



Studies on Fish Affected with Epizootic Ulceric Syndrome with Special Emphasis on Parasitic Infestation-A Project Report

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ABSTRACT: EUS is a seasonal epizootic condition of great importance in wild and farmed freshwater and estuarine fish. EUS is also known as red spot disease (RSD), mycotic granulomatosis (MG) and ulcerative mycosis (UM). Fish is an excellent source of food. Fish constitutes almost half of the total number of vertebrates in the world. Our country occupies 9th position in terms of fresh water mega biodiversity. During our study which was conducted from January to April 2015, we had come across a total of 21 different fish species which is infected with epizootic ulcerative syndrome (EUS) belonging to 6 order, 13 family, 18 genera. Four helminthes species were recorded during the investigation period like Acanthocephalan, Nematode, Trematode, and Cestodes.

Key words: EUS, Mycotic granulomatosis, Ulcerative mycosis, Acanthocephalan, Nematode, Trematode, Cestodes.

INTRODUCTION

Epizootic ulcerative syndrome or EUS is an infection caused by oomycete fungi known as *Aphanomyces invadans* or *A. piscicida*. *Aphanomyces* is a member of a group of organisms formerly commonly known as water moulds; they are currently recognized as belonging to the group of heterokonts or stramenopiles (OIE, 2006). EUS is also known by other names such as red spot disease (RSD), mycotic granulomatosis (MG), Ulcerative mycosis (UM) and in 2005 it was suggested to rename EUS as epizootic granulomatous aphanomycosis (EGA) (Baldock *et al.*, 2005). EUS causes ugly lesions in affected fish. Lesions can range from small pinpoint red spots, haemorrhagic spots, localized swelling, localized raised areas on the body surface, protruding scales, scale loss, skin erosion, reddened areas of the skin under the scales, exposure of underlying musculature, and ulceration. Ulcers can be found over a broad area with the center of the lesions being necrotic. Lesions are observed most often in the lateral surface but can also occur on any part of the body. Infection in fish occurs when motile spores of the fungi *Aphanomyces invadans* in the water or other carriers/ vectors are attracted to the skin of the fish. The

spores penetrate the skin and germinate, forming fungal filaments or hyphae. The hyphae invade widely into the surrounding skin and deeply into the underlying muscle tissues, resulting in extensive ulceration and destruction of tissues. Ulcerative skin lesions are common in freshwater and estuarine fishes. The presence of lesions often indicate contaminated or stressed aquatic environments and may be associated with a variety of infections including parasites, bacteria, viruses and fungi, as well as non-infectious causes such as for example toxic algae.

EUS was first described in farmed freshwater ayu (*Plecoglossus altivelis*) in Japan in 1971 and has since then spread across different countries of four continents including Asia, Australia, North America and Africa. The latest outbreak of EUS has been reported from Canada in a new susceptible species brown bullhead, *Ameiurus nebulosus*. It seems that the disease has potential to spread further, owing to the epizootic nature of the disease and broad susceptible host range. (Egusa and Masuda 1971).

In India, EUS was first reported in Tripura and by 1991 it had spread to several parts resulting in large-scale fish mortalities.

After the initial severe outbreaks during the 1990s, mortalities due to EUS in equivalent epidemic proportions have not been reported in spite of the endemic status of the disease. Following reports of heavy mortalities of cultured and wild fish species in wetland districts of Uttar Pradesh (UP), India in 2011, a thorough survey of the fish farms was carried out with the objectives to confirm the cause of large-scale mortalities and to document the severity and prevalence of the disease (Pradhan *et al.* 2014).

In the year 2010–11, outbreaks resulting in heavy fish mortality were recorded in wetland districts of Uttar Pradesh, India, and EUS was confirmed as the cause of mortality on the basis of histopathology, isolation of *Aphanomyces invadans*, bioassay and PCR. Epizootic ulcerative syndrome (EUS) has been reported in 26 countries across 4 continents. Till date, 94 fish species have been found to be naturally infected with EUS and its host range is gradually expanding.

Infection with *A. invadans* occurs mostly at water temperatures of 18–22°C and after periods of heavy rainfall (Bondad-Reantaso *et al.*, 1992). These conditions favour sporulation of *A. invadans* (Lumanlan-Mayo *et al.*, 1997), and temperatures of 17–19°C have been shown to delay the inflammatory response of fish to oomycete infection (Catap and Munday, 1998; Chinabut *et al.*, 1995). In some countries, outbreaks occur in wild fish first and then spread to fish ponds. Normally, a bath infection of *A. invadans* in healthy susceptible fish species does not result in clinical signs of disease. The *A. invadans* oomycete needs predisposing factors that lead to skin damage, such as parasites, bacteria or virus infection or acid water, to initiate the clinical signs of the disease (Lilley *et al.*, 1998). Helminths are multicellular eukaryotic animals that generally possess digestive, circulatory, nervous, excretory, and reproductive systems. Some are free-living in soil and water. Helminth infections differ from bacterial or protozoan infections because the worms do not usually increase in number in the host. Most individual fish in wild or cultivated populations are infested with parasites but in the great majority of cases no significant harm appears to be caused to the host fish. Although there are surprisingly few reports of parasites causing mortality or serious damage to wild fish populations such effects often perhaps go unnoticed. Parasites in wild fish are frequently only remarked upon when they are so obvious as to lead to rejection of fish by fishermen or consumers. In cultured fish populations, however, parasites often cause serious outbreaks of disease. Many parasite species are host specific to at least some degree and are capable of infecting one or only a limited number of host species. Individual parasite species may have widely differing effects on different

host species. Fishes being very important source of protein for human are consumed as white meat. Since humans utilize these fishes, it is important that they should be healthy and free of infection. Infections which are caused by viruses, bacteria and parasites among fishes in natural and manmade culture systems are harmful for fish health and growth. The parasitic infections are sometimes very fatal and can cause high mortalities (Ahmed, 1994) when intermediate hosts support their life cycles. The major parasitic groups found in freshwater fishes are trematode, cestodes, acanthocephalans and nematodes. Their infections not only deteriorate the muscle quality, stunt growth but even sometimes prove damage as a result of internal injury. The diversity in the parasitic fauna is bound to occur because many varieties of freshwater and hill stream fishes inhabit in Assam and Manipur. So, choosing a particular fish as habitat by the different forms of parasites shall be of great interest and may throw light upon new ideas to as to how diversified forms co-exist. Since 1886 there has been increased interest in academic parasitology as well as in medical and economic aspects of diseases caused by parasites. We agree with Rogers, although research on helminthes as important fish pathogens has lagged considerably behind that on birds and mammals as hosts. Williams (1964), mentioned by especially with regard to the nematodes of fish. He pointed out that until about 1950 most researchers on fish helminths had dismissed them as being of little or no importance as agents of disease. From this date, greater prominence has been given to fisheries helminthology and fish culture with an accompanying degree of caution in interpreting the effects of helminths on their fish host. It should, however be mentioned that there were notable exceptions amongst fish helminthologist during the first half of this century who also believed these helminths to be potentially dangerous. Some of this researcher was referred to in Williams (1964). Others, including Prost, M (1957), draw attention to the importance of the monogenians *Dactylogyrus*, *Gyrodactylus*, *Tetraonchus*, *Diplozoon* and *Octobothrium*; the tapeworms *Bothrocephalus*, *Caryophyllaus*, *Ligula* and *Schistocephalus*; many digenian and nematode species and several species of the acanthocephalan '*Echinorhynchus*'. Ward (1917) referred to T.H. Bean's plea to the American Fisheries Society in 1910 for a 'systemetic study of fish diseases' and stated that the breaking out of parasite epizootics among wild and cultured fish was already well known. He suggested that the vastness of oceans and the rapidity with which dead or dying fish are either consumed by ocean currents had, perhaps concealed evidence of epizootics due to parasites amongst oceanic fish.

Chubb (1979) seems to have the first to point out that the fish worms can modify the specific characters of a host fish to such an extent that infected individuals appear to represent a different species.

It was not until the 1950s and early 1960s that a full response to the view of Hof Hofer, Ward and Chubb was witnessed when helminthologist in the USSR, various European countries, Malaysia, Canada and the USA began stressing the need for full parasitological investigations not only of all fish but also of all animals closely associated with them. This is evident from a number of sources, including Dogiel (1958), Furtado (1963), Direct and circumstantial evidence from these and other publications and their cited references suggest that we should not accept that fish helminths are potentially dangerous pathogens even among fish in nature, but only with due regard to a number of influential biotic and abiotic factors which may trigger their pathogenicity and to whether the consequent effects are lethal or sub lethal. The following selected sources from about 1950 to 1986 support this view. An early and dramatic account of the effects of worms on fish from which one must conclude that natural mortality, directly or through predation, is inevitable. Miller refers to the pseudophyllidian tapeworm *Trianophorus crassus* and its great economic importance with regard to Canadian fishes. Work of Yamaguti (1958, 1959, 1961, 1962, 1963, and 1971), related to occurrence of helminth parasites in vertebrate host is of immense importance. Many works have been done in relation to seasonal occurrence of helminth parasites of fresh water fishes in many countries. Chubb (1977, 1979, 1980 and 1982) illustrated the studies of seasonal occurrence of helminthes in fresh water fishes of different climatic zones of the world. Madhavi (1979), Jha *et al.* (1992) and Shomorendra *et al.* (2005) worked on the occurrence of helminth parasites of freshwater fishes in India. Many workers like Bhalerao (1942), Gupta (1954), Srivastava (1960), made substantial contribution on the taxonomy of digenetic trematode parasites. Fish nematodes of Indian region have been studied by, Soota (1983). The Jha *et al.* (1989) studied on the characterization of parasito-fauna of the fishes of Muzzaffarpur, Bihar. Sinha and Misra, 2011 made substantial contribution on the study of seasonal fluctuations of parasites and effect of length and sex of fishes on incidence and intensity of the parasites. Surayawanshi *et al.*, 2010; Kadam Dhole, 2011; Pardeshi and Hiware, 2011 made significant contribution in the field of cestodes of fishes. Sarwat, 2011 made significant contribution in the studies of trematodes of fishes. Kadam Dhole, 2011; Pardeshi and Hiware, 2011 made significant contribution in the field

of cestodes of fishes. Sarwat, 2011 made significant contribution in the studies of trematodes of fishes. Moravec and Yooyen, 2011; contribution to the studies of nematode of fishes. Kar and Sen (2007), studied on the fish fauna of North-East (NE) India including diseases in fishes. Binky *et al.* (2011a) studied on the Ichthyospecies diversity of Karbhala Wetland in Cachar District of Assam. Shomorendra *et al.* (2009) studied the helminth parasites of air breathing fishes from Manipur. Shomorendra and Jha (2009) worked on acanthocephalan parasites of certain fishes from Manipur. Recently helminth parasites of the fishes of Manipur have been studied by some new workers like Zenith and Gambhir (2010), Geetarani *et al.* (2010), and Ranibala *et al.* (2010) and Sangeeta *et al.* (2010). Puinyabati *et al.* (2010, 2011), Singha *et al.* (2010, 2011), Binky *et al.* (2010, 2011b) studied on the helminth parasites of different regions of Assam. Singh, *et al.* (2013) made a detail study on helminth parasites diversity of Pumlun Lake, India. And Ngasepam, and Kar, (2014) also study the abundance of Parasites in Sone Beel, Assam, India.

MATERIAL AND METHOD

A. Study Site

The Barak Valley; located in the southern region of the Assam, India. (24°48'N to 24.800°N; 92°45' E to 92.750°E). The region is named after the Barak River. The Barak valley mainly consists of three administrative districts of Assam State *viz.*, Cachar, Karimganj, and Hailakandi (Fig. 1).



Fig. 1. Showing the Study Site (Sources www.google.com).

B. Collection of fish

Fish required for the present investigation were mostly caught by gill nets, cast nets, angling and occasionally through fishermen. Fishes were collected from time to time for four consecutive months from January to April 2015 from Barak valley, Assam. The fish samples were brought to the laboratory in polythene which contains water of the mention study sites for further study. The fishes were then kept in the refrigerator in order to avoid decay.

C. Examination of fish

Before examination of helminthes parasites, the weight, total length and sex of fish were entered as host data on an accession card with a reference number for each fish specimens. The fishes were examined thoroughly for external and internal helminth parasites. The sex of the fish was determined by inspecting the urino-genital papillae which is pointed and narrow in males and broad and square in females (Miller, 1984) and by observing the reproductive organs.

First of all a checklist of fish species present in and around Silchar will be prepared following with the help of standard taxonomic keys like Jayaram (2010), Vishwanath (2002), and Kar (2007). External and internal body organs were thoroughly examined for parasites following. Sex and spawning period of the fish will be taken to ascertain the infection of parasites

(Bylund *et al.*1980). Parasites collected were then preserved in respective recommended fixatives. The acanthocephalan were fixed and preserved in the AFA (alcohol-formalin-acetic acid), cestodes in 5% formalin, nematode stretched in glacial acetic acid and fixed in 70% alcohol and trematodes is fixed in A.F.A and preserved in 70% alcohol. To facilitate identification of worms cestodes is stained in alum carmine and mounted in Canada balsam while in acanthocephalan, the worms were cleared in Lactophenol and observed under microscope.

D. Examination of water

The physico-chemical properties of water like Free Carbon Dioxide (FCO₂), Dissolved oxygen (DO), Total alkalinity (TA), pH and Water temperature (APHA, 2005).

RESULTS

Table 1: Fish Diversity of Study Site.

SL NO.	Name of Fish	Order	Family
1	<i>Puntius sophore</i> (Hamilton, 1822)	Cypriniformes	Cyprinidae
2	<i>Labeo gonius</i> (Hamilton, 1822)	Cypriniformes	Cyprinidae
3	<i>Cyprinus carpio</i> Linnaeus, 1758	Cypriniformes	Cyprinidae
4	<i>Cabdio morar</i> (Hamilton, 1822)	Cypriniformes	Cyprinidae
5	<i>Chela sp.</i>	Cypriniformes	Cyprinidae
6	<i>Labeo rohita</i> (Hamilton, 1822)	Cypriniformes	Cyprinidae
7	<i>Lepidocephalichthys guntea</i> (Hamilton, 1822)	Cypriniformes	Cobitidae
8	<i>Channa punctata</i> (Bloch, 1793)	Perciformes	Channidae
9	<i>Channa striata</i> (Bloch, 1793)	Perciformes	Channidae
10	<i>Oreochromis mossambicus</i> (Peters, 1852)	Perciformes	Cichlidae
11	<i>Glossogobius giuris</i> (Hamilton, 1822)	Perciformes	Gobiidae
12	<i>Trichogaster fasciata</i> Bloch & Schneider, 1801	Perciformes	Osphronemidae
13	<i>Anabas testudineus</i> (Bloch, 1792)	Perciformes	Anabantidae
14	<i>Clarias batrachus</i> (Linnaeus, 1758)	Siluriformes	Clariidae
15	<i>Heteropneustes fossilis</i> (Bloch, 1794)	Siluriformes	Heteropneustidae
16	<i>Mystus cavasius</i> (Hamilton, 1822)	Siluriformes	Bagridae
	<i>Mystus tengara</i> (Hamilton, 1822)	Siluriformes	Bagridae
18	<i>Macrognathus aral</i> (Bloch & Schneider, 1801)	Synbranchiformes	Mastacembelidae
19	<i>Mastacembelus armatus</i> (Lacepède, 1800)	Synbranchiformes	Mastacembelidae
20	<i>Xenentodon cancila</i> (Hamilton, 1822)	Beloniformes	Belonidae
21	<i>Gudusia chapra</i> (Hamilton, 1822)	Clupiformes	Schilbeidae

RESULT AND DISCUSSION

During our study which was conducted from January to April 2015 we had come across a total of 21 different fish species belonging to 7 order, 11 family, and 18 genera. Order Cypriniformes contribute 7 different fish species, 6 different fish species under order Perciformes, 4 different fish species under order Siluriformes, 2 different fish species under Synbranchiformes and Beloniformes and Clupeiformes

contributed by 1 fish species (Table 1). Nematode and Acanthocephala were collected from EUS infected *Channa striata*, Acanthocephala also collected from infected *Channa punctata* and *Heteropneustes fossilis* were infected by Cestode parasites. (Table 2). The site of infection is very common i.e. intestine but in case of Trematode parasite are collected from body cavity (Table 3).

Table 2: Showing EUS Affected Fishes of Barak Valley.

SI No.	Name of The Host	Cestode	Acanthocephala	Trematode	Nematode
1	<i>Channa striata</i>	Absent	Present	Absent	Present
2	<i>Mastacembelus armatus</i>	Absent	Absent	Absent	Present
3	<i>Channa punctata</i>	Absent	Present	Absent	Absent
4	<i>Heteropneustes fossilis</i>	Present	Absent	Absent	Absent

Table 3: Showing Site of Infection of Affected Fishes.

Sl. No.	Fish host	Parasites groups	Site of infections
1	<i>Channa striata</i>	Acanthocephala, Nematode	Intestine
2	<i>Mastacembelus armatus</i>	Nematode	Intestine
3	<i>Channa punctata</i>	Acanthocephala	Intestine
4	<i>Heteropneustes fossilis</i>	Cestode	Intestine
5	<i>Trichogaster fasciata</i>	Trematode	Body cavity

Table 4: Showing Prevalence and Intensity of Helminth Parasite.

SL. NO.	Name of the fish	Total fish examined	Total no of fish infected	Total no parasites	Inten sity	Prevalence %	Parasite group
1	<i>Channa striata</i>	26	12	21	1.75	46%	Nematode Acanthocephala n
2	<i>Mastacembelus armatus</i>	16	9	21	2.33	56%	Nematode
3	<i>Channa punctata</i>	19	9	6	0.66	47%	Acanthocephala n
4	<i>Heteropneustes fossilis</i>	15	9	18	2	60%	Cestodes
5	<i>Trichogaster fasciata</i>	10	5	7	1.4	50%	Trematode
	Total	86	44	73	1.30	65.12%	

Table 5: Parasite Load of Study Site.

Parasite group	Total fish examined	Total fish infected	Total collected parasites
Cestodes	86	9	18
Nematode		21	39
Trematode		5	7
Acanthocephalan		21	9
Total		56	73

The percentage of prevalence is highest and lowest in *Mastacembelus armatus* and *Channa striata*. Intensity of infection is highest and lowest in *Mastacembelus armatus* and *Channa punctata*. Total prevalence infection percentage respectively is 65.12% and intensity is 1.3. Total percentage of prevalence is 65.12 and intensity is 1.30. 86 fishes were sacrificed for our study, out of that a total of 56 fishes were found infected and 73 numbers of helminth parasites were

recovered. Total infectious rate is 65.116%. (Table 4 & Table 5). The temperature, pH, DO (mg/L), FCO₂ (mg/L) and TA (mg/L) were recorded as 11.67±0.88, 6.63±0.12, 4.33±0.15, 10.29±1.62 and 35±0.58. The correlation between physiochemical parameter of study site, in that temperature is strongly correlated with FCO₂ (P < 0.01) which means increase and decrease of temperature fluctuate the value of FCO₂. (Table 6 & Table 7).

Table 6: Showing Physico-chemical Parameters of study site.

Months	Temperature (°C)	pH	DO(mg/l)	FCO ₂ (mg/l)	TA(mg/l)
January	10	6.8	4.3	13.26	34
February	12	6.4	4.1	9.9	35
March	13	6.7	4.6	7.7	36
*MSE	11.67±0.88	6.63±0.12	4.33±0.15	10.29±1.62	35±0.58

*MSE=Mean Standard Error

Table 7: Showing Correlation between Physico-chemical Parameters of study site.

		pH	DO	FCO ₂	TA	Temp
pH	Pearson Correlation	1				
DO	Pearson Correlation	.636	1			
FCO ₂	Pearson Correlation	.355	-.496	1		
TA	Pearson Correlation	-.240	.596	-.993	1	
Temp	Pearson Correlation	-.419	.434	0.998*	.982	1
** . Correlation is significant at the 0.01 level (2-tailed).						

Table 8: Effect of length of *Channa punctata* in helminth parasite infection.

Length Group (Mm)	No. of Fish Examined	No. of Fish Infected	No. of Parasites	Prevalence	Intensity
5-25	2	1	10	50%	10
26-46	10	6	12	60%	2
47-67	4	2	1	50%	0.5
68-88	6	1	1	16.6%	1
89-109	4	2	0	50%	0

Table 9: Effect of Weight of *Channa Punctata* in Helminth Parasite Infection.

Weight Group	No. of Fish Examined	No of Fish Infected	No of Parasites	Prevalence	Intensity
5-25	2	0	0	0	0
26-46	3	2	4	66.67%	1.3
47-67	12	6	10	50%	0.8
68-88	3	1	3	33.3%	1
89-109	6	2	0	33.3%	0

Percentage Prevalence of infection is highest in fish of length ranges from (26-46) mm and lowest in fish ranging from (68-88) mm whereas % prevalence of infection is highest in fish of weight from (26-46) g and lowest in (5-25) g.

Intensity of infection is highest in fish of length (50-25) mm and lowest in fish ranging from (89-109). Intensity of infection is highest in fish of weight (26-46) g and lowest in fish ranging from (5-25) g and (89-109) g. (Table: 8 & Table 9).

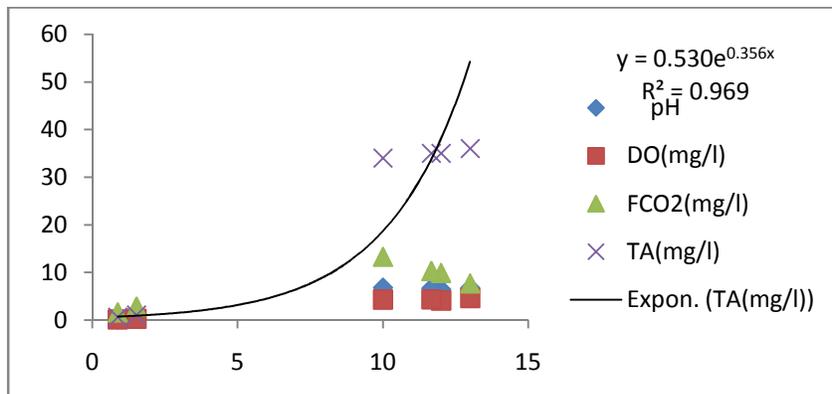


Fig. 2. Showing the linear equation graph of physico-chemical parameter.

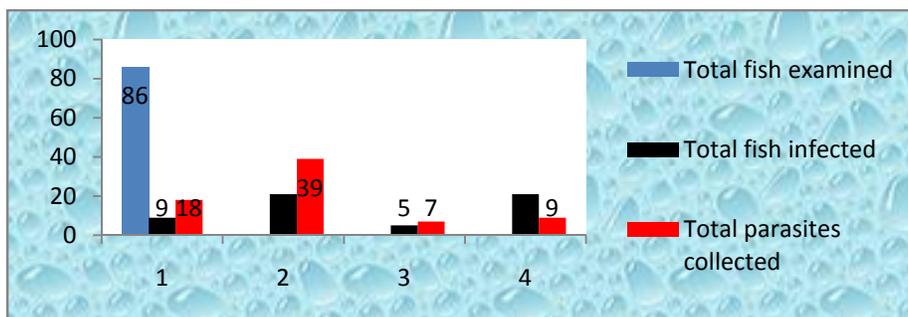


Fig. 3. Showing the Parasite Load of Study Site.

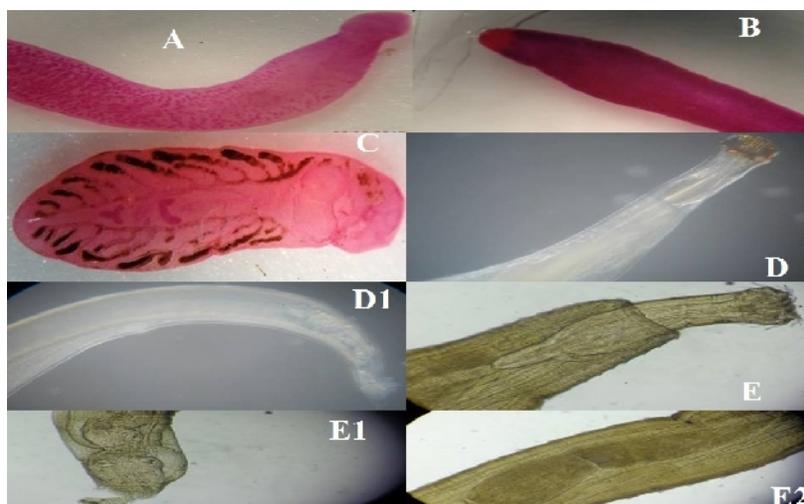


Fig. 4. Helminth parasites groups during study.

(A. Posterior portion of a Cestode Parasites B. Anterior portion of a Cestode Parasite C. Whole mouth of a Trematode Parasite D. Anterior portion of a Nematode Parasite D1. Posterior portion of a Nematode Parasite E. Anterior portion of Acanthocephalan Parasite E1. Posterior portion of Acanthocephalan Parasite E2. Middle portion of Acanthocephalan Parasite).



Fig. 5. EUS infected fishes of study sites.

(a. EUS infected *Clarias batrachus* b. EUS infected *Anabas testudineus* c. EUS infected *Channa punctata* d. EUS infected *Catla catla*. e. EUS infected *Puntius spp.* e. EUS infected *Oreochromis mossambicus* f. EUS infected *Channa punctata*).

CONCLUSION

Epizootic ulcerative syndrome (EUS) is one of the most important diseases affecting more than 100 species of wild and cultured finfish. There are no specific control measures in fish for EUS in natural environments. Fish from infected waterways, especially those with lesions of EUS, should not be relocated to other waterways. Effective control and treatment of EUS is a major problem today, because the primary pathogen has not yet been identified. Control of EUS in large natural water bodies may not be possible. However in view of the fact that TA of water acts as a predisposing 'stress' factor, application of lime in low alkaline water bodies, helps to control the spread of EUS. EUS today is a semi-global problem among the fresh water fishes. Unfortunately, in view of its complex infectious etiology, it is yet to be accurately defined.

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