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Preliminary Epidemiological Studies on Epizootic Ulcerative Syndrome (EUS) in Freshwater Fishes of Assam

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ABSTRACT

Preliminary epidemiological investigation was carried out to examine the status of Epizootic Ulcerative Syndrome among the freshwater (FW) fishes of Cachar district in Assam during the period from January 2013 to May 2015. Our study revealed fluctuation in the intensity of the disease in relation to species affected. A total of *c* 581 EUS-affected fishes were recorded during the present study period. The temperature of air and water of the wetlands were recorded as 17^o C to 35^o C and 19^o C to 28^o C respectively. pH was recorded as 5.5 to 7.0. The most prevalent symptoms of EUS disease, as reported by the farmers, were ulceration on the skin followed by tail and fin rot. The Case Fatality Ratio (CFR) has been calculated species wise and has been found to be high among *Cirrhinus mrigala* (83.72 %), followed by *Puntius ticto* (75 %), *Channa marulius* (70 %), *Mastacembelus armatus* 69.23 %), *Anabas testudineus* (53.85 %), and, so on. Further, the Specific Death Rate (SDR) due to EUS was found to be 57.89 %. Communicative nature of EUS revealed variation in time gap between fish and infection in different species. Financial loss to farmers due to fish disease was colossal, as was revealed through interviewing the Fish farmers.
Keywords: Fish, diseases, EUS, Epidemiology, Cachar, Assam, India.

I. INTRODUCTION

Disease, *per se,* is not an entity of an end in itself. Disease is the end result of an interaction between a noxious stimulus and biological system. In fact, it is necessary to understand all aspects of the biology of the species in order to understand a disease.

Severe outbreaks of disease may result from the introduction of parasites, pathogens, from malnutrition, from chemical and physical alterations of the water, from the genetic make-up of the fish; and, from the interrelationship of any or all these factors. This relationship could be expressed in the form of an equation as follows:

$$H + P + S^2 = D$$

Where 'H' represents the 'Host'; 'P', the 'Pathogen'; 'S', the 'Stress' caused by the environment; and, 'D', the resulting 'Disease'.

It may be noted here that, the stress caused by the changes in the environment may increase very rapidly after the acceptable range for a particular species of fish exceeds. As such, the effect of such a stress could be expressed as a quantity which increases exponentially; while, the concomitant changes in the environment could be represented arithmetically, e.g., temperature by degrees; Dissolved Oxygen (DO) by mg/lit; and, so on. The stress created by such changes and the resulting mortalities are likely to increase geometrically or logarithmically.

Fish forms a dependable source of animal protein for people of developing countries, like India, Bangladesh, Sri Lanka, and other countries of South and South-East Asia. Large-scale fish mortality often occurs due to environmental stress followed by parasitic afflictions. Fungi, bacteria and certain viruses are often found associated with such diseases which often become epizootic and epidemic.

Cutaneous ulcerative diseases, often involving a number of pathogens, are a common problem in wild and cultured fish. For the last two decades, a group of epizootic syndromes, all involving a severe ulcerative

mycosis, have been reported from Australia (Rodgers and Burke, 1981); South-East United States (Noga and Dykstra, 1986); and, Asia, stretching from the Philippines (Reantaso, 1990) in the East to India (Kar and Dey, 1988; Das *et al*., 1990) in the West.

Outbreaks of ulcerative disease have been affecting the freshwater and estuarine fishes over much of Asia and Australia since last > 25 years. The disease had been variously named as 'Mycotic Granulomatosis (MG) in Japan, Red Spot Disease (RSD) in Australia and Epizootic Ulcerative Syndrome (EUS) in South-East and South Asia. Some of the studies had shown that, the same pathogenic *Aphanomyces* fungus had been involved in each case; but, there are other workers who subscribe to its primary viral aetiology. An account of each of the three epidemic conditions is briefly given below.

II. METHODS AND MATERIAL

Mycotic Granulomatosis (MG)

The first report of an EUS-like condition came in Summer of 1971, in farmed ayu (*Plecoglossus altivelis)* in Oita Prefecture, Japan (Egusa and Masuda, 1971). The characteristic lesion, a granulomatous response to invasive hyphae, was described and the disease was named Mycotic Granulomatosis (Miyazaki and Egusa, 1972). It had rapidly spread to several other Prefectures and had affected various fish species, predominantly the cultured ayu and the goldfish (*Carassius carassius auratus)* , the Formosan snakehead (*Channa maculate)* and the grey mullet (*Mugil cephalus)* (Miyazaki and Egusa, 1972). However, the common carp (*Cyprinus carpio)* was not affected.

Red Spot Disease (RSD)

In 1972, outbreaks of a cutaneous ulcerative condition, called Red Spot Disease (RSD) affected the estuarine fishes, particularly, the grey mullet (*Mugil cephalus)*, in Queensland, Australia (McKenzie and Hall, 1976). The disease later progressed to affect the freshwater (FW) and estuarine fishes in coastal rivers in New South Wales (NSW) (Callinan, *et al*., 1989), Northern Terrritory (Pearce, 1990) and Western Australia (Callinan, 1994).

Epizootic Ulcerative Syndrome (EUS) Origin of the problem

The origin of EUS could be said to be a matter of controversy and speculation. Following the report of outbreak of MG in Japan during the 1970s, EUS was reported in Australia and in South-East and South Asia during the subsequent decades.

Chronology, Status and Major outbreaks in the World

Following the report of MG in Japan, sometime around 1971, the earliest report of EUS outbreak goes back to Australia during 1972 and Papua New Guinea during 1974; from where, EUS has been sweeping almost in a chronological manner through most of the South-East and South Asian countries, like Indonesia (1980), Malayasia (1979-83), Thailand (1985), Kampuchea and Lao PDR (1984); Myanmar (1984-85), Sri Lanka (1987); Bangladesh (March, 1988); until, EUS had reached India through the Barak valley region of Assam during July, 1988; and, has been sweeping the region, even today, causing large-scale mortality among the freshwater fishes.

In India, widespread initiation of outbreak of EUS started from Barak valley region of Assam since July, 1988 (Kar, 1999, 2005 d, 2007, 2010, 2013, 2014; Kar and Barbhuiya, 2013, Kar and Dey, S, 1988; Kar and Dey, SC, 1988 a, b; 1990 a, b, c; Kar et al., 1990 a, b; 1993, 1994, 1995 a, b, c; 1996 a, b, c; 1997; 1998 a, b, c, d; 1999 a, b; 2000 a, b, c, d; 2001 a, b; 2002 a, b, c, d; 2003 a, b, c, d, e, f, g ; 2004 a, b, c; Patil *et.al*., 2003). Outbreaks of EUS in India have been comprehensively reviewed at various Fora and workers (The Zoological Society of Assam, 1988; ICSF, 1992; Das and Das, 1993; Mohan and Shankar, 1994, *etc*.).

From Barak valley region of Assam (July, 1988), EUS has been sweeping, almost unabated and chronologically, through West Bengal (1989), Bihar (1989), Orissa (1989), UP (1990), Madhya Pradesh (1990), Maharashtra (1991-92), Karnataka (1993), Goa (1993), Tamil Nadu (1993), Andhra Pradesh (1992-93), Kerala (1994-95), till EUS had crossed India to other parts of the world.

Among some of the other Indian workers who had worked on EUS problems, are Bhaumik *et al.* (1990)

who had worked on the impact of Epizootic Ulcerative Syndrome on the Society. Chakraborty and Dastidar (1991) had repeatedly isolated chemoautotrophic nocardio form bacteria from fish affected by EUS. Chattopadhyay *et al.* (1990) had done microbiological investigations into EUS in fishes. Das *et al.,* (1990) had prepared a comprehensive account of EUS. Goswami *et al.,* (1988) had done studies on certain aspects of prevention and treatment of fish suffering from EUS. Jhingran (1991) had worked on strategy for containing EUS. Prasad and Sinha (1990) had prepared a status paper on the occurrence of EUS in fishes of Bihar. Purkait (1990) had done some case studies on the EUS of fishes in Chanditala of Hooghly district in West Bengal. Rahman *et al.,* (1988) had studied the role of EUS-affected fish on the health of ducks in Assam. CIFA (Central institute of Freshwater Aquaculture) (ICAR) developed the CIFAX which has been claimed to control EUS. Mohan and Shankar (1994), Vishwanath *et al.* (1997, a, b; 1998; Mohan *et al.*, 1999) did works revealing different aspects of EUS.

III. RESULTS AND DISCUSSION

Major species affected and Pattern of spread of EUS

In India, systematic study, conducted by us, since 1988, revealed wide scale attack among four species of fishes. These are: *Channa punctata, Macrognathus aral, Mystus vittatus, Puntius conchonius*

Other species affected by EUS during the same period, but not very widely, included *Aorichthys aor, Amblypharyngodon mola, Catla catla, Cirrhinus mrigala, Heteropneustes fossilis, Labeo rohita, Lepidocephalichthys guntea, Notopterus notopterus* and *Salmophasia bacaila.*

Our continued study revealed that, during the period 1992-94, the following species had been found to be very severely affected by EUS: *Parambassis ranga, Chanda nama, Nandus nandus, Glossogobius giuris* (Kar *et al*., 1994). Some of the other species affected by EUS to a lesser extent during this period, included *Mastacembelus armatus, Macrognathus pancalus, Xenentodon cancila, Colisa fasciatus,* etc.

Our recent studies (Kar et al., 1995, a,b,c; 1997; 1998 a,b,c; 1999 a; 2000 a; 2001 a; 2002 a; 2003 a; 2004 a) indicated that the following species of fishes have been severely-affected by EUS, since 1995, particularly, during the period Nov-Feb, causing large-scale mortality among them: *Channa striata, C. punctata, Anabas testudineus, Clarias batrachus.*

Thus, there has been a differential pattern of spread of EUS among different fish species during different seasons.

With regard to the Global scenario, more than 100 fish species have been reported to be affected by EUS (Lilley et al., 1992); but, only relatively few reports have been confirmed by demonstrating the presence of MG in histological section or by isolation of the pathogenic fungus, *Aphanomyces* sp., from tissues underlying ulcers.

Similarly, some commercially important species are considered to be particularly resistant to EUS. But, not much study has been done to confirm these observations and investigate the mechanism of resistance. Species reported to be unaffected by EUS outbreaks include the Chinese carps, tilapias and milkfish (*Chanos chanos)*. Hatai (1994) experimentally injected catfish (*Parasilurus asotus)*, loach (*Misgurnus anguillicausatus)* and eel (*Anguilla japonica)* with hyphae of *A. invadans* and found them to be refractory to infection. Wada et al (1994) and Shariffpour (1997) experimentally injected common carp (*Cyprinus carpio)* with zoospores of *Aphanomyces* from MG and EUS outbreaks respectively; and, demonstrated that fungal growth was suppressed by an intense inflammatory response.

Some authors have commented that the most severely affected species in natural outbreaks are generally bottom dwellers (Llobrera and Gacutan, 1987; Chondar and Rao, 1996) or the fishes which possess air-breathing organs (Roberts et al., 1994).

In the case of snakeheads, no particular size group appears to be more susceptible, with affected fish ranging from 40 g to 900 g (Cruz-Lacierda and Shariff, 1995). However, there is a possibility that, size or age may be significant in other species. For example, the IMCs suffer high mortalities as fingerlings (Roberts et al., 1989); but, larger fish, although appear ulcerated, are not reported as dying in large numbers (NACA, 1997).

Epidemiological Study

Epidemiology is the study of the distribution and determinants (*i.e*., causes) of disease in populations. Epidemiologists typically take a wide view of causal factors, defining them as any event, condition or characteristic which play an essential role in producing an occurrence of the disease'. By contrast, many pathologists and microbiologists may consider, for example, a particular infectious agent to be the cause of a disease and may relegate all other contributions to 'contributing' or 'predisposing' factors.

For most diseases, including EUS, there is a strong evidence that, outbreaks occur only when a number of causal factors combine together. Many of the causal factors which have been identified or suggested, on the basis of reasonable evidence for EUS, may be represented in a causal web. It may be noted here that, there are several levels within the web; and, that, a number of factors may act at the same level, but not necessarily at the same time or intensity. It may, further, be noted here that, for EUS to occur, combinations of causal factors must ultimately lead to exposure of dermis, attachment to it by *Aphanomyces invadans;* and, subsequent invasion of the dermis and muscle by the fungus. The resulting mycotic granulomatous dermatitis and myositis may be considered as expression due to EUS (Lilley et al., 1998).

The multifactorial nature of the cause(s) of EUS could also be represented by using the concepts of 'necessary cause, component cause and sufficient cause'. Each combination of various causal factors ('component causes') which together cause a disease, could collectively be regarded as a 'sufficient cause' for that disease to be initiated. It may be important to note here that, different combinations of 'component causes' may result in sufficient amount of causes for the initiation of a disease; which could, however, be different under different circumstances. Moreover, all sufficient causes for a particular disease have, in common, at least one component cause, known as a 'necessary cause', which must always be present, as expected, for that disease to prevail.

For EUS, studies have suggested that, there are a number of sufficient causes; each could make-up its component causes. It may be noted here that, each of these sufficient causes includes, amongst its component causes, one of the recognized necessary causes, viz., propagules of *A. invadans* (Lilley et al., 1998).
Notwithstanding the above, a unique feature of an epidemiologist is to test the aetiological hypothesis and identify the underlying causes (or risk factors) for the diseases. A number of factors have been hypothesized as either factors or determinants of EUS outbreak in India. So, in order to find out the exact causative organism, or the infectious aetiology, it is the need of the hour to study the epidemiological aspects of the disease.

A standard format had been designed by following Park (1997). This format had been used to collect different information related to epidemiology of EUS in the Barak valley region of Assam, where EUS had made its initiation in India. It has been covered from the angles of socio-economic impact of it, epidemiology of the disease and its impact on aquaculture farms.

Since 1988, EUS has been considered to be one of the most serious diseases affecting fresh water fishes in India. According to the present study, the high mortality rate had been found among *Cirrhinus mrigala, Channa stirata, Puntius ticto, Labeo gonius, Lepidocephalus guntea,* etc. When analysis is planned to throw light on the aetiology, it is essential to use Specific Death Rates (SDR) (Park, 1997). Therefore, attempts had been made to determine the SDR due to EUS.

$$\textbf{SDR due to EUS} = \frac{\textbf{Number of deaths during a year}}{\textbf{Mid year population (Total number of speices)}} \times \mathbf{100}$$

Attempts had further been made to determine the Case Fatality Ratio (CFR) in order to represent the killing power of the disease which is as follows:

$$\frac{\textbf{Total number of deaths due to EUS}}{\textbf{Total number of cases due to EUS}} \times \mathbf{100}$$

Further, a model related to the study of factors affecting the outbreak of EUS in farmed fish in Bangladesh, carried out by Ahmed and Rab (1995), in collaboration with ICLARM and the Department of Economics, Islamic University of Bangladesh at Kusthia, was replicated by us in the field in Assam University, Silchar, in which, three experimental ponds (*viz*., ponds A, B, C) were prepared of sizes 15 ft × 15 ft × 3 ft each. Lime

was applied during the post-stocking, generally, in the proportion of 0.027 kg, 0.032 kg and 0.043 kg respectively. Pond 'A 'had been seeded with five speices of fishes, viz., *Cirrhinus mrigala, Labeo rohita, Ctenopharyngodon idella, Oreochromis mossambicus* and *Channa punctatus.* Pond 'B' was seeded with only two species of fishes, *viz*., *Cirrhinus mrigala* and *Channa punctatus.* Pond 'C' was seeded with only a single speices of fish, *viz*., *Cirrhinus mrigala* only . No EUS-outbreak had been found to occur in any of the experimental ponds (*viz*., A, B, C) during the study period.

Our study had further revealed that, high mortality rate had been found among *Cirrhinus mrigala, Channa* spp., *Puntius* spp., *Labeo* spp., and *Clarias batrachus.* Specific Death Rate (SDR) due to EUS = No. of deaths during a year/ Mid-year population (total number of species) x 100= (22/38) x 100= 57.89 %. The same study had further revealed that, during the said period, the killing power of EUS was represented by the Case Fatality Ratio (CFR) (which is = Total No. of deaths due to EUS/Total No. of cases due to EUS × 100). CFR had been calculated species-wise and had been found to be high among *Cirrhinus mrigala* (83.72 %), followed by *Puntius ticto* (75 %), *Channa marulius* (70 %), *Mastacembelus armatus* 69.23 %), *Anabas testudineus* (53.85 %).

IV. CONCLUSION

EUS, today, is a semi-global problem among the freshwater fishes. However, in view of its complex infectious aetiology, it is yet to be accurately defined (OIE, 1997).

Various postulates have been put forward for this disease including physical factors, such as water pollution causing changes in physico-chemical characteristics and micro-nutrients. But these may just be the triggering factors. The origin of the disease is unknown and remains a matter of speculation even today. Attempts have been made in different laboratories to investigate the cause(s) of EUS and to correlate the outbreak of the epizootic with environmental parameters. The rapid seasonal depression of salinity and temperature as important environmental factors pre-disposing the fish to attack by EUS due to the stress condition created. No particular characteristic of soil fertility appear to be related to the occurrence of the EUS (Mackintosh and Phillips, 1986). *Aeromonas hydrophila* is one of the most commonly isolated bacterium involved in haemorrhagic fish diseases in the Indo-Pacific region (Roberts, 1989). A primary viral aetiology had been considered as a likely possibility for the rapid and uncontrollable spread of EUS (Frerichs *et al*., 1986; Ahne et al., 1988; Kar, 2007, 2013).

According to OIE (1997), EUS is a seasonal epizootic condition of wild and farmed fresh and brackish water fish of complex infectious aetiology. According to them, the most primary causative agent of EUS is the fungus *Aphanomyces invadans* often associated with bacterial septicaemia involving generally *Aeromonas hydrophila*. A variety of parasites have also been reported from the diseased fish; but, their presence is inconsistent. Associated viral infection also occurs frequently. It is believed that, the fungus, by itself, cannot normally invade fish; and, it is postulated that, certain co-factor, such as, epidermal damage (which could be initiated by an array of agents), severe environmental stress, or viral infection, is required to initiate this complex and exceedingly dangerous condition. EUS is said to be endemic in South-East and South Asia today.

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