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Histopathological lesions in *Corydoras* spp. (Siluriformes: Callychthyidae) caused by *Procamallanus (Spirocamallanus) pinto* (Nematoda: Camallanidae) from Iquitos, Peru

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Submitted: May 23rd, 2024. Accepted: November 8th, 2024.

Abstract

Callichthyidae is a family of armored catfishes commercially important in the Peruvian Amazon. Among these species, *Corydoras acutus*, *C. reticulatus*, and *C. virginiae* are stored for weeks or months and then exported to different countries worldwide, where they may present some sanitary problems along the way, such as parasitic infections. In the present study, it was reported the level of infection of *Procamallanus (Spirocamallanus) pinto* (Kohn and Fernandes, 1988), through the calculation of their parasitological indices and the description of their histopathological lesions in the intestinal tract. Sixty individuals of each species were acquired from fishermen of the District of Belén, in Iquitos, Peru. Fish were transported to the “Laboratorio de Parasitología y Sanidad Acuicola” from the “Instituto de Investigaciones de la Amazonía Peruana” (IIAP), in Iquitos-Peru. Parasitological examination allowed us to identify the nematode *P. pinto* infecting the intestinal tract of the fish. Histopathological analysis revealed: inflammatory cell infiltration (73.3%, 63.3%, and 60%), enterocyte hyperplasia (90%, 70%, and 63.3%), desquamation of epithelial cells (90%, 70%, and 63.3%), goblet cell hyperplasia (76.7%, 66.7%, and 50%) and necrosis (70%, 60%, and 66.7%), in *C. acutus*, *C. reticulatus*, and *C. virginiae* respectively for each lesion.

Keywords: Aquaculture, Amazon, histopathological lesions, necrosis, fish health.

Introduction

Of the approximately 2500-3000 fish species in the Amazon Basin (Swing 1985), 980 have been recorded in Peru (28), but the actual number is probably about 1200. Many of these species are fascinating, colorful, and possibly suitable for the ornamental aquarium trade (11, 17).

However, ornamental fish raised in aquariums are frequently infected with helminth parasites that perhaps cause high fish mortality (24).

Helminths are known to affect three main axes: economic, medical, and biological (30, 22). Parasitic nematodes affect their hosts in various ways, including mechanical damage such as irritation, tissue atrophy, obstruction of the alimentary canal, and blood vessels (39). Nematodes

belonging to the superfamily Camallanoidea are characterized by having a sclerotized buccal capsule, an organ that, while convenient for identification, damages the host when attached to the walls of the intestine by feeding on blood and causing anemia (25,35, 37, 38).

The Camallanoidea may also have spine hooks or ridges decorating the body cuticle (19). The host's body size is of biological importance because it affects the size of the likely parasite habitats it provides and thus contributes to the aggregate characteristics and composition of parasite infra communities (4, 7). Moreover, total species richness, abundance, and parasite prevalence are directly related to the size of the host (13, 32).

The objective of the present study was to determine the parasitological indices caused by *Procamallanus (Spirocamallanus) pintoii* (Kohn and Fernandes, 1988) and to recognize and classify their histopathological lesions in the intestinal tracts of *Corydoras acutus* (Cope 1872), *Corydoras reticulatus* (Fraser-Brunner 1938) and *Corydoras virginiae* (Burguess 1993) acquired in Iquitos, Peru.

Material and Methods

Ethics approval

The present study was approved by the Institutional Ethics Committee for the use of animals (CIEA) of the Universidad Peruana Cayetano Heredia, Lima, Peru, with approval number 204658.

Locality

Samples were acquired in the District of Belén, Iquitos, Peru, and then transported to the "Laboratorio de Parasitología y Sanidad Acuicola" of the "Instituto de Investigaciones de la Amazonía Peruana" (IIAP), Iquitos, for parasitological analyses.

Fish collection

Sampling was carried out between November 2020 and February 2021. Fishermen took 180 specimens, 60 of which were *C. acutus*, 60 were *C. reticulatus*, and 60 were *C. virginiae*. Thirty of the 60 specimens of each species were kept for parasitological analysis, and 30 were kept for histopathological analysis.

Fish were euthanized by immersion in eugenol (75 mg/l water) followed by a spinal cut using a scalpel-type stylet to desensitize them according to the ethical standards suggested for the sacrifice of fish (21).

Parasite collection fixation and identification

A cut was made in the abdominal cavity, the gastrointestinal tract (stomach and intestine) was removed, and lengths were placed in order in Petri dishes of distilled water. All the recovered nematodes were fixed and stored in hot 70% ethanol. Worms were cleared in lactic acid according to the technique described by Murrieta & Floríndez (26) and observed, using an optical microscope, for taxonomic study, following the method of Kohn and Fernández (16). Taxonomic identification was carried out with the aid of a Leica DM750 optimal light microscope. The taxonomic keys of Moravec (25) and Kohn and Fernández (16) were used to identify the parasite species. Photographs were taken with a Leica ICC50 digital camera, and using Leica LAS EZ software, the sheet with the sample was washed in 70% alcohol to remove the bleach to allow preservation and labeling.

The morphometric data of *P. pintoii* were taken from prepared specimens using a LEICA ICC50 W microscope incorporating an Axio Cam-type camera with Leica Application Suite software. The number of striae, length of the buccal cavity (mm), width of the oral cavity (mm), muscle esophagus length (mm), muscular esophagus width (mm), length of glandular esophagus (mm), width of glandular esophagus (mm), number of papillae in males and tail length (mm) were recorded.

Parasitological indices

Parasitological indices of prevalence (P%), mean intensity, and mean abundance of infection followed (5).

Histopathology

During necropsy, two posterior portions of the gastrointestinal tract were taken, one infected with *P. pintoii* and one not from each fish, so that comparisons could be made between apparently healthy (without parasites) and infected fish (parasites being visible through the skin). These samples were fixed in 10% formalin and processed following the conventional histological methods used in the Parasitology Laboratory of the Universidad Peruana Cayetano Heredia, Lima.

Worms were dehydrated in ascending concentrations of ethyl alcohol, cleared, and then embedded in paraffin. Tissue sections at 5µm thickness were taken and stained with hematoxylin and eosin (H&E). A total of 60 slides (20 slides of specimens from *C. acutus*, 20 from *C. reticulatus*, and 20 from *C. virginiae*) were examined using a LEICA ICC50 W phase microscope to discover any histological alterations, and microphotographs were taken. The histopathological lesions caused by *P. pintoii* were classified according to the degree of involvement by area in the examined tissue, using a graded scale ranging from 1-4: Degree I (Scarce): Little presence of a lesion, up to approximately 25%

in the entire sample studied, II (Mild): Presence of injuries, in more than 25%, up to 50% of the entire study sample, III (Moderate): Injuries found in more than 50%, up to 75% of the sample studied, IV (Severe): Affection in more than 75% and even reaching 100% of the study sample (33).

The histological lesions in the intestines of *C. acutus*, *C. reticulatus*, and *C. virginiae* were classified according to the type of alteration and its effects as inflammatory disorders, adaptation disorders, or degenerative disorders (10, 12).

Results

Parasite identification

Medium size nematodes with finely striated cuticles. Buccal capsule with inner spiral thickenings. Esophagus divided. Its anterior muscular portion is club-shaped, posterior glandular part is almost cylindrical, longer than the muscular portion. Female specimens are bigger than males (Fig. 1A). Male specimens with 3 mm of body length. Buccal capsule with 6-8 spiral thickenings (Fig. 1B); caudal papillae with 4 pairs

of preanal, and 2 pairs of postanal papillae, spicules short and similar (Fig. 1D). Female specimens with 22 mm body length, buccal capsule with 6-8 spiral thickenings (Fig. 1C); the tail is conical and pointed (Fig. 1E). Morphometric characteristics of *Procamallanus pintoi* belonging to *C. acutus*, *C. reticulatus*, and *C. virginiae* are presented in Table 1.

Parasitological indices

For *C. acutus* it was reported a prevalence of 100% with a total number of parasites of 37, mean intensity and mean abundance of infection of 1.23; *C. reticulatus* presented a prevalence of 87% with a total number of parasites of 33, mean intensity of 1.26 and mean abundance of 1.1; *C. virginiae* presented a prevalence of 80% with a total number of parasites of 26, mean intensity of 1.08 and mean abundance of 0.86.

Histopathology

Table 2 presents the frequency of histological alterations of the intestine, according to the type of lesions and degree of affection in *C. acutus*, *C. reticulatus*, and *C. virginiae*.



Figure 1. A- Female (big nematode) and male (small nematode) of *Procamallanus (Spirocamallanus) pintoi*. B. Buccal capsule of male specimen. C. Buccal capsule of female specimen. D. Tail of male. E. Tail of female.

Corydoras acutus

In the intestinal tract 70% (21/30) of the samples showed necrosis; hyperplasia of enterocytes (yellow circle) in 90% (27/30) (Fig. 2A, 2B); hyperplasia of goblet cells (asterisks) in 76.7% (23/30) (Fig. 2B); inflammatory cell infiltration (red circle) in 73.3% (22/30) (Fig. 2C); in 90% (27/30) desquamation of the epithelial cells (blue circle) (Fig. 2D).

Corydoras reticulatus

In the intestinal tract of *Corydoras reticulatus*, 60% (18/30) of the samples showed necrosis (red circle), and 70% (21/30) and desquamation of the epithelial cells (Fig. 3A); inflammatory cell infiltration (orange circles) was found in 63.3% (19/30) (Fig. 3B); hyperplasia of goblet cells (yellow circle) in 66.7% (20/30) (Fig. 3C); hyperplasia of enterocytes in 70% (21/30) of fish, and destruction of the mucosa (green circle) (Fig. 3D).

Corydoras virginiae

The mucosa of *C. virginiae* was made up of folds with few goblet cells observed. The submucosa consisted of loose connective tissue with a few isolated lymphocytes. In the intestinal tract of *C. virginiae* 66.7% (20/30) of the samples showed necrosis and 63.3% (19/30) desquamation of the epithelial cells (green circle) (Fig. 4A); inflammatory cell infiltration (red circle) was found in 60% (18/30) (Fig. 4B); hyperplasia of goblet cells (blue circle) in 50% (15/30) (Fig. 4B); hyperplasia of enterocytes in 63.3% (19/30) of

fish, and presence of macrophages and inflammatory cells (yellow circle) (Fig. 4B).

Discussion

Fish Identification

Nematodes, in particular, may cause large losses of infected fish. Representatives of the genera *Camallanus*, *Procamallanus*, and *Spirocamallanus* have been reported as causing health problems and histopathological damage in several species of fish in the Amazon region (25, 26, 34). Therefore, it is important that camallanid nematodes can be accurately identified to ensure that the pathological effects of infection are accurately determined for each fish-parasite species model and fish health models can be correctly applied. The morphometric variability of *P. pinto* was found when comparing our data with that of Kohn and Fernandes (16) and Ailan-Choke (1) these measurements were congruent with those reported by Kohn and Fernandes (16) in describing *P. pinto* from two *C. paleatus* species collected from Iguazu River basin although the buccal capsule (0.52-0.54 mm), and the glandular esophagus (587mm), were shorter than in this study. The measurements of *P. pinto* in this study were also similar to those of *P. pinto* from *C. micracanthus* (Regan 1912), *C. paleatus* (Jenyns 1842) from Cuenca del Rio Bermejo, Argent. Although, for example, the glandular esophagus, 0.924–0.990 mm long, of males reported by Ailan-Choke (1) was shorter than that of the males in this study, 0.986–1160 mm. It reinforces the need for accurate

Table 1. Morphological characterization of the nematode *Procamallanus (Spirocamallanus) pinto* in 30 specimens belonging to *Corydoras acutus* (Cope, 1872), *Corydoras reticulatus* (Fraser-Brunner, 1938) *Corydoras virginiae* (Burgess, 1993), Iquitos-Peru.

Anatomical and morphological data of <i>Procamallanus (Spirocamallanus) pinto</i>	<i>Corydoras acutus</i>		<i>Corydoras reticulatus</i>		<i>Corydoras virginiae</i>	
	Females	Males	Females	Males	Females	Males
Number of samples	5	5	10	10	5	5
Stretch mark numbers	9	8	10	6	9	6
Oral cavity length (mm) (average)	0.063	0.051	0.069	0.052	0.067	0.056
Oral cavity width (mm)(average)	0.053	0.052	0.056	0.054	0.055	0.054
Length of muscular esophagus (mm) (average)	0.263	0.203	0.293	0.353	0.394	0.338
Width of muscular esophagus (mm) (average)	0.121	0.237	0.154	0.197	0.152	0.140
Length of glandular esophagus (mm) (average)	1.160	0.924	1.113	0.990	0.986	0.939
Width of glandular esophagus (mm) (average)	0.329	0.258	0.341	0.197	0.269	0.263
Tail Length (mm) (average)	0.444	0.324	0.340	0.141	0.369	0.239
Tail Width(mm) (average)	0.213	0.167	0.295	0.168	0.1955	0.240
Papillae numbers in males	----	4 preanal pairs and 2 postanal pairs	----	4 preanal pairs and 2 postanal pairs	----	4 preanal pairs and 2 postanal pairs

species identification. To this end, it is recommended that an identification key for *Procamallanus* spp. in the Neotropical Region be developed to enhance accurate identification.

Histopathology

The range of histopathological responses to parasites can vary from parasite encapsulation by host cells to acute, chronic inflammation and necrosis, and intestinal parasites

can cause intestinal occlusion or affect the structure of the intestinal epithelium through attachment or feeding (9). The pathological effects and the immune responses of fish to infection are two important aspects of the parasite-host interaction (14). *P. pinto* has been shown to destroy intestinal villi, which, along with other degenerative changes including necrosis, together with an inflammatory response involving lymphocytic infiltration, hyperplasia, and an increase in the number of goblet cells, would negatively affect absorption through the intestinal wall. In some instances, more marked

Table 2. Frequency of histological alterations of the intestine, according to the type of lesions and degree of affection in *Corydoras acutus* (Cope, 1872), *Corydoras reticulatus* (Fraser-Brunner, 1938), *Corydoras virginiae* (Burgess, 1993) from the city of Iquitos-Peru.

TYPE OF INJURIES	DEGREE OF AFFECTION					TOTAL	ALTERATION PERCENTAGE (%)
	NORMAL	SCARCE I	MILD II	MODERATE III	SEVERE IV	Frequency	
<i>Corydoras acutus</i>							
INFLAMMATORY							
Transepithelial lymphocyte infiltration	6	3	9	11	1	24/30	80.0%
Inflammatory cell infiltration	8	3	5	11	3	22/30	73.3%
ADAPTATION							
Goblet cell hyperplasia	7	3	8	9	3	23/30	76.7%
Enterocyte hyperplasia	3	8	6	11	2	27/30	90.0%
DEGENERATIVES							
Necrosis	9	6	9	3	3	21/30	70.0%
Peeling of epithelial cells	3	9	9	7	2	27/30	90.0%
<i>Corydoras reticulatus</i>							
INFLAMMATORY							
Transepithelial lymphocyte infiltration	7	3	10	9	1	23/30	76.7%
Inflammatory cell infiltration	11	6	3	10	0	19/30	63.3%
ADAPTATION							
Goblet cell hyperplasia	10	3	6	9	2	20/30	66.7%
Enterocyte hyperplasia	9	1	6	11	3	21/30	70.0%
DEGENERATIVES							
Necrosis	12	3	3	9	3	18/30	60.0%
Peeling of epithelial cells	9	5	6	8	2	21/30	70.0%
<i>Corydoras virginiae</i>							
INFLAMMATORY							
Lymphocyte transepithelial infiltration	8	2	10	10	0	22/30	73.3%
Inflammatory cell infiltration	12	0	6	12	0	18/30	60.0%
ADAPTATION							
Goblet cell hyperplasia	15	0	2	12	1	15/30	50.0%
Enterocyte hyperplasia	11	1	6	11	1	19/30	63.3%
DEGENERATIVES							
Necrosis	10	4	6	8	2	20/30	66.7%
Peeling of epithelial cells	11	1	6	9	3	19/30	63.3%

lesions were observed, affecting the muscular layer in *C. micracanthus* and *Parachanna obscura* (Gunther 1861) (2, 3). Similarly, in our study of *C. acutus*, *C. reticulatus*, and *C. virginiae*, there was necrosis of the epithelium and destruction of the mucosa and submucosa. Moreover, damage to the intestinal wall occurred where the buccal capsules of the attached worms were confirmed by the study of Meguid and Eure (23). These authors showed that the mechanical damage in infected fish was caused mainly by the parasite's attachment to the intestinal wall through its buccal capsule. Variations in the morphology of the buccal capsule species of the Camallanidae are thought to determine the extent of the damage to the intestinal mucosa and submucosa (25). Further, the resulting lesions affect the mucosa and submucosa, producing a desquamation of epithelial cells.

The essential function of goblet cells is to prevent intestinal inflammation (27), and their production is activated when there is mechanical damage. Likewise, in our study, intestinal inflammatory reactions were triggered in parasitized

fish with inflammatory cell infiltration in frequency of 73.3%, 63.3%, and 60%, hyperplasia of enterocytes in frequency of 90%, 70%, and 63.3%, desquamation of epithelial cells in frequency of 90%, 70% and 63.3%, hyperplasia of goblet cells in frequency of 76.7%, 66.7% and 50% and necrosis in frequency of 70%, 60% and 66.7% in *C. acutus*, *C. reticulatus*, and *C. virginiae* respectively.

We also found mild to moderate hyperplasia of the intestinal epithelium, which may be due to prolonged fasting and/or stress caused by the capture and transfer of the fish, generating variations in intestinal permeability (8, 18). Hyperplasia of enterocytes and mucosal cells in infected fish could also be considered an adaptive immune response to infection (15). This alteration was confirmed in the findings of hyperplastic enterocytes and mucosal cells in the intestinal wall of infected fish in our study.

Some camallanids are known to ingest blood from the host intestine, causing anemia. Ruhela et al. (36), described macrocytic anemia, with a marked reduction in the total

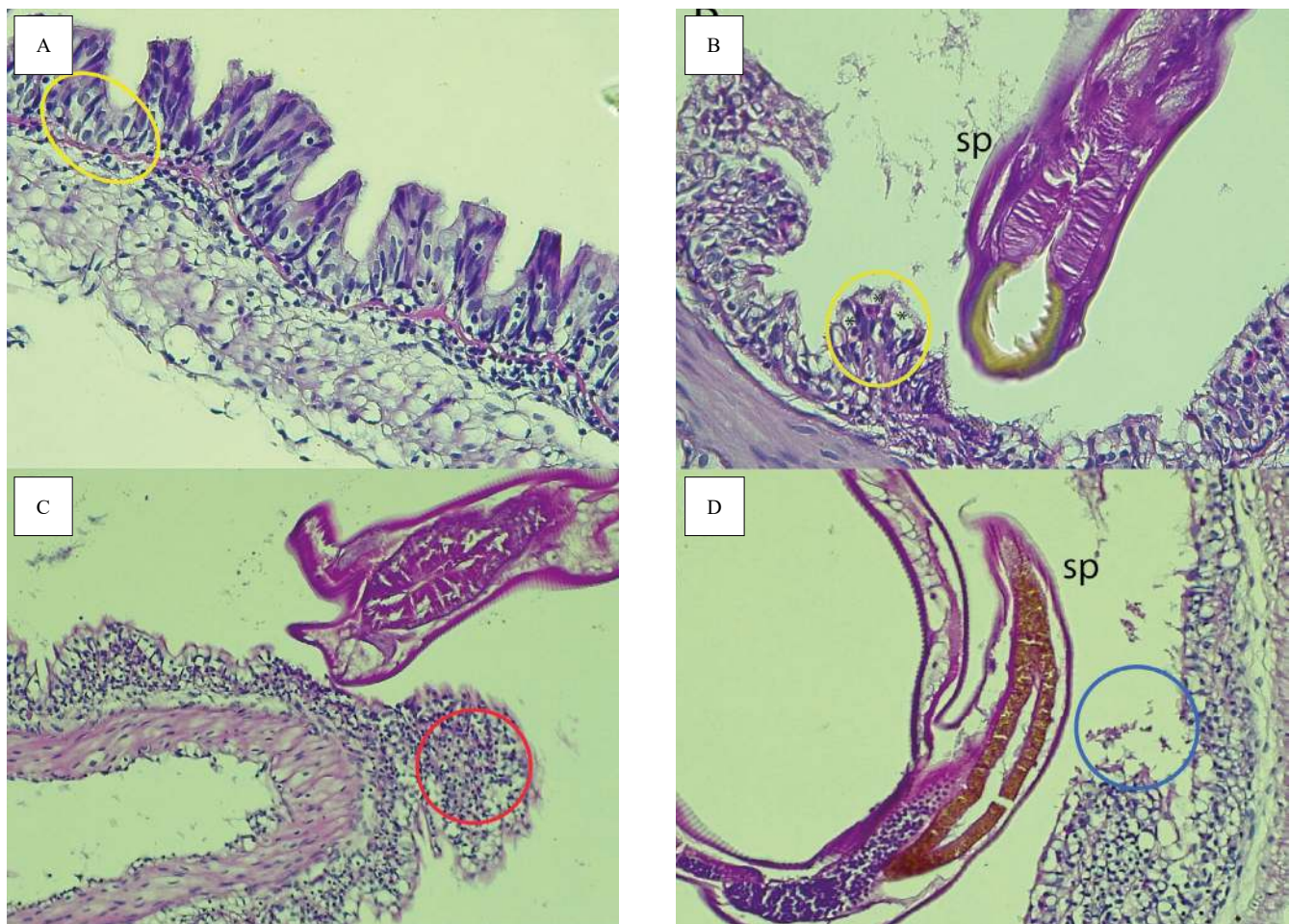


Figure 2. Histopathological lesions in *Corydoras acutus* caused by the infection of *Procamallanus (Spirocamallanus) pinto*. A- Hyperplasia of enterocytes (yellow circle). B- Hyperplasia of goblet cells (asterisks). C- Inflammatory cell infiltration (red circle). D- Desquamation of the epithelial cells (blue circle).

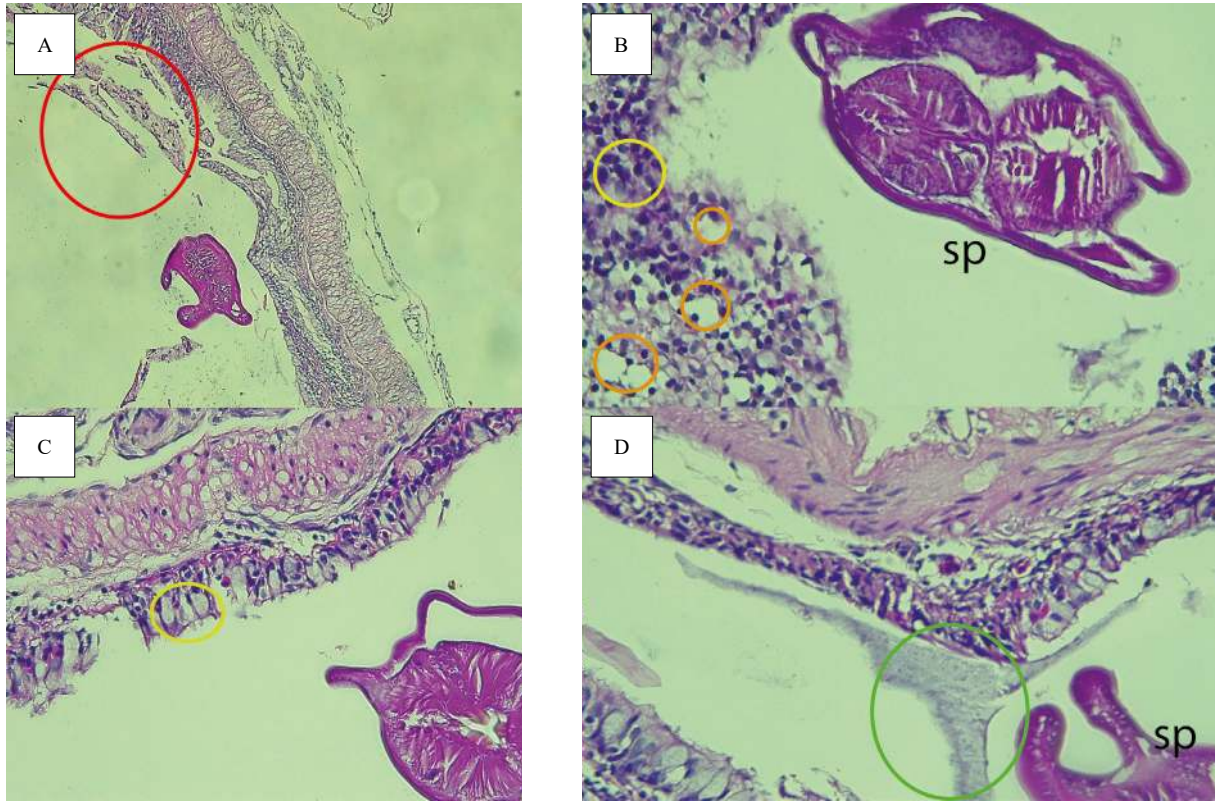


Figure 3. Histopathological lesions in *Corydoras reticulatus* caused by the infection of *Procamallanus (Spirocamallanus) pinto*. A- Necrosis (red circle) and desquamation of the epithelial cells. B- Inflammatory cell infiltration (orange circles). C- Hyperplasia of goblet cells (yellow circle). D- Destruction of the mucosa (green circle)

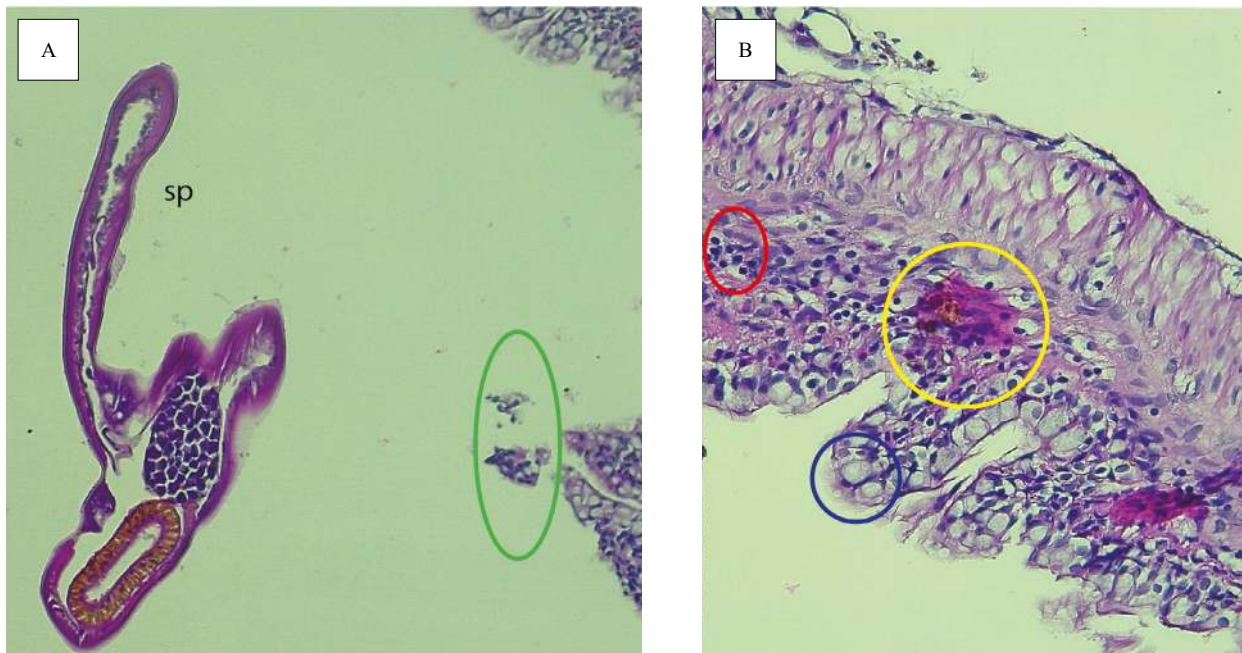


Figure 4. A- Histopathological lesions in *Corydoras virginiae* caused by the infection of *Procamallanus (Spirocamallanus) pinto*. A- Necrosis and desquamation of the epithelial cells (green circle). B- Inflammatory cell infiltration (red circle); hyperplasia of goblet cells (blue circle); hyperplasia of enterocytes, and presence of macrophages and inflammatory cells (yellow circle).

erythrocyte count and hemoglobin content in *Clarias batrachus* (Linn.) (Clariidae) infected by *Procamallanus*. We also observed hematophagous female *P. pinto* in our study.

Parasite indices

Piñeros et al. (31) reported that *P. pinto* was present at a high frequency (53.33 – 88.23%) in *Corydoras metae*. Murrieta & Florindez (26) reported infections of frequency of 90% and 85% for *C. reticulatus* and *C. virginiae*. Similarly, in our study, a frequency of 87%, 80%, and 67% was recorded in the samples of *C. acutus*, *C. reticulatus*, and *C. virginiae* respectively, with *C. acutus* presenting the most marked histopathological lesions in the intestine. These results support the contention of Piñeros et al. (31) that nematode infection imposes a health risk for fish during their confinement and transfer for export.

Parasite infection has, however, been found to disturb both the physiological system and the metabolic activities of the host (20). Infection with *P. pinto* may cause enough damage to affect the health status of the fish, depending on the size of the fish and the extent of the disease along the entire intestine (26). In our study of *C. acutus*, *C. reticulatus*, and *C. virginiae*, we confirmed that parasite infection causes lesions in the host's intestine, most frequently hyperplasia of enterocytes, hyperplasia of goblet cells, inflammatory cell infiltration, desquamation of enterocytes and tissue necrosis. We were able to classify these types of lesions on a scale of degrees from mild to severe (33), the most frequent lesions being moderate inflammatory cell infiltration. Because these lesions were mostly mild to moderate, they were sufficient neither to produce mortality of fish nor maintain a balance between the host and the parasite. In contrast, Wilson and Kakouli (40) mention that increasing the parasitic loads may cause fish deaths. However, the establishment of fish immunity may also lead to massive parasite infections (6, 21).

Ortega (29), in discussing the determinants of nematode infections in river fish, suggested high contamination of larvae and adults of *Contracaecum* sp. (Anisakidae), environmental changes, the presence of pathogens and sub-optimal conditions, as well as nutritional and immunological conditions were important. Likewise, it is documented that the negative effects of parasites reduce the commercial value of the fish by limiting their populations or causing losses in the volume of the catch because infected fish are discarded. We found, for example, that individuals of *C. acutus*, infected with *P. pinto* are not selected for commercialization because the nematode is easily visible in the abdominal cavity, thereby degrading the commercial value of the fish.

In conclusion, the histopathological analysis revealed moderate to high levels of alteration percentage from 50% to 90% in the intestine from *Corydoras acutus*, *Corydoras reticulatus*, *Corydoras virginiae* from Iquitos-Peru, in this way, this study provides us with an overview of how

endoparasitism does affect the health of the fish, with a resultant negative impact on market exports. We confirmed the findings of Murrieta & Florindez (26) that the actual and current health status of fish for exports, including *Corydoras* spp. is not known. It was also evident that antiparasitic control is scarce or non-existent, causing large losses of parasitized fish and threatening the viability of fish exports. Additionally, a study of the parasite's life cycle would clarify the situation by providing data on the residence time of the parasite in the fish.

Conflict of Interest

The authors declare no competing interests.

Funding

Financial support provided to the UPCH Master's Program in Aquaculture Health subsidized by FONDECYT of CONCYTEC (Convenio de Gestión No. 230-2015- FONDECYT-DE-PROMOCION 4).

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