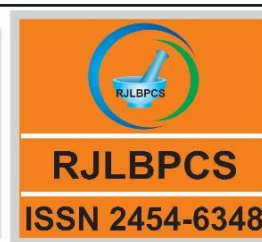




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Original Research Article

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ROLE OF VIRAL AGENTS IN THE INDUCTION OF EPIZOOTIC ULCERATIVE SYNDROME (EUS)

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ABSTRACT: Fisheries and aquaculture play an important role in economies around the world, the sector is threatened by external factors such as pollution, runoff and temperature fluctuations, could increase physiological stress on cultured stock affecting not only productivity but also increase vulnerability to diseases and, in turn, reduce returns to farmers. From an aquaculture point of view, the greatest concern is the rapid multiplication of the pathogen within the host and the danger of transfer to other individuals of the host population, which may result in an uncontrollable epizootic forms. Electron Microscopic study was carried out in naturally EUS affected fishes, showing heavy Parvovirus (DNA virus) infection at an area of red spot in *Channa punctatus* and Paramyxovirus (RNA virus) in *Clarias batrachus*. Bacteria, fungi, viruses are the pathogen responsible for ulcers in fishes leading to mortality, but penetration into the host is the first step for the microbial agent to multiply and invade the vital organs of its host. When a viral pathogen is able to penetrate the external barriers of an organism Thus follows the bacterial and fungal infection. EM section of ulcerated skin part from a red spot had showed a presence of virus indicates virus as an initial pathogen which is responsible for EUS outbreak.

KEYWORDS: EUS, EM, *Parvovirus*, *Paramyxovirus*, *Channa punctatus*, *Clarias batrachus*.

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1. INTRODUCTION

EUS is one of the listed diseases of fishes as per OIE [1], The disease has been found to infect more than 100 species of fishes and cause huge economic losses. EUS has hit India for last ten to fifteen years. It has become a matter of tremendous concern. From an aquaculture point of view, the greatest concern is the rapid multiplication of the pathogen within the host and the danger of transfer to other individuals of the host population, which may result in an uncontrollable epizootic form. Recent studies have shown that the same pathogenic *Aphanomyces* fungus is involved in each case. Prior to recent mycological findings, viruses were considered to be the most likely necessary infectious cause of EUS. Since 1983, several types of viruses have been observed and isolated from the fishes infected with EUS [2] and varying interpretations have been suggested that at least one of these species may be involved in some EUS outbreaks, particularly in Thailand, by predisposing fish to infection by *A. invadans*. It appears that in EUS, virus infection must be primary triggering the bacteria and fungi to secrete cytotoxins. Yadav et al., [3] also suggested that the progression of pathogenicity of EUS may be linked with the presence of cytolytic strains of *A. hydrophila* in the EUS lesions. Again, it was suggested that an initial virus infection may be necessary to make a fish host susceptible to fungal entry [4]. Pathogenicity trials with most EUS-associated viruses have usually demonstrated little more than scale damage or occasional development of minor skin lesions. Frerichs et al., [5] were unable to show any consistent lesion in snakeheads immersed or injected i.p. (Intra peritoneally) with an isolate of UDRV. Of the birnaviruses, only SHV has been tested in challenge studies. Saitanu et al., [6] reported that i.p. injections of SHV resulted in scale damage in 80% of small snakeheads, but not at all in larger fishes. John [7] demonstrated that the reovirus was not pathogenic to juvenile snakeheads in injection challenges. More work by Kanchanakhan [4] showed that rhabdovirus strain (T9412) can result in substantial lesions in striped snakeheads, particularly using challenges by i.m. (intra muscular) injection. Penetration into the host is the first step for microbial agent to multiply and invade the vital organs of its host. This normally happens through ingestion, rupture of the skin and transgression of gill lamellae or penetration of the egg membrane. The specific point of entry may have a decisive role on the virulence of the microbe.

2. MATERIALS AND METHODS

The present study was carried out by collecting the suspected diseased fishes from nearby EUS prone water bodies (Wadali and Malkhed lakes) with the help of fishermen and were brought to the laboratory and maintained in glass aquaria for a week to study the type of infection.

Electron Microscopical studies for detection of viral infection:

Specimen Preparation for TEM:

The tissues liver, kidney, skin and gills were dissected out aseptically in the laboratory, from the EUS affected fishes, trimmed to size 1.0-1.5mm thick and fixed into a fixative containing 2.5% Glutaraldehyde and 2% paraformaldehyde, made in 0.1 M sodium phosphate buffer (pH 7.4) for 6-12 hrs at 4 0C for 24 hrs. After fixation, the specimens were sent for detection of Viral infections, at AIIMS, New Delhi, where the ultra-thin sections were visualized under transmission electron microscope at different magnifications and photographed.

3. RESULTS AND DISCUSSION

Electron Microscopic alterations in EUS affected fishes:

Electron Microscopic study was carried out in naturally EUS affected fishes, showing heavy Parvovirus (DNA virus) infection at an area of red spot in *Channa punctatus* (Fig:1) and Paramyxovirus (RNA virus) in *Clarias batrachus* (Fig : 2).

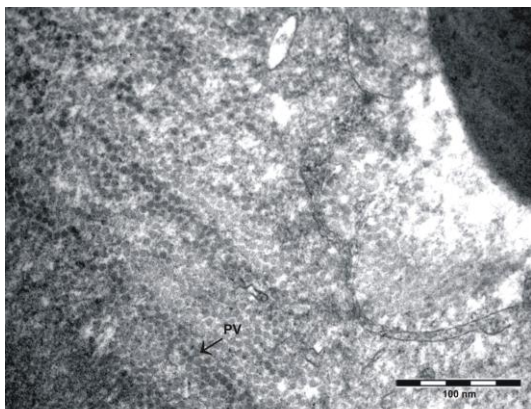


Fig: 1. EM showing heavy viral infection in the skin of *Channa punctatus* at an area near red spot showing *Parvovirus* (DNA virus). PV-Parvovirus

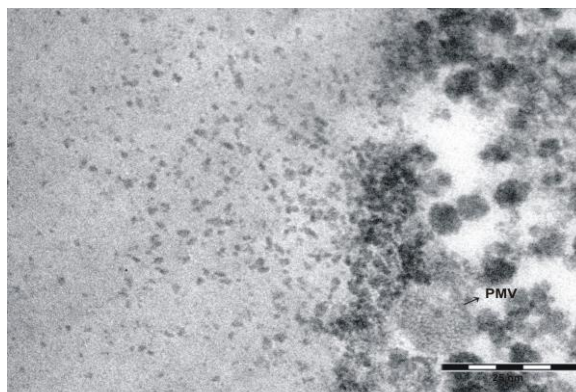


Fig: 2. *Paramyxovirus* (RNA virus) seen in the red spot region of naturally EUS affected *Clarias batrachus*. PMV-Paramyxovirus.

The EM structure of hepatic cell of naturally affected *Channa Punctatus* showed damage and breakdown of mitochondria and distortion of endoplasmic reticulum (ER), (Fig: 3). The breakdown of mitochondrial cristae and ER was also seen in hepatic cell of naturally EUS affected *Clarias batrachus*, Heavy infection of *Corn Snake Parvovirus* is evident in cytoplasm (Fig: 4).

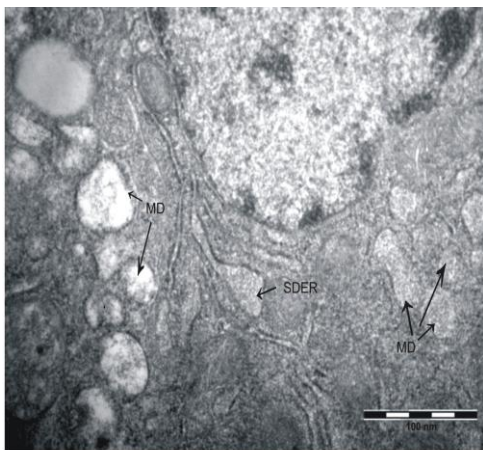


Fig: 3, Electron Microscopic image of a hepatic cell of EUS affected *Channa punctatus* showing mitochondrial damage and swelling and distortion of endoplasmic reticulum. SDR-Swelled and distorted endoplasmic reticulum, MD - Mitochondrial damage

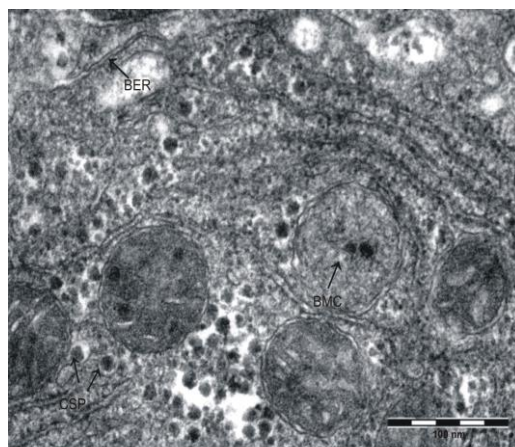


Fig: 4, Corn snake parvovirus seen in the cytoplasm of hepatic cell of naturally EUS affected *Clarias batrachus*. Breakdown of Mitochondrial cristae and ER is seen. CSP-Corn snake parvovirus, BMC-Breakdown of mitochondrial cristae, BER-Breakdown of endoplasmic reticulum.

Chloride cell of gill of EUS affected *Channa punctatus* and epithelial cells of gill lamellae of *Clarias batrachus* showed infection of Parvoviruses (DNA virus) distorted nucleus and disintegration of mitochondria (Figs.:5 and 6).

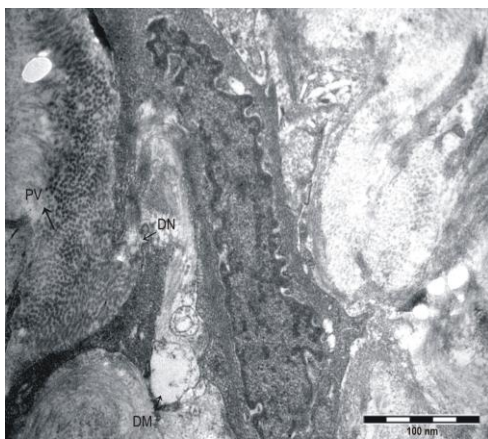


Fig: 5, EM showing heavy viral infection in the chloride cell of gill of EUS affected *Channa punctatus* showing Parvoviruses (DNA virus), distorted nucleus, and disintegration of mitochondria. DN-Distorted nucleus, DM-Disintegration of mitochondria, PV-Parvoviruses.

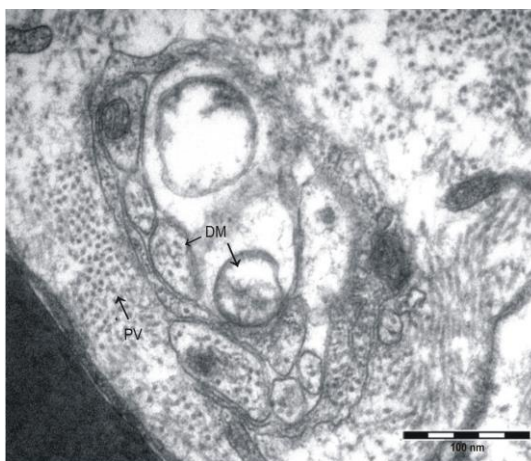


Fig:6, EM of Epithelial cell of gill lamella of EUS affected *Clarias batrachus* showing parvoviruses (DNA virus). Disintegrating mitochondria are also seen. DM-Disintegrating mitochondria

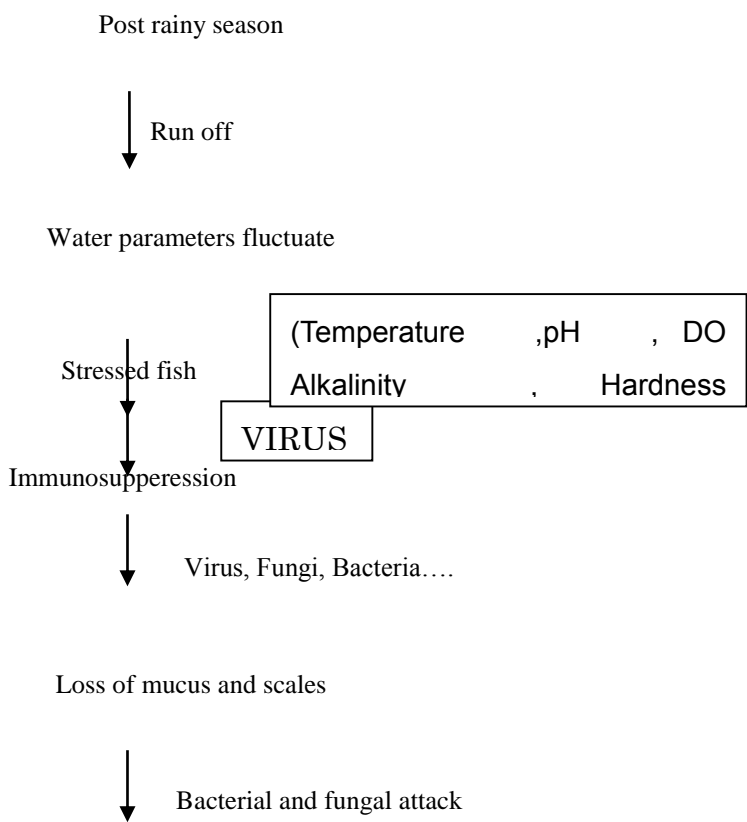
It is observed that bacteria, fungi, viruses are the pathogens responsible for ulcer in fishes leading to mortality. For confirmation of the presence of viruses in the EUS affected tissues; electron microscopic sections of skin, liver, kidney and gill were taken which are shown as (Figs: 1 to 6). Detailed electron microscopic studies done with ultrathin sections of EUS affected fish tissues revealed virus like inclusion bodies in the cytoplasm of hepatic cells and cells of gill lamellae. Large number of scientists is working on different aspects of EUS and its causative agents, but today also there is no concrete answer to it. Efforts are made to know about EUS through experimental infection trials since 1988 by various workers. Miller [8] ; Kanchanakhan [4 and 9]; Lio-Po et al., [10] suggested viral origin. Pradhan and Pal [11] and Shariff and Subhasinghe [12] opined bacterial origin of EUS and Roberts et al., [13] suggested fungal origin of EUS. When a

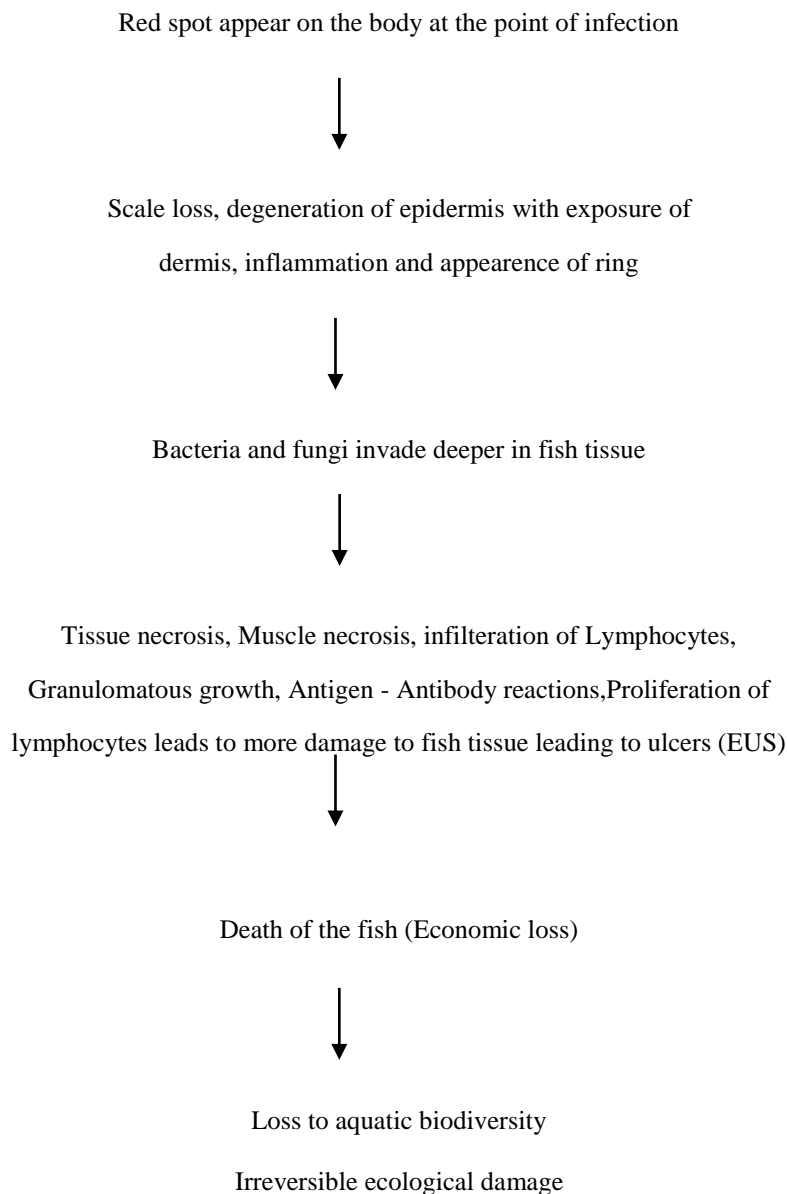
portion of skin tissue with red spot was removed for the electron microscopic studies, the EM showed presence of Paramyxovirus (Fig : 4.65). The present results indicate the appearance of red spot at the onset of EUS with viral infection. The present result is well in agreement with that of Kanchanakhan (5).

4. CONCLUSION

A general survey of fish market at Amravati was carried out to know the intensity of EUS affected fishes coming in the market. It was observed that the fisherman brought the EUS affected fishes from nearby lakes Wadali and Malkhed. EM section of ulcerated skin part from a red spot had showed a presence of virus indicates virus as an initial pathogen which is responsible for EUS outbreak. The results indicate that interaction between rainfall, deteriorating water quality and presence of pathogens could provide stressful conditions for fish, thereby inducing EUS lesions in susceptible fish populations. Thus it can be said that EUS virus Bacterial cell Fungus. It is also suggested that Viruses being crystalline in nature have cellular penetration power. This natural tendency of virus itself indicated that viruses are the only agents which can act as the initial causative agents for EUS.

The probable path of EUS





CONFLICT OF INTERESE

There is no conflict of interest

ACKNOWLEDGEMENTS

This Paper is dedicated to my guide Late Dr. G.N.Vankhede and I would also like to acknowledge department of TEM for detection and photography of viral infections, at AIIMS, New Delhi.

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