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One Health in the Brazilian Western Amazon: helminths in native fish and the food change of the Puruborá people

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ABSTRACT

The aim was to approach an analysis of fish parasites in the Manoel Correia and Caio Espínola Rivers, which border the Aldeia Aperoí, and the report of the Puruborá People on the use of fish as animal protein. Twenty-one fish were collected in the dry season and 15 in the rainy season, totaling 36 specimens, which dissipated cestodes, acanthocephala and nematodes encysted in the musculature, preventing their consumption. Six residents of Aldeia Aperoí were interviewed. The circulation of parasites with zoonotic potential was identified in fish native to the Manoel Correia and Caio Espínola rivers, which border the Aldeia Aperoí, in the Western Amazon of Brazil. It was verified the helplessness of the Puruborá people regarding their inquiries about the environmental imbalance, which causes the loss of fish diversity and, consequently, damage to their culture and their costumes.

Introduction

Foodborne diseases (FBD) are infections or intoxications caused by eating contaminated food and/or water that have an impact on public health¹. There are more than 250 types of FDB, and among them, those caused by parasitic infections due to fish consumption^{2,3}.

The identification of parasitic diseases associated with the consumption of fish in Brazil is not easy, as most are not mandatory to report. Additionally, due to the low severity of the symptoms and the little knowledge by the doctors on the subject, they go unnoticed⁴. Thus, the most appropriate approach to parasitic diseases in fish is in the context of one health, since ecological studies on freshwater fish parasites provide relevant data on the seasonality and susceptibility of the parasites and their hosts, while analyze the health status of fish, streams and indigenous people who use this source of protein as food, in addition to the quality of the water. Thus, there are answers regarding the imbalance of the environment and the possibility of zoonoses due to the consumption of parasitic fish⁵.

Fish are, worldwide, an important protein source for thousands of people; however, for indigenous and riverine people it does not only represent the food and economic base, but also the maintenance of customs and culture⁶, especially for groups that settled on the banks of rivers, as occurs in the Amazon. The Puruborá People on the state of Rondônia in the Western Amazon of Brazil is such an example.

The history of colonization and occupation of the state of Rondônia (which was created only in 1981), located in the western Brazilian Amazon, is full of disorderly occupations and invasions of forest spaces and indigenous lands. This is the result of several economic cycles: rubber, wood and precious metals. In all cycles, the destruction or extinction of indigenous ethnic groups was observed⁷.

The Puruborá people had their first contact with non-indigenous people in 1909, and from that moment on they began deconstructing themselves as a people/ethnicity. They suffered great miscigenation with the rubber tappers who occupied the area during the rubber cycle. Thus, the Puruborá people were expelled from their lands, and expropriated from their language and culture and were almost decimated by diseases⁸. Over the years they spread throughout the territory of Rondônia and for survival, they became an ethnic group with mixed race with blacks, whites, other indigenous peoples, in addition to Bolivians and quilombolas^{8,9}.

At the end of the 1990s the Puruborá were forced to start a new life on the banks of the BR-429, between the municipalities of Seringueiras and São Francisco do

Guaporé, in Rondônia, which was later known as Aperoí Village¹⁰. Currently, the Puruborá People try to recover their identity, language and customs¹¹.

However, the eating behavior based on fish protein has been modified due to environmental changes caused, among others, by damming and contamination of rivers, loss of ichthyofauna diversity and increase of parasites in fish. In relation to the latter, it is known that parasites can be bioindicators of environmental balance, since they present advantages over other organisms, such as rapid response to environmental changes, due to the interdependence of the ecological networks in which they live¹². In Brazil, ethnoichthyological studies under the view of health status with riverside and indigenous populations are scarce.

Thus, this study aimed to address the analysis of fish parasites in the Manoel Correia and Caio Espínola Rivers, which border the Aperoí Village and the Puruborá People's narratives on the use of fish as animal protein.

Results

Identification of parasites

In Aperoí Village fishing can be performed by men and women, and also by children, when accompanied by adults.

We identified in this work the following parasites: of the genus *Eustrongylides* sp., third-instar larvae of *Contracaecum* sp., metacercaria of *Ithyoclinostomum* sp. and *Neoechinorhynchus buttnerae* (Table 1).

Table 1. Prevalence, location and parasites identified in naturally infected native fish in the Manoel Correia and Caio Espínola rivers, municipality of Seringueiras, state of Rondônia, Brazil.

Sample	Scientific name	Common name	Parasite	Tissue	P*
N=16	<i>Hoplerythrinus</i> sp.	Jeju	<i>Eustrongylides</i> sp.	Skeletal muscle	100
			3rd-instar larvae of <i>Contracaecum</i> sp.	Encysted in the intestinal serosa	18
			Metacercaria of <i>Ithyoclinostomum</i> sp.	Skeletal muscle	6.25
N=17	<i>Hoplias</i> sp.	Traíra	<i>Neoechinorhynchus buttnerae</i>	Intestine	35.29

			<i>Eustrongylides</i> sp.	Skeletal muscle	100
			3rd-instar larvae of <i>Contracaecum</i> sp.	Stomach and mesentery serosa	41.17
			metacercaria of <i>Ithyoclinostomum</i> sp.	Skeletal muscle	11.76
N= 2	<i>Cichla</i> sp.	Tucunaré	<i>Eustrongylides</i> sp.	Skeletal muscle	50
N= 01	<i>Pimelodus</i> sp.	Mandim	-	-	0

*P – prevalence by fish species.

Transcript of interviews with indigenous residents of Aperoi village

We selected relevant excerpts regarding the dietary change in the use of fish protein in the past ten years in Aperoi Village. The researchers respected the way of speaking and the common name that the interviewees gave to objects and fish.

Researcher asked: Good morning! Could you please tell me how the fishes and the Manoel Correia and Caio Espínola rivers were like about ten years ago?

Interviewee 1: Regarding what we eat, we used a lot of fish. From about ten years ago our river has changed very much in the aspect of water volume – because before our river didn't get dry – so it was plenty of fish all the time and there wasn't a lot of fisherman. But then the number of fishermen increased and the river is now drying up. It is very harmful for the people who need this food, as we believe that some worms in fish are caused by the lack of water. When we catch and open the traíra it has worms. We normally take it out and consume it anyway, but when it has a lot of worms, we have to throw it away. The same thing is with the peacock bass, also with lots of worms during the dry season. But I don't know if it's because the water is still when the river is drying up, or if it's something that it uses as food. For example, we fish the pacu (*Piaractus mesopotamicus*) with longline (a heavy fishing line that may be many miles long and that has baited hooks in series) during the flood season, but it also comes with many worms. We don't know whether it is good or bad to eat these fish, but the river is a

very essential source of food for us, the Puruborá, it is really important.

Researcher asked: Do you believe there has been a great decrease in fish populations in the Manoel Correia River? And if so, in which species did you notice the greatest decreases and presence of parasites?

Interviewee 2: The peacock bass, piau (*Leporinus* sp.), piranha (*Pygocentrus* sp.) and catfish had no worms, it was nice then.

interviewee 3: The fish that we used to catch a lot in this river and now catch little are the peacock bass. In the dry season you, no way eats them because they are all full of worms. The white piranhas decreased a lot, I used to kill them with arrows and they were very clean. When I went fishing with my wife, I got a mandubé (carnivorous fish of the genus *Ageneiosus*) and when I opened its tripe it was full of worms and we had to throw it away.

Researcher asked: Have you and the community decreased or stopped eating fish due to parasites?

Interviewee 4: It really decreased, because in the past I used to catch fish that didn't have worms, but now fish have a lot of worms. People used to eat some of them and now they don't eat because of the worms. Nobody buys peacocks and traíras because of the worms. People buy more piau, which is now without worms. However, there was piau that had worms in the belly, but without getting into the skin, but I think there will be later on. And that affects sales of peacock bass: nobody wants to buy it because of the worms. I sell pacu more because it eats fruits; but in a while they will also have worms as even in fruits, we may find worms.

Interviewee 5: I believe so, when I talk to my brother-in-law, he says he is disgusted to eat our fish, because of lots of worms; ox dies by the river, so people are afraid to eat fish.

Researcher asked: Why do you think fish are so full of parasites?

Interviewee 3: [...] I believe it is just like my brother said, because we were born and raised here. From about 15 years ago on, things are getting more difficult every day. I also believe that this has to do with the tree felling and a lot of poison that farmers throw on

the land; To plant soy they throw a lot of poison, to harvest soy they throw more poison and we believe that all these poisons go to the river, so a lot of fish are dying this year. That's been happening for two years in the São Miguel River, which is almost our river, one is an affluent of the other. There was a lot of fish mortality, and in our river, there was also fish mortality. People believe that it is because of these poisons thrown in the fields and there are many streams here that throw these contaminated waters into our river. Our river is not so big here, so we can see this all happening.

Researcher asked: Are there any further considerations about the fishes, the river and the parasites that you would like to share?

Interviewee 6: Well, if they stopped using poison, I think these worms would decrease. We believe that poison and felling trees are making fish sick. There will come a time when we will not eat fish anymore, no one will want to eat a fish full of worms, people will no longer call friends to eat fish for when they try it, worms will appear and they'll say you gave them fish with worms to eat. One day we'll eat only meat.

Interviewee 6: First of all, I would like the authorities to take action on what is happening to our rivers. We had protection from IBAMA (Brazilian Institute of the Environment and Renewable Natural Resources), but now with this current government (The chieftain refers to the current Brazilian government of President Jair Bolsonaro) ... It's very sad for us who were born and raised here, it's been very difficult. But imagine how it will be in two, three years? We are sure that the children that are being born will not know the fish that we know.

Interviewee 7: What I believe that harms the fish are these farms on the banks of rivers and streams, the owners put machinery in and instead of pushing things for a place and burn it, they push everything into the water and it becomes rotten water. Then, when the flood season comes, that black, rotten water, looking like coffee, goes to the river.

Discussion

The history of the Puruborá ethnicity can be portrayed as an example of the modification of Brazilian forest spaces and the change in eating behavior, among others, due to the imbalance in the environment. In this research, several reports describe the decline of fishing in the Aperoí Village, of the Puruborá ethnicity. Among the problems mentioned are the presence of parasites in fish, and the main complaints are disgust and fear of becoming ill. In fact, some of the parasites identified in this study have zoonotic potential.

A large number (prevalence = 100%) of *Eustrongylides* sp. in the musculature of jeju, traíra and tucunaré, fish that are very popular in the region. This parasite has been reported in several parts of the world, in Europe¹³, in Asia¹⁴ and in the Americas^{15,16}.

Larvae of *Eustrongylides* sp. have been described mainly in traíra and jeju in several states in northern Brazil^{8,15-18}.

In the literature, there are records of infection in humans and the Center for Disease Control (CDC) in the United States of America (USA) has warned of these possible zoonoses since 1982¹⁹. According to the Brazilian Regulation for Industrial and Sanitary Inspection of Products of Animal Origin – RIISPOA³, fish with endoparasites in the muscle are inappropriate for human consumption, especially when they are raw. Also, the US Food and Drug Administration (FDA) Agency warns that all fish products that are not cooked or processed at temperatures less than 60° centigrade should be frozen at -35°C for more than 15 h, or at -23°C for a minimum period of 7 days²⁰.

However, it is common to consume raw fish fillet with lemon and salt during fishing¹⁵, not only by indigenous people and riverine people, but by sport fishing (personal communication).

The presence of *Eustrongylides* sp. in the musculature of the fish it gives a disgusting aspect, due to the large size of the larvae, reddish color and quantity found (as in the present study). It is important to emphasize that when fish are low (of parasites) they remove and ingest the meat (narrative of the interviewees). This highlights the importance of health education to the population regarding the risks of eating raw or undercooked fish. In reports of human infection, it was possible to observe that the larvae penetrate the digestive tract wall, causing perforation and severe pain²¹.

In addition, *Contracaecum* sp., a parasite of the Anisakidae family, also has zoonotic potential and has been frequently described in native fish in Brazil²², and generally in co-infection with *Eustrongylides*²³. There are no reports of parasitized

humans in Brazil; however, literature reveals them in other countries such as South Korea²⁴, Australia²⁵ and Japan¹⁴. We must add that descriptions of infections with *Contracaecum* larvae were of severe and painful condition in humans²⁶.

There has been no epidemiology work carried out at the Aperoí Village to identify possible cycles of *Eustrongylides* and *Contracaecum* within the human community yet. Accordingly, no case of illness has been reported without identifying the primary cause. However, as the signs of such parasitism are not specific, it can be inferred that if infection occurs in this population, it may go unnoticed. Another important point to make is that, due to the unpleasant aspect of parasites in the fish meat, it turns to be disagreeable for consumption, consequently reducing its possibility and therefore, of infection.

Another parasite identified in this study was *Ithyoclinostomum* sp., which is a digenetic trematode, and has no reports of zoonotic potential, but that the presence of cysts containing the metacercariae of this parasite causes disgust²⁷. This parasite has been found from north to south of Brazil^{28,29} and studies on the parasite-host relationship need to be conducted in order to understand the role of each host within the epidemiological cycle as well as the action of the parasite on the performance of the host.

The parasite *Neoechinorhynchus buttnerae*, Acanthocephala, which is located in the intestine of fish does not have zoonotic potential; however, it causes spoliation and in high parasitic loads can cause ulcerations, due to the penetration of the proboscis in the intestinal mucosa, and subsequent necrosis of the intestinal epithelium, reflecting the lack of development, appetite, and finally death²².

In Brazil, reports of human infection by parasites of native fish are scarce and attention to the health of indigenous people and the environment in which they live is poorly disclosed. It is well known that the environment, environmentalists and indigenous peoples are being severely threatened by the current Brazilian Federal Government. President Jair Bolsonaro publicly defends the opening of indigenous lands to miners and farmers, even if it increases deforestation rates in the country³⁰. In 2019, during the development of this research, there were several reports by the chief Hozana Puruborá of coercion and even of an attempt on her life, since she is an activist for indigenous rights, recognition of the Puruborá people and demarcation of their lands (personal communication).

The health of the indigenous people is closely linked to the health of the

environment; therefore, the use of One Health is fundamental when analyzing populations that are culturally linked to the environment and primarily depend on natural resources for their livelihood. It would also be fundamental to identify at which points of the eco-epidemiological chain of zoonotic parasites are out of balance and try to mitigate and prevent disease outbreaks. Indeed, we are aware that about 75% of human diseases emerging in recent years were caused by pathogens from animals³¹. The use of pesticides and the deforestation of riverbanks put at risk the diversity of fauna, which serves as food for indigenous peoples^{32,33}.

This fact was reported several times by the interviewees in this study, who observed that deforestation on the banks of the rivers around the village, the low water quality and the increase in fish parasites in the last ten years, have consequently forced them to choose another source of animal protein.

The indigenous peoples are called native peoples and are the guardians of Brazilian forests. Their defense, as well as that of their rights and health, is a responsibility of all Brazilians, and in general, of all countries in the world. Indeed, to preserve the health of the planet is to preserve human life. Thus, awareness of all the population and access to disease prevention information to indigenous peoples minimizes the risk of infections.

Methods

Ethics and procedures

This study was approved by the methods involving humans' subjects and were performed in accordance with relevant guidelines and criteria according to Research Ethics Committee (CEP) number 2689.242 of the Federal University of Rondônia. All participants from the indigenous community, are majority of age, agreed to the Terms of Free and Informed Consent (TCLE) and agreed that their answers are published. Informed consent was obtained from all the subjects and the study was performed in accordance with relevant guidelines. All fishes used in this study were handled in accordance with the Ethics Committee on the Use of Animals - CEUA, number 005/2018, approved by the Federal University of Rondônia. All analyzes performed with fish were in accordance with ARRIVE guidelines (<https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000411>), and regulations of this committee, and by Chico Mendes Institute of Biodiversity

Conservation (ICMBio), number 63086 (<https://www.gov.br/icmbio/pt-br/servicos/autorizacao-para-licenciamento-1>) for the study of fished native specimens.

Data collection location and sampling

The collections of native fish were carried out in the Manoel Correia and Caio Espínola Rivers, which border the Aperi Village, located in the municipality of Seringueiras, state of Rondônia, in the western Amazon of Brazil.

Four members of the indigenous community collected the fish in October 2019 and January 2020, according to traditional customs (nets, meshes of varying sizes, fishing rod and arrow), at four different points in the Manoel Correia and Caio Spinola Rivers: point 01: S 20° 27.261', W 061° 43.979' elevation 415 m; point 02: S 11° 56'24,180", W 063° 15'41.0440" elevation 166 m; point 03: S 11° 55.965', W 063° 15.296' elevation 500 m; and point 04: S 11° 46,302 ', W 061° 40,322' elevation 390 m.

Once the fish were collected, they were packed individually by the researchers in plastic bags identified with the date and place of collection and placed in isothermal boxes with ice for transportation (under 8.0°C), for 148.8 km, until the Laboratory of Parasitology, Entomology and Molecular Biology focused on One Health (LAPEMSU) at the Federal University of Rondônia, campus Rolim de Moura.

A number of 21 fish were collected in the dry period and 15 in the rainy season, totaling 36 specimens. The fish were weighed, measured (Table 2) and necropsied. For parasitic analysis, the organs were separated and observed under a stereomicroscope, namely: eyes, gills, intestines, liver, spleen, swimming vesicle and skeletal musculature.

The digestive tract was separated into portions and its contents were washed with 0.9% saline solution for analysis under a stereomicroscope to collect the parasites.

The nematode helminths, larvae and cysts were conditioned in 70% alcohol and the *Acanthocephali* were fixed in 5% hot formalin to distend the proboscis. The identification of the parasites was based on specific bibliographic consultation according to the findings and the methodologies described by Thatcher³⁴, Martins³⁵ and Eiras et al.³⁶.

Table 2. Fish species collected in Manoel Correia and Caio Espínola rivers, municipality of Seringueiras, state of Rondônia, Brazil.

Sample	Point	Scientific name	Common name	Length (cm)	Weight (g)
1	4	<i>Hoplerythrinus</i> sp.	Jeju	29	301
2	4	<i>Hoplerythrinus</i> sp.	Jeju	28	255
3	4	<i>Hoplerythrinus</i> sp.	Jeju	25	192
4	4	<i>Hoplerythrinus</i> sp.	Jeju	25	195
5	4	<i>Hoplerythrinus</i> sp.	Jeju	30	289
6	4	<i>Hoplerythrinus</i> sp.	Jeju	27	210
7	4	<i>Hoplerythrinus</i> sp.	Jeju	30	346
8	4	<i>Hoplerythrinus</i> sp.	Jeju	31	340
9	4	<i>Hoplerythrinus</i> sp.	Jeju	29	313
10	4	<i>Hoplerythrinus</i> sp.	Jeju	21	123
11	4	<i>Hoplerythrinus</i> sp.	Jeju	25	196
12	4	<i>Hoplerythrinus</i> sp.	Jeju	30	227
13	4	<i>Hoplerythrinus</i> sp.	Jeju	22	119
14	4	<i>Hoplerythrinus</i> sp.	Jeju	29	294
15	3	<i>Hoplerythrinus</i> sp.	Jeju	24	119
16	4	<i>Hoplerythrinus</i> sp.	Jeju	27	245
17	1	<i>Hoplias</i> sp.	Traíra	30	298
18	3	<i>Hoplias</i> sp.	Traíra	31	388
19	3	<i>Hoplias</i> sp.	Traíra	33	407
20	3	<i>Hoplias</i> sp.	Traíra	25	144
21	1	<i>Hoplias</i> sp.	Traíra	36	480
22	3	<i>Hoplias</i> sp.	Traíra	28	184
23	1	<i>Hoplias</i> sp.	Traíra	31	311
24	3	<i>Hoplias</i> sp.	Traíra	32	317
25	1	<i>Hoplias</i> sp.	Traíra	35	439
26	2	<i>Hoplias</i> sp.	Traíra	20	77
27	1	<i>Hoplias</i> sp.	Traíra	25	124
28	3	<i>Hoplias</i> sp.	Traíra	35	423
29	2	<i>Hoplias</i> sp.	Traíra	31	296
30	1	<i>Hoplias</i> sp.	Traíra	33	293
31	2	<i>Hoplias</i> sp.	Traíra	32	325

32	2	<i>Hoplias</i> sp.	Traíra	34	331
33	2	<i>Hoplias</i> sp.	Traíra	30	248
34	1	<i>Cichla</i> sp	Tucunaré	35	582
35	1	<i>Cichla</i> sp	Tucunaré	31	491
36	1	<i>Pimelodus</i> sp.	Mandim	30	130

Interviews with Puruborá indigenous people in Aperoi Village

We developed this investigation according to the research precepts described by Gil³⁷, as an exploratory study based on interviews. The researcher, also belonging to the Puruborá ethnic group, personally conducted six interviews with seven residents of Aperoi village (one of the interviews was conducted with two people: interviewee 1 and her brother interviewee 2. The names of the five remaining interviewees are: interviewee 3 (husband of interviewee 1), interviewee 4, interviewee 5, interviewee 6 and interviewee 7. The interviews were recorded in audio and then transcribed. The questionnaire presented five questions previously planned and structured and applied in the following order:

- 1 °) Could you please tell me how the fishes and the Manoel Correia and Caio Espínola rivers were like about ten years ago?
- 2 °) Do you believe there has been a great decrease in fish populations in the Manoel Correia River? And if so, in which species did you notice the greatest decreases and presence of parasites?
- 3 °) Have you and the community decreased or stopped eating fish due to parasites?
- 4 °) Why do you think fish are so full of parasites?
- 5 °) Are there any further considerations about the fishes, the river and the parasites that you would like to share?

In conclusions, the circulation of parasites with zoonotic potential was identified in fish native to the Manoel Correia and Caio Espínola rivers, which border the Aperoi Village, in the Western Amazon of Brazil.

The Puruborá People's helplessness was verified regarding their inquiries about the environmental imbalance, which causes loss of fish diversity and, consequently, damage to their culture and customs. And above all, from the exposure of the indigenous people of Aperoi Village to parasitic infections due to the prevalence of zoonotic parasites in native fish used as a source of animal protein in their diet.

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Author contributions

M.A.P.F., G.O.M., and W.G.M. contributed equally to this work, performed, reviewed, revised, and agreed with the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Ethics declarations

This study was approved by the Research Ethics Committee (CEP) number 2689.242 of the Federal University of Rondônia for the methods involving humans' subjects and were performed in accordance with relevant guidelines and criteria according to Research Ethics Committee (CEP) number 2689.242 of the Federal University of Rondônia.