UNIVERSITI PUTRA MALAYSIA

AETIOLOGIC AGENTS OF FRY MORTALITY SYNDROME IN THE RAINBOW TROUT *(Oncorhynchus mykiss)* IN IRAN

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SEYED MOHAMMAD EBRAHIM JALIL ZORRIEHZAHRA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in the Fulfilment of Requirements for the Degree of Doctor of Philosophy

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DEDICATION

WITH LOVE AND APPRECIATION TO:

My dear wife: Masoumeh Kohinejad

My dear son: Seyed Mohammad Ehsan

My dear brothers: Seyed Kamal, Seyed Jalal, Seyed Amir Ahmad and

Seyed Mohammad Mehdi

and

My dear sister: Eftekhar Sadat

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in the fulfilment of the requirements for the Degree of Doctor of Philosophy

AETIOLOGIC AGENTS OF FRY MORTALITY SYNDROME IN RAINBOW TROUT *(Oncorhynchus mykiss)* IN IRAN

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April 2008

Chairman: Associate Professor Dr Hassan Hj. Mohd Daud, Ph.D.

Faculty: Veterinary Medicine

An investigation was conducted in order to find out the etiological factors of Fry Mortality Syndrome (FMS) that causes serious economical loss in rainbow trout farms in Iran. In recent years obscure fry mortalities have been observed in many hatchery farms in Iran. It was reported that the rate of fry and juvenile mortality increased dramatically in some provinces e.g. 23 million fry were produced in hatchery centers of Chahar Mohal Bakhtiary province in 2002 but nearly 21 million fry (91.3%) in different stages of growth died before distribution to farmers. Also close to 23 million fry were produced in Mazandaran province, but 12 million fry equivalent to 52.12% of total fry production died mysteriously.This investigation was carried out with objectives of detecting and confirming the main causative agent that contribute to the occurrence of Fry Mortality Syndrome in Iran. During 32 months, from October of 2001 until May of 2004, 52 different hatchery centers and rearing farms of rainbow trout *(Oncorhynchus mykiss)* which were located in Tehran,

Mazandaran, Guilan, Fras, Markazi, Kerman and Kohkiloyeh Boyerahmad provinces, were visited and various samples from affected farms were collected. Collected samples consisted of ovarian fluid, milts, eggs, eyed-eggs, larvae, fry < 1 g and 1-3 g as well as internal organs from adult fishes. A total of 2,107 samples were collected from farms in six provinces and were examined by five methods such as virology (410 samples), bacteriology (899 samples), serology (consisted of IFAT: 392 samples and ELISA: 44 samples), histopathology (160 samples) and hematology (202 samples). Some of the mentioned approaches such as fish cell culture, ELISA and IFAT techniques were set-up and optimized for the first time in Iran.

The clinical signs of suspected fishes were darkening, exophthalmia, ascites, abnormal swimming and whirling. From 410 samples that of tissues inoculated on to cell cultures two samples showed CPE in EPC and BF-2 cell lines which were inoculated with ovarian fluid from broodstock obtained from hatchery farms in Mazandaran province. The CPE was similar to IHN virus induced. The CPE foci revealed dying cells congegrated as grape-like clusters (ballony performance with cytolysis).TEM findings in infected cells showed bullet-shaped particles having sizes of 130-180 nm in length and 65-70 nm in diameter. From the virion morphology it was suggested that observed particles were similar to Rhabdovirus. FAT examination revealed that all samples were examined with MAbs and PAbs against IPNV and VHSV were negative. On the other hand, two samples were positive when examined with MAbs and PAbs against IHNV. These smears were originated from samples that had showed CPE in EPC and BF-2 cell lines and bullet shaped particles in electron microscopy. ELISA findings (cut-off value, optical density and detection-level percentage) showed that IHNV had higher percentage of detection with 23.25%

in comparison with other relevant viral diseases i.e. IPNV with 7.31% and VHSV with 14.29%. Results of histopathological study on the sampled fry revealed that the target tissues in the kidney, liver, spleen, hepatopancreas, intestine and gills showed different degree of tissue changes beginning from cell degeneration to complete necrosis. There were also renal blood vessels congestion, marked degenerative changes in posterior kidney with tubular necrosis and interstitial hematopoeitic tissue degeneration. In addition, interstitial degeneration and oedema in anterior portion of kidney, focal necrosis in the tubular area and several stages of cell necrosis in the hematopoeitic tissue were the most important histopathological changes seen in kidney tissues examined. Hepatopancreatic tissues also revealed marked changes such as congestion, atrophy and necrosis of pancreatic acinar cells and Islets of Langerhans. Spleen samples revealed spleenic congestion, severe necrosis, hemosiderosis and increased presence of melanomacrophage centers (MMC). Gills tissue in sampled fry showed hyperplasia, clubbing and fusion of lamellae.

Hematological findings revealed that total white blood cell count, i.e. lymphocyte and neutrophil in investigated fish showed significant increased compared with the control fish ($p< 0.05$). On the contrary, all the samples showed a decreased in RBC, Hb and HCT values. In addition, MCHC and total protein plasma showed a marked decreased ($p<0.05$). In the blood serum components analysis, similarly it was revealed LDH and AST showed a significant decreased ($p<0.05$).

In conclusion, with marked clinical signs, cell culture observation and TEM findings, ELISA and IFAT results, histopathology and hematological findings (blood and biochemical parameters) seen in the current investigation lead to possibility of a viral disease agent infection as the cause of fry mortality syndrome in the hatchery

and rearing trout farms in Iran. From findings of the current study, it is concluded that IHN-like virus could be most probable etiologic of fry mortality syndrome in Iran.

Key words: Fry Mortality Syndrome, Rainbow trout, Cell culture, TEM, ELISA, IFAT, Histopathology, Hematology, IHNV, IPNV, VHSV, Iran

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

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SEYED MOHAMMAD EBRAHIM JALIL ZORRIEHZAHRA

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Pengerusi: Profesor Madya Dr. Hassan Hj. Mohd Daud, Ph.D.

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Satu penyiasatan telah dijalankan untuk menentukan faktor etiologik Sindrom Kematian Anak Ikan yang telah menyebabkan kehilangan ekonomi yang serius dalam ladang ikan trout pelangi di Iran. Dalam tahun kebelakangan ini, kematian anak ikan yang tidak di ketahui punca telah dilihat di banyak ladang penetasan di Iran. Di lapurkan bahawa kadar kematian anak ikan dan ikan juvenil telah meningkat secara mendadak di beberapa daerah, contohnya 23 juta anak ikan di keluarkan di pusat penetasan daerah Chahar Mohal Bakhtiary dalam tahun 2002, hampir 21 juta anak ikan (91.3%) dalam pelbagai peringkat pertumbuhan mati sebelum dapat diedarkan kepada penternak. Juga hampir 23 juta anak ikan yang dihasilkan di daerah Mazandaran, 12 juta iaitu 52.12% dalam jumlah keseluruhan anak ikan mati secara misteri.

Kajian ini dijalankan dengan objektif untuk mengesan dan mengesahkan agen utama penyebab kejadian sindrom kematian anak ikan di Iran. Dalam masa 32 bulan iaitu dari Oktober 2001 hingga Mei 2004, sejumlah 52 pusat penetasan dan ladang peliharaan ikan trout pelangi *(Oncorhynchus mykiss)* terletak di daerah Tehran,

Mazandaran, Guilan, Fras, Markazi, Kerman dan Kohkiloyeh Boyerahmad dilawati dan pelbagai sampel dikumpulkan. Sampel yang diambil termasuk cecair obari, cecair sperma, telur, telur bermata, larvae, ikan fri bersaiz < 1 gm dan bersaiz 1-3 gm dan juga organ dalaman dari ikan dewasa. Sebanyak 2,107 sampel telah dikumpul dari ladang di enam daerah dan disiasat menggunakan lima tatacara iaitu virologi (410 sampel), bakteriologi (899 sampel), serologi (terdiri dari IFAT: 392 sampel dan ELISA: 44 sampel), histopatologi (160 sampel) dan hematologi (202 sampel). Sesetengah dari prosedur seperti kultur sel ikan, ELISA dan IFAT teknik adalah pertama dibangunkan dan optimakan di Iran.

Tanda klinikal ikan yang dijangkiti adalah kehitaman, eksoptalmia, asites, berenang tidak normal dan berputar. Daripada 410 sampel yang diinokulasikan dalam kultur sel, dua sampel menunjukkan kesan sitopatik dalam sel turutan EPC dan BF-2 yang mana diinokulasi dengan cairan ovum dari induk berasal pusat penetasan di daerah Mazandaran. Kesan sitopatik itu adalah sama seperti cetusan virus IHN. Kawasan kesan sitopatik menunjukkan sel-sel nazak berkumpul seperti buah anggur (berbentuk belon dan sitolisis). Keputusan TEM menunjukkan partikel berbentuk peluru bersaiz 130-180 nm panjang dan 65-70 nm diameter. Dari morfologi partikel virion yang dilihat ianya adalah serupa seperti Rhabdovirus. Pemeriksaan IFAT menunjukkan kesemua sampel yang diuji dengan MAbs dan PAbs terhadap IPNV dan VHSV adalah negatif. Walaubagaimana pun dua sampel adalah positif apabila diuji dengan MAbs dan PAbs terhadap IHNV. Smer ini adalah berasal dari sampel yang menunjukkan kesan sitopatik dalam sel turutan EPC dan BF-2 dan partikel berbentuk peluru dalam TEM. Keputusan ELISA (titik penggalan, ketumpatan optik dan peratus aras pengesanan) menunjukkan bahawa IHNV mempunyai peratus

pengesanan setinggi 23.25% berbanding dengan penyakit virus relevan yang lain seperti IPNV dengan 7.31% dan VHSV dengan 14.29%. Keputusan kajian hematologi pada anak ikan menunjukkan bahawa tisu tumpuan dalam ginjal, hepar, limfa, hepatopankreas, usus dan insang memperlihatkan pelbagai tahap perubahan bermula dengan degenerasi sel hingga nekrosis penuh. Terdapat kongesi saluran darah renal, perubahan degeneratif nyata di ginjal posterior dengan nekrosis tubular dan degenerasi tisu perantaraan hematopoeitik. Tambahan pula , degenersasi tisu perantaraan dan edema dalam ginjal anterior, nekrosis fokus dalam kawasan tubul dan beberapa peringkat nekrosis sel dalam tisu hematopoeitik adalah perubahan histopatologi yang penting dalam tisu ginjal yang diperiksa. Tisu hepatopankreas mempamerkan juga perubahan nyata seperti kongesi, atrofi dan nekrosis dalam sel asinar pankreas dan Islets of Langerhans. Sampel limfa menunjukkan kongesi, nekrosis teruk, hemosiderosis dan penambahan kehadiran pusat melanomakrofaj (MMC). Tisu insang dalam anak ikan yang disampel menunjukkan hiperplasia, berbentuk ”club” dan percantuman lamella.

Keputusan hematologikal menunjukkan bahawa jumlah sel darah putih iaitu limfosit dan neutrofil dalam ikan yang disiasat menunjukkan keputusan yang bererti apabila dibandingkan dengan ikan kawalan ($p<0.05$). Ikan-ikan tersebut menunjukkan peningkatan dalam jumlah sel darah putih, limfosit dan neutrofil. Walaubagaimana pun kesemua sampel ikan menunjukkan penurunan nilai sel darah merah, hemoglobin dan hematokrit yang bererti ($p<0.05$). Tambahan lagi, MCHC dan jumlah protein plasma juga menunjukkan kekurangan yang nyata ($p<0.05$). Dalam analisis komponen serum, ia telah menunjukan bahawa LDH dan AST telah menurun dengan bererti ($p<0.05$).

Pada kesimpulannya, dengan tanda klinikal yang nyata, pemerhatian kultur sel, penemuan TEM, keputusan ELISA dan IFAT, serta penemuan histopatologi dan hematologi dalam kajian ini telah mengarah kepada kemungkinan bahawa jangkitan penyakit virus adalah penyebab sindrom kematian anak ikan dalam pusat penetasan dan ladang ternakan di Iran. Dari penemuan kajian sekarang, adalah disimpulkan bahawa virus serupa IHN adalah agen etiologik utama penyebab sindrom kematian anak ikan di Iran.

Perkataan kunci: Sindrom Kematian Anak Ikan, Trout Pelangi, Kultur sel, TEM, ELISA, IFAT, Histopatologi, Hematologi, IHNV, IPNV, VHSV, Iran

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I certify that an Examination Committee has met on 30 October 2008 to conduct the final examination of Seyed Mohammad Ebrahim Jalil Zorriehzahra on his Doctor of Philosophy thesis entitled "Aetiologic Agents of Fry Mortality Syndrome in the Rainbow Trout *(Oncorhynchus mykiss)* in Iran" in accordance with Universities and University Colleges 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 Doctor of Philosophy.

Members of the Examination Committee were as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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Date: 12 February 2009

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

SEYED MOHAMMAD EBRAHIM JALIL ZORRIEHZAHRA

Date :

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