UNIVERSITI PUTRA MALAYSIA

STUDIES ON *AEROMONAS HYDROPHILA* ISOLATED FROM EPIZOOTIC ULCERATIVE SYNDROME (EUS) POSITIVE FISH IN MALAYSIA

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by

H. A. Mahinda Kulathilaka

Thesis Submitted in Fulfilment of the
Requirement for the Degree of Master of Science
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To

My Mother,

Wife and Children

For Their Love and Inspiration

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LIST OF ABBREVIATIONS

APHA	- American Public Health Association
ATCC	- American Type Culture Collection
ECP	- Extracellular product
EDTA	- Ethylene diamine tetraacetic acid
ESC	- Enteric septicemia of catfish
EUS	- Epizootic Ulcerative Syndrome
FAT	- Fluorescent Antibody Technique
FCA	- Freund's complete adjuvant
FIA	- Freund's incomplete adjuvant
IFAT	- Indirect Fluorescent Antibody Technique
IM	- Intramuscular
IP	- Intraperitoneal
MMC	- Melanomacrophage centres
NACA	- Network of Aquaculture Centres in Asia
RS	- Rimler-Shotts medium
SRBC	- Sheep red blood cell
TSA	- Trypticase Soy Agar
UDS	- Ulcerative Disease Syndrome

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirement for the degree of Master of Science.

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January 1993

Supervisor : Assoc. Professor Dr Mohamed Shariff

Faculty : Fisheries and Marine Science

The present study indicates that the only portal of entry of *A. hydrophila* serovar I isolate V which induces EUS-like lesions in *C. batrachus* is the intramuscular injection and the effective dose is 6.5 X 10^6 CFU/fish. The lesions do not develop into putrefied necrotic ulcers but heals gradually within 14-18 days of initial injection. Immersion exposure and oral administration of *A. hydrophila* serovar I isolate V were incapable of inducing EUS-like lesions in *C. batrachus*.

Intramuscular injection of *A. hydrophila* serovar I strain V cause severe pathological changes in *C. batrachus*, only at the site of injection and did not produce systemic

infection. *Clarias batrachus* mounts a strong inflammatory response against *A. hydrophila* following intramuscular infection and is capable of eliminating bacteria from the infected tissues and efficient in fast wound healing and repair of the dermal lesions. *Aeromonas hydrophila* serovar I strain V does not have the capability to overcome the host response and manifest systemic infection in *C. batrachus* following intramuscular injection and oral administration under the conditions at which the experiments were conducted. Oral administration of *A. hydrophila* serovar I strain V can cause acute gastritis in *C. batrachus* but it is possible that the action of gastric secretions eliminate the bacteria within a very short period.

Aeromonas hydrophila serovar I strain V induces a higher agglutinating titre in *C. batrachus* following injection with formalin killed bacteria compared to that of live bacteria, following repeated injections. Although *C. batrachus* mounts an immunological memory, the action of *A. hydrophila* serovar I strain V toxins and proteases could cause localised inflammatory changes leading to a necrotic lesion, at least at the site of injection.

There is no evidence to conclude that $CaCO_3$ up to 400 ppm cause any significant stress on fish which made them more susceptible to *A. hydrophila*. However, during *in vitro* culture, 20 ppt NaCl appear to affect the virulence properties of *A. hydrophila* serovar I strain V. The low temperature also has an effect on the ability of intramuscularly injected *A. hydrophila* to cause mortality in *C. idella*.

It was concluded that *A. hydrophila* serovar I strain V is not a primary causative agent of EUS but is a secondary etiological agent of the syndrome.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia, sebagai memenuhi keperluan untuk mendapat Ijazah Master Sains

KAJIAN KE ATAS *AEROMONAS HYDROPHILA* DARI IKAN YANG POSITIF TERHADAP SINDROM EPIZOOTIK ULSERATIF (SEU) DI MALAYSIA

oleh

H.A. Mahinda Kulathilaka

Januari 1993

Penyelia : Profesor Madya Dr. Mohamed Shariff

Fakulti : Perikanan dan Sains Samudera

Kajian yang dijalankan ini menunjukkan bahawa jalan masuk *A. hydrophila* serovar I isolat V yang menyebabkan lesi serupa-SEU pada *C. batrachus* adalah suntikan intramaskular dan dos yang berkesan adalah 6.5 x 10^6 UPK/ikan. Lesi tidak berkembang menjadi ulser yang nekrotik dan putrefaktif tetapi mula sembuh dalam tempoh 14-18 hari selepas suntikan pertama. Pendedahan rendaman dan pemberian oral *A. hydrophila* serovar I isolat V tidak berkebolehan untuk mempengaruhi lesi sindrom serupa-SEU pada *C. batrachus*.

Suntikan intramaskular *A. hydrophila* serovar I isolat V menyebabkan beberapa perubahan patologi hanya pada kawasan suntikan dan tidak menghasilkan jangkitan sistemik pada *C. batrachus*. *Clarias batrachus* menunjukkan tindakbalas inflamasi yang kuat terhadap *A. hydrophila*