UNIVERSITI PUTRA MALAYSIA

PATHOGENICITY OF STREPTOCOCCUS AGALACTIAE IN JUVENILE RED TILAPIA (OREOCHROMIS SP.) FROM A FISH FARM IN SELANGOR, MALAYSIA

ALI FARAG ABUSELIANA

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2011

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By

ALI FARAG ABUSELIANA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science

March 2011

DEDICATION

With appreciation and respect, this thesis is dedicated

To my parents and family,

for their understanding, encouragement and constant support

To all my supervisors,

who ensured it all worthwhile

To all my friends

for their encouragement and moral assistance

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Veterinary Science

PATHOGENICITY OF *STREPTOCOCCUS AGALACTIAE* IN JUVENILE RED TILAPIA (*OREOCHROMIS* SP.) FROM A FISH FARM IN SELANGOR, MALAYSIA

By

ALI FARAG ABUSELIANA

March 2011

Chairman: Prof. Madya Hassan Hj Mohd Daud, PhD

Faculty : Veterinary Medicine

Streptococcal infection is one of the emerging bacterial diseases that was reported to cause significant mortality and high economical loss in freshwater and saltwater fish species including *Tilapia sp*., worldwide. Recently, a streptococcosis outbreak affecting Red tilapia (*Oreochromis* sp.) farm in Selangor was investigated. Affected fish showed loss of appetite, cornea opacity and serpentine swimming. Healthy and morbid fish were clinically examined. Samples from brain, liver, spleen and kidney were collected for causal agent isolation. Pure bacteria isolates were successfully isolated on trypticase soy agar (TSA) blood agar (BA) and brain heart infusion agar (BHIA). The colonies were of grayish white color, circular, convex, pin-head size and β-haemolytic. All isolates were gram-positive cocci, oxidase-negative and catalase-negative. They were identified as group B *Streptococcus agalactiae* (GBS) using a commercial identification kit (Streptococcal grouping kit, RapID™ STR System and BBL Crystal GP ID kit). Specific polymerase chain reaction (PCR) and 16S rRNA sequencing technique confirmed the isolates as GBS. The isolates were sensitive to amoxicillin, ampicillin, erythromycin, chloramphenicol, linomycin,

rifampicin, vancomycin, gentamicin, sulfamethoxazole + trimethoprime and tetracycline. On contrary, they were resistant to neomycin, amikacin, kanamycin and streptomycin. The 120 hours median lethal dose (LD_{50}) value in juvenile tilapia injected intraperitoneally (IP) was 1.5×10^5 cfu/mL. Experimental infections were carried out by bathing the fish for 30 minutes in water containing the bacteria and by intraperitoneal (IP) injection. It was observed that IP route was more potent to cause mortality to juvenile Red tilapia and produced clear clinical signs within five days. It was noted that the mortality started to reduce after five days and fish recovered after nine days post inoculation. In contrast, immersion route did not induce mortality, but produced moderate clinical signs such as lethargy and loss of appetite, and fish started to recover after six days. The findings of the current study indicated that *S. agalactiae* infection started to become an issue in tilapia farms and warrants focusing to formulate a suitable measure to prevent and control the disease before it becomes endemic in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk jatoh Master Sains Veterinar

KEPATOGENAN *STREPTOCOCCUS AGALACTIAE* TERHADAP IKAN TILAPIA MERAH JUVENIL (*OREOCHROMIS* SP.) DARIPADA SEBUAH LADANG IKAN DI SELANGOR, MALAYSIA

Oleh

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Jangkitan Streptococcal merupakan salah satu penyakit bakteria yang dilaporkan menyebabkan kematian dan kerugian ekonomi yang tinggi dalam spesies ikan air tawar dan masin termasuk spesies tilapia di seluruh dunia. Baru-baru ini, satu wabak penyakit streptokokosis melibatkan kultur ikan tilapia merah (*Oreochromis* sp.) di Selangor telah disiasat. Ikan yang dijangkiti menunjukkan tanda kehilangan selera makan , berenang bengkang-bengkok dan kekaburan kornea . Ikan yang sihat dan sakit disiasat secara klinikal dan sampel diambil dari otak, hati, limfa dan ginjal untuk pemencilan agen etiologiknya. Isolat kultur tulen berjaya dikultur di atas agar TSA dan darah. Koloni-koloni di atas agar TSA adalah berwarna putih kekelabuan, bulat, cembung, bersaiz pin dan β-hemolitik. Semua isolat adalah kokus, Gram positif, oksidase dan katalase negatif. Ia dikenalpasti sebagai kumpulan B *Streptococcus agalactiae* (GBS) menggunakan kit pengenalan komersil (Streptococcal grouping kit, RapID™ STR System dan BBL Crystal GP ID kit). Tindakbalas berantai polimerase (PCR) dan jujukan 16S rRNA mengesahkan isolat-

isolat sebagai GBS. Isolat-isolat adalah sensitif terhadap amoksisilin, ampisillin, eritromisin, kloramfenikol, linomisin, rifampisin, vancomisin, gentamisin, sulfamethoxazol + trimethoprim dan tetrasiklina. Sebaliknya, ia rentang terhadap neomisin, amikasin, kanamisin dan streptomisin. Nilai dos kematian median 120 jam (LD_{50}) dalam anak ikan tilapia merah yang disuntik secara intraperitoneal (IP) adalah 1.5×10^5 cfu/mL. Jangkitan eksperimen dicapai dengan merendam ikan selama 30 minit dalam air mengandungi bakteria dan secara suntikan intraperitoneal. Ia dapat dilihat bahawa jangkitan IP lebih berkuasa menyebabkan kematian terhadap anak ikan tilapia merah dan menunjukkan tanda-tanda klinikal yang jelas dalam masa lima hari. Ia dapat dilihat juga kematian mulai berkurangan selepas lima hari dan ikan pulih selepas sembilan hari pasca inokulasi. Sebaliknya, rendaman tidak mengaruhkan kematian, tetapi menghasilkan tanda-tanda klinikal sederhana seperti kelesuan dan kehilangan selera makan, dan ikan mulai pulih selepas enam hari. Hasil daripada kajian ini menunjukkan bahawa jangkitan *S. agalactiae* telah mula menjadi isu pada ternakan tilapia dan memerlukan kita mengambil langkah yang sesuai untuk mengelak dan mengawal jangkitan ini sebelum menjadi endemik pada masa hadapan.

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I certify that a Thesis Examination Committee has met on 24 March 2011 to conduct the final examination of Ali Farag Abuseliana on his thesis entitled "Pathogenicity of *Streptococcus agalactiae* in Juvenile Red Tilapia (*Oreochromis sp.*) from a Fish Farm in Selangor, Malaysia" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Veterinary Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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ALI FARAG ABUSELIANA

Date: 24 March 2011

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LIST OF ABBREVIATIONS

BA	Blood agar
B.C.	Before Christ
BHIA	Brain heart infusion agar
bp	Base pair
°C	Degree centigrade
CAMP	Christie, Atkins and Munch-Petersen
cfu	Colony-forming unit
cm	Centimeter
DDW	Double distilled water
DO	Dissolved oxygen
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphate
dpi	Days post inoculation
EB	Ethidium bromide
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
g	Gram
GBS	Group B *Streptococcus agalactiae*
H&E	Hematoxylin and eosin
hpi	Hours post inoculation
IP	Intraperitoneal
L	Liter
LD_{50}	Median lethal dose

mg	Milligram
MIC	Minimum inhibitory concentration
PCR	Polymerase chain reaction
ppt	Parts per thousand
rRNA	Ribosomal ribonucleic acid
TBE	Tris-borate-EDTA
TSA	Trypticase soy agar
UV	Ultra violet

CHAPTER 1

INTRODUCTION

Fish are an excellent source of food for people and considered as a healthy alternative to other animal meats. Fish flesh consists mainly of high grade protein and low in fat, besides, containing minerals, vitamins and essential fatty acids. Fish protein makes up around 15% of total world animal protein supplies, providing more than 2.9 billion people with 20% or more of their average daily protein intake. Nowadays, fish account for 30% human protein supply in Asia, 20% in Africa and 15% in Latin America and Caribbean (Bone and Moore, 2008; FAO, 2008).

Aquaculture is probably one of the most important and fastest growing food production sectors in the world. It is increasing much more rapidly than animal husbandry and capture fisheries (Lucas and Southgate, 2003). World aquaculture has grown significantly in all its forms during the last fifty years and now constitutes about 50% of world food fish production (De Silva and Davy, 2010). From an annual production of less than one million tonne in the 50's, production has increased to about 52 million tonnes in 2006, with an annual growth rate of nearly 7% and a value of around US$ 79 billion (FAO, 2008). Asia and Pacific regions are by far the world's leader in aquaculture production, producing more than 89% of the world's total aquaculture output in 2006. China is reported to be the world number one producer by producing 67% of the total quantity and 49% of the total value of world aquaculture production (FAO, 2008). With the increase in population expected over the next two decades and captured seafood production from fisheries

is at or near its peak, aquaculture is considered as the best solution to meet the growing demand for fish and other aquatic products. The ratio of per capita consumption and demand of aquatic products need to be maintained as global aquaculture production must increase by at least 40 million tonnes by 2030 (De Silva and Davy, 2010; FAO, 2008).

The aquaculture industry in Malaysia in 2007 contributed 268,500 tonnes, about 16% to national seafood supply with a value of about US$ 0.37 billion (Wing-Keong, 2009). Freshwater aquaculture contributed 70,064 tonnes and marine and brackish water contributed 198,449 tonnes of total Malaysian aquaculture production in 2007. Tilapia, catfish, carp, blood cockle, shrimp and seaweeds were considered the most important aquatic species cultured in Malaysia (Annual Fisheries Statistics, 2007). The first introduction of Red tilapia (*Oreochromis niloticus*) hybrid to Malaysia was in middle of 1980s and from that time Red tilapia production has increased dramatically (Siti-Zahrah *et al.*, 2008). In 2007, tilapia production occupied 46% of Malaysian total freshwater aquaculture production (Wing-Keong, 2009).

The rapid increase in global aquaculture industry have exposed to many diseases that were not known in aquaculture fields. In theory, the relationship between the fish, the pathogen and the manifestation of the disease is largely due to the phenomenon of stresses which causes negative affects on fish immune system and makes fish more susceptible to diseases. Similar to livestock, fish farms are also exposed to different types of diseases which can cause huge economical damages to farming activity. The etiology of these diseases may be of viral, bacterial, fungal,

parasitic, environmental, nutritional or genetic origin. However, bacteria were often associated with multifactorial nature of fish diseases which were responsible for most of the diseases that cause mortality and losses in fish farms (Yin, 2004; Plumb, 1999; Inglis *et al.*, 1993).

Fish streptococcosis is one of bacterial diseases affecting both captive and wild fish in freshwater, estuarine and marine environments. Moreover, it is one of the fish diseases that is reported in intensive aquaculture systems causing high economic loss to fish farmers, and can cause more than 50% mortality within one week (Yanong and Francis-Floyd, 2006; Lio-Po and Lim, 2002; Inglis *et al.*, 1993). It was noted that the occurrence of streptococcosis outbreak increased when fish were stressed such as due to changes in optimal culture environment (Buller, 2004; Shoemaker *et al.*, 2000). Furthermore, fish streptococcosis is reported to be potential zoonotic capable in causing disease in humans (Toranzo *et al.*, 2005).

Several species of Streptococci have been reported to cause infections in wild and cultured fish species. *Streptococcus agalactiae*, one of Streptococci species, has a broad host range, infecting both terrestrial and aquatic animals. The organisms have been isolated from numerous fish species in natural disease outbreaks and showed to be pathogenic to several fish species (Musa *et al.*, 2009; Toranzo *et al.*, 2005; Evans *et al.*, 2002; Elliott *et al.*, 1990).

Inevitably, streptococcosis in tilapia will be a major setback in fish production program in Malaysia especially with rapid growth in tilapia industry and intensification of culture system. Thus, there is a need to focus on effort to obtain

comprehensive knowledge about this infectious disease, its etiological agent and the infectivity status, so as to find the best biosecurity measures to prevent and control the disease before it becomes a major serious threat to aquaculture industry in Malaysia.

The hypothesis of this study was *Streptococcus agalactiae* isolated from infected fish is pathogenic to cultured Red tilapia (*Oreochromis niloticus*).

The objectives of the study were:

1. To isolate and identify *S. agalactiae* from diseased farmed tilapia.
2. To determine the antibiotic susceptibility patterns of *S. agalactiae* isolates.
3. To determine the median lethal dose (LD_{50}) of *S. agalactiae* to juvenile Red tilapia (*Oreochromis sp*.).
4. To determine the pathogenicity of *S. agalactiae* in juvenile Red tilapia (*Oreochromis sp*.).

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