Occurrence of Epizootic Ulcerative Syndrome in Pond Fish of Kapilvastu District of Nepal

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ABSTRACT

An interview-based questionnaire survey of 30 fish farmers randomly selected in Kapilvastu District of Nepal was carried out to study the prevalence of epizootic ulcerative syndrome (EUS) in pond fish. The survey was carried out during the EUS season (November 2004 to March 2005). At each pond site, 100 fish were examined for lesion, and one fish of each species with lesions was sampled for histological diagnosis. A fishpond was classified as affected with EUS if one or more fish of any species had a positive diagnosis based on the presence of characteristic mycotic granuloma in histological sections. A descriptive statistical tool-SPSS was used to analyze socio-demographic and pond characteristics. Out of 30 ponds examined for lesions, fish in 6 ponds (20 %) were confirmed to be EUS positive. In total, 3000 pond fish were examined and 291 samples with lesions were collected and processed in the histopathological unit of the Central Fisheries Laboratory, Balaju, and Teaching Hospital, Maharajgang, Kathmandu. Of 291 samples, 143 were confirmed to be EUS positive. A total of 17 species with lesions were sampled for histology. Among them, 13 fish species (two cultured and 11 wild) were confirmed as EUS-positive. However, the four cultured species, *i.e.* common carp, silver carp, grass carp and bighead carp had non-EUS lesions. Results indicate that ponds with high relative risks of EUS were characterized by the presence of wild fish, entry of flood water, and connection with paddy fields. Ponds that were not drained, limed and shared contaminated nets had high risk of getting EUS.

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INTRODUCTION

Epizootic ulcerative syndrome (EUS) was first officially recognized as a major problem at the FAO Expert Consultation in Bangkok in 1985 (Lilley *et al*., 1998). The use of the term "syndrome" highlights the complexity of the condition, which involves the interaction of a specific monoclonal fungus, a wide variety of environmental factors and a range of secondary invading pathogens, which differ with each outbreak. It is now recognized to be synonymous with the condition mycotic granuloma (MG) first described from Japan in 1971 and red spot disease (RSD) described from Australia in 1972 (Chinabut and Roberts, 1999). Epizootic ulcerative syndrome (EUS) is a seasonal epizootic condition of great importance in wild and farmed freshwater and estuarine fish (OIE, 2003). It was first reported in farmed ayu (*Plecoglossus altivelis*) in Japan in 1971 (Egusa and Masuda, 1971). It was later reported in estuarine fish, particularly grey mullet (*Mugil cephalus*) in eastern Australa in 1972 (McKenzie and Hall, 1976). The outbreak has extended its range through Papua New Guinea into SoutheEast and south Asia, and recently into west Asia, where it has now reached Pakistan (Lilley *et al*., 1998; Tonguthai, 1985). The disease with severe ulceration and causing heavy mortality of fishes from small and large water bodies has been a major concern almost all over the countries of the Asia Pacific region since 1972. So far no fish disease has been as virulent and menacing as EUS in fish (Chinabut, 1994).

Aquaculture is potentially an important sector of agriculture in Nepal (Pradhan and Shrestha, 1996). Since 1989, EUS has been considered the most serious disease affecting freshwater fish in Nepal (Shrestha, 1990). The initial outbreak of EUS in Nepal was reported in February 1989 from the eastern part of Nepal (Shrestha, 1994). The occurrence of EUS has been reported from Terai, mid-hills, and valleys, *e.g.* Pokhara and Kathmandu (Dahal, 2002; Dahal, 2003). However, the trend of severity in general seems comparatively lesser than in 1990s. Kapilvastu District was selected for the purpose of the present study, as this district was one of the EUS-affected in the country (DoFD, 2002).

The broad objective of this study was to assess the occurrence of EUS in pond fish in Kapilvastu District of Nepal; specific objectives were to identify fish species susceptible to EUS, to confirm the occurrence of EUS by histopathological diagnosis and to assess the relative risk of EUS outbreak with the pond management practices.

MATERIALS AND METHODS

Selection of study area

A list of village and the number of ponds in each village was obtained from the fisheries profile of the District Agriculture Development Office (DADO) in Kapilvastu in September 2004. Each pond was numbered chronologically. Out of 405 fish farmers in Kapilvastu District, 30 fish farmers with a pond was selected, in October 2004, using simple random sampling method. But if for some reasons, when we visited the farm, the farmer was not there, or did not co-operate, or the pond didn't contain susceptible fish, a nearby pond was selected.

Sources of information and data collection

The respondent fish farmers were the major source of primary data. The data were collected through interviews, observations, fish sampling, water quality testing, informal group discussions and key informant surveys. Technical reports, articles, books, proceedings, research report, etc. published by different institution were the major source of secondary data.

Field survey

The actual field survey was carried out from November 2004 to March 2005, recognized as EUS-season in Nepal. On arrival at the sampling pond site, information was gathered from the farmer and this was followed by measurement of water quality parameters. After the completion of the water quality measurement, at least 100 susceptible fish from each pond site were examined for EUS-lesions. At least one fish of each species recorded with lesions at each site was sampled for histology. During the study period of five months, the randomly selected pond was visited five times with monthly interval to observe for the occurrence of EUS in farmed fish.

Descriptive statistical tool

A descriptive statistical tool such as percentage and frequency distribution was used in analyzing fish pond management, socio-demographic and farm characters, which included condition of the pond dike, pre-stocking, stocking and post-stocking management, of the randomly selected ponds

Fish sample collection

Fishermen were used to net the pond and catch the fish. 100 fish were randomly selected from the hapas and examined individually for the presence of external lesions. One fish of each species with visible lesions was sampled for histopathology. Fish with lesion was killed and two pieces of muscle of size 1 cm^3 each taken from the lesion and surrounding muscle for histology study. Sample were fixed immediately in cold 10% formalin and labeled. Each sample was put in a separate container.

Water quality analysis

Four important water quality parameters, *i.e.* temperature, pH, dissolved oxygen and transparency, were measured monthly *in situ* using portable water analysis kit (Hack Kit).

Histopathological analysis

Processing of formalin fixed tissues was carried out at Central Fisheries Laloratory, Balaju, Kathmandu and Histopathological laboratory, Teaching Hospital, Maharajganj, Kathmandu. The processing as well as H&E staining procedure described by Chinabut and Roberts (1999) was followed.

Method and techniques of data analysis

Data were analyzed with the help of Statistical Packages for Social Sciences (SPSS) computer software package.

RESULTS

A total of 3000 fish samples were examined of which 291 samples with external lesions were processed for histopathology. Of the 291 fish with external lesions, 143 fish were confirmed as EUS-positive, based on the presence of mycotic granulomas. Of the total 17 fish species examined, 13 species (two cultured and 11 wild) were confirmed to be EUS-positive. The Chinese carps and common carp with external lesions similar to EUS were negative for EUS. A total of 752 wild fish found in ponds along with cultured species, were examined for lesions. Of these, 156 with external lesions were sampled for histology and 67 were confirmed as EUS- positive. The average prevalence of EUS in wild fish species was 8.3 percent with a range of 4.8 to 11.5 percent (Table 1). Photographs of EUS affected wild fish species are given in Figure 1a and b.

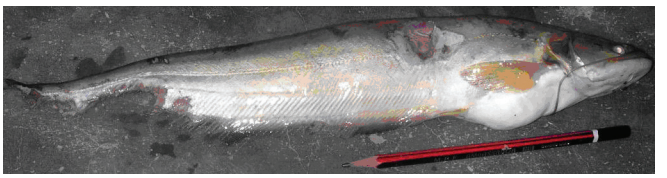
a. Naini (*Cirrhinus mrigala*) b. Rohu (*Labeo rohita*)

Figure 1 (a,b). Photographs of cultured fish species showing typical EUS lesions.

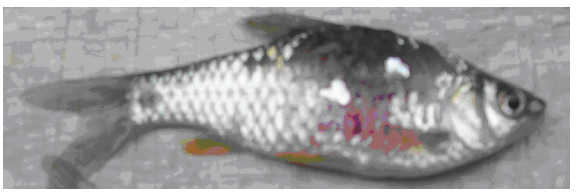
Table 1. Prevalence of EUS in wild fish species.

S.N.	Fish species	No. of fish examined			Confirmed cases (EUS-positive)	Percent infection of EUS
		Total	Healthy	With lesions		
1.	Tengri (*Mystus tengara*)	53	45	8	5	9.4
2.	Pothia (*Puntius ticto*)	55	35	20	5	9.0
3.	Darahi (*Puntius sarana*)	65	55	10	6	9.2
4.	Pothia (*Puntius chola*)	50	40	10	5	10.0
5.	Kabai/kotri (*Anabas testudineus)*	160	140	20	12	7.5
6.	Garahi (*Channa punctatus*)	139	104	39	16	11.5
7.	Buhari (*Wallago attu*)	12	07	05	01	8.3
8.	Mungri (*Clarias batrachus*)	51	39	12	5	5.8
9.	Dhebari (*Nandus nandus*)	60	48	12	5	8.3
10.	Gainchi (*Macrognathus aral*)	42	37	05	2	4.8
11.	Chelwa (*Salmostoma bacaila)*	65	50	15	5	7.7
	Total	752	600	156	67	Mean: 8.3

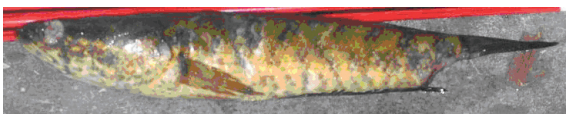
Amongst the cultured fish species, rohu and naini (major carps) were found to be affected by EUS. The total number of major carps examined was 704; of these rohu and naini were 312 and 392, respectively. The average occurrence of EUS was 9.9 and 11.5 in rohu and naini, respectively (Table 2). Photographs of EUS affected cultured fish species are given in Figure 2.



a. Buhari (*Wallago attu*)



b. Pothia (*Puntius ticto*)



c. Hile (*Channa punctatus*)

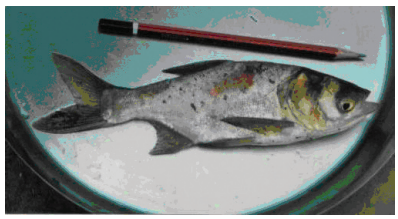
Figure 2 (a-c). Photographs of wild fish species showing typical EUS lesions, Kapilvastu (2005).

Table 2. Prevalence of EUS in cultivated fish species.

S.N.	Fish species	No. of fish examined			Confirmed cases (EUS-positive)	Percent infection of EUS
		Total	Healthy	With lesions		
1.	Rohu (*Labeo rohita*)	312	273	39	31	9.9
2.	Naini (*Cyprinus mrigala*)	392	347	45	45	11.5
	Total	704	620	84	76	Mean: 10.7

Cultured common carp and three Chinese carps were found with clinical signs (open dermal ulcers) similar to EUS. However, histopathological examination did not reveal the presence of mycotic granulomas in any of the samples (Table 3). Histopathologically, EUS affected fish showed the presence of distinctive MG in affected tissues caused by the oomycete fungus *Aphanomyces invadans* (Lilley *et al.*, 1998). Photographs of cultured fish species showing clinical signs similar to EUS, but negative for MG are given in Figure 3.

a. Common carp (*Cyprinus carpio*)



b. Bighead carp

c. Silver carp

Figure 3 (a-c). Photographs of cultured fish species showing clinical signs similar to EUS but negative for MG.

Table 3. Details of cultured fish species with clinical signs similar to EUS but negative for mycotic granulomas, the diagnostic feature of EUS.

SN	Fish species	No. of fish examined		
		Total	Healthy	With lesions
1.	Common carp (*Cyprinus carpio*)	397	379	18
2.	Silver carp (*Hypophthalmichthys molitrix*)	507	495	12
3.	Grass carp (*Ctenopharyngodon idella*)	291	275	16
4.	Bighead carp (*Aristichthys nobilis*)	349	340	09
	Total	1544	1489	55

The study showed that 36.4% of ponds in Kapilvastu District do not have permanent source of water and such ponds depended for water on rainfall. Ponds that received water from the canal connected to the rice field, river and reservoir and wetland showed relatively high risk of EUS while the occurrence of EUS was not observed in ponds that had received water only from rainfall or underground source. The study showed that the majority (60%) of the fishpond was not dried and 40 percent of the pond dried during the previous year. About 27.8 percent of EUS occurrence was confirmed in ponds that had not dried in the previous year. Majority (70%) of the fish growers in Kapilvastu district do not apply lime during pond drying and post stocking management. The occurrence of EUS was observed mostly in ponds that did not apply lime during the previous year. All of the EUS positive confirmed cases were collected from the ponds that were not limed before.

DISCUSSION

Occurrence of EUS in wild and cultured fishes in Kapilvastu District was confirmed through histopathological studies. Of the total of 17 fish species collected with EUS like lesions, only 13 (2 cultured and 11 wild species) were confirmed positive for EUS based on the presence of mycotic granulomas, the diagnostic characteristic of EUS. Amongst the commercially important cultivated species, Chinese carps (silver carp, bighead carp and grass carp) and common carp were observed with EUS like lesions, but did not have MG. Hatai (1994) had observed that Chinese carp and common carp were not affected during EUS outbreaks in Japan. Mechanisms of resistance in these species have been described by Wada et al. (1996). The study showed that ponds containing more wild fish were at high risk of EUS occurrence. Lilley *et al*. (1998) reported more than 100 wild species as susceptible to EUS in Asia Pacific region.

The present study showed that 36.4% of ponds in Kapilvastu district do not have permanent source of water and such ponds depended for water on rainfall. About 26.6 percent of ponds received water from the canal connected to the rice field while 20.0 percent received water from canal connected to river. Ponds that received water coming through rice field and river/ditch had showed high relative risk of EUS while the occurrences of this disease was less in ponds that had received water only from underground source. Ahmed and Rab (1995) indicated that wild fish in natural bodies might be the source of infection, and methods of excluding of wild fish and other potential carriers from pond are likely to be effective in reducing occurrence of EUS (Jha and Shrestha, 2003).

The information gathered showed that there is more chance of EUS occurring in culture ponds containing wild fish. There was also higher relative risk of EUS occurring on farmed fish when pond embankments were not high enough to prevent incoming water. Similarly, ponds that were repeatedly flooded that year also showed a higher relative risk. Fish farms directly connected to water bodies that allowed the entry of wild fishes also showed a higher relative risk of EUS.

In this study, 17 fish species were examined. Of these, 13 species were histopathologically confirmed as EUS positive, while common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idella*) were negative for EUS. It has been reported that EUS has no effect on common carp (Hatai and Egusa, 1978). Therefore, these species are considered as EUS resistant. Some scientists have commented that the severely affected species in natural outbreak are generally bottom dwellers (Llobrera and Gacutan, 1987). This comment seems true to some extent with the present study findings.

Failure to drain and lime ponds prior to stocking has a high risk of outbreak of EUS (Mohan and Shankar, 1994; Jha, 2002). In the present study, ponds that had not been dried and limed, showed higher prevalence of EUS. Pond watercolor indicating high levels of phytoplankton or zooplankton had no effect on EUS outbreak. Khan *et al.* (1999) opined that the vulnerability to the occurrence of EUS might be high in ponds having frequent contact of animals, people and other materials as compared to closed ponds. Most (90%) of the fish farmers did not remove the upper layer of pond bottom after draining pond. Old ponds that have heavy deposit of mud (humus) at the bottom were found vulnerable to the occurrence of EUS.

CONCLUSION

EUS was confirmed in Kapilvastu District. Only two out of seven cultured species were affected by EUS indicating that cultured species are less susceptible than indigenous/wild species. Eleven wild species were found affected by EUS indicating that indigenous species are more vulnerable to EUS. Wild fish in the natural bodies might act as the source of infection. Ponds with more wild fishes were at high risk of EUS. Removal of all susceptible species from the pond and obtaining water from EUS free sources might help to avoid EUS. Drying out and liming of pond prior to fish stocking might prevent the outbreak of EUS. Pond management practices such as repair of pond dike condition to prevent the entry of wild fishes along with flood-waters into the pond; mud removal from the pond bottom; application of lime during post stocking management; restriction on washing and cattle bath in the pond appear to be helpful in avoiding the occurrence of EUS. Stocking of less number of susceptible species and substituting resistant fishes such as Chinese carps and common carp having similar feeding niches might be helpful to avoid EUS in culture ponds.

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