Viruses associated with the epizootic ulcerative syndrome (EUS) of fish in south-east Asia

Gn Frerichs

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GN Frerichs

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK

Summary — A number of birnaviruses, rhabdoviruses and a reovirus have been isolated from occasional fish affected with the epizootic ulcerative syndrome (EUS) in the past decade. The heterogeneous nature of these isolates, together with a low and inconsistent level of recovery from diseased specimens, suggests that these viruses may only represent adventitious infections unrelated to outbreaks of EUS. Furthermore, experimental induction of the condition by direct exposure to cell culture isolated viruses has not been achieved. The significance, if any, of C-type retroviruses identified in cell cultures derived from EUS-susceptible fish species is not known.

birnavirus / reovirus / rhabdovirus

Résumé — Virus associés au syndrome ulcératif épizootique (SUE) des poissons en Asie du Sud-Est. *Plusieurs birnavirus, rhabdovirus et un réovirus ont été occasionnellement isolés à partir de sujets infectés par le syndrome ulcératif épizootique (SUE) depuis une dizaine d'années. Le caractère hétérogène de ces isolats et le taux d'isolement faible et inconstant fait penser que ces virus sont responsables seulement d'infections accidentelles, sans rapport avec le SUE. De plus, l'induction expérimentale de la maladie par exposition directe aux virus isolés en culture cellulaire n'a pas été réussie. L'importance, si tant est qu'il y en ait, des réovirus de «type C» identifiés sur des cultures de cellules obtenues à partir d'espèces de poissons sensibles au SUE reste inconnue.*

birnavirus / réovirus / rhabdovirus / Asie / SUE / poisson

INTRODUCTION

Epizootic ulcerative syndrome (EUS) has been recently and authoritatively defined as a seasonal epizootic condition of freshwater and estuarine warm water fish of complex infectious aetiology characterized by the presence of invasive *Aphanomyces* infection and necrotizing ulcerative lesions typically leading to a granulomatous response (ODA, 1994). The same seminar also concluded that the condition known as red spot disease (RSD) in Australia was indistinguishable from EUS and that the 2 conditions should now be regarded as synonymous. Available evidence also suggests that ulcerative mycosis affecting fishes in estuarine ecosystems of the western Atlantic seaboard of the USA and mycotic granulomatosis of cultured ayu *(Plecoglossus*

altivelis) in Japan are very similar to, if in fact not the same as EUS.

Epizootiological evidence accumulated over many years from numerous outbreaks of EUS covering a wide geographical area has established the infectious nature of the clinical disease. These field observations have been supported by a limited number of experimental disease transmission studies. Pearce (1989) first reported successful transmission in Northern Territory, Australia of EUS from diseased rainbow fish *(Melanotaenia splendida)* to healthy specimens by co-habitation. Balasuriya *et al* (1990) and Subasinghe (1993) have demonstrated transmissibility to snakeheads (*Ophicephalus* spp) in Sri Lanka by co-habitation, contact with water from an affected site and feeding of diseased prey fish. Cruz-Lacierda and Shariff (1993) confirmed these findings by inducing typical EUS lesions in healthy striped snakehead *(O striatus)* by co-habitation with diseased specimens or exposure to EUS-contaminated water from Laguna de Bay in the Philippines.

Although an *Aphanomyces* infection is now accepted as an essential element in the pathogenesis of clinical EUS, it is equally well recognised that there is a distinct early premycotic stage in the development of the disease. A primary viral infection has always been considered a possible underlying causative factor, either directly by initiating the early skin lesion or indirectly by suppressing the ability of the host to resist invasion by the specific *Aphanomyces* fungus (Roberts *et al,* 1994).

VISUALIZED VIRUSES

The earliest virological investigations into EUS comprised a series of electron microscope studies of tissues from striped snakeheads and walking catfish *(Clarias batrachus)* during early epizootics in central Thailand in 1983 and 1984. Virus-like particles were seen in hepatocytes, spleen, kidney and blood cells, and degenerative skeletal muscle myofibrils and capillary endothelium at the site of ulceration in diseased specimens. Similar particles were not identified in tissues from unaffected fish (Wattanavijarn *et al,* 1983a, b; 1984a, b, 1985). The particles were located in the cytoplasm of infected cells and were pleomorphic in form with round, oval, kidney and elongate shapes being seen. On the basis of morphological appearance, the particles were considered to resemble an orthomyxovirus, an arenavirus or a bunyavirus. Pearce (1989) reported the identification of rhabdovirus-like particles in skin lesion material from 2 diseased barramundi *(Lates calcarifer).*

In addition to the direct visualization of viruses in fish tissues, Wattanavijarn *et al* (1986a) also reported the observation of rhabdovirus, IPNV-like and other pleomorphic forms of particle in cell cultures inoculated with tissue extracts from diseased snakeheads. Large rhabdovirus-like particles have been similarly identified in cultures showing cytopathic effects following infection with tissue extract from barramundi and a variety of other diseased species (Humphrey and Langdon, 1986).

ISOLATED VIRUSES

Recorded accounts of viruses isolated in cell culture from fish affected with EUS are summarized in table I.

Birnaviruses

The first virus to be specifically identified in association with EUS was isolated from an ulcerated sand goby *(Oxyeleotris marmoratus)* in Thailand (Hedrick *et al,* 1986). Although typically belonging to the aquatic birnavirus group of viruses, the sand goby

virus (SGV) was culturally, antigenically and biochemically distinct from Sp, Ab and VR-299 reference strains of IPN virus of cold-water fish. Other birnaviruses were recovered at about the same time from diseased snakeheads *(O striatus)* by Saitanu *et al* (1986) and Wattanavijarn *et al* (1988). The former isolate (snakehead fish virus: SHV) was considered to be similar to IPNV on the basis of particle morphology and resistance to chloroform and heat treatment, but was not serologically examined. The latter isolate was specifically identified as the Sp serotype of IPNV. Most recently, the recovery of another birnavirus strain from the spleen of an ulcerated giant snakehead *(O micropeltes)* in Singapore has been reported (Subramaniam *et al,* 1993). Like SGV, this newest isolate showed differences in genomic RNA and structural polypeptide

Table I. Viruses isolated from epizootic ulcerative syndrome (EUS).

Viral agent	*Characterization*	*Reference*
Birnaviruses		
Sand goby virus (SGV) from ulcerated sand goby, Thailand	Related to but antigenically distinct from VR-299, Sp and Ab IPNV	Hedrick *et al* (1986)
Snakehead fish virus (SHV) from diseased fish, Thailand	Limited examination but considered similar to IPNV	Saitanu *et al* (1986)
IPNV from 3 diseased snakeheads and 1 eye-spot barb, Thailand/Burma	Sp serotype	Wattanavijarn *et al* (1988)
IPNV from spleen of ulcerated snakehead, Singapore	Genome segments migration pattern differs from VR-299, Sp and Ab serotypes	Subramaniam *et al* (1993)
Reoviruses		
Reovirus-like particles from diseased snakehead, Thailand	Characterization awaited	Roberts *et al* (1994)
Rhabdoviruses		
Rhabdovirus (UDRV) from 4 diseased snakeheads and 1 freshwater eel, Thailand/Burma	Antigenically distinct from 7 other pathogenic fish rhabdoviruses [a]	Frerichs *et al* (1986, 1989a)
Snakehead rhabdovirus (SHRV), Thailand	Antigenically distinct from 5 other pathogenic fish rhabdoviruses [a]	Wattanavijarn *et al* (1986b); Ahne *et al* (1988)
Rhabdovirus from diseased snakehead, Sri Lanka	Antigenically related to UDRV	Frerichs *et al* (1989b)
Rhabdovirus from diseased snakehead, Thailand	Antigenically and structurally distinct from UDRV	Lilley and Frerichs (1994)

[a] UDRV *(Vesiculovirus)* and SHRV *(Lyssavirus)* are structurally and antigenically different; Kasornchandra *et al* (1992).

migration patterns from the 3 classical IPNV reference serotypes, but no direct comparison of the 2 EUS associated viruses has yet been reported.

Reoviruses

The recovery of a reovirus-like agent from a characteristically diseased striped snakehead fish during the 1992 epizootic in Thailand has been reported, but the relationship of this isolate to the many other aquareoviruses from fish has yet to be determined (Roberts *et al,* 1994).

Rhabdoviruses

Rhabdoviruses have constituted the group of viral agents most frequently isolated from EUS-affected fish. The first isolates were obtained from diseased striped snakeheads and a freshwater eel *(Fluta alba)* in northern Thailand and Burma (Frerichs *et al,* 1986) and from a similarly affected snakehead in central Thailand (Wattanavijarn *et al,* 1986b). The northern Thailand/Burma group of viruses was shown by cross-neutralization to be serologically unrelated to 7 other fish pathogenic rhabdoviruses (Frerichs *et al,* 1989a) and the central Thailand isolate was similarly shown to be antigenically distinct from 5 other serotypes (Ahne *et al,* 1988). A subsequent study by Kasornchandra *et al* (1992) not only confirmed these early isolates to be serologically unrelated to other known fish rhabdoviruses, but also established that the northern Thailand strains (UDRV) and the central Thailand snakehead rhabdovirus (SHRV) represented 2 different serotypes. Furthermore, it was judged on the basis of structural polypeptide mobility patterns that the UDRV isolates exhibited a *Vesiculovirus*-type protein profile whereas the SHRV strain resembled a *Lyssavirus.*

Additional rhabdovirus isolations have been reported from a green snakehead *(O punctatus)* examined during the first disease outbreak in Sri Lanka (Frerichs *et al,* 1989b) and, more recently, from striped snakeheads in central Thailand in 1992 (Lilley and Frerichs, 1994) and 1994 (unpublished observation). Both sets of these newest Thailand isolates are culturally and serologically distinct from the early northern Thailand–Burma strains, and may equate antigenically with the original central region isolate, but direct comparisons with SHRV have yet to be carried out.

Cell culture viruses

The spontaneous production of C-type retrovirus particles has been identified in cell lines developed from healthy striped snakehead, snakeskin gourami *(Trichogaster pectoralis)* and climbing perch *(Anabas testudineus)* tissues (Frerichs *et al,* 1991). These fish species are all recognised as susceptible to EUS, but the significance of the cell culture expressed retroviruses in relation to the pathogenesis of the clinical disease is undetermined.

VIRUS PATHOGENICITY

Although the epizootiology of EUS, and the successful experimental transmission of the disease by exposure of susceptible fish to a naturally affected environment, have established the infectious nature of the condition, the role of any of the laboratory-isolated viruses in the pathogenesis of the disease remains uncertain. In the only experimental infectivity study reported to date, Frerichs *et al* (1993) were unable to induce any lesions indicative of EUS in snakeheads following intraperitoneal inoculation or bath exposure to an EUS rhabdovirus or snakehead cell line retrovirus.

DISCUSSION

EUS is recognised as a disease of complex aetiology, in which more than 1 infectious agent may interact with a number of seasonal environmental factors to give rise to a clinical outbreak. Replication of the appropriate environmental parameters and ecological balances of the natural habitat is difficult to achieve under laboratory conditions. Obtaining fully susceptible fish from the wild state in disease affected areas is also difficult and these 2 particular problems mitigate against experimental pathogenicity studies. It should not be considered surprising, therefore, that little appears to have been achieved in this respect.

The highly heterogeneous nature of the viral agents recovered over the years from outbreaks of EUS does not readily suggest a common causal agent. Isolates have been distributed not only between different families but also between different species and serotypes within these families. The very low rates of recovery of these viruses from outbreaks of disease are also inconsistent with a sudden, explosive, rapidly spreading condition such as EUS. Even the rhabdoviruses, which have been most frequently recovered, have not been isolated from more than 5% of sampled fish in any one outbreak. Indeed, in some major disease incidents, such as the first Bangladesh epizootic in 1988, no viral agent was recovered from any of a large number of fish examined. One possible explanation for these findings is that the isolated viruses were merely adventitious agents which would as likely have been isolated from the occasional healthy fish as the occasional diseased specimen.

Despite the investigations of the past 10 years, it is still not known what role any of the viruses so far isolated play in the pathogenesis of EUS, and a primary causal infectious agent has yet to be identified.

REFERENCES

Ahne W, Jorgensen PEV, Olesen NJ, Wattanavijarn W (1988) Serological examination of a rhabdovirus isolated from snakehead *(Ophicephalus striatus)* in Thailand with ulcerative syndrome. *J Appl Ichthyol* 4, 194-196

Balasuriya KSW, Kulathilake M, Subasinghe RP (1990) Preliminary investigation of ulcerative diseases syndrome in fish in Sri Lanka. *In: The Second Asian Fisheries Forum* (Hirano R, Hanyu I, eds) Asian Fisheries Society, Manila, Philippines, 659-662

Cruz-Lacierda ER, Shariff M (1993) Experimental transmission of epizootic ulcerative syndrome (EUS) in snakehead *(Ophicephalus striatus). In: Second Symposium on Diseases in Asian Aquaculture.* Asian Fisheries Society, Manila, Philippines [Abstract], p 42

Frerichs GN, Millar SD, Roberts RJ (1986) Ulcerative rhabdovirus in fish in South-East Asia. *Nature (Lond)* 322, 216

Frerichs GN, Hill BJ, Way K (1989a) Ulcerative disease rhabdovirus: cell-line susceptibility and serological comparison with other fish rhabdoviruses. *J Fish Dis* 12, 51-56

Frerichs GN, Millar SD, Alexander M (1989b) Rhabdovirus infection of ulcerated fish in South-East Asia. *In: Viruses of Lower Vertebrates* (W Ahne, E Kurstak, eds), Springer-Verlag, Heidelberg, Germany, 394-410

Frerichs GN, Morgan D, Hart D, Skerrow C, Roberts RJ, Onions DE (1991) Spontaneously productive C-type retrovirus infection of fish cell lines. *J Gen Virol* 72, 2537-2539

Frerichs GN, Millar SD, Chinabut S (1993) Clinical response of snakeheads *(Ophicephalus striatus)* to experimental infection with snakehead fish rhabdovirus and snakehead cell line retrovirus. *Aquaculture* 116, 297-301

Hedrick RP, Eaton WD, Fryer JL, Groberg WG, Boonyaratapalin S (1986) Characteristics of a birnavirus isolated from cultured sand goby *(Oxyeleotris marmoratus). Dis Aquat Org* 1, 219-225

Humphrey JD, Langdon JS (1986) Ulcerative disease in Northern Territory fish. *Internal Report, Australian Fish Health Reference Laboratory,* Benalla, Victoria, Australia, 13 p

Kasornchandra J, Engelking HM, Lannan CJ, Rohovec JS, Fryer JL (1992) Characteristics of three rhabdoviruses from snakehead fish *(Ophicephalus striatus). Dis Aquat Org* 13, 89-94

Lilley JH, Frerichs GN (1994) Comparison of rhabdoviruses associated with epizootic ulcerative syndrome (EUS) with respect to their structural proteins, cytopathology and serology. *J Fish Dis* 17, 513-522

ODA (1994) *ODA Regional Seminar on Epizootic Ulcerative Syndrome* (RJ Roberts, B Campbell, IH

MacRae, eds). Aquatic Animal Health Research Institute, Bangkok, Thailand, 282 p

Pearce M (1989) *Epizootic Ulcerative Syndrome Technical Report, Dec 1987–Sep 1989.* Fishery Report No 22, Northern Territory Dept of Primary Industry and Fisheries, Australia, 82 p

Roberts RJ, Frerichs GN, Tonguthai K, Chinabut S (1994) Epizootic ulcerative syndrome of farmed and wild fishes. *In: Recent Advances in Aquaculture V* (JF Muir, RJ Roberts, eds) Blackwell Science Ltd, Oxford, UK, 207-239

Saitanu K, Wongsawang S, Sunyasootcharee B, Sahaphong S (1986) Snakehead fish virus isolation and pathogenicity studies. *In: The First Asian Fisheries Forum* (LB Dizon, LV Hosillos, eds), Asian Fisheries Society, Manila, Philippines, 327-330

Subasinghe RP (1993) Effects of controlled infections of *Trichodina* sp on transmission of epizootic ulcerative syndrome (EUS) to naive snakehead, *Ophicephalus striatus* Bloch. *J Fish Dis* 16, 161-164

Subramaniam S, Chew-Lim M, Chong SY, Howe J, Ngoh GH, Chan YC (1993) Molecular and electron microscopic studies of infectious pancreatic necrosis virus from snakehead. IXth International Congress of Virology, Glasgow, UK (Abstract) p 368

Wattanavijarn W, Tesprateep T, Wattanodorn S, Sukolapong V, Vetchagarun S, Eongpakornkeaw A (1983a) Virus-like particle in the liver of snakehead fish. *Thai J Vet Med* 13, 51-8 (in Thai)

Wattanavijarn W, Rattanaphani R, Tesprateep T *et al* (1983b) Virus-like particles in the skeletal muscle, capillaries and spleen of sick snakehead fish *(Ophicephalus striatus)* during a disease epidemic. *Thai J Vet Med* 13, 122-30

Wattanavijarn W, Rattanaphani R, Tesprateep T *et al* (1984a) Examination of sick snakehead fish during a disease epidemic by scanning and transmission electron microscopy. *Thai J Vet Med* 14, 13-22 (in Thai)

Wattanavijarn W, Tangtronpiros J, Rattanaphani R, Sukolapong V, Eongpakornkeaw A, Vetchagarun S (1984b) Examination of sick catfishes by scanning and transmission electron microscopy. *Thai J Vet Med* 14, 31-9 (in Thai)

Wattanavijarn W, Rattanaphani R, Tesprateep T, Tangtrongpiros J, Thirapatsakun T (1985) Detection of virus in snakehead fish during a disease epizootic by light and electron microscopy. *Nat His Bull Siam Soc* 33, 47-52

Wattanavijarn W, Wattanodorn S, Hunnak P, Tangtrongpiros J, Rattanaphani R (1986a) Viruses of ulcerative diseased fish. *Electron Microsc Soc Thailand Newsl* 3, 20-3

Wattanavijarn W, Tangtrongpiros J, Wattanadorn K (1986b) Viruses of ulcerative diseased fish in Thailand, Burma, and Laos. *In: First International Conference on the Impact of Viral Diseases on the Development of Asian Countries*, WHO, Geneva (Abstract), p 121

Wattanavijarn W, Torchy C, Tangtrongpiros J, de Kinkelin P (1988) Isolation of a birnavirus belonging to Sp serotype, from south east Asia fishes. *Bull Eur Ass Fish Pathol* 8, 106-107