found in several mollusk involved in the tilapia chain production. The main data is reported from Asia. For example, in Vietnam, where ponds and rice plots converge, at least 15 or more species of gastropods have been described, such as *Melanoides tuberculata*, *Bithynia fuchsiana* and *Stenothyra messengeri* with a cercarial infection prevalence of 8.9%, 6.4% and 1.5%, respectively (Madsen *et al.*, 2015). The trematodes *Haplorchis pumilio* has been isolated in fish ponds with presence of five gastropods species, one of which belongs to the *Viviparidae* family (*Angulyagra polyzonata*) and the other four to the *Thiaridae* family: *Melanoides tuberculata*, *Thiara scabra*, *Tarebia granifera* and *Sermyla requetii* (Van Phan *et al.*, 2010). Cercariae of the genus *Procerovum* have been recorded in the gastropods *Melanoides tuberculata* (0.92%) and *Bithynia fuchsiana* (0.11%). *Opisthorchis viverrini* can be found in the gastropods *Bithynia siamensis goniomphalos* (0.86%) and *B. funiculata* (0.14%) (Dao *et al.*, 2017; Hung *et al.*, 2015). Cercariae of *Clonorchis sinensis* have been recorded in the families *Hydrobiidae*, *Bithyniidae*, *Melaniidae*, *Assimineidae*



and *Thiaridae* (Zhao-Rong *et al.*, 2005), particularly in the species *Alocinma longicornis* (27%), *Bithynia fuchsianus* (8%) and *Parafossarulus striatulus* (8%) (Petney *et al.*, 2013; Zhao-Rong *et al.*, 2005). The cercariae of *Heterophyidae* and *Echinostomatidae* families are established in the mollusks *Pomacea canaliculata*, *Bellamya aeruginosa* and *Cipangopaludina Oncomelania* (Kang *et al.*, 2013). *Heterophyes heterophyes* cercariae is related to four families of gastropods as primary hosts (*Potamididae*, *Melaniidae*, *Pleuroceridae* and *Littorinidae*), although the most recurrent specie is *Pirenella conica* (Chai, 2014; Chai & Jung, 2017). Similarly, loads of *Clinostomun complanatum* have been reported in *Radix swinhoei* as the primary host, with a prevalence of 0.62 (Wang *et al.*, 2017<sup>a</sup>). In America, reports of cercariae were found from Costa Rica, where the gastropods *Melanoides turricula*, *Pomacea flagellata*, *Haitia cubensis* and the bivalve *Anodontiles luteola* have been recorded as intermediate hosts for *Centrocestus formosanus* (Cortés *et al.*, 2010).

**Table 1. Prevalence of cercariae associated with the production chain and capture of tilapia for consumption**

Fluke	Primary intermediary host	Continent distribution	Prevalence of cercariae	Secondary intermediary host	Reference
<b><i>Opisthorchis viverrini</i></b>	<i>Bithynia siamensis</i> <i>goniomphalos</i> <i>Bithynia funiculata</i>	Asia (Vietnam)	0.14	<i>Oreochromis niloticus</i>	(Dao <i>et al.</i> , 2017)
			0.86		
<b><i>Clonorchis sinensis</i></b>	<i>Parafossarulus striatulus</i>	Asia (China)	3-8	<i>Tilapia mossambica</i>	(Zhao-Rong <i>et al.</i> , 2005)
	<i>Alocinma longicornis</i>		27	<i>Oreochromis mossambicus</i>	(Petney <i>et al.</i> , 2013)
	<i>Bithynia fuchsianus</i>		8	<i>Oreochromis mossambicus</i>	
<b><i>Haplorchis sp.</i>, <i>Procerovum varium e</i> <i>Indefinidos</i></b>	<i>Bithynia fuchsiana</i> y <i>Melanoides tuberculata</i>	Asia (Vietnam)	0.11-0.92	<i>Oreochromis niloticus</i>	(Hung <i>et al.</i> , 2015)
<b><i>Haplorchis pumilio</i></b>	<i>Melanoides tuberculata</i> , <i>Bithynia fuchsiana</i> y <i>Stenothyra messengeri</i>	Asia (Vietnam)	1.5-8.9	<i>Oreochromis niloticus</i>	(Madsen <i>et al.</i> , 2015)
<b><i>Clinostomum complanatum</i></b>	<i>Radix swinhoei</i>	Asia (China)	0.62	<i>Tilapia zillii</i>	(Wang <i>et al.</i> , 2017 <sup>a</sup> )

The study of gastropods mollusks are important, since this organism are extremely resistant to abiotic factors, such as *Pirenella conica*, which is resistant to salinity between 15 and 80 ppm (Chai, 2014; Chai & Jung, 2017; Hung *et al.*, 2013). One of the most recurrent primary hosts is the gastropod mollusk *Melanoides tuberculata* (Cortés *et al.*, 2010; Hung *et al.*, 2013; Petney *et al.*, 2013; Pinto *et al.*, 2014; Zhao-Rong *et al.*, 2005),



which is resistant to desiccation, low oxygen levels, and extreme salinity, also, resist a temperature lower than 18 °C (Fleming *et al.*, 2011). In culture ponds, the vegetation surrounding and the oligotrophic environments promote the establishment of different populations of gastropods (Chi *et al.*, 2008; Cortés *et al.*, 2010). Due to these characteristics, the sanitary management of gastropods mollusks requires special attention.

### **Metacercaria stage isolated in fishing tilapia**

Tilapia has been reported as a transmitter of zoonotic flukes, becoming a public health problem and, in some cases, spoils the commercial perception of aquaculture (Adugna *et al.*, 2020; Chibwana *et al.*, 2020). Table 2 shows the prevalence of metacercariae in different tilapia species; the table also shows that cercariae have no predilection for the anatomical region of the fish. The analyzed reports include different trematode species, such as, *Heterophyes heterophyes* metacercariae which is parasite of *Tilapia simonis*, *T. nilotica* and *T. zillii* (Chai, 2014; Chai & Jung, 2017). In natural water bodies, metacercariae of *Opisthorchis felinus*, *O. viverrini* and *Clonorchis sinensis* have been collected from *Oreochromis* and *Tilapia* (Petney *et al.*, 2013; Wang *et al.*, 2018; Williams *et al.*, 2020<sup>a</sup>; Zhao-Rong *et al.*, 2005), while metacercariae of *Centrocestus formosanus* have been recovered from *Oreochromis niloticus* produced in a recreational artificial lake in Belo Horizonte, Brazil (Pinto *et al.*, 2014). Prevalence of *Opisthorchis viverrini* metacercariae in *Oreochromis niloticus* young tilapia inhabiting of a lake in Binh Dinh province, Vietnam, was of 18.8% (Dao *et al.*, 2017), while in a dam from Zimbabwe, the prevalence of *Clinostomum metacercariae* was of 62.8% in *Oreochromis mossambicus* (Mutengu *et al.*, 2018). In another context, in the Lake Agulu, Nigeria, the prevalence of *Clinostomum tilapiae* metacercariae in *Tilapia zillii* was 1.54% (Okoye *et al.*, 2014).

### **Metacercariae and concomitant parasite infections in tilapia fish**

Co-infections in tilapia by different taxonomic groups is a common event. The study of concomitant infections is pertinent because the risk of morbidity and mortality in the production of fish for commercial and consumption purposes. Concomitant infection between two etiological agents from different taxonomic groups or multiple etiological agents from the same taxonomic group have been documented. In the first case, we can mention as an example, the infection between the ciliated protozoa *Trichodina heterodontata* or *Ichthyophthirius multifiliis* with the bacterium *Streptococcus iniae* (Abdel-Latif *et al.*, 2020). Regard to helminths, the co-infection between the nematode *Contraecaecum multipapillatum* (51.8% prevalence) and the trematode *Heterophyes* sp. (19.6% prevalence) in *Tilapia zillii* or *Oreochromis leucostictus* was documented in Lake Neivasha in Kenya (Otachi *et al.*, 2014). In addition, concomitant infection between larvae of *Contraecaecum* sp. nematode (5.48%) and metacecariae of *Clinostomum* sp. (27.39%)



were found in the mesentery, pericardial area and branchial cavity of *Oreochromis niloticus* resident of the Koka water reserve in Ethiopia (Gulelat *et al.*, 2013). In the case of multiparasitism, the findings reported in *Tilapia nilotica* and *Tilapia zillii* from the Lake Manzala (brackish water) and from the Nile River (fresh water) can be cited as example. In the first case, the frequency of infection was 64.9%, while in the second case it was 17.6% and the metacercariae collected were from the genera *Heterophyes heterophyes*, *H. aequalis*, *Pygidiopsis genata*, *Haplorchis yokogawai*, *H. pumilio*, *Phagicola ascolonga* and *Stictodora tridactyla* (Elsheikha & Elshazly, 2008<sup>b</sup>; Hegazi & Abo-elkheir, 2014). These data suggest that tilapia is an organism that tolerates multiparasitism. Also, some parasites such as *Clinostomum* sp. showed a higher prevalence in concomitant infection than when recovered individually, this may be because some microorganisms arise as opportunistic infections in organisms that have primary infections (Fajer-Ávila *et al.*, 2017).

**Table 2. Reports of metacercariae in tilapia. Geographic and anatomical distribution of parasites, only the reports that have prevalence are included**

Tilapia	Fluke	Distribution	Prevalence of metacercariae	Anatomical distribution	Reference
<b><i>Oreochromis niloticus</i></b>	<i>Centrocestus formosanus</i>	Vietnam,	11.8%-12.5%	ca, mu, pi, al, br, es	(Chi <i>et al.</i> , 2008)
	<i>Haplorchis pumilo</i>	China			
<b><i>Oreochromis niloticus</i></b>	<i>Opisthorchis viverrini</i>	Thailand,	18.8%	-	(Dao <i>et al.</i> , 2017)
		Cambodia, Laos, Vietnam			
<b><i>Oreochromis niloticus</i>, <i>T. zillii</i></b>	<i>Heterophyes heterophyes</i> , <i>H. aequalis</i> , <i>Pygidiopsis genata</i> , <i>Phagicola</i> sp., <i>Haplorchis</i> sp., <i>Stictodora</i> sp.	Egypt	16.4%-17.6%	mu	(Elsheikha & Elshazly, 2008 <sup>a</sup> )
<b><i>Oreochromis niloticus</i></b>	Heterophyidae Echinostomatidae	China	1.5%	ca, br, mu, al, pi, es	(Kang <i>et al.</i> , 2013)
<b><i>Oreochromis niloticus</i>, <i>T. zillii</i></b>	<i>Heterophyes heterophyes</i> , <i>H. aequalis</i> ,	Egypt	30% -33.3%	mu, ca	(Lobna <i>et al.</i> , 2010)



	<i>Pygidiopsis genata,</i>					
	<i>Ascocotyle (Phagicola) ascolonga,</i>					
	<i>Haplorchis yokogawi</i>					
<b><i>Oreochromis niloticus</i></b>	<i>Centrocestus</i> sp.	Egypt Brazil,	10%	br		(Mahmoud <i>et al.</i> , 2016)
<b><i>Oreochromis niloticus</i></b>	<i>Centrocestus formosanus</i>	Egypt, Vietnam, Saudi Arabia	31.1%	br		(Pinto <i>et al.</i> , 2014)
<b><i>Oreochromis niloticus</i></b>	<i>Clonorchis sinensis,</i> <i>Haplorchis pumilio,</i> <i>H. taichui,</i>	Vietnam, Korea, China,	2%-10%	-		(Van De <i>et al.</i> , 2012)
<b><i>Oreochromis niloticus,</i></b>	<i>Centrocestus formosanus</i>	Thailand				
<b><i>Oreochromis niloticus,</i></b>	<i>Stellantchasmus falcatus,</i>	Thailand, Lao,				
<b><i>Oreochromis niloticus</i></b>	<i>Haplorchis pumilio,</i> <i>Procerovum varium</i>	Cambodia, Vietnam	2%-50%	al		(Wiriya <i>et al.</i> , 2013)
<b><i>Oreochromis niloticus</i></b>	<i>Centrocestus formosanus</i>	Costa Rica	1026 (total number recovered)	br, al y te		(Cortés <i>et al.</i> , 2010)
<b><i>Oreochromis niloticus</i></b>	<i>Heterophyes</i> sp., <i>Pygidiopsis genata,</i>	Egypt, Palestine,				
<b><i>Oreochromis niloticus</i></b>	<i>Haplorchis pumilio,</i>	Hawaii,	42.6% 64.9%	-		(Hegazi & Abo-elkheir, 2014)
<b><i>T. zillii</i></b>	<i>Phagicola</i> sp., <i>Stictodora tridactyla</i>	Ukraine, Canada, Alaska				
<b><i>Oreochromis niloticus,</i></b>	<i>Haplorchis taichui</i>	Laos, Thailand, Cambodia, Vietnam	0%	-		(Kopolrat & Sithithaworn, 2015)
<b><i>O. mossambicus</i></b>						
<b><i>Tilapia</i> sp.</b>	<i>Clonorchis sinensis</i>	China	0%	mu		(Wang <i>et al.</i> , 2017 <sup>b</sup> )





<b><i>Oreochromis niloticus</i></b>	<i>Heterophyes</i> sp.	Kenya	6%-8%	-	(Ojwala <i>et al.</i> , 2018)
<b><i>Oreochromis</i> sp.</b>	<i>Haplorchis pumilio</i>	Vietnam, China	3%-15.6%	mu, hu, pi, ca, cau	(Chi <i>et al.</i> , 2009)
<b><i>Oreochromis aureus</i></b>	<i>Centrocestus formosanus</i>	United States	0%	-	(Fleming <i>et al.</i> , 2011)
<b><i>Oreochromis niloticus</i></b>	<i>Haplorchis</i> sp., <i>Procerovum varium</i>	Vietnam	2.19%-23%	-	(Hung <i>et al.</i> , 2015)
<b><i>Oreochromis niloticus</i></b>	<i>Haplorchis pumilio</i>	Vietnam	32%	-	(Madsen <i>et al.</i> , 2015)
<b><i>Oreochromis mossambicus</i></b>	<i>Clinostomum</i> sp.	Zimbabwe	62.80%	cb, pi, oj	(Mutengu <i>et al.</i> , 2018)
<b><i>Oreochromis leucostictus</i></b> <b><i>Tilapia zillii</i></b>	<i>Heterophyes</i> sp.	Kenya	19.6-51.8%	cp, br	(Otachi <i>et al.</i> , 2014)
<b><i>Oreochromis niloticus</i></b>	<i>Haplochis taichui</i>	Vietnam	24%	-	(Van Phan <i>et al.</i> , 2010)
<b><i>Oreochromis niloticus</i></b>	<i>Clinostomum</i> sp.	Ethiopia	32.4%-58.8%	cbr, cp	(Adugna <i>et al.</i> , 2020)
<b><i>Tilapia guinensis</i></b>	<i>Clinostomum complanatum</i>	Nigeria, Korea, Japan Nigeria,	39.99%	cb, cbr, hu, oj, mu, cp, cab, mes, vis, vn	(Echi <i>et al.</i> , 2009 <sup>b</sup> )
<b><i>Sarotherodon melanotheron</i></b>	<i>Clinostomum complanatum</i>	Korea, Japan, Ghana	20.80%	cb, pi, oj	(Echi <i>et al.</i> , 2009 <sup>a</sup> )
<b><i>Oreochromis niloticus</i></b>	<i>Clinostomum</i> sp.	Ethiopia	5.48-27.39%	mes, cp, cbr	(Gulelat <i>et al.</i> , 2013)
<b><i>Oreochromis niloticus</i></b>	<i>Clinostomum</i> sp.	Benin	6.17%	Pi, br, in, cb	(Sèdogbo <i>et al.</i> , 2019)
<b><i>Tilapia zillii</i></b>	<i>Clinostomum</i> sp.	Nigeria	1.54%	cab	(Okoye <i>et al.</i> , 2014)
<b><i>Oreochromis niloticus</i></b>	<i>Clinostomum</i> sp.	Uganda	22%	pi, es	(Walakira <i>et al.</i> , 2014)

Abbreviations: ca (head), mu (muscle), pi (skin), al (fins), br (gills), es (scales), est (stomach), in (intestines), te (integument), hi (liver), ri (kidney), hu (bone), cau (caudal region), cb (oral cavity), oj (eyes), cbr (branchial cavity) cp (pericardial cavity), cab (abdominal cavity), mes (mesentery), vis (viscera) and vn (swim bladder)



## Metacercariae in tilapia fish farms

Prevalence studies in fish farms are of particular interest to determine the prevalence and distribution of infectious agents that could represent a risk to cause disease in humans or domestic animals. Most of the articles analyzed showed data on *Oreochromis tilapia* from Asian countries. In Vietnam, a 32% prevalence of metacercariae of *Haplorchis pumilio* was found in adult and hatchlings (Chi *et al.*, 2008; Madsen *et al.*, 2015) and a 24% of prevalence was found for *H. taichui* during the months of December and January (Van Phan *et al.*, 2010). Metacercariae of the genus *Clinostomum* were found in the skin, gills, intestine and oral cavity of tilapia from Africa, particularly in the Republic of Benin (6.17%), Uganda (22%) and Ethiopia (32.4%), where *Clinostomium* was found concomitantly (58.8%) with the nematode *Contracaecum* sp. larvae (Adugna *et al.*, 2020; Cortés *et al.*, 2010; Sèdogbo *et al.*, 2019; Walakira *et al.*, 2014). The presence of metacercariae of *Centrocestus formosanus* has been documented in young fish (fingerlings) with a counting of 1,026 larvae in Costa Rica (Cortés *et al.*, 2010) and in Vietnam with a prevalence of 11.8%, where *C. formosanus* also occurred in co-infection with *Haplorchis pumilio* (Chi *et al.*, 2008). Other metacercariae, from the *Heterophyidae* and *Echinostomatidae* families, have been found with a prevalence of 1.5% in mono- and polyculture ponds in Guangdong, China (Kang *et al.*, 2013).

## Comparative studies between fishing and aquaculture systems

There are numerous studies in the field of parasitology to try to define the behavior of parasitosis in free-living populations and crowded populations. However, it is striking that only was found some studies carried out in Vietnam and Thailand that address this comparison. In these works, *Oreochromis niloticus* tilapia were in captivity in farms or aquaculture ponds and the obtained data were compared with those obtained from free-living fish. In three studies (Vietnam), fish were found to have single infections with *Haplorchis pumilio* or concomitant infections with *Procerovum varium* or *Centrocestus formosanus*. The prevalence of infection in free-living animals was 14.3%, while in farms it was 52.8%, considering that the prevalence found in the farm was always higher (Hung *et al.*, 2015). In contrast, in a study carried out in Thailand, three species of metacercariae (*Stellantchasmus falcatus*, *Haplorchis pumilio*, and *Procerovum varium*) were recorded in free-living tilapia, while no trematodes were found in fish collected from cages and ponds (Wiriya *et al.*, 2013). The high prevalence of aquaculture populations may be due to the high stocking density that is managed in some systems.

## Definitive hosts of trematodes transmitted by tilapia

The presence of definitive hosts that live around the bodies of water are indicators that the life cycle of trematodes can be completed and perpetuated in the environment (Horak



*et al.*, 2019). In the life cycle of trematodes, where tilapias intervene as secondary intermediate host, numerous species of piscivorous birds have been identified as definitive host. For example, the African aninga bird (*Anhinga rufa*) is the definitive host of *Clinostomum* (Mutengu *et al.*, 2018). Another type of definitive hosts are the so-called accidental. Prevalence reports of trematodes associated to tilapia in accidental hosts is unusual; in most cases, the finding of adult worms in unusual hosts is fortuitous.

**Table 3. Prevalence of zoonotic trematodes in accidental hosts that are associated with the production and capture of tilapia**

Trematode	Secondary intermediary host	Host	Adult parasite prevalence	Distribution	Reference
<i>Heterophyes heterophyes</i>	<i>Tilapia nilótica,</i> <i>Tilapia zillii</i>	Jackal,	14.2%,	Korea	(Chai, 2014)
		Fox,	33.3%,		
		Dog	2.5%		
<i>Clonorchis sinensis</i>	<i>Oreochromis</i> sp. <i>Tilapia</i>	Shrimp	3%	Australia	(Wang <i>et al.</i> , 2018)
<i>Heterophyes heterophyes,</i> <i>H. aequalis,</i> <i>Pygidiopsis genata,</i> <i>Haplorchis yokogawai</i> <i>Phagicola ascolonga</i>	<i>Tilapia nilotica,</i> <i>T. zillii</i>	Dog	19.4%, 15.4%, 18%, 12% and 11.4% (Respectively for the reported parasites)	Egypt	(Elsheikha & Elshazly, 2008 <sup>b</sup> )
<i>Haplorchis</i> sp., <i>Procerovum varium</i>	<i>Oreochromis niloticus</i>	Dog, cat, pig	32.7%, 49%, 13%	Vietnam	(Hung <i>et al.</i> , 2015)
<i>Haplochis taichui</i>	<i>Oreochromis niloticus</i>	Cat, dog	70.2% y 56.9%	Vietnam	(Van Phan <i>et al.</i> , 2010)

Table 3 shows the prevalence reports of zoonotic trematodes in accidental hosts that are associated with the production and capture of tilapia. Vertebrate wildlife animals, as well as domestic animals that occasionally include tilapia in their diet, can accidentally become a definitive host by consuming metacercariae that may be found in tilapia (Chai, 2014; Elsheikha & Elshazly, 2008<sup>b</sup>; Wang *et al.*, 2017<sup>b</sup>). In general, it has been observed that the pig (*Sus scrofa*) can be definitive host of *Haplorchis taichui* and *Procerovum varium* (Hung *et al.*, 2015; Van Phan *et al.*, 2010). One of the most studied domestic animals is



the dog (*Canis familiaris*), perhaps due to its social closeness to humans. Dog puppies can act as hosts for *Heterophyes heterophyes*, *H. aequalis*, *Pygidiopsis genata*, *Haplorchis* sp., *Phagicola* sp., *Stictodora* sp., *Ascocotyle (Phagicola) ascolonga*, and *Haplorchis yokogawai* (Elsheikha & Elshazly, 2008<sup>a</sup>; Hung *et al.*, 2015; Lobna *et al.*, 2010; Van Phan *et al.*, 2010). The identification of the final hosts in the transmission of zoonotic parasites is a useful tool for the comprehensive control of parasite loads.

### Geographic distribution of trematodes transmitted by tilapia

The worldwide distribution of trematodes transmitted by the consumption of tilapia meat is shown in figure 1. Undoubtedly, several studies have been carried out in the Asian continent to determine the prevalence and distribution of trematodes with zoonotic potential, perhaps motivated because the consumption of raw fish meat is common. In Vietnam, China, Thailand, Laos, Philippines and India, the genera *Centrocestus*, *Clonorchis*, *Echinostoma*, *Haplorchis*, *Heterophyes*, *Opisthorchis*, *Phagicola*, *Procerovum*, *Pygidiopsis*, *Stellantchasmus* and *Stictodora* have been described (Chi *et al.*, 2008; Dao *et al.*, 2017; Hegazi & Abo-elkheir, 2014; Hung *et al.*, 2015; Kang *et al.*, 2013; Van De *et al.*, 2012; Wang *et al.*, 2017<sup>b</sup>; Wiriya *et al.*, 2013). In the African continent, usually characterized by its abundant biodiversity, the trematodes *Ascocotyle (Phagicola) ascolonga*, *Haplorchis pumilio*, *H. yokogawai*, *Heterophyes aequalis*, *H. heterophyes*, *Pygidiopsis genata* and *Stictodora tridactyla* have been recorded in Egypt (Elsheikha & Elshazly, 2008<sup>b</sup>, 2008<sup>a</sup>; Hegazi & Abo-elkheir, 2014; Lobna *et al.*, 2010); while, in Nigeria, Kenya, Zimbabwe, Ethiopia, Benin and Uganda, it has been documented the presence of the genera *Clinostomum* (Adugna *et al.*, 2020; Echi *et al.*, 2009<sup>a</sup>; Mutengu *et al.*, 2018; Okoye *et al.*, 2014; Sèdogbo *et al.*, 2019; Walakira *et al.*, 2014), *Centrocestus* (Mahmoud *et al.*, 2016) and *Heterophyes* (Ojwala *et al.*, 2018; Otachi *et al.*, 2015). In the European continent, *Clonorchis sinensis*, *Heterophyes dispar* and *H. heterophyes* are distributed in Greece, Italy, Turkey, France, Spain, Russia and Ukraine (Chai, 2014; Chai & Jung, 2017; Hegazi & Abo-elkheir, 2014; Hung *et al.*, 2013; Wang *et al.*, 2018). In the American continent, the epidemiological studies of trematodes on fish are still insufficient and, the occurrence of zoonotic parasites is documented in gastronomic food of Asian origin (Castellanos-Garzón *et al.*, 2019; Leroy *et al.*, 2017); so, the need of conducting safety studies of fish meat used as food source is important (Gutiérrez-Jiménez *et al.*, 2019). There are reports of *Centrocestus formosanus* in Brazil, Costa Rica and the United States (Cortés *et al.*, 2010; Fleming *et al.*, 2011; Pinto *et al.*, 2018). In Mexico, the studies of parasites recovered from fish are focus at the southwestern region of the country. In the states of Veracruz, Oaxaca and Puebla, 39 families of helminths have been described that can infect 35 species of fish. *Centrocestus formosanus* can develop in 16 species of fish, including the cichlids *Astatherops robertsoni*, *Cichlasoma fenestratum*, *Cichlasoma urophthalmus* and *Vieja synspila*. In Chiapas, 72 species of helminths have been identified



in 54 species of freshwater fish, 10 of which are cichlids, and among them the trematode *Clinostomum complanatum* was identified in fish of the genus *Vieja* (Salgado-Maldonado *et al.*, 2005, 2011).

### Influence of socioeconomic activities on trematode distribution

The distribution of zoonotic parasites that infect tilapia (figure 1), is dependent on multiple factors that can influence their dispersion. This is relevant because fishing and aquaculture of tilapia take place in more than 100 countries around the world. The main tilapia producing countries are China with 1.8 million tons per year, Indonesia with 1.1 million tons and Egypt with 875 million tons (Abdel-Latif *et al.*, 2020). However, the prevalence and biodiversity of trematode flukes are associated with the geographic areas where intermediate and definitive hosts converge in the same trophic chain. Socio-economic activities, such as polyculture, wet markets, the unregulated sale of fish hatchlings (Tesana *et al.*, 2014), the consumption of raw food, tourism and migratory flow (Chai & Jung, 2017), among others, promote the occurrence and dispersal of zoonotic parasites (Carrique-Mas & Bryant, 2013). An example of the influence of humans on parasitic dispersal is associated with the import/export of animals for zootechnical purposes.

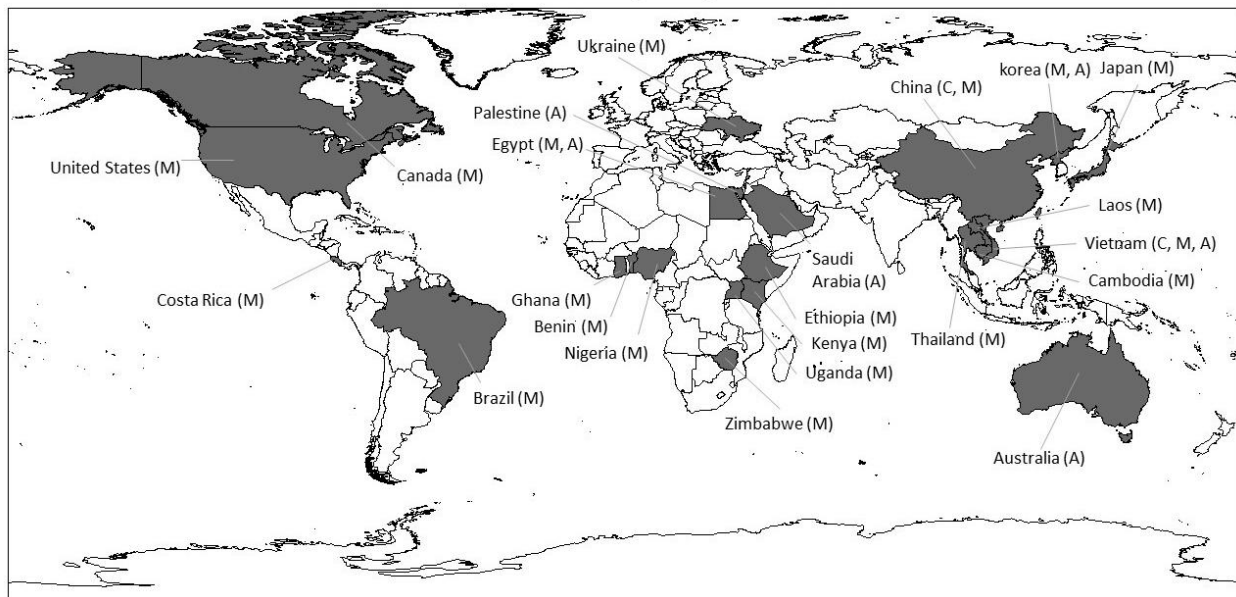


Figure 1. Worldwide distribution of the record of trematodes associated with tilapia with socioeconomic importance. The map shows the presence of cercariae-infected gastropods (C), metacercaria-infected tilapia (M), and adults in definitive hosts (A)



## Trematode pathology in aquaculture of tilapia

Parasites, as their definition indicates, develop pathological problems in the host and, fish are no exception to the rule. The course of pathologies caused by parasites can culminate in economic losses for the producer (Gulelat *et al.*, 2013) and, the severe clinic cases put at risk the food security of some areas with high fish consumption. Fish that present parasitosis are more likely to present secondary pathologies due to viruses, bacteria, or fungi (Mutengu *et al.*, 2018). Tilapias are not exempt from presenting a clinical signs in the presence of larvae of trematodes (Echi *et al.*, 2009<sup>b</sup>), which can be the cause of deficit in the growth of fish, culminating in some morbidity and even mortality (Adugna *et al.*, 2020; Fang *et al.*, 2018). For example, the *Centrocestus formosanus* metacercariae cause swimming alterations (curved, erratic or spiral) of tilapia (Cortés *et al.*, 2010), in addition to edema, hemorrhage, loss of respiratory epithelium, fusion of primary lamellae, destruction of lamellae secondary disorders and distortion due to hyperplasia of the branchial cartilage (Fleming *et al.*, 2011; Mahmoud *et al.*, 2016), causing a decrease in respiratory capacity and death. The infection of *Clinostomum complanatum* metacercariae in *Tilapia guineensis*, trigger hemorrhages and skin damage due to penetration of the cercariae, different degrees of damage to the eyes, from exophthalmia, necrotic cells and ulceration of the coating membranes to blindness (Echi *et al.*, 2009<sup>b</sup>).

### Diagnosis of metacercariae in fish

The lifetime that a metacercaria can be in hypobiosis at muscle of fishes is variable. *Haplorchis pumilio* metacercariae are viable for nine weeks in *Sarotherodon spilurus*, but *Clinostomun complanatum* metacercariae can be present throughout the life of *Tilapia guineensis*, which is 2 to 3 years (Boerlage *et al.*, 2013; Echi *et al.*, 2009<sup>b</sup>). Due to this variability, the diagnosis in fish's hosts is a difficult task. Also, diagnostic tools in aquatic organisms are frequently applied in fish farms than in wild life fish. The tools can be classified in macroscopic, microscopic, histological, microbiological, immunological and molecular, which allow follow-up from the presumptive diagnosis to the isolation and identification of the etiological agent (Sitjà-Bobadilla & Oidtmann, 2017). Regarding parasitological diagnosis, gill biopsies, skin cytology, fecal examination, and post-mortem studies are common within 6 to 8 hours after death. For endo-parasite recovery, liver, spleen and digestive tract are examined, among others (Mjakakhamis & Sagweorina, 2017). The recovery and preservation of parasites require their deposition in 70% ethanol solution for taxonomic identification, generally through morphological characteristics (Elsheikha & Elshazly, 2008<sup>b</sup>, 2008<sup>a</sup>; Sepulveda & Kinsella, 2013). The recovery of metacercariae includes the observation of meat between two glass plates against the light, using a light source of 100 watts. The isolation is achieved from an artificial digestion with pepsin, the resulting material is filtered and washed with 0.85% physiological saline solution (Diaz Camacho *et al.*, 2002). On the other hand, it is well known that the use of



molecular diagnosis is essential for the confirmation of cases and identification of the etiological agents. However, there are still insufficient diagnostic studies in tilapia that have used molecular methodology, such as the polymerase chain reaction in the identification of zoonotic trematodes. For example, in Thailand, three trematodes, *Stellantchasmus falcatus*, *Haplorchis pumilio* and *Procerovum varium*, were identified in Nile tilapia from the amplification of the 28S rDNA gene and the use of primers LSU-5, 1500R and 900F (Wiriya *et al.*, 2013).

### Pathology in humans

Anthropocentric changes, such as the urbanization of wilderness areas, the intensification of food production and the modernization of the market increase the risk of human exposure to unusual pathogens (Carrique-Mas & Bryant, 2013; Chi *et al.*, 2009; Pinheiro *et al.*, 2019; Wang *et al.*, 2017<sup>b</sup>). Even the change or the neglect of human eating habits can be a risk in the transmission of zoonotic diseases. Consuming raw or undercooked tilapia meat can be the source of infection of helminth larvae (Wang *et al.*, 2017<sup>b</sup>), when the human being consumes metacercariae, these larvae will develop into adult worms and, ultimately, the adults will be the origin of the pathology in humans. The manifestations of the disease can range from subclinical to polysymptomatic with different degrees of severity, depending on some factors such as parasite load, immune status, and previous exposures to the etiological agent (Chai, 2014). In general, the infection by adult worms of *Clonorchis sinensis*, *Opisthorchis viverrini* and *O. felinus* are associated with peripheral eosinophilia with intermittent jaundice and leukocytosis in 40% of clinical cases (Zhao-Rong *et al.*, 2005). In early infection, suppurative cholangitis extended to the parenchyma of the liver tissue is observed, causing hepatitis with the formation of micro- and macro-abscesses, whereas in chronic infection there may be cholangiocarcinoma. *O. viverrini* adults are associated with adenomatous hyperplasia of the biliary epithelium and thickening of the walls with fibrous connective tissue, hypertrophy and dilation of the bile ducts (Hung *et al.*, 2013). *Clonorchis sinensis* parasitosis is characterized by hyperplasia and metaplasia of the intrahepatic bile epithelium, followed by periductal fibrosis (Dao *et al.*, 2017; Hung *et al.*, 2015; Wang *et al.*, 2018). *Clinostomum* adults damage the pharynx, in the arytenoid region, oropharyngeal wall and lateral lymphatic band, causing discomfort in the throat, pain when eating food, bloody phlegm and fever (Acosta *et al.*, 2016), becoming diagnosed as pharyngitis, laryngitis or the clinical syndrome called halzoun (Echi *et al.*, 2009<sup>a</sup>; Fang *et al.*, 2018; Williams *et al.*, 2020<sup>b</sup>). Infections with adult worms from trematodes of *Echinostomatidae* family manifest different degrees of focal necrosis and inflammation of the intestinal mucosa, severe infections can cause eosinophilia, abdominal pain, severe diarrhea, anemia, and anorexia (Petney & Taraschewski, 2011). Infection with *Heterophyes heterophyes*, like other flukes of the *Heterophyidae* family, for example, *Metagonimus*, which, transmitted by freshwater carp, cause diarrhea and



abdominal pain, lethargy, anorexia and weight loss, in addition, there may be erratic or extraintestinal parasitism in the heart, brain, and spinal cord (Chai, 2014; Wiriya *et al.*, 2013). The eggs of heterophyid flukes can be carried through the bloodstream to unusual ectopic sites, producing eosinophilic granuloma in the heart, brain, and spine (Elsheikha & Elshazly, 2008<sup>b</sup>, 2008<sup>a</sup>; Hegazi & Abo-elkheir, 2014; Kang *et al.*, 2013). An epidemiological study in Egypt documented that the risk factors for infection with *Heterophyes aequalis*, *H. heterophyes*, *Pygidiopsis genata*, *Ascocotyle (Phagicola) ascolonga* or *Haplorchis yokogawaik* were associated with (1) female sex (odds-ratio (OR) = 1.59), (2) be fisher (OR = 1.39) or homemaker (OR = 1.24) or (3) being in the age groups of 15 to 45 years (OR = 2.22) or 5 to 14 (OR = 1.29) (Lobna *et al.*, 2010). In Vietnam it was found that the frequency of infection is higher in people over 19 years of age (4.2 to 53.8%) compared to those under 19 years of age (1.4 to 13.4%), likewise, the occurrence by gender was 31.1% in men, while in women it was 13% (Hung *et al.*, 2015). In Vietnam, during the evaluation of the prevalence of *Haplorchis taichui* in ponds where the tilapia *Oreochromis niloticus* is produced, a negative effect of the ignorance of the inhabitants on the sanitary quality of the water with which the fish produce was documented, the occurrence of the parasites was 76% in ponds fed with channel water and 82% in ponds where the feed water had contact with drainage discharges (Van Phan *et al.*, 2010).

### Intervention strategies

One of the recurring activities in aquaculture to avoid parasitic infections is the elimination of intermediate hosts. The use of anthelmintic drugs in aquaculture is common; however, the development of drugs for the exclusive use of aquaculture is still insufficient. The use of 70% niclosamide directly in the water body is one strategy to parasite control by elimination of gastropod mollusks, which is the intermediary host of *Opisthorchis viverrini* (Tesana *et al.*, 2014). Regarding the treatment against helminths in aquaculture, only a few drugs such as praziquantel have been used in *Oreochromis niloticus* (Bader *et al.*, 2019). Praziquantel has also been used at a dose of 2.5 mg/L as an antiparasitic in target pufferfish (*Sphoeroides annulatus*), where a combination of praziquantel, ivermectin, pyrantel and fenbendazo was also used (Morales-Serna *et al.*, 2018). Praziquantel has also been used at doses of 2 to 40 mg/L dispensed in feed for catfish, carp, and trout (Bader *et al.*, 2019). Albendanzaol has also been used as an anthelmintic treatment in tilapia, however more studies are needed regarding the use of anthelmintics in aquatic organisms (Portela *et al.*, 2020).

### CONCLUSION

In this work, we described that the biodiversity of trematodes with zoonotic potential that can be transmitted by tilapia is abundant. Most of these trematodes have been described with greater occurrence in Asia, Africa and Europe, but prevalence and distribution studies





are insufficient in America. It is also clear that human activities in the food industry also influence the spread of trematodes in the world, without taking into account that climatic changes and the condition of the host can also influence the prevalence of trematodes in fish. Finally, in this work we document that tilapia, a fish of high socio-economic value, without proper sanitary management can become a transmitter of trematode parasites with zoonotic potential. New studies should be carried out in tilapia with another taxonomic group of helminths, such as nematodes and cestodes to have a complete picture of the biodiversity of helminths and the risk of being transmitted to humans.

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