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Research Article

Epitheliocystis prevalence and histopathological alterations in gills of Nile tilapia *Oreochromis niloticus* Linnaeus cultured in southwestern Mexico

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ABSTRACT. Epitheliocystis is an emergent infectious disease affecting fish gills and skin worldwide. Few studies document their distribution in wild and cultured fish in Latin America. This study reports the epitheliocystis distribution and prevalence, histopathological index (HI), and its possible relation with other observed pathologies in the gills of cultured tilapias *Oreochromis niloticus* in ponds and cages of three states of southwestern Mexico. In Guerrero, the number of affected fishes was less (29.2%) compared to Oaxaca (39.8%) and Chiapas (49.1%), with significant difference (G_{adj} = 13.39, df = 2, P = 0.01). The size of the cysts was between 5.37 to 52.96 µm, and most of the analyzed fish showed a low number of cysts per gill arch. The prevalence by regions was varied (0 to 75%) and no correlation (r = -0.002, P = 0.659, n = 474) between epitheliocystis prevalence and type culture. The fish had a low infestation of *Trichodina* sp. and monogeneans plus different pathologies such as inflammation, eosinophilic cells, rodlet cells, telangiectasia, edema, and thrombosis. The correlation analyses show a low inverse correlation (r = -0.281, P = 0.000, n = 474) between epitheliocystis prevalence and fish weight; and a low correlation (r = 0.372, P = 0.000, n = 474) between epitheliocystis prevalence *vs.* HI. The presence of the pathologies registered in this study seems to be possibly associated with other conditions like water quality or toxicants. This research is the first scientific study documenting the presence and distribution of epitheliocystis in Mexico.

Keywords: aquaculture; epitheliocystis; histopathology; pond and cages; tilapia; aquaculture; southwestern Mexico

INTRODUCTION

Epitheliocystis is an emergent infectious disease with adverse effects in the aquaculture industry (Blandford et al. 2018, Novacovsky et al. 2021, Cascarano et al. 2022). Bacteria cysts in the gills and skin of about 90 species of marine and freshwater fish worldwide have been identified (Stride et al. 2014). Various studies in cultured fish show the pathogenic capacity of these agents, although apparently, the mortality rate is highly variable (Mitchell et al. 2010). The causative agents are intracellular bacteria. Most probably, *Chlamydia* like organisms or *Rickettzia* -like organisms of phylum Chlamydiae mainly cause this affectation (Groff et al. 1996, Crespo et al. 1999, Kim et al. 2005, Guevara-Soto et al. 2016a) as *Candidatus Piscichlamydia salmonis* (Draghi et al. 2004) and *Candidatus Clavichlamydia salmonicola* (Karlsen et al. 2008), in salmonids were identified. These bacterial species seem to be specific for salmonids. Although in the last years also, other groups of bacteria identified as β and γ Proteobacteria have been associated with this pathology (Toenshoff et al. 2012, Mendoza et al. 2013, Mitchell et al. 2013, Contador et al. 2016, Cascarano et al. 2022).

In recent years genomic studies have identified and characterized a high variability of aetiological agents that cause the epitheliocystis (Fehr et al. 2013, Blandford et al. 2018, Cascarano et al. 2022). The aetiological agents of epitheliocystis disease are intracellular pleomorphic microorganisms only appreciable with an electronic microscope (Crespo et al. 1999, Seth-Smith et al. 2016). At optic microscope, histological preparations show the presence of hypertrophied cells with granular basophilic inclusions inside the infected cells of gill filaments. In advanced

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stages the infected cells increase their basophilic granular content limited by an eosinophilic wall (Paperna & Sabnai 1980, Seth-Smith et al. 2016, Cascarano et al. 2022). The epitheliocystis infection is associated with a subclinical infection (chronic) with small numbers of inclusions scattered throughout the gills arch; until hyper-infections that involve approximately 80% of inter-lamellar spaces with more than 100 inclusions per gill arch (Camus et al. 2013, Guevara-Soto et al. 2016b).

Few studies exist that document their distribution in wild and cultured fish in Latin America (Venizelos & Benetti 1996, Mendoza et al. 2013, Pádua et al. 2015, Novacovsky et al. 2021) even though in the last years, the number of affected fishes has increased globally (Crespo et al. 1999, Nowak & LaPatra 2006, Guevara-Soto et al. 2016b, Blandford et al. 2018, Cascarano et al. 2022). There are no reports on the economic losses caused by epitheliocystis; however, the aquaculture industry may be impacted due to the recent increase in reported cases worldwide. Therefore, it is necessary to know its distribution and better understand its pathogenesis and potential impact on commercial aquaculture.

The world production of farmed fish increased 7.6% yearly, from less than 900.000 t in 1990 to nearly 7 million tons in 2018 (FAO 2020). Aquaculture was the main driving force behind the impressive growth, with the share of aquaculture in the total world tilapia *Oreochromis* spp. production increasing from 43 to 88% between 1990 and 2018 (FAO 2020, Martínez-Cordero et al. 2021). Tilapia, after shrimp culture, is the second-largest species group in Mexico's aquaculture and, in 2018, produced 53,000 t. Mexico is the second-largest tilapia capture fisheries country, with 116 t in the same year, and the second-largest international market for tilapia products. In 2018, Mexico imported the equivalent of 228,000 t of live weight of tilapia, which was higher than its domestic production. Something important to mention is that the average per capita apparent tilapia consumption in Mexico was 3.08 kg (around one-fifth of its total fish consumption) in 2018, which was much higher than the 0.9 kg world average (Martínez-Cordero et al. 2021). Its study is essential to understand the disease, avoid its distribution as much as possible, and avoid considerable damage to their cultivation and fisheries, considering the species' importance to the country and the scant knowledge of this pathology. In this sense, the present work evaluates (a) the distribution and prevalence of epitheliocystis in tilapia cultures from southwestern Mexico, (b) whether the prevalence varies depending on the type of culture (ponds or cages), and (c) whether the infection by epitheliocystis is associated with an increase in damage to gill tissue.

MATERIALS AND METHODS

Tilapias *Oreochromis niloticus* of different sizes were collected from ponds and cages of 35 aquaculture production units (APUs) of the states of Guerrero (11 APUs), Oaxaca (12 APUs), and Chiapas (12 APUs), located in 14 different socioeconomics regions of the southwestern of Mexico (Fig. 1). Ten to 15 fish were collected, measured, and weighed from each APU. The sampled fish were obtained at random directly from the ponds (P) or cages (C) and killed by a puncture in the head to be dissected (OIE 2019). A total 474 fish were sampled, 154 (P = 41, C = 113) from Guerrero; 153 (P = 124, C = 29) from Oaxaca and 167 (P = 109, C = 58) from Chiapas. Macroscopically, fish gills did not show apparent clinical signs of disease.

Four-gill arches were taken for histopathological analysis, fixed in neutralized formalin at 10%, and processed by the conventional histological methods of Tonguthai et al. (1999). Sections of 5 µm of the gill tissue were obtained with a manual microtome (Leica 820) and stained with hematoxylin-eosin stains (Luna 1968). The histological slides were analyzed with an optic microscope (Nikon Eclipse E200). One gill arch to identify the presence of epitheliocystis cysts was used. The cyst area (µm^2) and diameter (µm) were measured with ImageJ software, and the cysts prevalence was determined by the percentage of the frequency with the following formula:

P = (number of positive organisms) / (number of analyzed organisms) × 100

Additionally, other pathologies observed in each gill include necrosis, telangiectasia, hyperplasia, lamellar fusion, inflammation, thrombosis, rodlet cells, eosinophilic cells, and parasite infestation *Trichodina* sp., and monogeneans were also evaluated. The pathological alterations and parasite infestation were classified as scores from 0 to 4 based only on the extension of the injury; each pathology was considered by ranking the severity of tissue lesion in grade 0: no pathological alterations; grade 1: focal alterations, indicating that the damage is not extended and is located only in one or two small sections of the gills; grade 2-3: mild to moderate alterations, when the changes observed can affect a significant part of the gills, but still, there are functionally sections; and grade 4: extended severe alterations when the damage cover all the organ or tissue. The scores were utilized to calculate the histopathological index (HI) based on two methods: the mean assessment values (MAV) modified

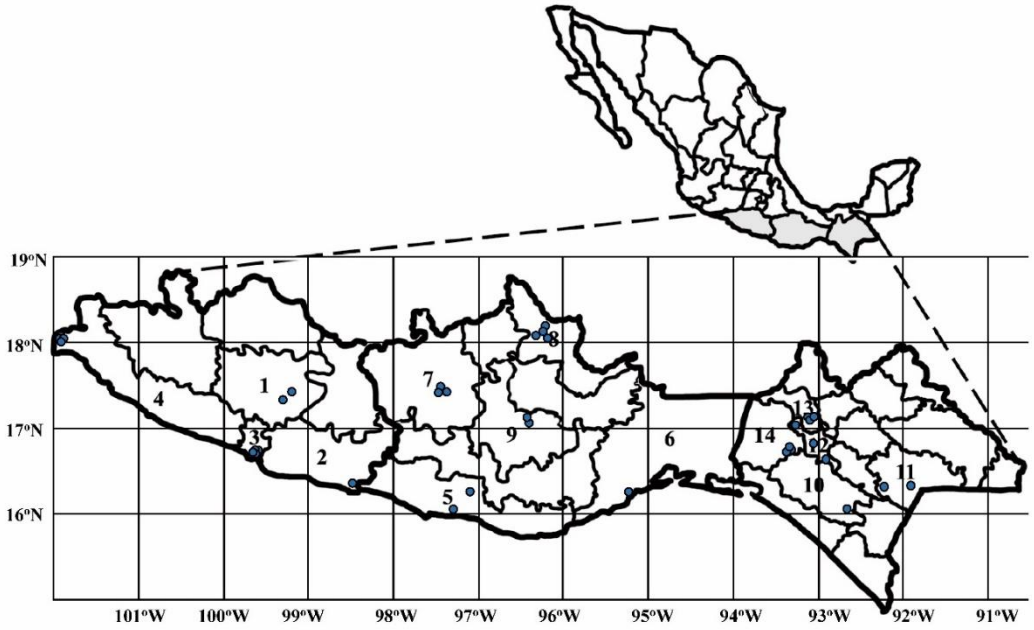

Figure 1. Distribution and coordinates of the sampled tilapia *Oreochromis niloticus* farms in the regions of the three southwestern states of Mexico. Guerrero State: 1) Region Centro, 2) Costa Chica, 3) Acapulco, 4) Costa Grande. Oaxaca State: 5) Costa, 6) Itsmo, 7) Mixteca, 8) Papaloapam, 9) Valles Centrales. Chiapas State: 10) Frailesca, 11) Meseta, 12) Metropolitana, 13) Mexcalapa, 14) Valles Zoque.

method by Schwaiger et al. (1997) and the degree of tissue change (DTC) (Poleksic & Mitrovic-Tutundzic 1994). The MAV reflects the extension of present damage in tissue; while which, the DTC assumes that there is an exponential growth in the kinetics of the tissue changes. At each identified pathology, three different degrees of progressive changes (Table 1) were registered:

I. The first stage changes without damage to the gill function, but the organ becomes normal if the stress factor is eliminated.

II. The second stage change affects the gill function, but if the stress factor is eliminated, the damage is reversible, allowing the tissues and organs to return to normality.

III. The third stage change implies the presence of irreversible alterations in the tissues, affecting the function, even if the stress factor is eliminated.

Each pathology was cataloged as a state of change; afterward, the DTC values obtained were added according to the following formula: $\mathrm{DTC} = (1 \times \sum \mathrm{I}) + (10 \times \sum \mathrm{II}) + (100 \times \sum \mathrm{III})$.

HI was calculated from the MAV and DTC values as follows HI = (MAV × DTC).

The HI was interpreted using the value scale proposed by Poleksić & Mitrović-Tutundžić (1994) with modifications:

Table 1. Values of the degree of progressive change assigned to each pathology.

State change	Pathology in gills
I	Inflammation
I	Eosinophilic cells
I	Rodlet cells
II	Hyperplasia
II	Telangiectasia
II	Thrombosis
II	Parasites infestation
III	Necrosis

0-10: incipient damage without physiological compromise, that is, at the onset of stimuli, injury, or disturbance of homeostasis, some cells of the tissue or organ respond with acute cellular inflammation without affecting their function, which may be reversible if the stimuli disappear.

11-20: light damage and light physiological compromise, when tissues respond with alterations such as light hyperplasia, atrophy, edema, hydropic degeneration, lipidosis, and presence of melanomacrophages, which are considered positive adaptation and may return to the normal stage if the disturbance disappears.

21-50: light to moderate physiological damage and compromise, when the mentioned changes cover most tissues but the tissue or organ still functions.

51-100: moderate to severe physiological damage and compromise; and >100:

Irreparable damage and lethal physiological compromise.

Shapiro-Wilk normality test was probed, and a Spearman's rank correlation coefficient was used.

The epitheliocystis prevalence (dependent variable) in the states, regions, and culture types were analyzed with the G test of independence with the William correction. In addition, epitheliocystis prevalence was correlated with fish size, culture type, HI, and parasite infestation.

The cysts diameter by states, the epitheliocystis intensity and HI by regions of each state were analyzed with Kruskal-Wallis one-way analysis of variance (ANOVA) on Ranks because the normality test failed in SigmaPlot 11.0 software. The significance level was $P = 0.05$ in all cases.

The epitheliocystis intensity and the HI data distributions were analyzed with Kolmogorov-Smirnov & Bartlett's tests; however, they were not normal nor homoscedastic. Therefore, R1 rank transformation (Conover & Iman 1981, Conover 2012) was used. Then the epitheliocystis intensity and HI data were analyzed independently using two-way ANOVA and Holm Sidak multiple comparison tests. The independent variables were the states and the culture type in both cases. The significance level was $P = 0.05$.

The authors confirm that the Aquatic Animal Health Code standards (OIE 2019) were followed during the handling and collecting of wild animals.

RESULTS

The gills of cultured tilapias *Oreochromis niloticus* in Guerrero, Oaxaca, and Chiapas states, presented cysts with similar morphology, ranging from ovoid to circular shape (Fig. 2). The cysts showed characteristic basophilic granular material associated with the proliferation of bacteria and delimited by a thin hyaline eosinophilic capsule. The cysts were located indistinctly in different regions of the primary lamella (Fig. 2a) and the middle and apical sections of the secondary lamellae (Figs. 2b-c). However, smaller cysts tended to be located at the tip of the secondary lamellae (Fig. 2b). Cellular response adjacent to most cysts was not observed, although the development of hyperplasia and lamellar fusion was detected (Fig. 2d). The cysts diameters in tilapias of Oaxaca showed the highest value (26.66 ± 6.87 µm) compared with those observed in the Guerrero and Chiapas, which diameters were similar (22.48 ± 9.98 and 21.13 ± 10.06 µm). The statistical results of cysts diameters in the states showed a significant difference only between Oaxaca and Chiapas ($P < 0.05$).

In the three states, the fish sizes were variable, and the number of cysts was low (1 to 5 cysts per gill arch), though there were fish with more than 24 cysts per gill arch. The epitheliocystis intensity analyzed by two-way ANOVA shows statistical differences between Guerrero *vs*. Oaxaca and Chiapas ($P < 0.05$), and the culture type analyzed by each state had significant differences in the three states ($P < 0.05$) (Fig. 3a). On the other hand, the one-way ANOVA applied to the intensity of this pathogen by regions shows a significant difference between Acapulco with Costa Chica, Costa Grande, and Centro in Guerrero ($P < 0.05$). In Oaxaca, there were differences only in Papaloapam with Mixteca ($P < 0.05$). The higher values in the Frailesca, Meseta, and Mexcalapa zone in Chiapas have significant differences from Valle Zoque ($P < 0.05$) (Fig. 3b).

The analysis of cases with epitheliocystis shows an inverse and low correlation between epitheliocystis intensity ($r = -0.334$, $P = 0.000$, n = 474) *vs*. fish size (Fig. 4a), and no correlation between epitheliocystis prevalence and culture type (ponds or cage) (r = - 0.020, $P = 0.659$, n = 474).

The number of fishes with epitheliocystis in Guerrero was less (29.2%, 45/154 fishes) than in Oaxaca (39.8%, 61/153 fishes) and Chiapas (49.1%, 82/167 fishes). The G test comparison shows a significant difference ($G_{adj} = 13.39$, df = 2, $P = 0.01$) between prevalence and state (Fig. 4b). The culture type analysis (pond and cages) was no significant statistically ($G_{adj} = 0.19$, df = 1, $P = 0.05$), however during the comparison of culture type by each state, all shows significant differences (Chiapas: $G_{adj} = 11.71$, df = 1, $P = 0.01$; Oaxaca: $G_{adj} = 15.38$, df = 1, $P = 0.01$; and Guerrero: $G_{adj} = 4.21$, df = 1, $P = 0.05$); with the higher prevalence in cages of Oaxaca (72%) and Guerrero (34%); and ponds of Chiapas (59%) (Fig. 5a).

In Guerrero and Oaxaca, the distribution of this pathogen by regions shows a statistically significant difference between the sampled regions ($G_{adj} = 40.02$, df = 3, $P = 0.01$ and $G_{adj} = 15.88$, df = 4, $P = 0.01$ respectively), with highest prevalence in Acapulco, Guerrero (58%), and in Papaloapam, Oaxaca (61%). In Chiapas, significant differences ($G_{adj} = 22.85$, df = 4, $P = 0.01$) were observed, with higher values of prevalence in Frailesca (75%), Meseta (69%) and Mexcalapa (64%) (Fig. 5b).

On the other hand, the low prevalence of *Trichodina* sp. (1-23 %) and monogeneans (0-19 %; Fig. 6a) observed in the samples, no shows correlation with the presence of epitheliocystis (*Trichodina* sp.: r = 0.061,

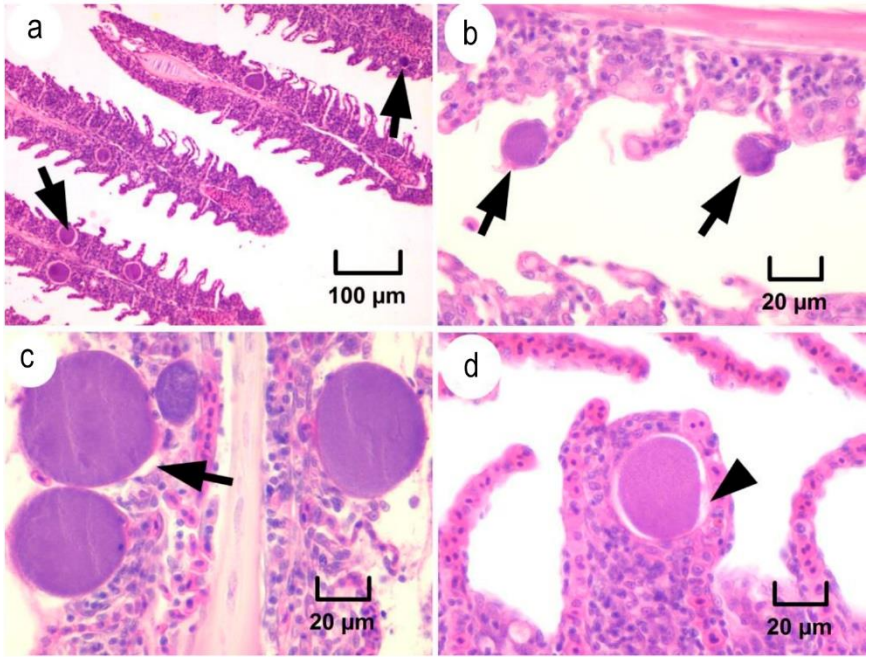

Figure 2. Microphotography of gill filaments of tilapia *Oreochromis niloticus* cultivated in southwestern Mexico. a-c) The cysts of various sizes contain basophilic granular material associated with the development of intracellular bacteria (arrows); d) cyst in the secondary filament with hyperplasia and mild inflammation in adjacent tissue, surrounded by an eosinophilic hyaline capsule (arrowhead).

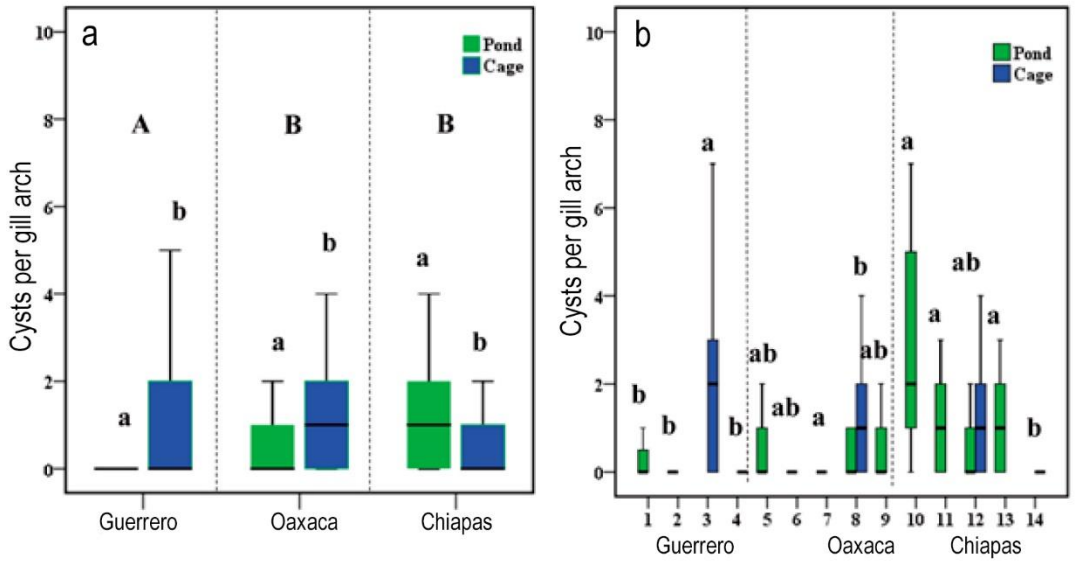

Figure 3. Box plot of epitheliocystis intensity values in tilapia cultured in ponds and cages in a) three states, and b) regions of three states in southwestern Mexico. Guerrero: 1) Centro, 2) Costa Chica, 3) Acapulco, 4) Costa Grande. Oaxaca: 5) Costa, 6) Itsmo, 7) Mixteca, 8) Papalopam, 9) Valles Centrales. Chiapas: 10) Frailesca, 11) Meseta, 12) Metropolitana, 13) Mexcalapa, 14) Valles Zoque. Capital letters represent differences between states. Similar letters indicate no statistically significant differences between states or regions of the same state.

P = 0.184, n = 474; monogeneans: r = 0.077, P = 0.093, n = 474). Other histopathological changes, such as inflammation (Fig. 6b), eosinophilic cells (Fig. 6c), telangiectasia (Fig. 6d), edema, thrombosis, and rodlet cells, represented numerically in the HI, were present indistinctly in the gills of the affected or no affected fish by epitheliocystis. The correlation between epitheliocystis prevalence and the values calculated of HI in gills

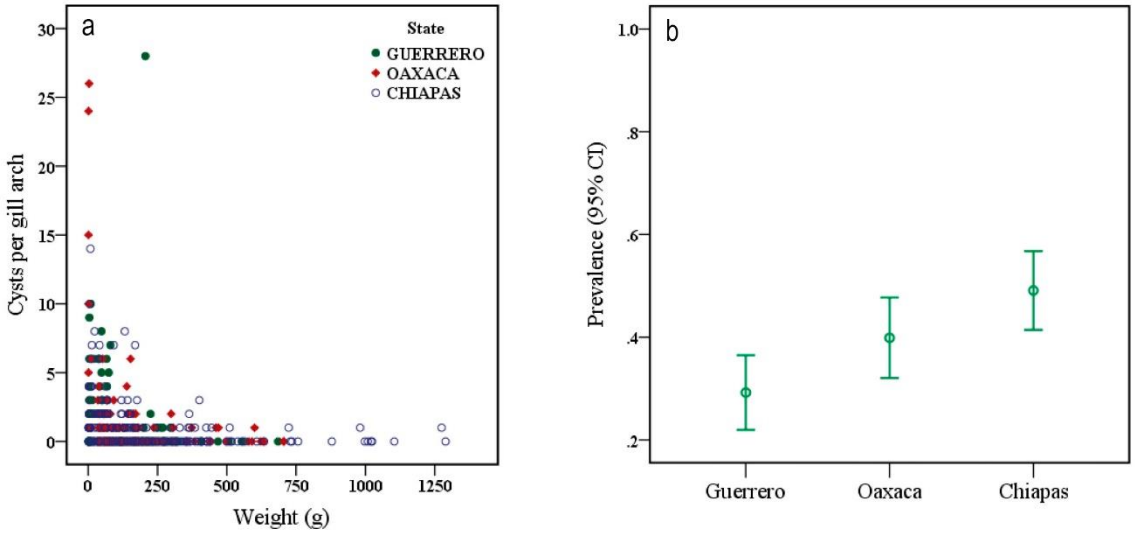

Figure 4. a) Scatter plot of epitheliocystis point data (cysts per gill arch) and fish size (weight) in the three states and b) graph of prevalence by state with significant difference (P = 0.01).

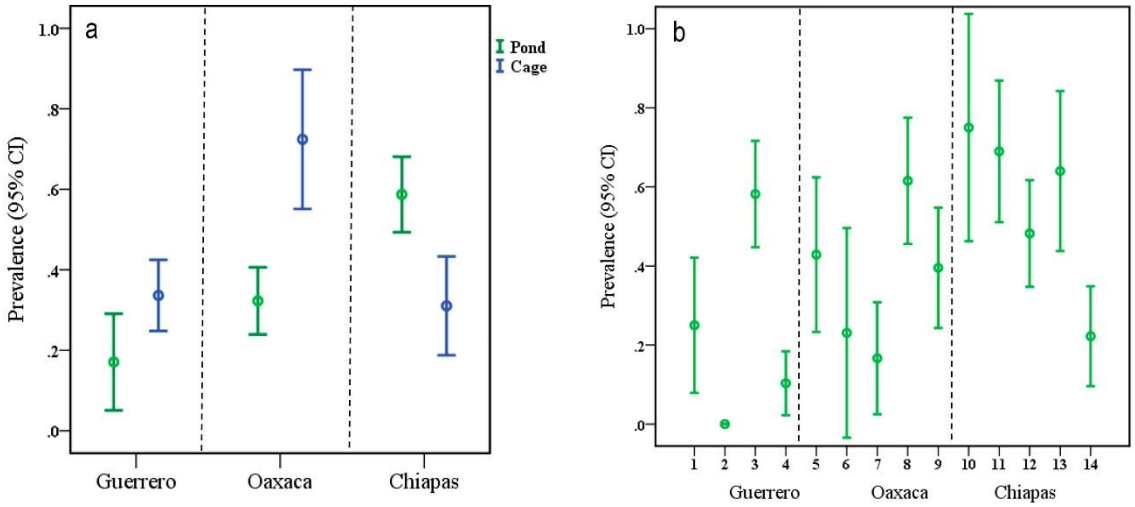

Figure 5. a) Graph of epitheliocystis prevalence by culture type with significant difference and b) prevalence by regions from southwestern states of Mexico with significant difference. Guerrero: 1) Centro, 2) Costa Chica, 3) Acapulco, 4) Costa Grande. Oaxaca: 5) Costa, 6) Itsmo, 7) Mixteca, 8) Papalopam, 9) Valles Centrales. Chiapas: 10) Frailesca, 11) Meseta, 12) Metropolitana, 13) Mexcalapa, 14) Valles Zoque.

shows a low correlation (r = 0.372, P = 0.000, n = 474) in the three states.

The results of HI analyzed with two-way ANOVA by states show significant differences (P < 0.05) between the three states (Fig. 7). In contrast, the culture type analyzed by each state had significant differences in Chiapas and Guerrero (P < 0.05). The comparison by mean of one-way ANOVA of HI in the Chiapas regions shows that tilapias cultured in Frailesca presented the highest average values of HI (76 HI) and had significant differences with Valles Zoque, Mexcalapa, and Meseta (P < 0.05) and between Metropolitana *vs*. Valles Zoque (P < 0.05). In Oaxaca and Guerrero, the HI was less than 10 in all the regions. In Oaxaca, statistical differences (P < 0.05) were observed between the Costa, Valles Centrales, and Papaloapam *vs.* Itsmo and Mixteca. No significant differences were observed in the Guerrero regions (Fig. 7).

DISCUSSION

In the present study, the morphologic characteristic of cysts in tilapia *Oreochromis niloticus* gills was similar in all regions of southwestern Mexico. These results

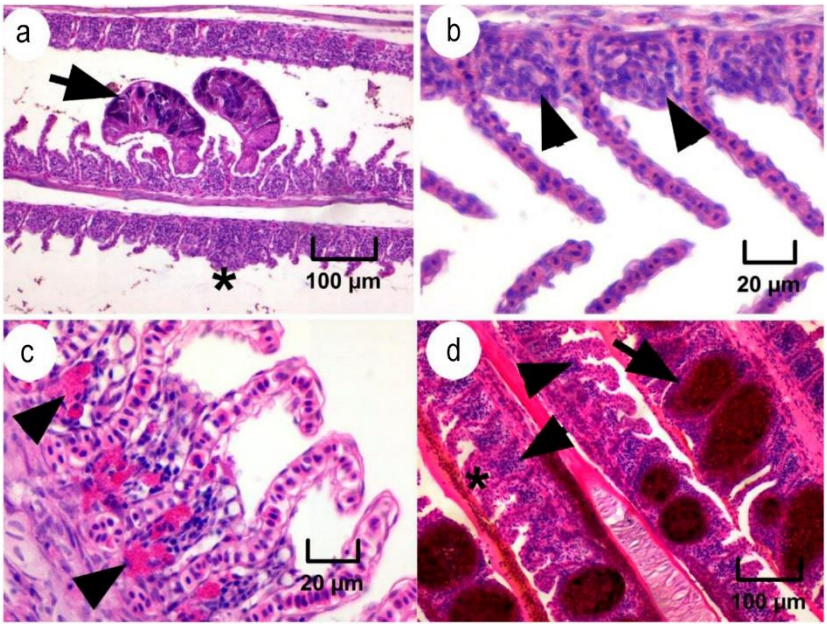

Figure 6. Microphotography of gill filaments of tilapia *Oreochromis niloticus* cultured in southwestern Mexico. a) Gill tissue with monogenean infestations (arrow) and focal hyperplasia (*); b) secondary filaments with mild inflammation (arrowhead); c) secondary filaments with eosinophilic cells (arrowhead), focal hyperplasia, and mild edema; d) secondary gill filaments with telangiectasia (arrow), hyperplasia (*), and inflammation (arrowhead).

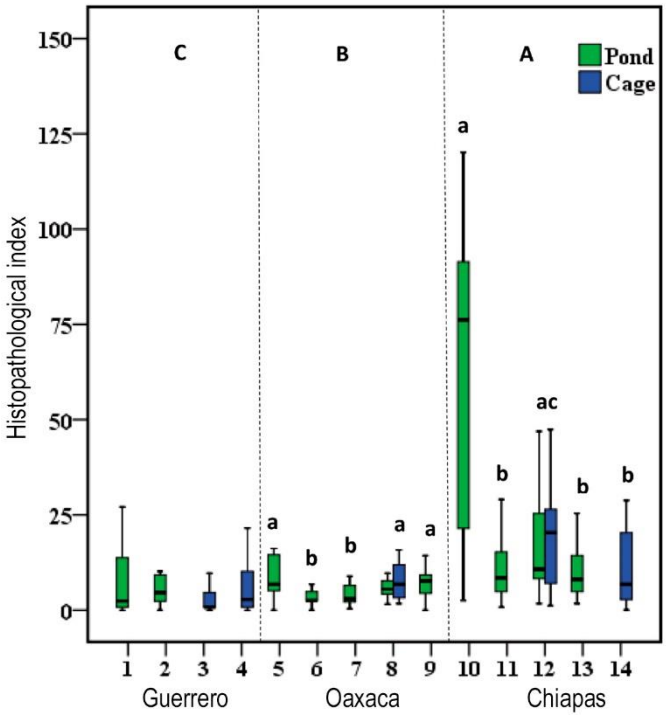

Figure 7. Box plot of the histopathological index values in tilapia *Oreochromis niloticus* cultured in ponds and cages in southwestern Mexico. Guerrero State: 1) Centro, 2) Costa Chica, 3) Acapulco, 4) Costa Grande. Oaxaca State: 5) Costa, 6) Itsmo, 7) Mixteca, 8) Papalopam, 9) Valles Centrales. Chiapas State: 10) Frailesca, 11) Meseta, 12) Metropolitana, 13) Mexcalapa, 14) Valles Zoque. Similar letters indicate no statistically significant differences between regions of the same state. Capital letters represent differences between states.

were consistent with previously reported epitheliocystis cases in tilapia (Pádua et al. 2015) and those reported in other species of fish (Nylund et al. 1998, Nowak & Clark 1999, Camus et al. 2013).

High variability in cyst size ranging from a few microns to several tens of microns has been reported (Nowak & LaPatra 2006). However, the presence of cysts up to 300 µm has recently been reported in broadnosed pipefish, *Syngnathus typhle* (Fehr et al. 2013). In this study, the size of the cysts (diameters) was highly variable; however, the mean values diameter was low, as also was reported in *Cyprinus carpio* (Miyazaki et al. 1986, Kim et al. 2005) and *Dicentrarchus labrax* (Crespo et al. 2001). Lai et al. (2013) found epitheliocystis cysts with slightly larger diameters (50.7 ± 0.50 µm) in striped juvenile trumpeter *Latris lineata*, while another study with gilthead seabream (*Sparus aurata*) observed epitheliocystis cysts bigger with diameters up to 100 µm. Fishes from Oaxaca showed epitheliocystis cysts bigger with a significant difference (26.66 ± 6.87 µm, $P < 0.05$) compared with fish from Chiapas and Guerrero (21.13 ± 10.06 and 22.48 ± 9.98 µm, respectively).

In recent years, high variability of bacterial strains in the development of epitheliocystis through genomic studies has been evidenced, including bacterial phylum Chlamydiae, β and γ Proteobacteria (Blandford et al. 2018, Cascarano et al. 2022). The evidence shows that the causative agents of epitheliocystis reside and replicate within epithelial cells leading to the formation and increased cyst size (Fehr et al. 2013, Guevara-Soto et al. 2016a, Seth-Smith et al. 2016). In this sense, a cyst can contain more intracellular bacteria in its core, while a higher number of cysts indicates that more cells were infected in the fish gills. Most of the fish in this study showed low infected epithelial cells due to the low number of cysts (1 to 5 per gill arch). Pádua et al. (2015) observed a similar number of epitheliocystis cysts of tilapia *O. niloticus* cultured in Brazil, although too observed fish with more than 30 cysts per gill arch. In this sense, it could be that an increase in the number or size of cysts in infected fish can compromise oxygen exchange in the fish, increase susceptibility to other pathogens, or even cause death, as were informed in another species (Seth-Smith et al. 2016, Cascarano et al. 2022).

It is known that the epitheliocystis can vary from low infection (subclinical, chronic) to hyper infection (Camus et al. 2013, Guevara-Soto et al. 2016b); however, the development of this pathological condition and transmission mode is still unclear (Cascarano et al. 2022). Contador et al. (2016) observed during an outbreak of epitheliocystis in lake trout *Salvelinus namaycush* a significant increase in the number of cysts after and during the mortality peaks, determining that the number and size of the cysts present in the fish reflected the degree and evolution of the infection. Recently Cascarano et al. (2022) reported an intensity higher of epitheliocystis in greater amberjack of until 35 cysts per gill filament, although infections higher than 100 cysts per gill filament have also been reported (Camus et al. 2013, Guevara et al. 2016b). In our study, most of the analyzed fish showed a lower cysts number (1-5) per branchial arch, except three fish (one fish in Guerrero and two in Oaxaca), which presented more than 24 cysts per branchial arch. These results suggest that the infection was present in the three states, possibly at a subclinical or chronic stage.

No clinical signs of the disease or mortality were observed before or during the sampling. The pathogenesis of epitheliocystis is unknown; however, some studies informed that the bacteria infection persists in fish long after the first outbreak (Seth-Smith et al. 2016, Cascarano et al. 2022). More studies are necessary through targeted sampling to detect active outbreaks, identify the causative agent through molecular tools, and characterize and quantify the intensity of cysts during the tilapia culture.

Recent studies indicate that water temperature, fish size, or pollution can increase disease development and prevalence (Novacovsky et al. 2021, Cascarano et al. 2022). An inverse correlation ($r = -0.334$, $P = 0.000$) was observed between the fish size and the epitheliocystis intensity, suggesting that smaller fish may present greater susceptibility. These results agreed with the recent papers on tilapia *O. niloticus* (Dang et al. 2022) and greater amberjack and gilthead seabream (Cascarano et al. 2022). Cascarano et al. (2022), in their study, observed that the size of the fish seems to be a significant predictor of the epitheliocystis presence. Although in our research, it is also possible that larger fish managed to eliminate the pathogen through their immune system. A more directed study will be necessary to evaluate whether the fish size is a predisposing factor for epitheliocystis infection in tilapia.

The epitheliocystis prevalence in Chiapas was higher (49.1%) than in Oaxaca and Guerrero (39.8 and 29.2%, respectively), while the prevalence by regions varied from 0 to 75%. The G test analysis the number of positive cases of epitheliocystis according to the type of production (ponds or cages), not show significant differences ($P = 0.05$), but analysis by single states shows significant differences ($P < 0.05$), with a higher prevalence in the tilapias cultured in cages of Guerrero and Oaxaca and ponds of Chiapas. These results suggest that the presence of epitheliocystis can be related to other factors, such as aspects of crop

management practices, larvae origin, or water contamination. Guevara-Soto et al. (2016b), in their research with wild and cultured brown trout, observed similar variability in the prevalence (0 to 60%) of epitheliocystis in the trout population of two effluents of the Geneva Switzerland River. However, the authors did not find differences between basins and the tributaries. The present study recorded the highest prevalence in a water body with a history of pollution as the Acapulco region in Guerrero State (Dimas et al. 2019, Sierra-Cortés et al. 2019). Dimas et al. (2019) reported high contamination in its water bodies due to wastewater, drainage, and solid waste from urban areas, hotels, and industry. Novacovsky et al. (2021) observed significant epitheliocystis prevalence in wild fishes from contaminated sites, suggesting that anthropogenic pollution predisposes individuals to this disease. In this context, the epitheliocystis prevalence variation in the present study may also be related to increasing anthropogenic contamination of the affluent used in the tilapia culture.

There is evidence that cultured fish with epitheliocystis can develop hyper infections that severely damage the epithelial cells of the branchial filaments (Camus et al. 2013, Cascarano et al. 2022), characterized by inducing two types of the host response to infection: proliferative (hyperplasia) and non-proliferative. These damages cause serious compromise in oxygen exchange and osmoregulation in affected fish (Crespo et al. 2001, Camus et al. 2013, Lai et al. 2013, Seth-Smith et al. 2016). In addition, the type of response can increase the susceptibility of fish to other pathogens or even lead to death in cultured fish (Mendoza et al. 2013). However, these responses appear not to depend on species or geographic location (Nowak & LaPatra 2006). Proliferative response was not developed in the tissue section where most cysts were located in the present study.

In some cases, the fusion of secondary lamellae was evident due to the development of hyperplasia of the branchial tissue adjacent to the cyst. These results agreed with the observed by Pádua et al. (2015). Crespo et al. (2001) also reported a non-proliferative response in sea bass, *Dicentrarchus labrax* hyper infected with epitheliocystis.

In our study, the proliferative response seems not to be associated with the cysts observed in the fish since hyperplasia was also present with other pathologies perceived in the sampled fish as activation of the immune response in areas where no epitheliocystis were located, indicating that other factors cause these alterations. Disease causal agents such as *Trichodina* sp. and monogeneans were present in gill samples. Still, these pathogens had low prevalence (1-23 and 0-19%, respectively), and there was no correlation with the presence and intensity of epitheliocystis in none of the studied states. Moreover, epitheliocystis prevalence shows a low correlation with HI; therefore, the presence of the pathologies registered in the present study seems to be associated with other agents. It could be possible that other agents such as pollution, unfavorable water quality, or factors combination could mediate the presence of pathologies observed in our sampled tilapias.

Water pollution is a global problem that has a significant concern due to industrial and agricultural progress and many anthropogenic activities. Ibrahim (2020), in its review article on diseases of Nile tilapia, provides different types of water pollution and their relation to fish diseases. In this study, other pathological alterations such as telangiectasia, edema, and thrombosis were present in fish without or with epitheliocystis. These pathological alterations are not due to the pathogens mentioned above or parasitic infestations; however, they have been associated with water pollution (Camargo & Martínez 2007, Camus et al. 2013, Baiomy 2016, Carvalho et al. 2020). Monteiro et al. (2008) observed histopathological changes such as edema, lamellar fusion, vasodilation, and aneurisms in tilapia gills exposure to copper. Edema and aneurysms presented a positive and significant correlation with the acute exposure to copper, while lamellar fusion was correlated with chronic exposure. Abdel-Moneim et al. (2012) observed that the main histopathological alterations in gills were epithelial lifting, hyperplasia, hypertrophy of the respiratory epithelium, lamellar fusion, and aneurysms in polluted Lake Mariut and Lake Edku, Egypt.

To date, few studies show the level of contamination in the water bodies of southwestern Mexico (Guerrero, Oaxaca, and Chiapas); however, the anthropogenic activities that cause pollution in rivers, dams, and watersheds are well known. (Häder et al. 2020). Musálem-Castillejos et al. (2018) detected the presence of Hg and As in the Grijalva River, particularly on the border between Chiapas and Tabasco during the dry season, with Hg values higher than those allowed in the Official Mexican Norm. Laino-Guanes et al. (2015) also mention in their study of the Grijalva River in Mexico-Guatemala limits that Hg was detected at higher values than those allowed in the Mexico and Canada norms. The concentrations of As, Cd, Cr, Cu, Ni, Pb, and Zn did not exceed the limits of the Mexican norm, although some exceeded the Canadian or NOAA norms.

Interestingly, the statistical analysis of the HI values quantified of all registered gill pathologies in the three states indicates that Chiapas had the highest values with

significant differences compared to data from Oaxaca and Guerrero. In Oaxaca and Guerrero states, although they also have significant differences ($P < 0.05$) between them, the HI values were less than 10 HI in all the regions. All the analyzed gills in Guerrero and Oaxaca had incipient damage (HI < 10), and none of them compromised any risk in the cultured fish. Similar results were reported by Steckert et al. (2018). Despite presenting pathological alterations in gills such as hyperplasia, the fusion of gill filaments, edema, and telangiectasia, among other alterations, they observed that the Nile tilapias cultured in southern Brazil do not affect the condition factor of these fish.

The present work shows that epitheliocystis disease is widely distributed in the Mexican southwestern with a higher prevalence in Chiapas. The prevalence by region was varied in the three states, and no correlation between epitheliocystis prevalence and culture type (ponds or cages) was observed. A low correlation between epitheliocystis prevalence and values of HI was observed, suggesting that epitheliocystis can be influenced by other factors, such as water contamination. It will be necessary to monitor and identify whether the conditions allow the development of the disease in different zones of tilapia cultivation. To date, no scientific studies document the presence of this disease in Mexico. Future studies will characterize the agent or the causal agents involved in this pathology. It is also important to include this pathogen in current monitoring and surveillance protocols in production systems to reduce the spread of epitheliocystis.

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