Original Article

Prevalence of *Henneguya* Infections on Cage-reared Channel Catfish *Ictalurus punctatus* (Rafinesque) in Mexico

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Abstract

The aim of the present study was to determine the type of infection, species, the period and annual spatial prevalence, the effect of site, period and host size on prevalence of *Henneguya* infections in floating cages-raised channel catfish. *Henneguya* infections were detected in gills, fin adipose and skin, with mature spores measuring 58 to 66 µm and 16 to 17 µm TL/BL. *H. exilis* was identified as the causing infection agent based on fresh cyst morphology and spore measurements. In addition, mature spores measuring from 45 to 60 µm in TL and 14 to 19 µm in BL, were observed in the adipose tissue infections; *H. adipose* was identified as the infective agent in the adipose tissue, whereas the skin infection may be related to *H. sutherlandi*. Histological changes showed the fusion and displacement of gill lamellae. In regard to *Henneguya* infections seasonal prevalence, it was shown that the highest (Maria Soto la Marina dam) and lowest (Soto la Marina River) prevalence were during the periods March-April and July-August.

Keywords: cage raising, channel catfish, *Henneguya*, infection

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บทคัดย่อ

ความชุกของโรค *Henneguya* ในปลากดหลวงเลี้ยงกระชัง (*Ictalurus punctatus*, Rafinesque) ในประเทศเม็กซิโก

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วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อหาชนิดของการติดเชื้อ สายพันธุ์ ระยะเวลาและความชุกในรอบปี จุดก่อโรค ระยะเวลา และขนาดของปลาที่มีต่อความชุกของโรค *Henneguya* ในปลากดหลวงเลี้ยงกระชัง การติดเชื้อ *Henneguya* พบได้ที่เหงือก เนื้อเยื่อไขมันบริเวณครีบและผิวหนัง โดยขนาดของสปอร์เต็มวัยอยู่ระหว่าง 58 ถึง 66 ไมครอน และ 16 ถึง 17 ไมครอน (ความยาวและความกว้าง) โดยตรวจพบสปีชีส์ *H. exilis* เป็นสปีชีส์ก่อโรคเมื่อพิจารณาจากรูปร่างของซิสต์และขนาดของสปอร์เป็นหลัก นอกจากนั้นยังพบว่าสปอร์เต็มวัยขนาดความยาว 45-60 ไมครอน และกว้าง 14-19 ไมครอน ในบริเวณเนื้อเยื่อไขมันที่มีการติดเชื้อ ในขณะที่ *H. adipose* เป็นตัวก่อโรคที่เนื้อเยื่อไขมัน แต่พบว่าการติดเชื้อที่ผิวหนังสัมพันธ์กับ H. sutherlandi ผลการตรวจทางจุลพยาธิวิทยาพบว่ามีการเชื่อมกันและอยู่ผิดที่ของเส้นเหงือก เมื่อดูจากฤดูที่มีความชุกของการติดเชื้อ *Henneguya* พบการติดเชื้อสูงที่สุดที่เขื่อน Maria Soto la Marina และต่ำสุดที่แม่น้ำ Soto la Marina อยู่ในช่วงเดือนมีนาคมถึงเมษายน และเดือนกรกฎาคมถึงสิงหาคม

คำสำคัญ: ปลาเลี้ยงกระชัง ปลากดหลวง Henneguya การติดเชื้อ

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Introduction

The channel catfish, *Ictalurus punctatus* R., is one of the most important freshwater fish intensively cultured worldwide, being U.S.A. the main producer. It has been easily adapted to Mexico, where it is mainly cultured in floating cages, reaching 970 metric tons in 2008 (Comisión Nacional de Acuacultura y Pesca, 2008), which are placed in open freshwater such as dams and rivers. This would be a very important environment difference that could contribute greatly to the prevalence of pathogens. Fish in floating cages are exposed to abiotic and biotic factors, and because of the higher population densities compared to other systems such as earth ponds, disease transmission is a major concern. Several studies have shown temporal and spatial variations of pathogens from cage cultured fish (Zagmutt-Vergara et al., 2005; Vagianous et al., 2006), however, most of them have been done in cold sea water species, whereas, few efforts have been made to investigate diseases in ponds and cage culture in tropics (Lio-Po and Lim, 2002). Parasitic infections, particularly protozoans, on intensive culture systems in channel catfish, are a serious concern by the economic losses (Thune 1993), where one of most common pathogens is *Henneguya*, which produces infections in gill, skin and internal organs (Piper et al., 1982), and particularly the species *H. ictaluri* and *H. exilis* cause important economic losses (Feist and Longshaw, 2006). To date, to our knowledge there are not reports on *Henneguya* infections in cage reared channel catfish in Mexico. The aim of the present study was to determine the species of *Henneguya* presents, the lesions, the period and site annual prevalence, the effect of site, period and host size on *Henneguya* infections in floating cage-raised channel catfish.

Materials and Methods

From July 2009 to May 2010, 809 channel catfish (*I. punctatus*) were randomly collected every two months from five commercial catfish farms, engaged to the production of food size, located in four dams, María Soto la Marina, (24º24'N 98º59"O); La Loba (24º21'N 98º37'O); Vicente Guerrero (23º57'N 98º44'O) and Emilio Portes Gil (22º57'N 98º47'O) and the river: Soto la Marina (24º01'N 98º 25'O) in

Tamaulipas, Mexico. All farms use standard fish cages (6.9 m^3: 2.4x2.4x1.2 m) and stock the fish to of densities around 330 fish/m^3, using similar production protocols. Fish were collected with a hand net, stored in an individual plastic bag, placed on ice, and transported in a polystyrene box in less than two hours to the Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, where fish were measured (furcal length in mm). External and internal examinations of organ (skin, fins, right gill archs, stomach, intestine, liver, etc.) and cavities of fish were performed by naked eye, magnificent glass, stereoscope (LW Scientific, Inc®, USA) and light microscopy (Revelation III LW Scientific®, USA), following standard parasitology methods (Pritchard and Kruse, 1982; Secretaría de Pesca, 1994; Hoffman, 1999). *Henneguya* cysts detected were measured (mm) and crushed to obtain spores, which were immediately measured. Other spores were air dried, fixed in methanol (5 min) stained with a 3.5% methylene blue solution (30 min), dehydrated in ethanol series of 70, 80, 90 and 100% (5 min each one), cleared in xylene (3 min), mounted in synthetic resin, and photographed with digital cameras (Photosmart® R817, Canon®, Powershot G6 PC 1089). Total length of spore (TL), length of the spore (BL) and width of the spore measures (in µm) of fresh mature spores were obtained with an occular micrometer (Zeiss®, 464023-9901, CPL, W10X/18, Germany) and were measured according to Hoffman (1999); characterization of infections and identification of *Henneguya* species including host, target tissue, cyst morphology and spores measurements (Kudo, 1969; Noga, 1996; Hoffman, 1999; Eiras, 2002; Griffin et al., 2009). Prevalence (P) was expressed in percentage (%), as the number of individuals of a host species infected with a particular parasite/number of hosts examined (Margolis et al., 1982). Samples of infected organs were fixed, processed according to routine histological techniques, cut at 5 µm, stained with hematoxiline and eosine (H&E), observed and photographed under a light microscope (Zeizz–Axiostar Plus®). A Spearman's test was used to correlate host size and prevalence, and a Kruskal-Wallis test was used to indicate the effect of the season and site on the parasite prevalence. Statistical analyses were carried out with the aid of the MedCal® software (Belgium) and differences were considered significant at $p<0.05$. To obtain annual average, results of prevalence were grouped by period and site. For statistical analysis, only those species with prevalence ≥ 10% were taken into account (Iannacone et al., 1999).

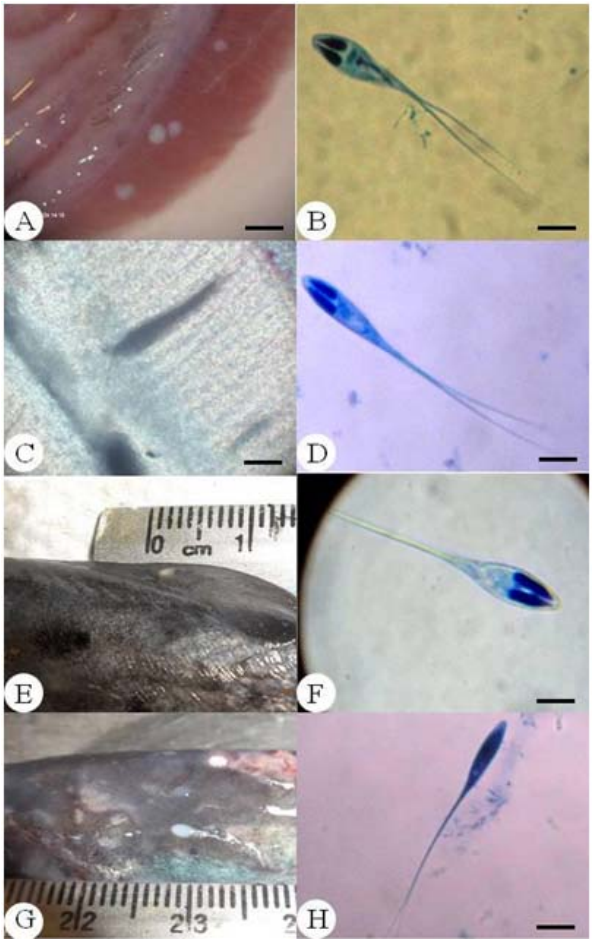

Figure 1 Macro and microscopic views of *Henneguya* spp. infections detected in cage-raised channel catfish Ictalurus punctatus R. in Mexico. A-B) Gill visible cysts and spore (bar=0.5 mm and 5 µm, respectively); C-D) Interlamellar cyst and spore (bar=0.1 mm and 5 µm, respectively); E-F) Adipose fin and spore (bar = 5 µm); G-H) Skin cyst and spore (bar = 10 µm). Photographs B, D, F and H were taken from spores stained with methylene blue solution.

Table 1 Fresh mature spores (mean ±SD) and cysts of *Henneguya* detected in cage-raised channel catfish *Ictalurus punctatus* R. in Mexico.

Type of infection	Spore size in µm (minimum to maximum size)			Cyst (mm)
	Body length	Width view	Total length	
Gill visible	16.75±1.18	5.75±0.91	65.8±6.79	0.2-1.3
(n=19)	(15-19)	(5-8)	(55-84)	
Interlamellar	15.94±1.80	3.82±0.88	58.52±6.96	0.04-0.1 x 0.25-0.80
(n=12)	(13-20)	(3-5)	(44-71)	
Adipose fin	14.50±3.66	4.43±0.73	59.86±5.03	1.0-1.3
(n=12)	15 (11-19)	(4-6)	62.5 (55-70)	
Skin	19.14±1.25	4.17±0.37	45.71±0.88	1.1-1.5
(n=7)	(17-21)	(4-5)	(45-47)	

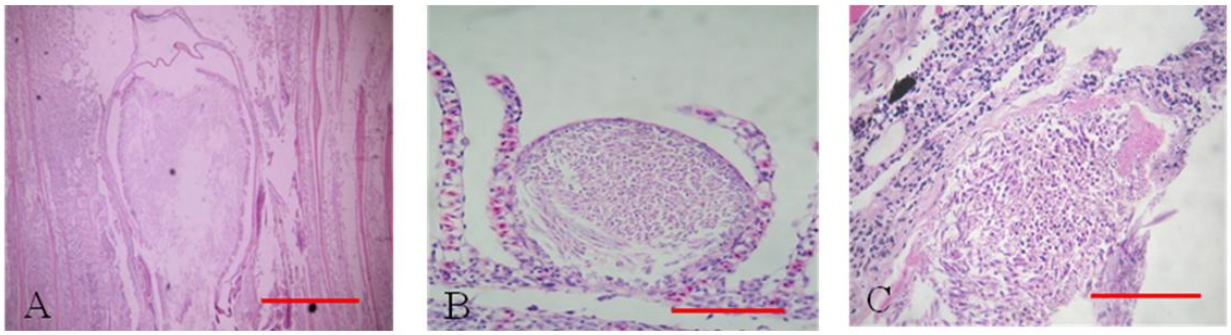

Figure 2 Histopathological changes due to *Henneguya* infections. A) Cyst of H. exilis between primary lamellae. Lamellar fusion of secondary lamellae can be observed. Bar= 1 mm, H&E staining; B) Cyst of *H. exilis* between secondary lamellae. Primary lamellae displacement is shown. Bar= 100 µm, H&E staining; C) Cyst of H. adiposa surrounding by connective tissue. Bar 100=µm, H&E staining.

Results

The mean furcal length of fish examined was 18.8 cm (± 4.6). Four types of *Henneguya* infections in channel catfish were detected, two of them in gills and two other on integument system: adipose fin and skin. One of the gill infections was observed as round white cysts were visible (Fig 1A), with mature spores measuring 65.8±6.79 µm and 16.75±1.18 µm TL/BL respectively (Table 1) (Fig 1B). The other infection – interlamellar- contained elongated and gray cysts (Fig 1C), with mature spores of 58.52±6.96 µm and 15.94±1.8 µm TL/BL respectively (Table 1; Fig 1D). Cyst morphology and measures of spores correspond to *H. exilis* in both gill infections. In the adipose fin and skin lateral skin of fish, visible cyst infections were observed (Figs 1E and G). Adipose and skin lesions displayed mature spores ranging in size from 59.86±5.03 and 45.71±0.88 µm TL and 14.5±3.66 and 19.14±1.25 µm in BL respectively (Table 1; Figs 1F and H). Adipose infection seems to correspond to the specie *H. adipose* whereas skin infection may correspond to *H. sutherlandi*. *Henneguya* infections were not detected in internal organs. Under stereoscope, the visible gill infection on gill showed distension of primary lamella, but in interlamellar infection they were not observed lesions, whereas adipose fin and skin infections showed cysts with mucus secretion. In the histological analysis, findings were mainly observed in gills, which consisted in the fusion and displacement of secondary lamellae, and on visible and interlamellar gill infections respectively (Fig 2).

Henneguya infections were detected all year without significance difference between periods (Table 2). However, the highest and lowest prevalence was detected in the periods of March-April and July-August respectively (Fig 3). The highest prevalence site of *Henneguya* infections corresponded to Maria Soto la Marina dam and the lowest to the farm on the river Soto la Marina (Fig 4), but the *Henneguya* infections did not show significant difference between site of catfish farm location (Table 2). Moreover, no significant differences were detected on host size on prevalence of interlamellar infection of *H. exilis* (Table 2). Annual prevalence of infections is showed in Table 3, where the highest infection (16.4 %) corresponded to interlamellar type and the lowest to adipose fin type (0.2%).

Table 2 Kruskal-Wallis and correlation Spearman tests to determinate the effect of period, site and host size (cm) on *Henneguya exilis* prevalence (%) on channel catfish raised in cages.

	Test	
	Kruskal-Wallis	Spearman
Period	p=0.363	
Site	p=0.877	
Host size (18.8±4.6 cm)		*Rho* = - 0.007

Table 3 Annual total prevalence (%) of *Henneguya* infections detected in channel catfish *Ictalurus punctatus* raised in floating cages.

Infections	Annual prevalence (%)
Interlamellar	16.4
Visible cysts in gills	0.9
Skin	0.9
Adipose fin	0.2

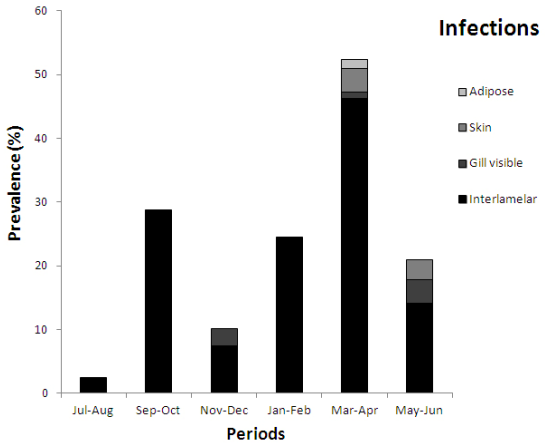

Figure 3 Period prevalence (%) of Henneguya infections in five sites on cage cultured channel catfish Ictalurus punctatus in five localities in Tamaulipas 2009-2010.

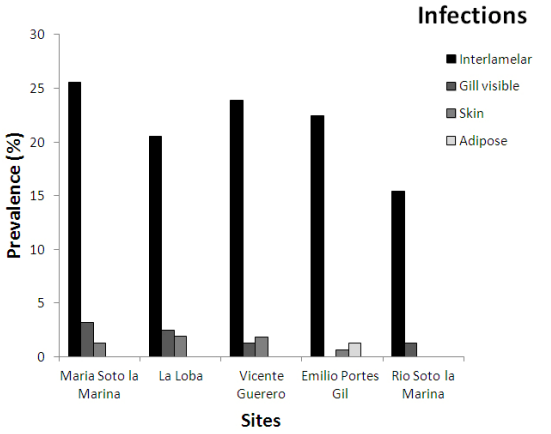

Figure 4 Annual spatial prevalence of Henneguya infections on cage cultured channel catfish Ictalurus punctatus in five localities in Tamaulipas during 2009-2010.

Discussion

Most members of the Class Myxosporea are typically site specific, and infect only certain target organs (Noga, 1996). *Henneguya*, which belong to this class is a common pathogen in cultured and wild fish around the world; in channel catfish infections are categorized according to target tissue and spore morphology (Minchew, 1977).

Different researches have established that interlamellar infection is caused by *H. postexilis* (Moore et al., 1984; Thune, 1993); nevertheless our results found that morphology of fresh cyst (Fig. 1), is more consistent with *H. exilis* (Hoffman, 1999). In the case of the adipose fin infections, host, cyst location and spore characteristics are consistent with *H. adipose* (Table 1; Fig 1), whereas the cyst locations and the morphologic characteristics of spores of skin infections seems to correspond to *H. sutherlandi* (Table 1; Fig 1).

Lesions in fish gill can produce a decrease in the respiratory capacity (Naldoni et al., 2009). In our study, gill physiology could be affected by the fusion and displacement of lamellae. Adriano et al. (2005) reported similar lesions of the intralamellar infection of *H. piaractus*, and that a massive infection may compromise the gill activity. Site prevalence did not show any statistical differences of *H. exilis* in interlamelar infection, which is consistent with reports with other myxosporidians (Su and White, 1996; Sanaullah and Ahmed, 1980). Period did not show an effect on *Henneguya exilis* prevalence, however one study (Adriano et al., 2005[a]) found significant differences in season prevalence in other *Henneguya* species, such as *H. piaractus* infecting gills of cultured fish in Brazil.

In our study, the peak of prevalence was observed during the period of March-April, and this finding is similar to the season of year of outbreaks of *H. ictaluri* in USA, where high prevalence in spring have been observed (Griffin et al., 2009), and other studies where the highest rates of infections have also been found in spring (Sabri et al., 2010; Abdel-Baki et al., 2011). Our results showed that annual prevalence of *H. exilis* was higher as compared with reports of the presence of *Henneguya spp.* in the Americas, with an overall prevalence from 6.5 to 7% in Chile and Venezuela respectively (Olmos et al., 2003; Centeno et al., 2004), but lower prevalence of reports in Brazil with percentages from 26.1 to 100% (Barrasa et al., 2003; Adriano et al., 2005b; Martins and Onaka, 2006; Feijo et al., 2008).

Statistical analysis did not show a correlation between host size and prevalence of the interlamellar infection of *H. exilis*. Some studies showed significant different in the gill parasite *H. piaractus* (Adriano et al., 2005a) of prevalence of the parasite in relation with host size, but the others did not in H. caudalongula in cultured fishes (Adriano et al., 2005b).

Our results show that *Henneguya* is a common parasite, that it is distributed in farms located in body waters in the center and south of Tamaulipas state, and that it has a constant presence throughout the year. In the case of lamellar disease produced by *H. exilis* which is considered a dangerous disease in USA. It seems that infection of this specie in Tamaulipas do not reach a high level of infection to express a severe disease. In USA, longer periods of growth in mud bottoms, probably influence the severity of this disease in earth ponds, contrary to the floating cage cultures in Mexico where continuous exchange of water, shorter periods of growth to marketable size because warmer temperatures, and distance of several meters between down side of cages and bottom of body waters could avoid a high grade of infection. Feist and Longshaw (2008) mention that pathogenicity of parasite in gills depends on the intensity of infection and that the number of cysts influences the respiratory function. Additionally, cycle of life of *H. exilis* is indirect, and transmitted by a bottom oligochate, *Dero digitata* (Feist and Longshaw, 2006). Further investigation should continue to assess the water quality in the sites, which have lotic and lentic environments, because this worm is more abundant in eutrophic zones (Krodkiewska and Michalik-Kucharz, 2009). To our knowledge, this is the first report of *H. exilis* and *H. adiposa* on channel catfish, *I. punctatus* raised in cages in Mexico.

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