



Suez Canal University Faculty of Veterinary Medicine Department of Fish Diseases and Management

STUDIES ON PREVAILING BACTERIAL DISEASES IN FRESHWATER CRAYFISH IN RELATION TO WILD NILE TILAPIA

Thesis presented by

Inas Rafat El-Saeed Mohamed

M.V.Sc. Cairo University, 2005

For

The degree of Ph.D of Veterinary Medical Science

(Fish Diseases and Management)

Thesis submitted to

Dept. of Fish Diseases and Management Faculty of Veterinary Medicine Suez Canal University

2015

CONTENTS

SUBJECT	page
INTRODUCTION	1
REVIEW OF LITERATURE	5
MATERIALS AND METHODS	42
RESULTS	63
DISCUSSION	99
CONCLUSION	112
SUMMARY	113
REFERENCES	115
ARABIC SUMMARY	i-ii

LIST OF TABLES

TABLENo.	Title	page
Table (1)	primer sequence of <i>A. hydrophila</i> lip gene with random DNA fragments	48
Table (2)	Sources and types of crayfish samples	52
Table (3)	Prevalence of different isolated pathogens from clinically diseased and apparently healthy crayfish during late summer only	70
Table (4)	Prevalence of different isolated pathogens from clinically diseased and apparently healthy Nile tilapia during late summer and early winter	71
Table (5)	Prevalence of <i>A. hydrophila</i> during early winter and late summer	72
Table (6)	The morbidity and mortality rates of experimentally infected tilapia and control group.	76

LIST OF PHOTO

Photo No.	Title	page
Photo(1)	PCR fragments of lip gene in <i>A. hydrophila</i> isolated from all examined strains.(a): Lane M: 100bp ladder marker, lane 1: control negative, lanes 2-7: <i>A. hydrophila</i> isolated from crayfish. lanes 8-13: <i>A. hydrophila</i> isolated from tilapia fish	73
Photo(2)	RAPD pattern of <i>A. hydrophila</i> isolates generated by primer OPA-01. Lane M: 0.1-10kb, Ladder marker	74
Photo(3)	RAPD pattern of <i>A. hydrophila</i> isolates generated by primer OPA-03. Lane M: 0.1-10kb, Ladder marker	74
Photo(4)	RAPD pattern of A. hydrophila isolates generated by primer OPA-10. Lane M: 0.1- 10kb, Ladder marker	75
Photo(5)	Gills of <i>P. clarkii</i> showing heamolysis and edema with inflammatory cells. H&E X200	87
Photo(6)	Gills of <i>P. clarkii</i> showing edema and inflammatory cells, H&E X200	87
Photo(7)	tips of secondary lamellae of gills of <i>P</i> . <i>clarkii</i> showing bifurcation, inflammatory cells and heamolysis, H&E X200	88
Photo(8)	Musculature of <i>P. clarkii</i> showing degeneration of muscle fibers with vacular degeneration, H&E X400	88
Photo(9)	Musculature of <i>P. clarkii</i> showing splitting of its fiber, H&E X400	89
Photo(10)	Musculature of <i>P. clarkii</i> showing disorganization and oedema in between muscle fibers, H&E X400	89
Photo(11)	Hepatopancreas of <i>P. clarkii</i> showing ballooning structure of its acinus, H&E X400	90

Photo(12)	Hepatopancreas of <i>P. clarkii</i> showing star- like appearance with severe vacuolar degeneration, H&E X400	90
Photo(13)	Hepatopanceas of <i>P. clarkii</i> showing severe vacuolar degeneration with cell remnants and somewhat inflammatory cells, H&E X 400	91
Photo(14)	Gills of <i>Oreochromis niloticus</i> showing dilatation and severe congestion of branchial blood vessels, H&E X 200	91
Photo(15)	Gill of <i>Oreochromis niloticus</i> showing fusion with club formation and oedema in between secondary lamellae, H&E X400	92
Photo(16)	Gills of <i>Oreochromis niloticus</i> with desquamation of their epithelium in between secondary lamellae, H&E X400	92
Photo(17)	Liver of <i>Oreochromis niloticus</i> showing congestion and dilatation of blood vessels, H&E X400	93
Photo(18)	Liver of <i>Oreochromis niloticus</i> showing disorganization of hepatic cords with adenoid formation, H&E X650	93
Photo(19)	Liver of <i>Oreochromis niloticus</i> showing acinus formation with minute haemolytic areas, H&E X650	94
Photo(20)	Spleen of <i>Oreochromis niloticus</i> showing severe depletion of lymphoid elements with melanomacrophage centers, H&E X200	94
Photo(21)	Spleen of <i>Oreochromis niloticus</i> showing diffuse melanomacrophage cells with haemocedrosis. H&E X400	95
Photo(22)	Spleen of <i>Oreochromis niloticus</i> showing melanomacrophage around splenic blood vessels with heamocidrosis. H&E X 100	95

Photo(23)	Kidney of <i>Oreochromis niloticus</i> <i>Oreochromis niloticus</i> showing cystic formation with degeneration, necrosis and clusters of cells in its center. H&E X400	96
Photo(24)	Kidney of <i>Oreochromis niloticus</i> showing glomerulonephrosis. H&E X650	96
Photo(25)	Kidney of <i>Oreochromis niloticus</i> showing sluphing of epithelial lining of renal tubules making star formation. H&E X400	97
Photo(26)	Dorsal musculature of <i>Oreochromis</i> <i>niloticus</i> showing oedema between muscle fibers. H&E X400	97
Photo(27)	Dorsal musculature of <i>Oreochromis</i> <i>niloticus</i> showing Zinker necrosis and accumulation of lymphocytes (start of splitting). H&E X650	98
Photo(28)	Musculature of <i>Oreochromis niloticus</i> showing splitting. H&E X650	98

LIST OF PLATES

plate No.	Title	page
plate (1)	<pre>showing apparently health crayfish and diseased a: apparently healthy P. clarkii with intact cuticle b: erosion of legs c: red patches of cuticle</pre>	64
plate (2)	<pre>showing naturally infected P. clarkii with A. hydrophila a: lethergy b: recumbent c:darkening and softening of hepatopancreas</pre>	65
plate (3)	 showing naturally infected <i>O. niloticus</i> with <i>A. hydrophila</i>: A: shallow oval ulcer on the outer surface. B: diffuse heamorrahges C: Redness around vent, sciatic and sunken eye D: focal heamorrahge E: sloughing scales and tail fin erosion 	66
plate (4)	<pre>showing postmortem lesions of naturally infected O. niloticus with A. hydrophila: A: enlarged viscera B: congested liver and spleen</pre>	67
plate (5)	showing O. niloticus on the 3 rd day post injection with A. hydrophila A: heamorrahge of caudal trunk B: progressing ulcer under dorsal fin	77
plate (6)	 showing O. niloticus on 4th day post injection with A. hydrophila A: focal heamorrahge and ascites B: partial desquamation of scales C: congestion of liver and spleen 	78

plate (7)	 showing O. niloticus on the 5th day post injection with A. hydrophila A: heamorrahge and ascites of viscera B: erosion of caudal fin and opaque eye C: ulceration of caudal peduncle and sunken eye 	79
plate (8)	showing O. niloticus on the 6 th day post injection with A. hydrophila A: darkness of whole body B: Sunken eye and emaciation	80
plate (9)	showing <i>O. niloticus</i> on the 7th day postinjection with <i>A. hydrophila</i>A: pale gills and progressing skin ulcersB: ulceration of skin and caudal fin erosion	81

Auther	Inas Rafat El-Saeed Mohamed
Title	Studies on prevailing bacterial diseases in freshwater
	crayfish in relation to wild Nile tilapia
Faculty	Veterinary Medicine- Suez Canal University
Department	Fish Diseases and Management
Location	Ismailia - Egypt
Degree	Ph. D. Fish Diseases and Management
Date	-
Language	English
Supervision	Prof. Dr. Ismail Abd El-Monem Eissa
committee	Prof. of Fish Diseases and Management Faculty of
	Veterinary Medicine, Suez Canal University
	Dr. Maather Mohemed El-Lamie
	Lecturer of Fish Diseases and Management, Faculty of
	Veterinary Medicine, Suez Canal University
	Prof. Dr. Ahmed Mohamed El-Gamal
	Head researcher Bacteriology, Animal Health Research
	Institute, Mansoura Provincial Laboratory
	Summary

A total of 200 freshwater Crayfish *procambarus clarkii* were collected during late summer from different natural water resources of River Nile, Dakahlia governorate. The diseased crayfish showed focal heamorrahge on cuticle, liquifacation of hepatopancreas and/or congestion. A total of 258 Nile tilapia *Oreochromis niloticus* were collected from same water resources of crayfish during late summer and early winter. 60 fish that of healthy appearance were directed for pathogenicity test.

The clinical findings and post mortem lesions of naturally infected crayfish were lethergey and erosions associated with softening and darkening of hepatopancreas, while tilapia showed shallow ulcers, diffuse heamorrahges associated with congested vissera. The detected isolates from crayfish were *Escherichia coli, Pseudomonas aeruginosa*, Proteus spp. and *Aeromonas hydrophila* which were of higher prevalence. The rate of isolation in winter seasons was 0%, while in summer, it was 35%. In contrast, the rate in tilapia was 8.08 and 17.17% in winter and summer respectively. Molecular identification revealed that *A. hydrophila* harbor the lip gene at 760bp, while the genetic diversity using RAPD-PCR OPA-10 primer revealed that 0.9kb and 1.4kb amplicons were common to all isolated Aeromonas with 12 different amplicons of size ranging from 0.5 to 1.9kb. Histopathological findings were varied from severe to mild degenerative changes.

Key word Procumbarus clarkii, Oreochromis niloticus, Aeromonas hydrophila