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Farmer-based Investigation of Treatments for Ulcerative Disease in Polyculture Carp Ponds in Bangladesh

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Abstract

Ulcerative disease was identified by polyculture carp farmers in two districts (Rajshahi and Kishoregonj) of Bangladesh participating in the CARE project, Locally Intensified Farming Enterprises (LIFE), as the most significant constraint to higher production. In order to address this problem, six candidate prophylactic treatments were identified through consultation with researchers and farmers. These comprised of: ash, lime, salt, salt+lime, neem branches (*Azadirachta indica*), and fertilizer. A total of 233 farmers participated in the trial. Each farmer selected one of the treatments for his experimental pond. Lime and ash were the most popular selections. For each treatment except neem, fortnightly applications were advised over the study period from 1 October 1999 to 28 February 2000. However, at the end of the study, the number of applications of these treatments varied from 1 to 7 times. The occurrence of ulcerative disease was reported by farmers in a structured questionnaire at the end of the study. A proportion of affected sites were visited by CARE staff during the study and 30 fish samples were taken to determine whether the disease was epizootic ulcerative syndrome (EUS), using presence of mycotic granulomas in histological section as the diagnostic feature of EUS. Seventy percent of the samples could be confirmed as EUS-positive.

Farmers from all treatment groups reported a lower incidence of ulcerative disease than farmers with control ponds. The number of ponds affected by ulcerative disease under different treatments showed significant difference with control (one-way ANOVA, $p<0.05$). No farmer applying fertilizer reported disease, but only three farmers adopted this treatment that reduced the significance of the results. Other than fertilizer treatment, best result was obtained from lime treatment followed by ash, salt, salt+lime and neem treatments where, as reported by farmers, 3.3%, 9.8%, 10.0%, 15.4% and 19.0% of the treatment ponds were affected by ulcerative disease, whereas 61.9% of the control ponds were affected by the disease. Neem appeared to be the least effective of the treatments. Of the farmers that adopted one of the treatments, in total, 96% indicated they were satisfied with the treatment results. Treatment-wise analysis showed that all the farmers under lime, ash and fertilizer treatments were satisfied while 90% of the farmers adopting salt and neem treatments and 88% of the farmers under salt+lime treatment were satisfied with the treatment results. The majority (86.40%) of the satisfied farmers indicated that this was due to "no occurrence of ulcerative disease". Other pond variables were recorded to check whether they were associated with the occurrence of ulcerative disease in the experimental ponds. The entry of wild fish in ponds and the occurrence of ulcerative disease in nearby ponds were significant risk factors for ulcerative disease in Kishoregonj and Rajshahi districts respectively. The problems in obtaining reliable quantitative data from farm-level studies are discussed. However, it is suggested that farmer participatory research is an important means of identifying treatments that are acceptable to farmers.

Introduction

The Locally Intensified Farming Enterprises (LIFE) project run by CARE – Bangladesh is operating at Rajshahi and Kishoregonj districts located in the northern part of the country to enhance food security through improving farmer's knowledge and skills with regard to major agricultural activities. Farmers working with the LIFE project identified EUS (epizootic ulcerative syndrome) as the main problem they encounter in fish cultivation. The LIFE project undertook an initiative to address the EUS problem. Four possible EUS treatments (ash, lime, salt, and salt+lime) were identified through farmer participation during the 1998^{-1}999 season and these treatments were found to reduce the incidence and intensity of the disease (Nandeesha et al. 2002). To investigate the treatments further, the project continued the study during the 1999-2000 season, in collaboration with the Department for International Development (DFID)-funded regional EUS project. In addition to the previous treatments, leaves and stems of the neem tree (*Azadirachta indica*) and fertilizer treatments were also studied. The study aimed to determine whether the treatments reduced the incidence of EUS as reported by farmers, and to assess whether the farmers were satisfied with the treatments.

Materials and Methods

Formation of participatory action research groups (PARGs)

Participatory action research groups (PARGs) were formed at the village level. PARGs were formed by organising a general meeting of village farmers, informing them of project objectives and principles, and inviting interested farmers to become members. PARG members also had to satisfy certain selection criteria. They had to be defined as "food insecure" and show interest in solving problems using available resources. The LIFE project did not provide credit or any other incentives other than enhancing farmer knowledge and skills. Each PARG consisted of 25-30 farmers with either male-only or female-only members.

Participatory needs assessment (PNA)

After PARG formation, farmers' needs, or problems associated with agricultural activities, were identified through PNA (participatory needs assessment) sessions. In the majority of the PARGs, EUS was identified as the major problem in production in ponds.

Trial design and implementation

A learning session was conducted for the farmers of those PARGs where EUS was recognized as a major problem. The session included the history of EUS, known causes, and possible control measures using locally available resources. Based on these discussions, six possible preventative treatments were identified for the study. Farmers were urged to adopt one of the following treatments, and to make the first treatment application early in the cold season and to continue the treatment application up to the end of the study period. The farmers were advised to apply different treatments as follows.

Ash treatment

Applications of ash were advised at an initial dose of 3 kg•decimal^{-1} (741 kg•ha^{-1}), followed by 1.5 kg•decimal^{-1} (371 kg•ha^{-1}) at fortnightly intervals throughout the experimental period. The application procedure involved sieving the required quantity of ash, dissolving it in water, and then spreading it over the pond.

Lime treatment

This treatment comprised of an initial dose of 1 kg•decimal^{-1} (247 kg•ha^{-1}) lime followed by 0.5 kg•decimal^{-1} (124 kg•ha^{-1}) lime at fortnightly intervals throughout the experimental period. Farmers were instructed that the lime should be dissolved in water, cooled, and the resulting solution further diluted and then dispersed throughout the pond.

Salt treatment

This treatment comprised of an initial dose of 1 kg•decimal^{-1} (247 kg•ha^{-1}) table salt (NaCl) followed by 0.5 kg•decimal^{-1} (124 kg•ha^{-1}) at fortnightly intervals throughout the experimental period. The required amount of salt was dissolved in water before dispersal in the pond.

Salt+lime treatment

Ponds under this treatment were given an initial dose of 0.5 kg•decimal^{-1} (124 kg•ha^{-1}) of both salt and lime. Farmers were instructed that subsequent doses of 0.25 kg•decimal^{-1} (62 kg•ha^{-1}) of both salt and lime should be applied at fortnightly intervals throughout the experimental period. The salt and lime were dissolved separately, the lime solution was cooled and mixed with salt solution, and the mixture was spread over the pond.

Neem treatment

Farmers adopting this treatment inserted neem stems with leaves into the pond. Farmers applied a variable number of neem stems (between 75^{-1}400 stems per hectare) in their ponds (Table 3). Number of neem application varied from 1 to 3 during the experimental period (Table 2).

Fertilizer treatment

This treatment comprised of 2 kg•decimal^{-1} (494 kg•ha^{-1}) decomposed cow dung, 200g decimal^{-1} (49 kg•ha^{-1}) urea, 200g decimal^{-1} (49 kg•ha^{-1}) TSP and 250g decimal^{-1} (62 kg•ha^{-1}) mustard oil cake at fortnightly intervals. The number of applications varied from 3 to 6 (Table 2) depending on the level of phytoplankton in the pond water i.e. the farmers applied the fertilisers when water transparency was above 30cm.

Control

Participating farmers who did not want to follow a treatment were designated as control farmers. A total of 42 farmers remained as control farmers.

In order to satisfy the criteria for selection in the study, all the study ponds contained at least one of the species that has been listed as being susceptible to EUS by Lilley et al. (1998). The susceptible species may have been stocked and/or wild species. The trial was conducted in the cold season from 1st October 1999 to 28th February 2000, when EUS is more prevalent. Farmers normally looked for clinical signs on fish on a daily basis, but in addition, they agreed to check fish at least once every fifteen days using a cast net. Farmers identified EUS-affected fish by the presence of clinical lesions. A proportion of the farmers that reported EUS outbreaks to LIFE field trainers were visited by the authors to sample affected fish for later histological confirmation at Bangladesh Agricultural University (BAU).

Questionnaire and focus group discussions

At the end of the study, a series of workshops was arranged in Rajshahi and Kishoregonj to bring farmers together to discuss the treatments they had adopted. A structured questionnaire was used to gather results from each of the farmers.

Statistical analysis

Differences between treatments and controls were analyzed using one-way ANOVA followed by pair-wise multiple comparisons using the least significant difference method. Significantly different groups are shown at an alpha level of 0.05.

Results

A total of 233 ponds in 82 different PARGs in Rajshahi and Kishoregonj districts were used to study the impact of EUS treatment strategies for the prevention of EUS during the 1999-2000 season. Average area and depth of these ponds was 915 ± 1032m2 and 2.2 ± 2.1m respectively. The treatment selected by these farmers, and the number of applications of each treatment, are listed in Tables 1 and 2 respectively. Lime and ash were the most popular treatments and fertilizer was the least popular treatment. The number of application of different treatments varied from 1 to 7 while most of the farmers applied 3 times throughout the experimental period (Table 2). The majority of the neem treatment group (50% of total farmer under neem treatment) made one application of $75^{-1}400$ stems ha^{-1} at the beginning of the study period (Tables 2 and 3).

Effect of treatment on the prevention of ulcerative disease

According to data on disease occurrence obtained from farmer, all six treatments had a significant effect in the prevention of ulcerative disease (Table 4). The number of ponds affected by ulcerative disease under different treatments showed significant difference with control (one-way ANOVA, $p<0.05$). No farmers applying fertilizer reported disease, but only three farmers adopted this treatment. Other than fertilizer

Table 1. Number of experimental ponds under different treatments

Treatment	Pond number
Salt	20
Lime	60
Salt+lime	26
Neem	21
Ash	61
Fertilizer	3
Control	42
Total	233

Table 2. Application frequency of preventative treatments over the study period

Treatment	Number of ponds							
	Once applied	2 times applied	3 times applied	4 times applied	5 times applied	6 times applied	7 times applied	Total
Salt	1	2	6	6	2	3	0	20
Lime	8	16	18	10	5	3	0	60
Salt+lime	4	3	7	8	2	2	0	26
Neem	15	3	3	0	0	0	0	21
Ash	1	5	22	9	10	13	1	61
Fertilizer	0	0	1	1	0	1	0	3
Total	29	29	57	34	19	22	1	191

treatment, best result was obtained from lime treatment followed by ash, salt, salt+lime and neem treatments. Only two (3.3%) of the 60 farmers adopting lime treatment reported ulcerative disease in their ponds while only six (9.8%) of the 61 farmers adopting ash treatment reported the disease. In the case of the other treatments, 10% of salt farmers and 15.4% of salt+lime farmers reported the disease. Neem was the least effective of the treatments, with 19.0% of farmers reporting disease (Table 4).

Species affected

There was a high variability in species combinations and stocking densities in the study ponds as farmers added fish to the ponds according to their resources and the species available. Table 5 shows the species that farmers reported to be affected by ulcerative disease. Most of the species listed are considered to be EUS-susceptible. However in 13 cases, farmers identified fish that are considered to be EUS-resistant (Lilley et al. 1998) as "EUS-positive", i.e. tilapia, common carp, grass carp and silver carp. A common carp sample taken for histology was indeed found to be negative. However, in all cases where farmers identified affected resistant fish, they also recorded affected susceptible fish.

Of the 30 samples taken for histological confirmation of EUS, 70% were found to be EUS-positive by demonstration of invasive hyphae and associated granulomatous response (Table 5). This indicates that most, although not all, of the diseased fish that the farmers had diagnosed as "EUS-positive", were positive according to the pathological definition. The samples comprised of both stocked and wild fish, and were taken from a variety of treatment ponds.

Table 3. Application of neem

	Neem application (no. of stems ha^{-1})
Affected pond (Mean ± SE)	833.63 ± 231.89
Unaffected pond (Mean ± SE)	515.58 ± 75.03
Total pond (Mean ± SE)	576.16 ± 77.21
Neem application range	75 – 1400

Table 4. Preventive effects of the treatments compared to control

Treatment	Total number of pond	Number of affected pond	% affected
Salt	20	2^a	10.0^a
Lime	60	2^a	3.3^a
Salt+lime	26	4^a	15.4^a
Neem	21	4^a	a19.0
Ash	61	6^a	9.8^a
Fertilizer	3	0^a	0^a
Control	42	26	61.9

Figures with superscript show significant difference with control (one-way ANOVA, $p<0.05$)

Table 5. Species affected by ulcerative disease

Treatment	Kishoregonj	
	Cultured species	Wild species
Salt	Catla (*Ca. catla*)	Snakehead (*Ch. punctatus*)
	Rohu (*L. rohita*)*	Striped snakehead (*Ch. striatus*)
	Mrigal (*C. mrigala*) *(1-ve)	Catfish (*My. tengara*) *(1+ve)
	Silver carp (*Hy. molitrix*)*	Stinging catfish (*H. fossilis*)
	Silver barb (*B. gonionotus*) *(1+ve)	Walking catfish (*Cl. batrachus*)
	Gonia (*L. gonia*)	Puti (*P. sophore*)*
	Tilapia (*O. nilotica*)	
Lime	Catla (*Ca. catla*) *(2+ve)	Snakehead (*Ch. punctatus*)
	Rohu (*L. rohita*)*	Striped snakehead (*Ch. striatus*)
	Mrigal (*C. mrigala*) *(3+ve, 3-ve)	Catfish (*My. tengara*)
	Silver carp (*Hy. molitrix*)	Puti (*P. sophore*)
	Silver barb (*B. gonionotus*)	Climbing perch *(An. testudine*us) *(1+ve)
	Gonia (*L. gonia*)	Mola (*A. mola*)
	Common carp (*Cy. carpio*)	Flying barb (*E. danrica*)
	Calbaush (*L. calbasu*) *(1-ve)	Chanda (*Cha. nama*)
Salt+lime	Catla (*Ca. catla*)	Snakehead (*Ch. punctatus*)
	Mrigal (*C. mrigala*) *(1+ve, 1-ve)	Puti (*P. sophore*)*
	Silver barb (*B. gonionotus*)*	Flying barb (*E. danrica*) *(1+ve)
	Rohu (*L. rohita*)	
	Grass carp (*Ct. idella*)	
Neem	Catla (*Ca. catla*)	Snakehead (*Ch. punctatus*)
	Rohu (*L. rohita*)	Puti (*P. sophore*)
	Mrigal (*C. mrigala*)	Climbing perch *(An. testudine*us)
	Silver barb (*B. gonionotus*)	Wallago (*W. attu*)
	Tilapia (*O. nilotica*)	
	Grass carp (*Ct. idella*)	
Ash	Catla (*Ca. catla*)*	Snakehead (*Ch. punctatus*)
	Mrigal (*C. mrigala*) *(1-ve)	Armed spiny eel (*M. armatus*)*
	Silver barb (*B. gonionotus*) *(1+ve)	Striped snakehead (*Ch. striatus*)
	Common carp (*Cy. carpio*) *(1-ve)	Puti (*P. sophore*)
	Rohu (*L. rohita*)	Catfish (*M. vittatus*) *(1+ve)
Fertilizer	Not affected	Not affected
Control	Catla (*Ca. catla*)	Snakehead (*Ch. punctatus*) *(1+ve)
	Rohu (*L. rohita*) *(1+ve)	Puti (*P. sophore*)
	Mrigal (*C. mrigala*) *(4+ve, 1-ve)	Climbing perch (*A. testudineus*) *(2+ve)
	Silver carp (*Hy. molitrix*)	Catfish (*M. tengara*) *(1+ve)
	Silver barb (*B. gonionotus*)	Kholsa (*Co. fasciata*)*
	Gonia (*L. gonia*)	Striped snakehead (*Ch. striatus*)

All fish listed had lesions and were recorded as diseased for study analyses. Samples of fish shown here with asterisks were taken for histological diagnosis. Only a proportion of samples were processed.

-ve Fish sample found to be EUS-negative by histology, the number of samples examined is indicated

+ve Fish sample found to be EUS-positive by histology, the number of samples examined is indicated

Key:

A.= *Amblypharyngodon*, An.= *Anabas*, B.= *Barbodes*, C.= *Cirrhina*, Ca.= *Catla*, Ch.= *Channa*, Cha.= *Chanda*, Cl.= *Clarias*, Co.= *Colisa*, Ct.= *Ctenopharygodon*, Cy.= *Cyprinus*, E.= *Esomus*, H.= *Heteropneustes*, Hy.= *Hypophthalmichthys*, L.= *Labeo*, M.= *Mastacembelus*, My.= *Mystus*, O.= *Oreochromis*, P.= *Puntius*, W.= *Wallago*

Attitude of farmers to treatment results

Among the farmers who adopted one of the treatments, 96% indicated their satisfaction with the results (Table 6). Treatment-wise analysis showed that all the farmers under lime, ash and fertilizer treatments were satisfied while 90% of the farmers adopting salt and neem treatments and 88% of the farmers under salt+lime treatment were satisfied with the treatment results (Table 6). The majority (86.40%) of the satisfied farmers indicated that this was due to "no occurrence of ulcerative disease", although "better fish growth" was another important reason for satisfaction with the treatment reported by 10.33% of the farmers. Some farmers (1.09%) indicated that the treatment helped the fish recover from disease (Table 7).

Other factors influencing occurrence of disease

Other pond variables were recorded to check whether they were associated with the occurrence of ulcerative disease in the experimental ponds. The entry of wild fish in ponds, and the occurrence of ulcerative disease in nearby ponds, were significant risk factors for ulcerative disease in Kishoregonj and Rajshahi districts respectively (Table 8). If the data is recalculated for relative risk (RR), presence of wild fish in the experimental pond almost doubled the likelihood of disease in Kishoregonj ponds (RR=1.74), and presence of ulcerated fish in nearby ponds increased the risk of disease in Rajshahi ponds by over four times (RR=4.29).

Table 6. Satisfaction with treatment results

Treatment	Number of farmers	
	Happy	Unhappy
Salt	18 (90%)	2 (10%)
Lime	60 (100%)	0 (0%)
Salt+lime	23 (88%)	3 (12%)
Neem	19 (90%)	2 (10%)
Ash	61 (100%)	0 (0%)
Fertilizer	3 (100%)	0 (0%)
Total	184 (96%)	7 (4%)

Table 7. Reasons for satisfaction with treatment results

Reasons	Number of farmers	Percentage
No disease occurrence	159	86.41
Better fish growth	19	10.33
Recovered from EUS	2	1.09
Got higher price of fish	1	0.54
Got more production	3	1.63

Discussion

The study showed that EUS remains a common problem, and is an issue of concern to farmers in particular areas of Bangladesh. Khan and Lilley (2002) reported that 50% of 64 ponds sampled from each district of Bangladesh had ulcerative disease, and of these ponds, 94% contained fish diagnosed as EUS-positive. Of the control ponds in the present study, 61.9% had the ulcerative disease which is much higher than any of the preventative treatments (Table

Table 8. Possible confounding factors influencing ulcerative disease occurrence in ponds

Variable	Rajshahi		Kishoregonj	
	Chi square	P value	Chi square	P value
Pond was dried prior to stocking	0.01	0.934	0.02	0.879
Lime was applied prior to stocking	0.46	0.500	1.40	0.237
Pond is connected to external water source	0.06	0.771	1.20	0.273
Wild fishes were removed prior to stocking	0.07	0.797	0.20	0.656
Source of seed (Patilwala, Hatchery or Nursery)	2.92	0.232	0.69	0.709
Fertilization after stocking (Regular, Irregular or Never)	0.33	0.848	2.33	0.312
Supplementary feeding (Regular, Irregular or Never applied)	2.30	0.317	1.85	0.397
Wild fish entered the pond during culture period	0.98	0.321	4.44	0.035*
Net used in fish catching/harvesting (Own net or Borrowed net)	0.04	0.833	0.51	0.477
Pond was flooded in 1998-99	0.03	0.860	0.53	0.466
Cirrhinus mrigala present in the pond	0.03	0.863	1.24	0.265
Barbodes gonionotus present in the pond	0.09	0.769	1.28	0.257
Ulcerative disease present in nearby ponds	5.15	0.023*	1.26	0.261

*Significant difference ($P<0.05$)

4). However, of the 30 samples taken for histological diagnosis, a slightly lower proportion (70%) was found to be EUS-positive.

In this study, farmers who adopted any of the six suggested preventative treatments reported a lower incidence of disease in their ponds than control farmers. The lime treated ponds had the lowest reported incidence of disease (excluding the three fertilizer ponds) (Table 4). Liming is commonly used to stabilize water quality, particularly in areas susceptible to low pH. Khan and Lilley (2002) reported that lime applications, both before and after stocking, reduce the risk of EUS, and Ahmed and Rab (1995) showed that post-stocking applications reduce the severity of EUS. The lime that farmers are able to purchase is variable in terms of chemical composition. Both studies described above noted that farmers used lime that was predominantly calcium carbonate ($CaCO_3$). This has been shown to have little or no anti-fungal activity (Campbell et al. 2001). So its effect is probably in reducing the susceptibility of fish to infection by stabilising water quality.

The ash treatment ponds had the second lowest reported incidence of disease (Table 4). Experimental studies have shown that ash has very little in vitro fungicidal activity on the EUS fungal pathogen Aphanomyces invadans (Campbell et al. 2001). However, it is possible that it may be acting to improve water quality and prevent fish becoming predisposed to infection. Boyd (1990) reported that wood ash has 30-40% value of agricultural lime in terms of stabilising water pH.

The salt treatment ponds had a low (10%) reported incidence of disease (Table 4). Salt at 2 ppt has been shown to inhibit sporulation of the EUS pathogen, A. invadans (Fraser et al. 1992); however it is unlikely that salinity levels in experimental ponds reached these levels. It is probable, therefore, that the salt treatment may have had a greater effect on improving water conditions for the fish, than on treating the fungus, or any opportunistic pathogens.

The salt+lime group had a low (15.4%) reported incidence of disease (Table 4). Combined salt and lime treatments are commonly used as preventative and therapeutic treatments for EUS, but there are no controlled studies that have positively demonstrated a treatment effect.

Neem was one of the least effective treatments, but reports of disease in these ponds were still substantially lower than controls (Table 4). Neem was identified by farmers in India and Pakistan as a treatment for lesions on fish, but it is also used as a piscicide, so care should be taken in its application. In pond trials, fish challenged with *A. invadans* and treated with $50^{-1}25$ ppm dried, ground neem seeds demonstrated a lower incidence of EUS than untreated fish (unpublished data). In addition, Campbell et al. (2001) showed that a commercial seed extract containing 0.27% active ingredient (azadirachtin) inhibited *A. invadans* zoospore motility after 1 hour exposure at 10ppm (100 $kg \cdot ha^{-1}$), but neem leaf extract had little or no effect. Neem leaves are known to have much lower azadirachtin levels than seeds (Schmutterer 1995) but during the present study, it was apparent that farmers would not have the resources to collect and process an adequate number of seeds, and therefore stems and leaves were used. There was a very wide range in the number of stems added to each treatment pond (Table 3). While it is unlikely that the very low application rates used had any effect on the occurrence of disease, Table 3 shows that the neem-treated affected ponds actually had a higher number of stems (833.63±231.89) per hectare than unaffected ponds (515.58 ± 75.03). This suggests that very high application rates would increase the risk of disease. A later study (unpublished data) showed that application of neem stems (each 1.5-2 m high with 4-5 leafy branches) at the rate of 2 stems per decimal (494 stems ha^{-1}) significantly reduced the occurrence of ulcerative disease in ponds, although further work is needed.

Fertilizer was adopted by only three farmers (Table 4), none of whom reported an outbreak of disease during the study period but the low number of ponds in this group reduces the significance of the results. The use of fertilizer was suggested in order to induce production of high levels of algae in the ponds. Lilley (1992) has shown that there are lower fungal counts in pond water containing high levels of phytoplankton, and Khan and Lilley (2002) have shown that there is a lower incidence of EUS in ponds in Bangladesh with green (high phytoplankton) or red (high zooplankton) water. Although this treatment warrants further study, it will not be possible to prescribe a particular type and dose of fertilizer in order to achieve optimal water colour, as fertilization levels should be adjusted according to the development of the algal bloom.

The reported reduction in disease compared to control (Table 4) may be due to management advice given to farmers by project staff during PARG sessions. The high level of satisfaction expressed by farmers (Table 6), particularly with ash and lime treatments, are indicators that uptake of these treatments would be high among the wider population of Rajshahi and Kishoregonj district farmers. The fact that the project did not provide the farmers with any of the treatments helped to ensure that farmer's uptake of the treatment was sustainable, and motivated by a genuine concern for the health of the fish.

It is important to be aware that the high level of satisfaction that the farmers expressed may, in itself, be a cause for bias in the study. Attempts were made during the course of the study to verify information provided by individual farmers, but given the number of farmer participating in the study, it was not possible for the authors to visit all the sites. It is suggested that future studies should provide the farmers with a better means of obtaining the data themselves.

The variability between small-scale carp polyculture ponds in Bangladesh makes it very difficult to undertake a controlled, replicated treatment study. An attempt was made here to investigate possible compounding factors that may influence the occurrence of disease to a greater extent than the adoption of a treatment. Similar to findings by Khan and Lilley (2002), presence of wild fish in the pond, and presence of ulcerated fish in nearby water bodies, increased the risk of outbreaks. It is suggested that more stringent selection criteria should be used in future studies, to ensure that experimental ponds are as similar as possible. It is particularly important that participating farmers adopting a particular treatment apply the same treatment dose and number of treatment applications over the entire study period. If enough farmers participate in the study, it may be possible to exclude data from particular ponds that do not conform to study criteria.

Treatments should be pretested in controlled pond trials to ensure that they are capable of yielding positive benefits for the farmer. In this case, treatments were tested in pond trials in Thailand and Bangladesh and found to reduce the number of snakeheads and mrigal affected by EUS, after challenge with *A. invadans* (unpublished data). The success of the treatments as reported by farmers in the present farm-based study was even greater than was indicated by the previous pond studies. Despite the problems concerned with accurately assessing the impacts of the treatments on ulcerative disease in field situations, it is suggested that farmer participatory research is an important means of enabling and assessing uptake of these treatments by farmers.

Conclusion

The present study is a preliminary attempt to explore some preventative measures for the ulcerative disease, Epizootic Ulcerative Syndrome (EUS) on farm conditions. More research is needed to identify appropriate preventative measures against EUS through continued and modification of the treatments used in the present study. Application frequency and doses of different treatments could be examined further on a number of combinations to identify effective preventative measures.

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