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Naturally infected channel catfish (*Ictalurus punctatus*) concurrently transmit *Ichthyophthirius multifiliis* and *Edwardsiella ictaluri* to naïve channel catfish

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ABSTRACT

There is no information available whether fish naturally coinfected with *Ichthyophthirius multifiliis* (Ich) and *Edwardsiella ictaluri* can concurrently transmit both pathogens to naïve fish. The objective of this study was to expose naïve channel catfish (*Ictalurus punctatus*) to naturally infected fish that carried Ich and *E. ictaluri* to provide clinical evidence for transmission of both pathogens. Three tanks of fish were exposed to naturally coinfected fish and two tanks were utilized as mock-infected controls in each of two trials. In trial I, 34 out of 60 fish (56.7%) exposed to two infected fish per tank died at day one. All remaining fish died two days post exposure. Of the dead fish, all showed heavy Ich infection and *E. ictaluri* was isolated from the kidney of 60% of the dead catfish. In trial II, the cumulative mortality in fish exposed to 2 coinfected fish per tank was less than 20% during days 1–7 post exposure. Most of the fish died from 8 to 14 days post exposure to the coinfected fish. Ninety-six percent of fish were positive for both Ich and *E. ictaluri* in trial II. The results demonstrated that fish naturally coinfected with Ich and *E. ictaluri* could concomitantly transmit both pathogens to naïve fish. In aquaculture management, precaution is needed to thoroughly examine fish prior to shipment or purchase to prevent the spread of aquatic animal pathogens.

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1. Introduction

Channel catfish, *Ictalurus punctatus* (Rafinesque), is the most important cultured fish in the United States (Tucker and Hargreaves, 2004). Diseases are a major problem for catfish farming and can result in high mortality, increased production costs and decreased profits. Two important diseases of catfish are enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri* (Hawke et al., 1981) and ichthyophthiriasis (Ich) caused by the ciliated protozoan *Ichthyophthirius multifiliis* (Dickerson, 2006). In 2002, 50.5% and 44.3% of all catfish operations (approximately 1000 total in the USA) had losses caused by ESC and by Ich, respectively (Hanson et al., 2008). Published records of the occurrence of concurrent infections (i.e., bacteria and parasites simultaneously) from field and/or diagnostic cases are limited. Anecdotal evidence and personal communications with fish health diagnosticians suggest concurrent infections do occur. However, often fish diagnosticians may observe clinical Ich and do little if any further analysis due to the typical outcome of a severe Ich infection (i.e., death). Studies in our laboratories have focused on coinfections and the potential vectoring role of Ich in transmission of *E. ictaluri* (Shoemaker et al., 2012; Xu et al., 2012a,b). Although, our data supported the hypothesis of enhanced mortality and transmission due to coinfection, evidence from the field was lacking. In this study, the occurrence of both Ich and *E. ictaluri* in fish purchased from a commercial (supplier) farm is documented. We further provide clinical evidence of concurrent transmission of Ich and *E. ictaluri* to naïve fish under laboratory conditions using cohabitation with the naturally coinfected fish.

2. Materials and methods

2.1. Fish and water quality

The fish used in the two experimental trials were from different sources and strains that varied in size. Channel catfish (Industry pool strain) used in trial I were obtained from disease-free stock from the USDA-ARS Catfish Genetic Research Unit, Stoneville, MS and reared to experimental size (3.3 ± 0.4 cm in length and 0.5 ± 0.1 g in weight) in indoor tanks at the USDA-ARS Aquatic Animal Health Research Unit, Auburn, AL. Channel catfish used in trial II were Marion strain with an average total length of 11.6 ± 0.9 cm and weight of 9.9 ± 1.0 g from the E.W. Shell Fisheries Center, Auburn University, AL.

During the trials, dissolved oxygen in tanks was measured using a YSI 85 oxygen meter (Yellow Spring Instrument, Yellow Springs, OH). The pH, hardness, ammonia and nitrite were determined using Hach CEL/890 Advanced Portable Laboratory (Loveland, Colorado). The mean ± standard error of water quality were: dissolved oxygen 6.5 ± 0.2 mg/L; pH 7.0 ± 0.1, ammonia 0.24 ± 0.1 mg/L, hardness

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95.5 ± 7.9 mg/L, nitrite concentration below detection level and water temperature 25.0 ± 0.2 °C, measured daily.

2.2. Naturally coinfected catfish

Channel catfish fingerlings (15.5 ± 1.0 cm in length and 23.6 ± 6.0 g in weight) bought from a commercial farm were found to be naturally coinfected by both Ich and *E. ictaluri*. Most fish showed a light Ich infection with 4–10 spots per fish and hemorrhage was also noted at the base of fins suggesting an *E. ictaluri* infection (Fig. 1). Ten catfish were examined microscopically for Ich and cultured microbiologically for bacteria. Ich was observed in skin or gill from all 10 fish and *E. ictaluri* was isolated aseptically from kidney samples in 9 of the 10 fish. Twelve morbid coinfected fish were used in two infection trials.

2.3. Experimental design

Two trials were conducted to determine whether channel catfish naturally coinfected with Ich and *E. ictaluri* could concurrently transmit both pathogens to naïve catfish. A total of 110 channel catfish fingerlings were distributed into 5 tanks with 20 fish per tank for trial I. Three tanks of fish were exposed to 2 morbid fish naturally infected with Ich and *E. ictaluri*. Two tanks were maintained as mock-infected controls. A similar trial was conducted in trial II with 80 catfish. Fish were distributed into 5 tanks with 15 fish per tank. Three tanks of fish were exposed to 2 morbid infected fish and two tanks were used as mock-infected control. Ten naïve catfish in trial I and 5 fish in trial II were examined and cultured to verify pathogen free status of parasites and bacteria prior to the trial. All fish were negative for Ich and *E. ictaluri*.

Fish mortality was monitored daily after exposure to the fish infected with Ich and *E. ictaluri* in trial I. The dead fish were examined for parasite and *E. ictaluri* twice daily for 7 days post exposure (DPE) to the coinfected fish. In trial II, three fish were sampled from each tank to determine the parasite infection percentage, infection levels and *E. ictaluri* in catfish 4 DPE to the infected fish. The mortality of 12 remaining fish in each tank was recorded and dead fish were examined for parasite and *E. ictaluri* twice daily for 17 DPE to the infected fish. All fish treatment protocols were approved by an Institutional Animal Care and Use Committee at the Aquatic Animal Health Research Unit prior to the trials.

2.4. Parasite infection level

After fish were anesthetized with 300 mg/L MS-222, a skin wet mount sample was prepared by scraping 2 × 2 cm^2 lateral body surface and a gill sample by cutting several filaments from each fish. The samples were viewed under an Olympus BH-2 microscope (100×) and numbers of Ich trophonts within three viewing areas were counted. Then, numbers of trophonts on the fish skin and gills were evaluated using a semi-quantitative scale. The infection level was assessed by assigning scores of 0, 1 (light), 2 (moderate) and 3 (heavy) to fish that showed no infection, <5, 6–10, and >10 trophonts per viewing area, respectively.

2.5. Bacterial isolation

A selective medium, *E. ictaluri* medium (EIM) was used for the isolation of *E. ictaluri* from dead or sampled fish. The medium inhibits the growth of most gram-negative bacteria and some isolates of *Aeromonas hydrophila* (Shotts and Waltman, 1990). Other bacteria that grow on the EIM are differentiated from *E. ictaluri* based on colony color and morphology. *E. ictaluri* was confirmed as described by Panangala et al. (2005).

3. Results

In trial I, 20 naïve catfish in each treatment tank were exposed to 2 coinfected fish for an infection ratio of 1:10. Thirty-four of 60 fish (56.7%) in the treatment group died at day one and all remaining fish died at 2 DPE (100% mortality) to the moribund fish infected with Ich and *E. ictaluri*. The mean day to death (MDD) was 1.4 ± 0.1 day. During post-mortem examination, all examined fish showed moderate to heavy Ich infection. Sixty percent of the examined fish showed *E. ictaluri* and no *E. ictaluri* was isolated from the remaining 40% of fish examined (Table 1). No parasites or bacteria were detected from the mock infected fish. The fish used for trial I were less than 1 g and Ich trophonts were observed in every fish with moderate to heavy infection suggesting that the innate immune response was overwhelmed.

In trial II, 15 naïve catfish in each treatment tank were exposed to 2 coinfected fish at an infection ratio of 1:7.5. Four DPE to the coinfected fish in trial II, most fish in the treated tanks showed Ich trophonts on skin and gills. *E. ictaluri* was detected from kidney of 77.8% of the fish sampled (Table 2). Fish started to show mortality 4 DPE to the coinfected fish. The mortality increased greatly from 8 to 14 DPE to the coinfected fish (Fig. 2) with a MDD of 10.3 ± 1.5 day. Among dead fish examined, 80% (1–7 DPE) and 100% (8–14 DPE) of fish were positive for Ich (Table 3). During the same period of trial, *E. ictaluri* was isolated from the kidney of 80% and 96% of examined fish, respectively (Table 3). Typical clinical signs of *E. ictaluri* infection

Fig. 1. A channel catfish showing white spots (arrows) with trophonts of *Ichthyophthirius multifiliis* located within the skin epithelial layers and hemorrhage underside of head and at bases of fins, a symptom of enteric septicemia of catfish caused by the bacterium *Edwardsiella ictaluri*. Bar = 10 mm.