Short Communication

White necrotic tail tips in estuary seahorses, *Hippocampus kuda*, Bleeker

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Seahorses are classified as threatened species. They are popular in global aquarium trade and are additionally used in Asian countries in traditional Chinese medicines (Tendencia 2004; Smith et al. 2011; Vincent et al. 2011). Captive seahorses may succumb rapidly to a variety of parasitic, fungal and bacterial ailments (Raj, Lipton & Chauhan 2010). Peer-reviewed literature on seahorse diseases is scarce, and a handful of reports mainly deal with vibriosis caused by *Vibrio harveyi* (Alcaide et al. 2001; Tendencia 2004; Raj et al. 2010), *V. alginolyticus* or *V. splendidus* (Balcázar et al. 2011). White discolouration of the tail is a frequently occurring phenomenon that is well known amongst seahorse keepers and has been associated with an infection with *Vibrio sp.* (Balcázar, Planas & Pintado 2011) and a novel *Mycobacterium* sp. (Balcázar et al. 2011). However, its full aetiological profile remains to be elucidated, compromising an efficient control strategy. In this report, a case of necrotic tail tips in common seahorses, *Hippocampus kuda*, is described, the various steps in the diagnostic process for this condition listed, and the alleged aetiology discussed.

Common seahorses, kept by a commercial supplier of ornamental marine life, displayed white necrotic tails transforming into white patches covering the distal part of the tail. The breeder noticed at first the seahorses showed agitated swimming behaviour, scraped with the tail and had an increased breathing ratio. Gradually, the soft tissue of the distal part of the tail disappeared and in some cases led to the exposure of the vertebral column, consequently resulting in the death of the animal. The seahorses were fed live mysis during their stay at the supplier. Seven seahorses were brought to the Faculty of Veterinary Medicine at Ghent University. Four seahorses were already fixed in 4% phosphate-buffered formaldehyde upon arrival. Three of these did not reveal macroscopic abnormalities, whereas at the level of the tail of the fourth seahorse, the vertebral column was exposed (Fig. 1). Upon clinical examination of the three affected living seahorses, milky white patches on the distal part of the tail and greyish-white necrotic tail tips were noted (Fig. 2).

The edges of the necrotic lesions of the latter three animals were sampled using a sterile, cotton
swab, which was smeared onto both marine agar and Flexibacter maritimus medium (Pazos et al. 1996; Avendaño-Herrera et al. 2005). The plates were incubated aerobically for 72 h at 24 °C. After 36 h of incubation of the bacteriological media, numerous pale-orange colonies with smooth edges appeared in the plates of two of three sampled seahorse tails. A Gram stain revealed Gram-negative, long, slender bacterial cells of up to eight micrometres in length. The retrieved bacterial cells were non-fermenting, oxidase and catalase positive, motile and resistant to the vibriostatic agent 0/129. Further identification of the retrieved bacteria was carried out using 16S rRNA gene sequencing. Genomic DNA was extracted according to procedures used by Declercq et al. (2013). The 16S rRNA gene was amplified using the protocol as described by Smet et al. (2012). Sequences were compared with those in NCBI/GenBank and Greengenes using the BLAST search tool. The 16S rRNA gene sequence of the isolate showed 100% similarity with that of Tenacibaculum aestuarii strain D7025 as deposited in GenBank.

Wet mount preparations, made from skin scrapings of the three seahorses that were alive upon arrival, were observed microscopically to screen for parasites. Upon inspecting the skin samples, ovoid to elongated ciliated protozoans were noted with a morphology resembling that of species belonging to the genus Uronema (Scuticociliatida).

Subsequently, the three seahorses were euthanized by immersion in an overdose of a benzocaine (ethylaminobenzoate; Sigma) solution in acetone (10 g per 100 mL) and necropsied under a stereomicroscope (SZX7, Olympus with a colour View I Camera of Soft Imaging System). Necropsy did not reveal internal abnormalities in any of these animals. The tail region of all seahorses was cut-off and processed for histological examination.

The seahorse tails were placed in 4% phosphate-buffered formaldehyde for 24 h, dehydrated in an alcohol-xylene series and embedded in paraffin wax. All tissues were sectioned (8 µm) and stained with haematoxylin and eosin (H&E), Ziehl–Neelsen and Giemsa stain. Histological examination of the tail of two animals that were alive upon arrival and from which T. aestuarii was isolated, showed desquamation/disappearance of the epidermis and an ulcerative dermatitis covered with necrotic debris (Figs 3 & 4). The latter contained numerous clusters of long (up to 8 µm in length) slender bacteria, which had invaded the subcutaneous tissue. At these same sites, an
increase in proteoglycans, mild proliferation of blood vessels and limited to absent inflammation were noted. In areas where the epidermis was still intact, neutrophilic pustular lesions without bacterial infiltration were visible. Furthermore, necrotic muscle tissue was present underneath a necrotic epidermis in places where bacterial invasion was seen. No acid-fast bacteria were noted in the tissues after Ziehl–Neelsen staining. In the third seahorse that was alive upon arrival, the damaged tail presented a granulomatous dermatitis showing pustular lesions filled with neutrophils, macrophages and giant cells. Bacterial cells were not observed. The most severe histological lesions were seen in the tail of one of the animals already fixed upon reception. Focally, the epithelium had disappeared completely and was replaced by necrotic debris with an influx of macrophages. The muscle tissue in this site showed coagulation necrosis and focal dystrophic calcification and was covered with numerous, long and slender bacteria. Furthermore, an influx of inflammatory cells in the blood vessels was noted. The tails of the other three already fixed sea horses did not reveal any histopathological lesions.

Scuticociliates may, under certain circumstances, become opportunistic histophagous parasites, causing severe infections in marine fish, crustaceans and molluscs (Iglesias et al. 2002; Kim et al. 2004; Ramos et al. 2007). Scuticociliatosis has been recognized as one of the most important parasitological problems affecting cultured marine fish worldwide (Harikrishnan, Balasundaram & Heo 2010). The presence of these ciliates in fish tissues has been associated with a number of pathological signs, including skin ulcers spreading into muscular tissue and even exposing the fin rays (Azad et al. 2007; Ramos et al. 2007).

The fact that massive clusters of long, slender bacterial cells of up to eight micrometres in length – exhibiting the same morphology as the retrieved T. aestuarii isolate – were observed invading the subcutaneous tissue raises questions about their potentially pathogenic nature. This Tenacibaculum species, originally isolated from a tidal flat sediment in Korea (Jung, Oh & Yoon 2006), has never before been associated with neither disease signs nor mortality. However, various members belonging to the family Flavobacteriaceae are notorious pathogens in the aquaculture industry and the ornamental fish sector, as exemplified by Tenacibaculum maritimum, a well-known marine fish pathogen (van Gelderen et al. 2010). Additional studies are required to rectify or dismantle the above hypothesis that both scuticociliatosis and Tenacibaculum rank amongst the causative agents of white tail disease. In the fourth seahorse tail, major lesions of dermatitis were seen with the presence of a massive inflammatory infiltrate mainly consisting of macrophages and giant cells, but bacteria were not discernible. This might be ascribed to a strong immune response that may have induced phagocytosis of possibly present bacteria, which consequently were no longer visible at the time the tail was histologically examined.

To conclude, T. aestuarii was retrieved from necrotic tail tips in seahorses. A Uronema-like parasite was likewise noted upon microscopic examination of the affected tissues. Both agents may have played an aetiological role in the development of the skin lesions. However, further research is imperative because any pathological association between the observed lesions and the above agents at this stage is merely speculative. This case study in this respect is to be regarded as a plea for further research on diseases in seahorses and dissemination of the resulting findings in peer-reviewed literature.

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