PANAMJAS
Pan-American Journal of Aquatic Sciences

Treatment of a caseous infection caused by *Enterobacter aerogenes* (Enterobacteriaceae) in a captive longsnout seahorse, *Hippocampus reidi* (Actinopterygii, Syngnathidae)

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Abstract. A caseous lesion was recorded for the first time in a captive longsnout seahorse (*Hippocampus reidi* Ginsburg, 1933). The infection was caused by *Enterobacter aerogenes* and a secondary infection by *Klebsiella pneumoniae* caused the animal's death.

Keywords: cutaneous lesion, Enterobacteriaceae, ciprofloxacin, aquaculture.

Tratamento de infecção caseosa causada por *Enterobacter aerogenes* (Enterobacteriaceae) no cavalo-marinho-de-focinho-longo, *Hippocampus reidi* (Actinopterygii, Syngnathidae) de cativeiro. Resumo: Uma lesão caseosa foi registrada pela primeira vez no cavalo-marinho do focinho longo em cativeiro (*Hippocampus reidi* Ginsburg, 1933). A infecção foi causada por *Enterobacter aerogenes* e o animal foi a óbito devido a uma infecção secundária por *Klebsiella pneumoniae*.

Palavras-chave: Lesão cutânea, Enterobacteriaceae, ciprofloxacina, aquicultura.

Introduction

The bacteria of the family Enterobacteriaceae have a worldwide distribution and can be found in a range of different environments and niches, as well as forming part of the intestinal microbiota of many animal species, including fish (Abdel-Latif & Sedeek 2017). However, when an animal is exposed to stressful conditions, these bacteria may cause clinical infections, either as primary or even opportunistic pathogens, which often lead to the death of the animal (Zheng *et al*. 2004). Infection by enterobacteria is very common in fish farming operations, mainly in open systems, that may be affected by hospital, agricultural and industrial discharges (Cabello *et al.* 2016, Tacconelli *et al.* 2018), causing either major economic losses and/or the contamination of human consumers (Oliveira *et al.* 2017).

One of the many enterobacterial species is *Enterobacter aerogenes*, a Gram-negative bacterium, which is optionally anaerobic and catalase positive (Obi 2017). In aquatic animals, such as *Chanos chanos* and *Istiophorus* sp., infection by *E. aerogenes* has been associated with the production of histamine, which has caused intoxication (Tsai *et al*. 2004). More recent research indicates that *E. aerogenes* may be an emerging pathogen, given that it is known to cause enteritis in the catfish *Ictalurus punctatus* (Cao *et al*. 2017). However, it is unclear to what extent *E. aerogenes* may be a natural component of the intestinal microbiota of marine fish, including seahorses, and whether it does actually cause infections.

Seahorses are threatened by many human activities, and most species are assigned to some degree of threat in the IUCN Red List (IUCN 2021). The longsnout seahorse, *Hippocampus reidi* Ginsburg, 1933, can be identified according to some specific features, as the proportion of the snout length relative to the total head length (ranging from 2.0 to 2.6), as well as by the dorsal fin rays (17 to 19), anal fin rays (2 to 3) and the two pectoral fins rays (15 to 17 each) (Lourie *et al*. 2004). It is one of the species exploited most intensively worldwide (Koning & Hoeksema 2021), and a number of studies have investigated the potential for the captive rearing of this species as a sustainable means of supplying stock for the aquarium trade (Planas *et al*. 2021). Seahorse farming is a new industry, which is still only beginning to deal with its many challenges, in particular the control of diseases (Wang *et al*. 2016). In other words, there are still many obstacles to the successful, large-scale production of seahorses, in particular related to the understanding of the many pathogens that affect these fish.

The present study describes the first known case of an infection caused by *Enterobacter aerogenes* in a captive longsnout seahorse, *Hippocampus reidi*, but succumbed subsequently to an infection by *Klebsiella pneumoniae*. In December 2020, a caseous lesion was identified on the skin of a captive *H. reidi* being reared in the Laboratory of Animal Behavior and Conservation at Santa Úrsula University in Rio de Janeiro, Brazil. The lesion was located on the right ventral portion of the body, between the 7th and 9th rings, while a subcutaneous gas bubble was also found on the left side of the 8th ring. This individual was being raised together with another 30 *H. reidi* and was being fed daily with live, freshly-caught shrimp (*Mysis* sp.) or frozen *Artemia salina*, supplemented twice a week with the fauna associated with the bryozoans *Amathia verticillata* and *Bugula* sp.. This extra food was offered in an attempt to provide a wider range of nutrients that may have aided the recovery of the individual. The seahorse aquaculture project was authorized by the Brazilian government through license number 46586-8, issued by the Chico Mendes Institute (ICMBio), and followed the captive care protocol of the Association for the Study of Animal Behaviour.

As soon as the caseous lesion appeared on the skin of the seahorse, it was isolated in a 7-L aquarium, which contained two artificial plants. The quality of the water was verified daily and adjusted to maintain salinity (25–30 ppt), temperature (21.5–24.0°C), pH (8.0–8.2), toxic ammonia (< 0.25 ppm), and nitrite (< 1.00 ppm) levels within adequate limits.

The lesion was swabbed once, and the sample was preserved in Stuart's medium for analysis on the same day at the SM Clinical Analysis Laboratory. The sample was seeded directly onto a triplate covered with MacConkey, chocolate and blood agar separately (1/3 each) (Laborclin), which was used as the enriched growth media for the isolation of the bacterium. The blood agar was composed of 5% defibrinated sheep's blood and the medium was incubated in CO_2 for 24 hours at 35–37ºC. The species was identified based on the diagnostic morphological features of the colony.

The antibiogram was assessed using the disk diffusion method. The samples were diluted to match the 0.5 McFarland turbidity standard, following the CLSI protocol (CLSI 2018). The antibiotics were selected based on the results of the culture.

The initial analysis detected *Enterobacter aerogenes* in the tissue of the caseous lesion. This bacterium was sensitive to some of the antibiotics tested and resistant to others (Table I). We selected one of these antibiotics to which *E. aerogenes* was sensitive (Ciprofloxacin) to treat the infected seahorse.

Table I. Sensitivity of *Enterobacter aerogenes* to the different antibiotics tested in the present study.

Antibiotic	Sensitivity
Amikacin	**Sensitive**
Amoxicillin Clavulanate	Resistant
Ampicillin	Resistant
Aztreonam	**Sensitive**
Cephalexin	Resistant
Cefepime	**Sensitive**
Cefoxitin	Resistant
Ceftazidime	**Sensitive**
Ceftiofur	**Sensitive**
Cefovecin	**Sensitive**
Ceftriaxone	**Sensitive**
Ciprofloxacin	**Sensitive**
Enrofloxacin	**Sensitive**
Gentamicin	**Sensitive**
Imipenem	**Sensitive**
Marbofloxacin	**Sensitive**
Meropenem	**Sensitive**
Piperacillin Tazobactam	**Sensitive**

A seven-day course of antibiotic therapy was applied twice, with a five-day interval between treatments. During the treatment, one-eighth of a 500-mg tablet of Ciprofloxacin (Legrand) was added once a day, to a one-liter tank filled with seawater, and once it was completely dissolved, the seahorse was immersed in the water for 60 minutes. Following this daily bath treatment, a topical gentamicin-sulfadimidine based healing cream (®Vetaglos brand in Brazil) was applied to the lesion. The cream was applied once a day, continuously, during three months. The lesion recovered gradually during the course of this treatment (Fig. 1).

Following its recovery from the ventral lesions, the seahorse presented an edema on its prehensile tail with visibly caseous subcutaneous tissue (Fig. 2). This tissue was swabbed and analyzed following the procedure described above (culture in MacConkey/chocolate/blood agar [Laborclin] and disk diffusion for the antibiogram) for the identification of the bacterium and its known response to different antibiotics.

The culture revealed a mixed infection of *Enterobacter aerogenes* and *Klebsiella pneumoniae.* As the antibiogram indicated that both these bacteria are sensitive to Ciprofloxacin (Table II), we used the same treatment schedule as that applied to the previous lesion, with daily antibiotic baths being followed by the application of the healing cream. In this case, however, the animal did not present any signs of recovery and died on the second day of the second course of treatment.

Ciprofloxacin has been used to treat bacterial infections in a number of different fish species (Nouws *et al*. 1988, Kumar *et al*. 2015, Okoroafor *et al*. 2017), although it is important to note certain specific aspects of the present study. The first infection was well succeeded using ciprofloxacin. Although during the treatment of *Cyprinus carpio*, for example, Kumar *et al.* (2015) reported a loss of appetite, whereas in the present case, the seahorse continued to feed normally, and responded well to the full course of treatment. Even so, the secondary mixed infection was intense, and led to the death of the individual. El-Gahny *et al.* (2014) also tested ciprofloxacin for two weeks in mixed infection of discus *Symphysodon* sp. and the results reported mortality of 50% of the fish, in contrast to those treated with Metronidazole, which fully recovered. Grillon *et al.* (2016) noted that ciprofloxacin is more effective for some strains, having a less bactericidal activity than other antibiotics. Re-epithelization was satisfactory following the treatment with antibiotics and the application of the gentamicin-sulfadimidine based healing cream, which was shown to be an effective treatment for cutaneous lesions in the present study. None of the other 30 captive seahorses housed together with the infected individual treated in the present study presented any type of skin lesion that would indicate infection by either *Enterobacter aerogenes* or *Klebsiella pneumoniae.*

Both these bacterial species are known to cause opportunistic infections (Adeshina *et al*. 2016, Cao *et al*. 2017). *Enterobacter* is known to be a pathogen in humans (Kanemitsu *et al*. 2007) and other vertebrates, and has been reported infecting some farmed fish species, such as the catfish *Pangasianodon hypophthalmus* (Kumar *et al.* 2013) and *Ictalurus punctatus* (Cao *et al.* 2017). Cao *et al*. (2016) concluded that humans may be infected with this bacterium by consuming contaminated seafood, which is a concern for fish farming. *Klebsiella pneumoniae* is also an opportunistic, Gram-negative bacterium, which may cause disease and even death, if not treated promptly. This bacteria was observed in Indian Major Carp *Labeo rohita* and in clownfish, *Amphiprion nigripes*, which caused hemorrhages and ulcers (Gopi *et al*. 2016, Das *et al*. 2018) as we observed in seahorse in this study. Both bacteria are known to act as pathogens in fish following stressful

Figure 1. Clinical evolution of the cutaneous lesion provoked by *Enterobacter aerogenes* in a captive longsnout seahorse *Hippocampus reidi*: (a) caseous lesion on the right ventral portion of the skin on day 1 of the treatment; (b) subcutaneous gas bubble on the left 8th ring on day 1 of the treatment; (c) the caseous lesion shown in (a) on day 8 of the treatment; (d) the subcutaneous gas bubble shown in (b) on day 8 of the treatment, and (e) the seahorse on day 15 of the treatment.

Figure 2. Secondary infection in the captive longsnout seahorse, *Hippocampus reidi*, shown in Figure 1, showing: (a) the edema on the prehensile tail and (b) the visibly caseous subcutaneous tissue resulting from the mixed infection by *Enterobacter aerogenes* and *Klebsiella pneumoniae*.

events, as observed in the nishikigoi (carp) *Cyprinus carpio*, when handled inadequately (Oliveira *et al*. 2014) and in the clownfish, *Amphiprion nigripes* (Gopi *et al*. 2016). According to Gopi *et al.* (2016), the high ammonia level in captivity can cause stress, affecting the gills and restricting the respiration, which can improve susceptibility to pathogens, as a secondary intruder. In this study, the seahorse was maintained in ammonia below 0.25 ppm, however could lead to the secondary infection. Other causes may be related to bacterial infection, such as poor environmental quality, nutritional deficiency and overstocking (Sandeep *et al.* 2016). In the present study, water quality was weekly evaluated and stabilized, if not proper. Stocking density (0.04 in d.L), although proper to seahorses (Fonseca *et al.* 2015), was above their usual density in nature (0.2 ind.m^{-2}), probably intensifying interaction and competition for partner and food, turning them susceptible to infection by those stressful interactions.

Neither of the bacteria recorded in the present study have been reported previously in seahorses. *Vibrio* is the bacterium found most often in captive seahorses. The species include *Vibrio alginolyticus*, which is known to infect *H. reidi*, *Hippocampus guttulatus*, and *Hippocampus hippocampus* (Balcázar 2010a, Martins *et al*. 2010), *Vibrio splendidus*, recorded in *H. guttulatus* and *H. hippocampus* (Balcázar *et al*. 2010b), *Vibrio harveyi* and *Vibrio vulnificus* in *Hippocampus kuda* (Jiang *et al*. 2020, Xie *et al.* 2020), *Vibrio parahaemolyticus* in *Hippocampus kelloggi* (Yang *et al.* 2006), *Vibrio fortis* in *Hippocampus erectus* (Wang *et al.* 2016), and *Vibrio ponticus* and *Vibrio neptunius*, which are known to cause intestinal disease in *Hippocampus trimaculatus*, *H. erectus*, and *Hippocampus spinosissimus* (Li *et al*. 2015).

Table II. Sensitivity of *Enterobacter aerogenes* and *Klebsiella pneumoniae* to different antibiotics.

Antibiotic	*Enterobacter aerogenes*	*Klebsiella pneumoniae*
Amikacin	**Sensitive**	**Sensitive**
Amoxicillin Clavulanate	Resistant	**Sensitive**
Ampicillin	Resistant	Resistant
Aztreonam	-	**Sensitive**
Cephalexin	Resistant	Resistant
Cefoxitin	Resistant	Resistant
Ceftazidima	**Sensitive**	**Sensitive**
Cefovecin	**Sensitive**	**Sensitive**
Ceftriaxona	**Sensitive**	**Sensitive**
Ciprofloxacin	**Sensitive**	**Sensitive**
Enrofloxacin	Resistant	**Sensitive**
Ertapenem	**Sensitive**	**Sensitive**

Antibiotic	*Enterobacter aerogenes*	*Klebsiella pneumoniae*
Gentamicin	**Sensitive**	**Sensitive**
Imipenem	**Sensitive**	**Sensitive**
Marbofloxacin	Resistant	**Sensitive**
Meropenem	**Sensitive**	**Sensitive**
Piperacillin Tazobactam	**Sensitive**	**Sensitive**
Tetracycline	Resistant	Resistant
Tobramicina	Resistant	Resistant

Table II. Continued from previous page.

The present study is the first to report the infection of captive *H. reidi* by *E. aerogenes* and *K. pneumoniae*. This was also the first attempt to treat a bacterial infection in a fish species using antibiotics associated with the gentamicin-sulfadimidine based healing cream. Seahorse farming is intended to contribute to the conservation of wild populations, although a number of aspects of this process, including the prevention of disease and the control of pathogens, require further research. While Ciprofloxacin was effective for the treatment of the *E. aerogenes* infection, it did not appear to be as effective against *Klebsiella pneumoniae.* The healing cream used here (the Vetaglos brand in Brazil) did appear to be highly effective for the re-epithelization of the cutaneous lesions in the seahorse, however.

Acknowledgements

We thank Universidade Santa Úrsula for logistic support; Amanda Vaccani and Gabriela Cabiró for collaborating with the seahorse care in the laboratory. And also Solange Fonseca for the bacteriological analysis.

Ethics statement

Collection and/or manipulation of fish were conducted following all applicable ethical regulations regarding experimentation with animals.

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Received: January 2022
Accepted: April 2022
Published: August 2022