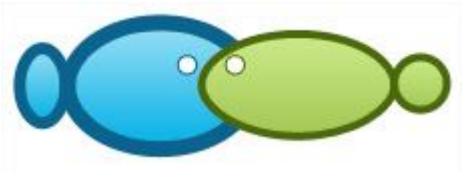

Susceptibility of hybrid grouper (*Epinephelus fuscogutattus* ♀ × *Epinephelus lanceolatus* ♂) to *Vibrio harveyi* VHJR7

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Abstract. The susceptibility of hybrid grouper (*Epinenephelus fuscoguttatus* × *Epinephelus lanceolatus*) to *Vibrio harveyi* VHJR7 at three different concentrations of bacteria (8.4×10^4 (T1), 1.3×10^5 (T2), and 1.7×10^5 (T3) c.f.u. g^{-1} body weight, respectively) was investigated. Fish were cultured in 150 L tank with 7 individuals per tank and duplicated. It was then observed for any signs of abnormalities due to infections and mortalities were recorded up to 10 days of post-challenge. Cumulative mortality of hybrid grouper ranged from 0 to 57.1%. The lethal dose 50% (LD_{50}) of *V. harveyi* to hybrid grouper was 1.6 x 10^5 c.f.u g^{-1} body weight. Red blood cell (RBC) and white blood cell (WBC) count of fish decreased as the bacterium concentration injected increases. The hybrid grouper is susceptible to *V. harveyi* VHJR7 at high doses.

Key Words: susceptibility, hybrid grouper, *Epinenephelus fuscoguttatus* × *Epinephelus lanceolatus*, *Vibrio harveyi* VHJR7, lethal dose.

Introduction. The hybrid grouper (*Epinenephelus fuscoguttatus*♀ × *Epinephelus lanceolatus* ♂) was first produced in Borneo Marine Research Institute (Ch'ng & Senoo 2008). The hybrid grouper has become popular in the Southeast Asian countries and has high aquaculture demand due to their high egg hatching rate and larval survival, faster growth, strong tolerance to the environmental factors, and resilience to diseases than its parental species (Mustafa et al 2013; Othman et al 2015; Koh et al 2016; Bunlipatanon & U-taynapun 2017; Arrokhman et al 2017). Like any other intensive mariculture, this fish is usually cultured in net cages during grow-out period and in high stocking density. Therefore, in such condition, this fish is directly exposed to poor handling, stress, and pathogen infection.

Vibrio harveyi is considered as one of the main pathogens affecting a wide range of marine fish species (Zhang & Austin 2000). It has been reported to cause mass mortalities in several grouper aquaculture farms (Saeed 1995; Yii et al 1997; Lee et al 2002; Sivaram et al 2004; Albert & Ransangan 2013; Ransangan & Mustafa 2013) and eventually lead to losses in aquaculture production. Moreover, this disease outbreak was also reported to affect crustacean which includes crabs and shrimps (Flegel & Alday-Sanz 1998; Zhang et al 2014).

To date, a number of studies reported on the susceptibility of marine fish species to various strains of *V. harveyi* (Liu et al 2004; Won & Park 2008; Ransangan et al 2012). Despite being a popular fish in Asian countries, there is limited information on the susceptibility of hybrid grouper to *V. harveyi*. The results obtained from other grouper species may not represent the hybrid grouper because susceptibility is species-specific (Gomez-Gill et al 1998). Furthermore, the hybrid species could have acquired heterosis advantages resulting from the hybridization. The present study investigated the susceptibility of the hybrid grouper to a fish pathogenic strain of *V. harveyi* VHJR7.

Material and Method

Experimental fish. Hybrid grouper juveniles were obtained from a local fish hatchery in Tawau, Sabah, Malaysia. The fish were acclimatized for 2 weeks in the fish hatchery and fed with commercial marine fish feed (Leong Hup Feedmill Sdn. Bhd). This study was conducted in May 2014.

V. harveyi VHJR7. *V. harveyi* VHJR7 (Ransangan et al 2012), which was used in this experiment was obtained from Fish Disease and Microbiology Lab of Borneo Marine Research Institute, Universiti Malaysia Sabah, Malaysia. The bacterium was cultured in triptic soy broth (Difco; +2% NaCl) for 24 hours at room temperature. The bacterial cells were harvested by centrifugation and washed three times, then resuspended in phosphate buffer saline (PBS) solution for experimental infection.

Challenge test. A total of 56 fish with the mean weight of 71.4±13g (mean±SD) were used in this experiment and were distributed into 8 tanks with 7 individuals per tank. Fish were cultured in 150 L constantly aerated tanks. The fish were starved for 24 hours before the challenge test and anaesthetized with Transmore® (Nika Trading, Puchong, Malaysia). Duplicate fish groups from each treatment were injected with three doses of the bacterium (8.4×10^4 (T1), 1.3×10^5 (T2), and 1.7×10^5 (T3) c.f.u. g^{-1} body weight) and the control group were injected with sterile PBS solution.

During the challenge test, fish tanks were cleaned and 60% of the water was changed daily. No feed was given to the experimental fish and water parameters were monitored throughout the challenge test. Fish were observed for any signs of abnormalities, which may be due to infection. Mortalities were recorded up to 10 days of post-challenge and the LD_{50} value was determined according to Reed & Muench (1938).

Blood parameters. At the end of the challenge test, blood was drawn at the fish caudal peduncle using syringe and stored into vacuette tubes containing EDTA. The bloods were then subjected to red blood cell (RBC) and white blood cell (WBC) counts and packed cell volume (PCV, %) test.

Results. Cumulative percentage of mortality (%) of the hybrid grouper (ranging from 0 to 57.1%) was recorded daily for 10 days (Table 1). The cumulative mortality of the fish had increased proportionally to the bacterial dosage injected. The first mortality was recorded after 24 hours in fish groups T2 (28.6%) and T3 (50%), respectively. After 72 hours of post-challenge, the fish in group T1 encountered first mortalities of 21.4%, and there was an increased mortality in fish group T3 (increased to 57.1%). There was no mortality recorded in control group up to 10 days.

Table 1

Fish mortality after 72h of infection with different concentrations of *V. harveyi*, VHJR7

Treatment (bacteria concentration)	*No. of fish (0h)*	*No. of fish (24h)*	*No. of fish (72h)*
Control	14	14	14
T1 (8.4×10^4)	14	14	11
T2 (1.3×10^5)	14	10	10
T3 (1.7×10^5)	14	7	6

Values of mortalities (%) are mean of duplicate groups and presented as mean±SD, n = 2.

In the present study, fish in groups T1, T2 and T3 exhibited symptoms such as sluggish swimming behavior within 12 hours of post-challenge. Simultaneously, fish in T3 group appeared to release excessive mucus. There was a noticeable mucosa on the surface and in the water column. In addition, morbid fish in group T3 also displayed abnormal movement, erratic swimming, lethargic, and reddening at anus area. Based on the

mortality data, the LD_{50} value of *V. harveyi* VHJR7 to hybrid grouper was 1.6 x 10^5 c.f.u g^{-1} body weight.

The RBC count, WBC count, and PCV (%) value of hybrid grouper are presented in Table 2. The RBC count in fish group control, T1, T2, and T3 were 2.15±0.21, 1.70±0.10, 1.30±0.42 and 1.25±0.21 (10^6 mm^{-3}), respectively. The lowest RBC count was observed in group T3 which was 41.9% lower compared to the control group. The WBC count of fish ranged from 362.30 to 426.20 (10^6 mm^{-3}). The highest WBC count was observed in T1 group, 426.20±19.23 (10^3 mm^{-3}). The PCV value in control group was the highest 36.0±1.4 (%), while, PCV value in treated group T1, T2 and T3 were much lower (25.0±1.40, 23.5±10.6 and 20.5±6.36 %, respectively).

Table 2

Changes in hematological parameters of fish infected with different dosage of *V. harveyi*, VHJR7

Treatments	*RBC (10^6 mm^{-3})*	*WBC (10^3 mm^{-3})*	*PCV (%)*
Control	2.15±0.21	406.15±56.78	36.0±1.40
T1	1.70±0.10	426.20±19.23	25.00±1.40
T2	1.30±0.42	362.30±19.09	23.50±10.60
T3	1.25±0.21	362.35±19.87	20.50±6.36

Values are mean of duplicate groups and presented as mean ± SD, n=2.

Discussion. In previous studies, *V. harveyi* were described to be highly virulent to marine fish species including humpback grouper, *Cromileptis altivelis* (LD_{50} = 8.33 × 10^3 c.f.u g^{-1} body weight), Asian seabass, *Lates calcarifer* (LD_{50} = 1.14 × 10^3 c.f.u g^{-1} body weight), and orange spotted grouper, *E. coioides* (LD_{50} = 8.7 × 10^3 c.f.u g^{-1} body weight), respectively (Ransangan et al 2012; Xu et al 2017). Nevertheless, in other findings, it was identified that *V. harveyi*, was low virulent to brown spotted grouper, *E. tauvina* (LD_{50} = 1.56 × 10^9 c.f.u g^{-1} body weight) (Saeed 1995). Based on the cumulative mortalities for 10 days without feeding activity, it appeared that *V. harveyi* VHJR7 was moderately virulent to hybrid grouper (*Epinenephelus fuscoguttatus* ♀× *Epinephelus lanceolatus* ♂) with LD_{50} value of 1.6 × 10^5 c.f.u g^{-1} body weight.

The effects of *V. harveyi* to fish are varied among species due to the differences in resistance and immune system. The present study showed that hybrid grouper was more resistant to *V. harveyi* than its maternal species, tiger grouper, *Epinephelus fuscoguttatus.* Apines-Amar et al (2012) had conducted a preliminary study and reported that the LD_{50} of *V. harveyi* to tiger grouper (*E. fuscoguttatus*) is $10^{6.33}$ c.f.u ml^{-1}. In addition, the size of fish used in their study ranged from 43.24-100.92g body weight, therefore, the LD_{50} of *V. harveyi* to the tiger grouper is around 10^4 c.f.u g^{-1} body weight. Meanwhile, in another study conducted by Xu et al (2012) reported that the LD_{50} of *V. harveyi* to tiger grouper is 5.8 x 10^2 c.f.u g^{-1} body weight. Although the LD_{50} values reported varied among authors, which could be due to the different strains of *V. harveyi*, both results are still lower compared to the hybrid grouper LD_{50} values. The resistance of hybrid grouper towards bacterial infection was possibly due to the hybrid vigor acquired during hybridization. Hybridization of fish has been shown to attain vigorness in term of growth rate, increased environmental and disease tolerance (Bartley et al 2000).

Fish infected with *V. harveyi* can be characterized with signs of swollen abdomen filled with opaque liquid, gastroenteritis, and reddening around anus area (Yii et al 1997; Soffientino et al 1999; Lee et al 2002). In the present study, the moribund fish expressed dark skin, lethargic, spiral swimming behavior and loss of balance prior to dying. Similar behaviors and clinical signs were also distinguished in Asian seabass and humpback grouper when infected with *V. harveyi* VHJR7 (Ransangan et al 2012). However, no fin rot or skin ulcers were noticed in the present study. The absence of fin rot and skin ulcers could be attributed to the virulence characteristic of *V. harveyi* VHJR7.

The *V. harveyi* VHJR7 affected the RBC and WBC counts, and PCV (%) after 10 days of challenge test. Fish produced WBC as a mechanism to combat bacterial infection. In the present study, highest WBC count was observed in T1 fish group may due to the least dosage of bacteria given during the challenge test, thus making the infection less

severe than T2 and T3 fish groups. Severe bacterial infections may cause the WBC in the T2 and T3 fish groups been used up faster than they can be produced. Decrease in RBC counts is inversely proportional to the increase of bacteria concentration. The results may indicate that level of fish stress increases as the bacterial concentration increases. Similar findings were also reported in channel catfish which infected with *Ichthyophthirius multifiliis* parasitism and previously exposed to *Edwardsiella ictaluri* (Shoemaker et al 2012). The decrease of RBC count in infected fish may be caused by the leucocytosis activity and stress induced by the pathogen following erythroblastosis which leads to severe anemic condition (Sabri et al 2009). The anemic condition in the fish was evident by the lower PCV (%) values in treatment fish. The PCV of less than 20% in teleosts fish are usually related to anemia (Clauss et al 2008).

Conclusions. The hybrid grouper (*Epinenephelus fuscoguttatus* ♀× *Epinephelus lanceolatus* ♂) is susceptible to *V. harveyi* VHJR7 at high doses with LD_{50} of 1.6 x 10^5 c.f.u g^{-1} body weight. The LD_{50} value was higher compared to previous recorded data in grouper species (dosage of less than 10^5 c.f.u g^{-1} body weight) including its maternal species, tiger grouper. Such attribute could have been contributed by the hybrid vigor during hybridization. However, further studies are necessary to elucidate disease resistance mechanism of hybrid grouper.

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