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Short Communication

An Outbreak of *Vibrio alginolyticus* Infection in Juvenile Sea Cucumbers *Holothuria scabra* in Sabah, Malaysia

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ABSTRACT

Sea cucumber breeding was carried out in Universiti Malaysia Sabah Hatchery Facilities in April 2015. Approximately 690,000 eggs were fertilised with a hatching rate of 9%, an estimated 60,000 larvae were produced. In June, the surviving 359 sea cucumber juveniles were sorted according to size. In late July, it was found that only 30 juvenile sea cucumbers had survived, and the remaining were dead, with multiple ulcers on the skin and excessive mucus covering the entire body. In December 2015, a total of 20 juvenile sea cucumbers were sampled for bacterial isolation and histopathological examinations. *Vibrio alginolyticus* was isolated and histopathological examinations revealed peracute inflammation and accumulation of extensive mucus surrounding the body.

Keywords: *Vibrio alginolyticus*, outbreak, sea cucumbers, *Holothuria scabra*

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INTRODUCTION

Sea cucumber, *Holothuria scabra*, is a marine organism that is popular in Malaysia for its medicinal values (Choo, 2004). One study reported that sea cucumbers contain high levels of protein and certain bioactive components such as mucopolysaccharides, chondroitin sulfate and antioxidant compounds (Bordbar, Anwar, & Saari,

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2011). A total of 52 morphospecies of sea cucumber from four orders comprising 12 genera have been identified in Malaysia; 38 species were reported in Sabah, 24 species in Peninsular Malaysia and 10 species in both regions (Kamarudin et al., 2015). Sea cucumber fisheries are exploited off the coastal waters of Sabah in East Malaysia and Langkawi Island in West Malaysia. In Sabah, annual landings of sea cucumber in 1980s were between 400 and 500 tonnes but this declined in the 1990s to approximately 100 tonnes. Sea cucumber is an endangered species and until now, sea cucumbers in Malaysia are harvested from the wild (Choo, 2012), thus severe overfishing is leading to a significant decrease in the natural sea cucumber population (Conand, 2004). Therefore, breeding and seeding of sea cucumber in Malaysia was initiated in 2012 (Mazlan & Hashim, 2015).

Most studies on sea cucumber involve the Japanese sea cucumber, *Apostichopus japonicus* (Yang, Sun, & Xu, 2015), and infection by *Vibrio splendidus* (Zhao et al., 2011). The infection leads to skin ulceration syndrome, which limits the development of the sea cucumber culture industry (Gao et al., 2015). This paper reports the first outbreak of *V. alginolyticus* infection in juvenile sea cucumber, *H. scabra*, in Malaysia. It describes the isolation and pathological changes in cultured juvenile sea cucumber naturally infected by *V. alginolyticus*.

First breeding of sea cucumber was carried out in the Universiti Malaysia Sabah hatchery facilities in April 2015. Large (250-300 g) adult and healthy sea cucumber brood-stocks were brought to the hatchery and stocked in one-tonne tanks with a flow-through water system. The bottom of the tank was covered with sand of about 10 cm in thickness from the specimens' natural habitat and the sand was changed twice a month. The seawater supply for the brood-stocks was direct from the nearby seabed but was filtered using a coral filter. The brood-stocks' stocking density was 20 individuals per 1000 L. During spawning induction, 30 sea cucumbers from the brood-stocks were placed in a flat-bottomed spawning tank filled with filtered seawater and subjected to thermal stimulation (Kubota & Tomari, 1998). The eggs of the sea cucumber were placed in a separate larval rearing tank containing seawater that was filtered using a 1-µm filter and treated with UV light. Egg development, fertilisation and hatching rates were recorded.

Approximately 690,000 eggs were fertilised and approximately 60,000 larvae were harvested. By late June, the surviving 359 sea cucumber juveniles were sorted according to size; a total of 113 juveniles were of the size $\geq$1 cm while 246 juveniles were <1 cm. By late July, only 30 juvenile sea cucumbers had survived. The juveniles were dead after showing inactivity with multiple small ulcers on the skin that started as a white spot on the skin followed by excessive mucus covering the entire body (Figure 1). Eventually, by December 2015 the remaining 15 surviving juvenile sea cucumbers were showing similar clinical signs.

Figure 1. Gross lesions that started as small white spot on the skin (left picture; arrow) that eventually led to mucus surrounding almost the entire body (right picture)

A total of 20 dead and moribund juvenile sea cucumbers were sampled for bacterial isolation. Swab samples were collected from the body ulcer and the mucus, and hemolymph and organs were streaked directly onto Tryptic Soy Agar (Merck, Germany) and incubated at 30^0C for 24 to 48 h. Pure cultures were obtained before the colonies were subjected to Gram staining to determine morphology. Bacterial cultures were further identified using biochemical characterisation of API-20E (bioMérieux, France) and were confirmed using PCR (Nehlah, Ina-Salwany, & Zulperi, 2016). The entire body of juvenile sea cucumbers was then fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin (HE) for histopathological examinations.

Pure bacterial cultures were observed after 24 h of incubation. They were Gram-negative short rods that were confirmed as *V. alginolyticus,* and were successfully isolated from all 20 (100%) sampled *H. scabra* juveniles. The biochemical characterisation of API-20E identified as *V. alginolyticus* (Gonzalez-Escalano, Blackstone, & DePaola, 2006) and PCR (Nehlah, Ina-Salwany & Zulperi, 2016) revealed the amplification of the 846 bp band (Figure 2). Histopathological examinations revealed that peracute inflammation of the blood vessels

consisted of polymorphonuclear cells and accumulation of extensive mucus surrounding the body with total destruction of the epidermis that exposed the connective tissue. The blood vessels of the organs were hyperaemic, while the internal organs were severely congested.

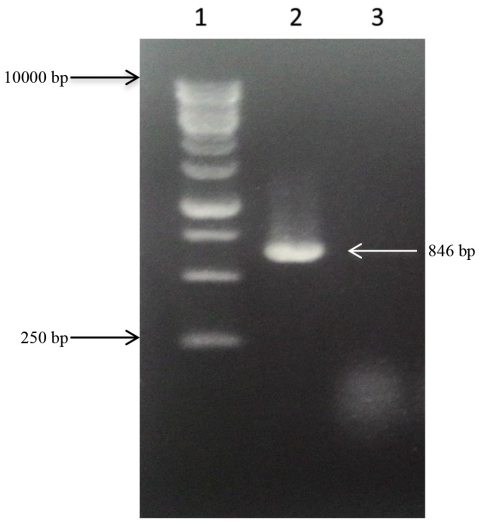

Figure 2. Polymerase chain reaction of the outer membrane protein, ompK, gene of *Vibrio* isolate RFZ-IPMB-16. Amplification targetted the DNA at 846-bp. Lane 1. DNA GeneRuler, 1kb DNA Ladder (Thermo Scientific, USA); Lane 2. ompK gene of *Vibrio* isolate RFZ-IPMB-2016; Lane 3. Negative control

It was concluded that the mortalities of the juvenile sea cucumber, *H. scabra*, in a sea cucumber hatchery facility at UMS Sabah, Malaysia were due to *V. alginolyticus* infection, which affected 100% of the juveniles between the ages of 2 and 8 months old. The source of *V. alginolyticus* is believed to have been the sea water (Lavilla-Pitogo et al., 1990; Becker et al., 2004) and ultraviolet treatment of the sea water seemed to have been ineffective (Shikongo-Nambabi, Kachigunda, & Venter, 2010). Affected sea cucumbers showed typical lesions of vibriosis, particularly skin ulcerations. Yang, Hamel and Mercier (2015) identified infection by *V. splendidus* and *V. alginolyticus* in Japanese sea cucumber as a skin ulcerative syndrome that frequently affects and kills young rather than mature *A. japonicus* sea cucumber. This hampers attempts to artificially breed and cultivate sea cucumber. Similarly, Becker et al. (2004) reported a skin ulceration disease of juvenile *H. scabra*, the widely marketed edible sea cucumber in Madagascar. The first sign of the infection is a white spot on the integument close to the cloacal aperture that spreads quickly to the whole integument, leading to the death of individual sea cucumbers within three days. Infection by *V. alginolyticus* in juvenile *A.*

japonicus sea cucumber has been reported by Wang et al. (2005) as producing skin ulceration syndrome.

This is believed to be the first report of the outbreak of *V. alginolyticus* infection in juvenile *H. scabra.* The infection killed all the juveniles quickly within three days, producing typical peracute lesions of skin ulceration syndrome. However, the excessive mucus production that covered the affected individuals observed in this outbreak has not been previously reported.

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