Cross Sectional Screening of Red Tilapia Health Status in Green Algae Pond

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Abstract: This study was carried out to determine the health status of red tilapia cultured in green algae pond that does not show any signs of abnormality. A total of 30 red tilapia were sampled from indoor green algae pond located in Perak. The overall gross examination showed 29% of red tilapia have clinical signs such as haemorrhage, pale gill, pop eye and redness while enlarged liver, pale liver and enlarged spleen were observed at internal organs. No ectoparasite was found from gill and body smear examination. Bacteriology showed prevalence 28.5% infection comprising of *Plesiomonas shillegoides*, *Pseudomonas putida*, *Edwarsiella tarda*, *Aeromonas sobria* and *Aeromonas hydrophila*. Analysis on Tilapia Lake Virus (TiLV) using semi-nested RT-PCR showed 80% were positive and 62.5% of positive sample having similarity sequence of nucleotide DNA with TiLV. Sequencing analysis on nucleotide DNA showed 98% are identical to the strain from Thailand (KY381578) and 95% from Israel (KU751816). Histopathology of liver showed presence of 40% hepatocytes resembling giant cells which contained multiple nuclei and is a typical pathology for TiLV. Indicating that, red tilapia currently used for green algae production had bacterial infections and high prevalence of TiLV. Although no mortalities were reported so far, we believe that under sudden or prolong exposure changes may potentially cause mortality in red tilapia particularly those having positive TiLV.

Keyword: green algae, tilapia, health, disease

Abstrak: Kajian ini dijalankan untuk menentukan status semasa kesihatan ikan tilapia merah dalam penggunaan kultur alga hijau di kolam yang tidak menunjukkan tanda-tanda abnormal. Sebanyak 30 ekor tilapia merah telah diambil daripada kolam alga hijau yang terletak di Perak untuk penyampelan. Pemeriksaan kasar menunjukkan 29% tanda-tanda klinikal seperti reput sirip dan pendarahan, insang pucat, mata terbonjol dan kemerahan, manakala hati besar, limpa besar dan hati pucat diperhatikan pada organ dalaman. Tiada ektoparasit dijumpai daripada pemeriksaan calitan insang dan badan. Pemeriksaan bakteriologi menunjukkan prevalen sebanyak 28.5% jangkitan yang terdiri daripada *Plesiomonas shillegoides*, *Pseudomonas putida*, *Edwarsiella tarda*, *Aeromonas sobria* and *Aeromonas hydrophila*. Analisis terhadap Tilapia Lake Virus (TiLV) menggunakan *semi-nested* RT-PCR menunjukkan 80% adalah positif dan 62.5% positif sampel mempunyai kesamaan turutan nucleotida DNA dengan TiLV. Analisis jujukan terhadap nukleotida DNA menunjukkan 98% adalah sama dengan TiLV daripada Thailand (KY381578) dan 95% daripada Israel (KU751816). Histopatologi terhadap hati menunjukkan 40% kehadiran hepatosit menyerupai sel gergasi yang mengandungi pelbagai nucleus dan ianya adalah patologi yang tipikal untuk TiLV. Hasil daripada kajian ini menunjukkan bahawa tilapia merah yang kini digunakan untuk pengeluaran alga hijau mempunyai jangkitan bakteria dan prevalen yang rendah TiLV. Walaupun tiada kematian yang dilaporkan setakat ini, kami percaya dengan perubahan faktor secara tiba-tiba atau berpanjangan terutamanya yang mempunyai TiLV positif berpotensi boleh menyebabkan kematian dalam tilapia merah.

Introduction

The aquaculture industry is a fast growing sector and its production has been increasing steadily. Increased aquaculture production may contributed by few factors and one of it was through the application of high quality feed at early stages of culturing through the use of green water technology (GWT). Green Water Technology is an integrated culture of economically important herbivorous fishes in pond. Green algae has been commonly used as a natural food for fish and other aquatic animals in aquaculture field. Many herbivorous fish can ingest algae as a natural food source. According to Battaglene and Cobcroft (2007), adding of microalgae to larval fish culture tanks confers a number of benefits, such as preventing bumping against the walls of the tanks. Besides that, it also enhancing predation on zooplankton, enhancing the nutritional value of zooplankton and as well as improving larval digestive (Cahu *et al.* 1998) and immune functions. Algae was also used in formulated fish feeds to assess their nutritional value and many have shown to be beneficial like Chlorella or *Scenedesmus* fed to Tilapia (Tartiel et al. 2008); Chlorella fed to Korean rockfish (Bai *et al.* 2001) and *Undaria* or *Ascophyllum* fed to Sea Bream (Yone *et al.* 1986).

GWT using red tilapia in Malaysia is very effective to produce green water within few days. According to Habiba *et al.,* (2017), tilapia grown in green water pond showed high productivity and can reached a mean body weight of 150.99 ± 0.5 gm within 120 days with no fertilization and feeding costs. This technology will benefit small farmers due to the lower production cost and high productivity. Tilapia is one of the important culture fish species and has important role in aquaculture and fisheries economics in the world. Tilapia is commonly known to tolerate the adverse environmental conditions, fast growth and could be cultured at high stocking density (El-Sayed AFM, 1999).

Disease is considered as a primary constraint to the culture of each aquatic species, including tilapia. The impact cause by disease can impede both economic and social development in many countries. So far, some important infectious diseases such as bacterial and viral diseases could be considered as one of the major challenges in aquaculture development. Some Herpes-like virus, Iridoviral disease (IVD) and Viral Nervous Necrosis (VNN) could pose more threats in tilapia industry (Zorriehzahra M.E.J. *et al*, 2015). Recently, a novel RNA virus termed Tilapia Lake Virus (TiLV) has been identified and caused massive mortalities of wild and pond-cultured tilapia all over Israel (Eyngor M., 2014). It was also reported that some red tilapia without showing abnormal symptoms were found to be infected with TiLV (Saengchan S., 2018). Although tilapia has been widely utilised in controlling green algae bloom, little is known on its health status. Hence, the objective of this study is to determine the health status of red tilapia in green algae pond through gross examination, parasitology, bacteriology, virology and histopathology.

Materials and Methods

Gross Observation

A total of 30 red tilapias were sampled from indoor green algae pond located at a hatchery in Perak. Live fish were clinically examined for abnormalities on the external body surface (skin, gills, eyes and mouth), external lesions like wounds, petechial haemorrhage, ulcers, emaciation, sloughing of scales from the skin or eroded fins and cysts according to the methods described by Austin and Austin (1987) and Noga (2010).

Parasitological examination

Body and gill smears were obtained by scraping the outer layer of gill filaments and body surface and spread them on clean glass slides. A drop of normal saline was placed on each smear and covered with a clean cover slip prior to microscopy examination (Lucky, 1977).

Histopathology

Samples of tissue from brain, kidney, liver and spleen were collected and preserved in 10% buffered formalin (Saengchan S. *et al*, 2018). All samples were brought back to National Fish Health Research Division (NaFisH) for further analysis. The samples were processed according to the standard histology procedures described by Puntanat T., *et al*, (2017). Briefly, the samples were processed using automatic tissue processor before they were embedded and sectioned. The sections were stained with Hematoxylin and Eosin (H & E) and viewed under microscope.

Bacteriology

Samples were aseptically taken from brain, eye and kidney using sterile loop and streaked onto tryptic soy agar (Oxoid, Hampshire, UK) for bacteriology. The media plates were incubated at 30°C for 24–48 hours. Biochemical analysis was performed using API 20E, API 20NE and API 20STAPH (Biomérieux, France).

Detection of TiLV by semi-nested RT-PCR

The liver were collected and preserved in 95% ethanol. Total nucleic acid was extracted using viral TaCo^{TM} DNA/RNA extraction kits. For TiLV, total nucleic acid was first reverse transcribed using MyTaq one step RT-PCR kit (Bioline). The first step semi nested RT-PCR was carried out using a pair of primers namely Nested -Ext1 (TAT GCA GTA CTT TCC CTG CC) and ME1 (GTT GGG CAC AAG GCA TCC TA) and followed by semi nested RT-PCR with primer pair ME1 (GTT GGG CAC AAG GCA TCC TA) and ME2 (TAT CAC GTG CGT ACT CGT TCA GT) (Dong *et al.,* 2017b). All the PCR products were electrophoresed on 1.5% agarose gel prepared in TBE buffer, at 100 V for 30 mins. The results were viewed under bio-imaging system (Syngene, Cambrige, UK) and TiLV positive were determined at 250 bp. The positive PCR products were later sent for sequencing.

Results and Discussion

Gross examination of 30 fish (mean weight ±143g) showed 29% having clinical signs while 71% were without any abnormality. The clinical signs seen at external body were haemorrhage, pale gill, pop eye and redness while enlarged liver, pale liver and enlarged spleen were observed at internal organs. Parasite examination indicated that no ectoparasites were found from gill and body smear. However, 100% red tilapia was infected with bacteria *Plesiomonas shigelloides, Pseudomonas putida, Edwardsiella tarda, Aeromonas sobria* and *Aeromonas hydrophila*. While only 28.5% have multiple bacterial infection.

These bacteria are opportunistic pathogens that cause disease when fish are exposed to risk factors such as deterioration of water quality, high density and rough handling. *Pseudomonas putida* is not a common pathogen in aquaculture and was first isolated from ayu, *Plecoglossus altivelis altivelis* and yellowtail, *Seriola quinqueradiata*, in Japan and later from rainbow trout in Turkey (Altinok *et al*., 2006). Infected fish shows ulcerative disease and haemorrhagic ascites. Meanwhile, *Aeromonas hydrophila* can causes skin ulcers on the fish at any site and often they are surrounded by a bright red rim of tissue. Two species (*E. ictaluri* and *E. tarda)* of genus Edwardsiella were recognized as opportunistic pathogens and known as human pathogen. *E. tarda* can cause red disease of eels and fish gangrene of catfish. The disease was first isolated from cultured eel in Asia (Rafet C.

Ö. & İlhan A., 2014). *E. tarda* can cause serious mortality in marine and freshwater fish, including catfish, carp, eel, flounder, seabream tilapia and yellowtail (Plumb and Hanson, 2011).

Apart from having multiple bacterial infections, the samples were detected positive with TiLV. Detection of TiLV using semi-nested RT-PCR showed 80% samples were positive and 62.5% of positive sample having similarity sequence of nucleotide DNA with TiLV.

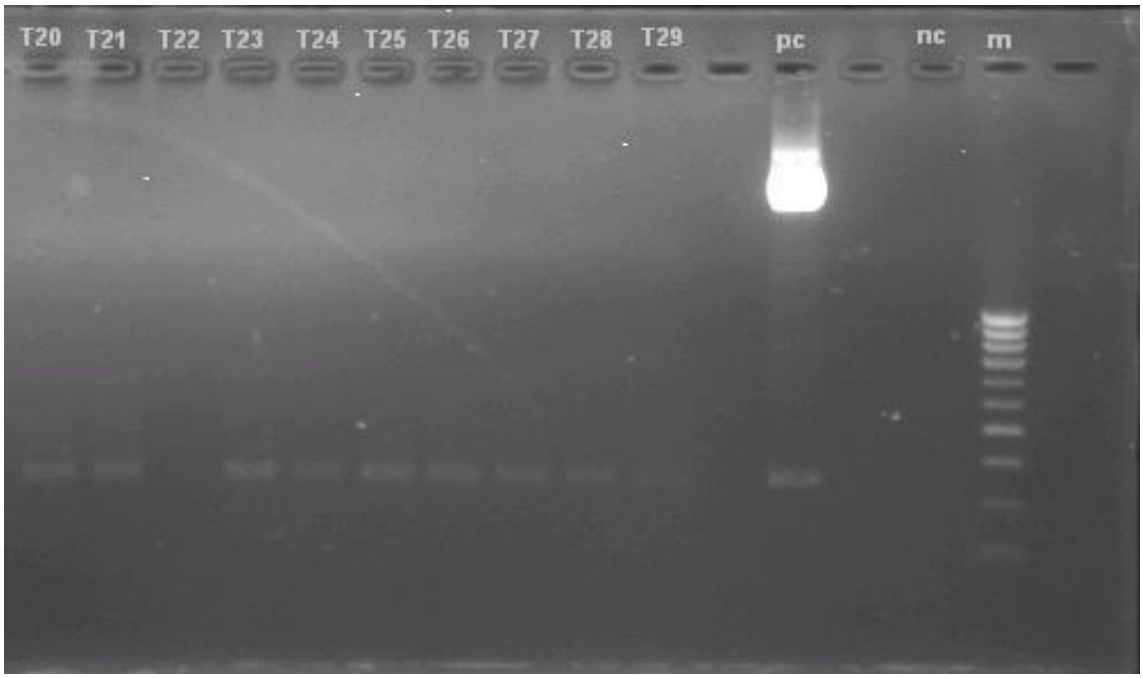

Figure 1: Agarose gels of TiLV detection using semi-nested RT-PCR assay

Sequencing analysis on nucleotide DNA–showed 98% are identical to the strain from Thailand (KY381578.1) and 95% revealed to the prototype TiLV from Israel (GenBank accession no. KU751816). Histopathological observation from 10 fishes showed formation of giant, multinucleated cells in 40% of the fish liver. Saengchan S., *et al*., (2018) reported a similar pathology was seen in healthy farmed tilapia which was detected positive for TiLV in Thailand. Apart from Thailand, several countries like Egypt, Chinese Taipei and Malaysia also reported lower mortalities tilapia that having positive TiLV, ranging from 0.71 to 15% (Saengchan S. *et al*., 2018).

In this study, tilapia samples were found to be TiLV positive without showing abnormal symptoms or unusual mortality. An unapparent infection and a wide range of mortality rates might theoretically suggest the existence of a genetic variability of TiLV as also described in a fish orthomyxovirus called infectious salmon anemia virus (ISAV) having more than 20 variants with differences in pathogenicity (Christiansen *et al*., 2011; Cottet *et al*., 2011). In this case, TiLV investigation on virus genotypes and their correlation with virulence should be a priority. Molecular diagnosis and control measures could then emphasize on highly pathogenic variant(s). On the other hand, the presence of TiLV in tilapia indirectly showed the current tilapia are in a carrier state or chronic state that can be a potential reservoir source for viral transmission. (Saengchan S. *et al*., 2018)

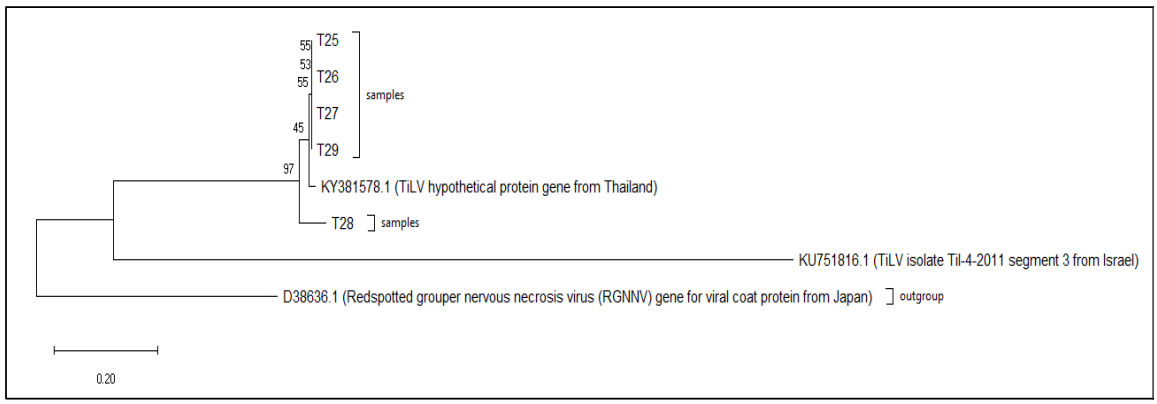

Figure 2: Phylogenetic tree of TiLV based on segment 3 nucleotide sequences. Phylogenetic tree was generated using Neighbor-Joining method by the MEGA X software.

Low clinical signs (29%) of tilapia were seen in indoor-green algae pond culture. However, 28.9% of tilapia was infected with multiple bacterial infection. Despite having multiple bacterial infection and high TiLV, there was no mortality recorded.

Futher study on the properties of numerous algae in GWT is necessary as green algae can be used as an additional feed. According to Ebtehal (2017), up to 5% algae can be added into Nile tilapia diet without any adverse effect or abnormalities of fish tissue. Besides, it can also improve growth rate, feed utilization, physiological indicators, stress response and disease resistance.

Conclusion

Low clinical signs (29%) of red tilapia were seen in indoor-green algae pond culture. However, 28.9% of red tilapia was infected with multiple bacterial infections (*Plesiomonas shillegoides*, *Pseudomonas putida*, *Edwarsiella tarda*, *Aeromonas sobria* and *Aeromonas hydrophila*) and high prevalence (80%) of TilV. Despite having multiple bacterial infection and high TiLV, there was no mortality in red tilapia. Further study on the properties of numerous algae in GWT is necessary as green algae can be used as an additional feed. Hence, this study provide an important information on health status of red tilapia where under sudden or prolong exposure changes may potentially cause mortality particularly for those having positive TiLV.

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