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Research Article

Emergence of the Epizootic Ulcerative Syndrome in Pond Reared Carp Fish (Cyprinids) and It's Control Measure in Chitwan, Central Nepal

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Abstract

A survey on epizootic ulcerative syndrome (EUS) was carried in carp fish (cyprinids) reared in ponds during January 2016 to January 2017 at six locations of Chitwan, Nepal. Outbreaks of EUS were observed in 39.6% of the carp during winter. EUS infection was the most for *Cirrhinus mrigala* (47.3% of the 170 samples) and the least for *Labeo rohita* (12.3% of the 65 samples). EUS infected fish weighing range between 15g-300g were characterized as extensive deep ulceration on head, dorsal and lateral part of body and at caudal peduncle region. Histopathology examination of affected tissue of skin with adjacent muscles showed mycotic granulomas indicated a positive diagnosis for EUS. Survey results indicate that poor water quality in undrained ponds, uncontrolled entry of flooded water into the pond, runoff water from adjacent paddy field and use of contaminated equipment and seine nets were found to trigger risk for EUS infection in carp pond. Impact assessment of EUS showed that average economic loss due to EUS fish disease was 2257.92 kg/ha/year (33.01%) of the expected production. The highest economic loss of 1432.14 kg/ ha/year (53.53%) was estimated for large farm and lowest loss of 950 kg/ha/year (5%) for medium size farm. The simultaneous application of Ciphalexin in feed at 80mg.kg^{-1} biomass of fish for 15 consecutive days and two applications of Kohrsolin-TH at 900 ml/ha (1 m pond depth water) at weekly interval was found effective to control EUS infection in carps. The combined efficacy of these two drugs was estimated to be 97.7% control of EUS infection in carp fish.

Keywords: Cyprinid; EUS; ulceration; granuloma; histology; drugs

Introduction

Aquaculture is often referred as the fastest growing primary production sector in Nepal in the last three decades, having witnessed an annual rate of growth of nearly 11.6 percent (Wagle *et al*., 2011). Demand of fish is very high in comparison to the production level and in general the production is consumed locally. The demand for aquaculture and fisheries to provide increasing fish needs in the country is estimated to be around 155000 tons by 2027 in which aquaculture has envisaged to contribute by 56% and the rest is from capture fisheries (Mishra and Upadhyaya, 2010). Intensification of aquaculture systems

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for warm and coldwater fishes is occurring significantly in the country in order to encompass increasing domestic demand for fish. Sustainable aquaculture production can only occur when fish are healthy and free from disease. In the country, infectious disease Epizootic Ulcerative Syndrome (EUS) is one of the major problem causing loss to fish farmers.

EUS is an infection caused by an oomycete fungi known as *Aphanomyces invadans* or *A. piscicida* has been reported to be one of the most destructive diseases both for farmed and wild fishes of fresh and brackish water origin (OIE, 2006; OIE, 2013) and caused serious losses in many freshwater fish populations in Asia, Australia and the USA since 1970's (Baldock, 2005). It is widely accepted that presumptive diagnosis of EUS is characterized by the presence of ulcerative dermal lesions (Bondad-Reantaso *et al*., 2001). For EUS confirmation is required mycotic granulomas (MG) in a histological section (Lilley *et al*., 1998; OIE, 2003).

EUS has been reported in more than 94 fish species spread across 26 countries (Lilley *et al*., 1998; Andrew *et al*. 2008; Saylor *et al*. 2010; Boys *et al*. 2012; Huchzermeyer *et al* 2012; Oidtmann, 2012). EUS was spread across Asia from Japan and Australia to Pakistan in 1996 and to southern Africa in 2006 (Baldock *et al.*, 2005). The disease spread through several Asian countries including Malaysia, Thailand, China, Vietnam, Lao PDR, Myanmar, Cambodia, Philippines, Sri Lanka, Bangladesh, Hong Kong, India, Bhutan, Nepal, Pakistan and Singapore throughout the eighties (Lilley *et al*. 1998; John and George, 2012).

In Nepal, EUS was reported first time in eastern Terai in February 1989 (Phillips, 1989; Shrestha, 1994). Reoccurring of EUS in the country during 1991 caused 15-20% (average 17.2%) loss of total fish production and economic loss from fish mortalities was estimated at about NRs 30 million (ADB/NACA, 1991). Spread of EUS in fish was reported mostly from flat Terai region and mid hills of central and western Nepal (Phillips, 1989; Dahal, 2002; Dahal, 2003; Dahal *et al*, 2008). A representative study carried out in Kapilvastu district of Western Nepal revealed that the average prevalence of EUS was 6.5%, irrespective of fish species - susceptible or resistant (Dahal *et al*, 2008). Recently, Chitwan district has been reported as EUS emerging site to cyprinids (Baidya and Prasad, 2013). During the course of culture operations, many farmers reported, the occurrence of EUS. The EUS outbreaks took place during winter (January 2016 to January 2017). Therefore, the objective of this study was to assess the emergence and prevalence rate of the epizootic ulcerative syndrome in cyprinid species in Chitwan district and find the effective control measure against EUS.

Materials and Methods

A survey was carried out on the occurrence and prevalence rate of EUS in cultured cyprinid fishes during January 2016 and January 2017 in Chitwan district. Six fish farms scattered in four Village Development Committee (Karaiya=2, Lalparsa=2, Panchkanaya=1 and Khaireni=1) within geographic range between Altitude N 27º34.643'-N27º39.039' and longitude E84º29.193'-E 84º32.047' at 120 msl to 196 msl. GPS coordinates of survey sites were obtained, using a Garmin GPS meter. For the examination of EUS outbreak, fish showing clinical signs of EUS and red spot on the body were caught. Samples of skin with adjacent muscles section including the edge of the lesion and gills were immediately fixed in 10% formalin for histopathological study following OIE procedure (OIE, 2013). Processing and staining (haematoxylin and eosin) of tissue sections was carried out at Animal Health Research Division, Khumaltar, Lalitpur. EUS was confirmed based on examination of stained tissue sections under a light microscope for mycotic granulomas according to OIE guidelines (OIE, 2013).

This study to test the efficacy of Ciphalexin (anhydrous Cephalexin 75 mg/g) and Kohrsolin-TH (100 g contains Glutaraldehyde 10g, 1,6- Dihydroxy 2,5-Dioxahexane Polymethyl Derivatives) against EUS infestation was performed on *Cirrhinus mrigala* and *Labeo rohita* fish. In a trial on drug efficacy two earthen ponds; Trial pond A (Panchkanaya VDC) and B (Khaireni VDC) were used, with 131 and 50 infected fish amongst the other cultivated fish (Table 1). Both trial ponds were received a combination of doses of Ciphalexin (80 mg/kg body weight in feed) for 15 convictive days and two applications of Kohrsolin-TH at 900 ml/ha (1 meter pond depth water) at weekly interval.

As a statistical analysis the efficacy of combination of Ciphalexin and Kohrsolin-TH against EUS was assessed using the equation: Efficacy % = C-T/C x 100; where C is the mean of the EUS infected fish in untreated fish (control group) and T is the mean number of EUS infected fish in treated fish (Wang *et al*., 2009).

A semi-structured questionnaire was used to collect the information related to past history of disease outbreak and realized economic loss due to EUS. A sum 15 respondent participated in the survey. The economic loss due to outbreak of EUS disease was estimated by the differences between the expected production value and the actual production value.

Risk for the potential outbreak of EUS was also assessed through the analysis of respondent's information and the visual observation of general condition of ponds during the field visits. Water temperature ºC, pH, dissolved oxygen mg/L, turbidity (transparency) NTU and conductivity µs/sec were recorded in infected ponds using a Water Verniuer Analogue Instrument (Model Labquest-2) and

alkalinity mg/L, hardness mg/L were recorded using Exact EcoCheck Kit (Digital Water Testing Kit).

Results

Survey and field observations carried out during winter season (January 2016 and January 2017) showed that 39.57 % of the 235 sampled fish (cyprinids) were infected with epizootic ulcerative syndrome (EUS). The outbreak of EUS in sampled cyprinids was evident in all six surveyed locations of Chitwan district. Study indicates the EUS infection was the most for *Cirrhinus mrigala* (47.26% of the 170 samples) and the least for *Labeo rohita* (12.31% of the 65 samples) (Table 1).

Water quality of surveyed ponds varied considerably except water temperature. Water quality parameters during the study period (winter season) were ranged between 17.4-19°C water temperature, 4.5-6 mg/L dissolved oxygen, 6.0-7.1 pH, 20-80 mg/L alkalinity, 24.0-80.0 mg/L hardness, 82.0-97.0 µs/cm conductivity and 90.2-209.2 NTU - turbidity.

Cirrhinus mrigala of sizes 15-300 g and *Labeo rohita* of sizes 15-20 g were appeared affected with extensive deep ulceration on head, dorsal and lateral part of body including at caudal peduncle region (Fig. 1). For EUS confirmation, tissues of affected skin with adjacent muscles of the infected fish were processed. Histopathology examination of affected tissue of skin with adjacent muscles showed mycotic granulomas inflammation indicated a positive diagnosis for EUS (Fig. 2). Fusion of gill lamellae due to hyperplasia (increase in the number of cells) and hypertrophy (increase in the size of cells) was observed (Fig. 3).

Impact assessment of EUS showed that the average economic loss due to EUS fish disease was 2257.92 kg/ ha/year (33.01%) of the expected production. The highest economic loss of 1432.14 kg/ ha/year (53.53%) was estimated for large farm and the lowest loss of 950 kg/ha/year (5%) for medium size farm (Table 2).

Efficacy of drugs combination of Ciphalexin and Kohrsolin-TH was calculated by comparing post-treated EUS infected fish counts with baseline data collected at the time of the clinic treatment (control group) (Table 3). Combination of Ciphalexin and Kohrsolin-TH was found highly effective in reducing EUS infected fish counts from pre treatment levels under field conditions. Based on geometric mean EUS infected cyprinids fish counts, were reduced by 97.7 percent on study day 25 (Table 3**).** This study suggests that combination of Ciphalexin in fish feed and Kohrsolin-TH/ha in pond water were very effective in the control of EUS infesting cyprinids.

Survey results of visual observation and respondent information during the field visits of the six fish farms (Karaiya=2, Lalparsa=2, Panchkanaya=1 and Khaireni=1) showed that poor water quality in undrained ponds, runoff water from adjacent paddy field to pond and the use of contaminated equipment and seine nets were found to trigger the risk for EUS infection in carp pond.

Table 1: Location wise sample size of fish, number of fish infected with epizootic ulcerative syndrome (EUS) and prevalence rate of EUS at surveyed sites (n=6) in Chitwan district

Cyprinid species	Body weight (g)	Karaiya (n=2)	Lalparsa (n=2)	Panchkanaya (n=1)	Khaireni (n=1)	Total (n=6)	Prevalence
Cirrhinus mrigala	15-300	30/30	5/20,	40/121	20/30	85/170	47.26
Labeo rohita	15-20	2/20,	1/15,	3/10,	2/20,	8/65,	12.31
Total		32/50	6/35,	43/131	22/50	93/235	39.57
Percentage		64	17.14	32.82	44	39.57	

*Values in parentheses indicate the number of fish farms surveyed.

Table 2: Average expected and actual production value and economic losses (ha^{-1} Yr^{-1}) in cyprinid fishes due to incidence of EUS outbreak

Farm category (Ha)	Farm category	Expected production (Kg/Ha)	Actual Production (Kg/Ha)	Fish Loss (Kg/Ha)	Economic loss %	Expected value (Rs.)	Actual value (Rs)	Economic loss value (Rs)
<0.5	Small	6521.74	1304.35	5217.39	20	1402174	280434.78	1121739.1
0.5-1	Medium	1000	50	950	5	215000	10750	204250
>1	Large	3071.08	1638.94	1432.14	53.53	660282.2	352373.03	307909.18
Average		3415.97	1158.06	2257.92	33.01	734434.6	248982.71	485451.87
SD		2290.01	766.22	1995.24	25.03	492353	164737.6	428977.07

Table 3: Prevalence rate of EUS disease in control and post treated cyprinids (*Cirrhinus mrigala* and *Labeo rohita*) on oral and pond administration of combination of drugs Ciphalexin and Kohrsolin-TH

Trial ponds	Fish weight (g)	No of infected fish	Ciphalexin (mg/kg fish bw)	Kohrsolin-TH (900 ml/ha (1 m pond depth water)	Prevalence rate	
					(no)	(%)
Trial pond A (control)	15-300	131	0	0	43	32.8
Trial pond A (treated)	15-300	131	80	900	3	2.3
Trial pond B (control)	15-200	50	0	0	22	44
Trial pond B (treated)	15-200	50	80	900	0	0

Fig. 1: EUS infected ulceration on head, dorsal and lateral part of body including at caudal peduncle region of collected cyprinid fish (*Cirrhinus mrigala* and *Labeo rohita*) at farmers field, Chitwan

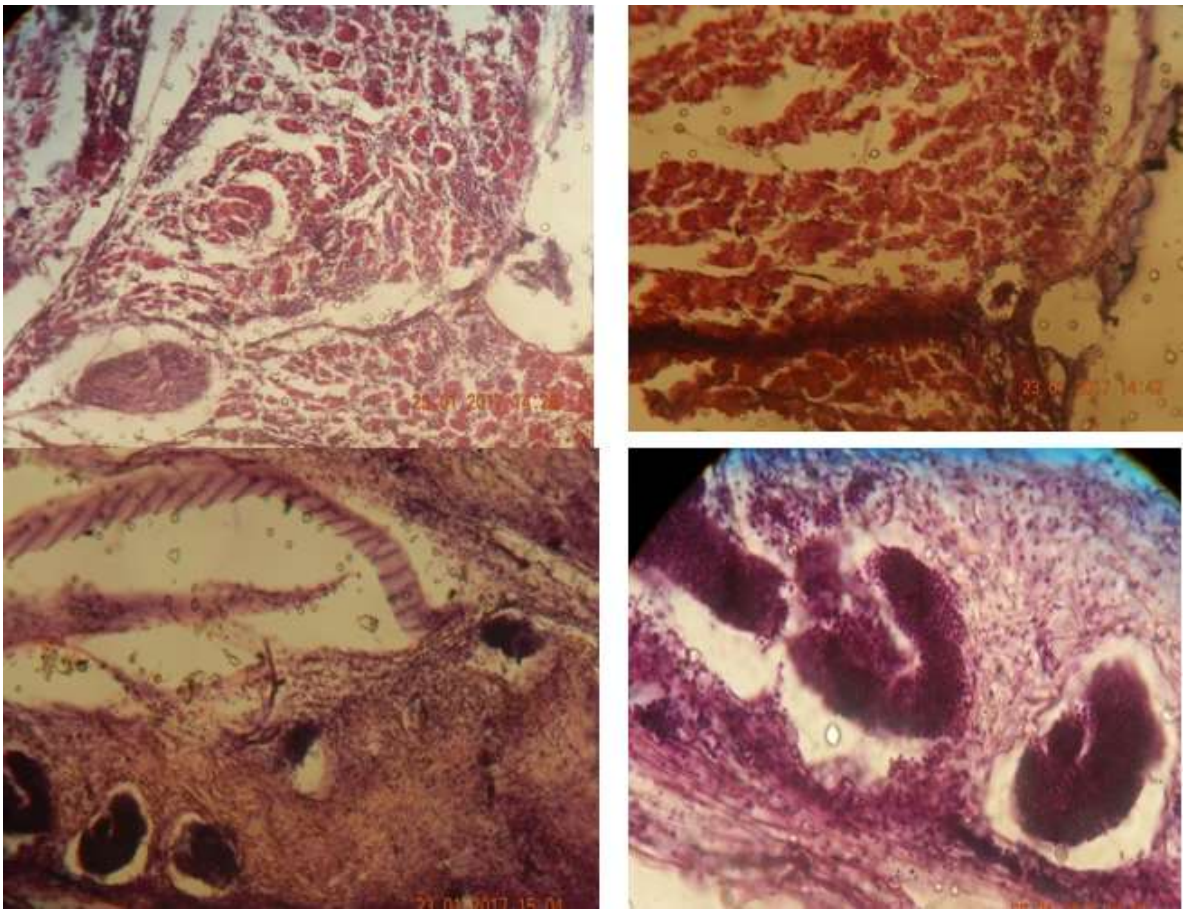

Fig. 2: Histopathology of affected tissue of skin with adjacent muscles of EUS infected fish samples collected from farmers fish pond, Chitwan

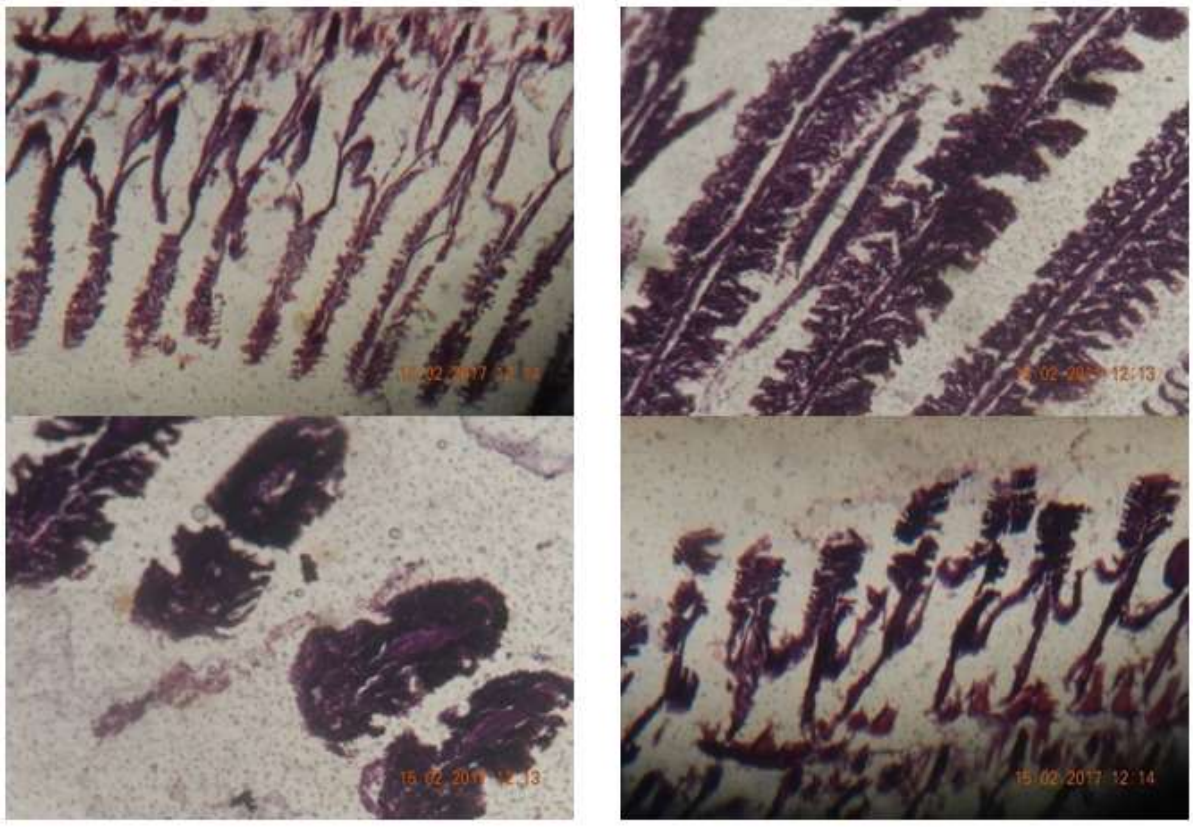

Fig. 3: Fusion between gill lamellae due to hyperplasia and hypertrophy of EUS infected fish

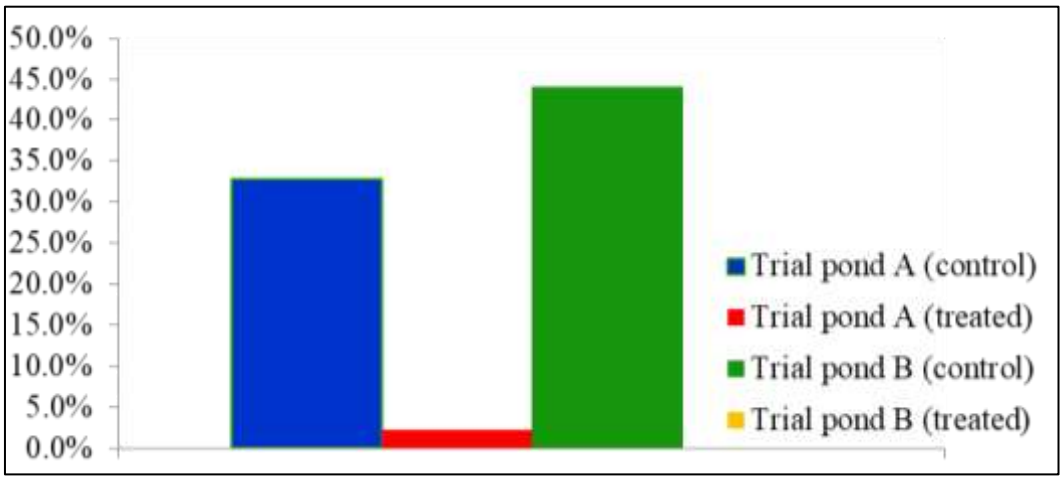

Figure 4: Comparison of EUS prevalence rate on carp fishes before and after treatment with Ciphalexin and Kohrsolin-TH at farmer's pond in Chitwan district

Discussion

The occurrence of EUS caused alarm among the fish farmers, consumers and governments (Shariff, 1998). Outbreaks of epizootic ulcerative syndrome (EUS) have become a common phenomenon both in high density commercial and less intensive farm aquaculture in Asia, adding an element of risk to investment (Ahmed and Rab, 1995). The economic impact of fish lost cause of disease outbreaks has been severe in recent years in Bangladesh, Thailand and Philippines (Liley *et al*., 1992). Losses due to EUS are difficult to estimate because of its widespread occurrence in many countries and inadequacy of record keeping. Thailand indicated losses in catfish and snakehead culture amounting to US$8.7 million in 1982-1983 outbreaks (Chinabut, 1994). In Bangladesh, the losses in fish mortality amounted to about US$5.6 million (ADB/NACA, 1991). A number of comprehensive reviews on economic loss and impact aspect of EUS were reported (Roberts *et al*., 1993; Noga, 1993; Chinabut, *et al*., 1995; Roberts, 1997; Chinabut, 1998; Lilley *et al*. 1998), besides EUS has listed by the Network of Aquaculture Centers in Asia-pacific (NACA) and internationally by the World Organization for Animal Health (Office International des Epizooties or OIE) (AFFA, 2000; FAO, 2009; Pradhan *et al*., 2014).

EUS in Nepal is an invasive epidemic threat in mid hill to terai region of Nepal and in current study the epizootic ulcerative syndrome was confirmed by the presence of MG in tissues of affected skin with adjacent muscles of *Cirrhinus mrigala* and *Labeo rohita* collected from farmers pond in Chitwan district using the OIE-recommended diagnostic tests (histopathology of infected tissue). During the present study has achieved promising drugs with a combination of Ciphalexin and Kohrsolin-TH to control epidemic of EUS (removal of EUS 97.9%) in cultured carp fish. The application of above revealed drugs has not previously reported in treating fish against EUS. Though, attempts at using ash, turmeric, neem (*Azadirachta*) seeds, dried banana leaves, CIFAX (an Indian propriety product), for prophylactic and therapeutic treatments of the EUS infected fish have shown variable results (Campbell *et al*., 2001). Water quality variable monitored at the time of fish sampling were not below acceptable trigger values for aquatic ecosystems. Though temperature is a critical factor determining the severity of EUS outbreaks and most mortalities occur when water temperatures are relatively low (Chinabut *et al*., 1995). Low water temperatures (<16^{o}C) and rapid decreases in temperature are immunosuppressive and induce changes to the epidermis including loss of mucus that induce changes to the epidermis, including loss of mucus that predispose fish to infection (Bly and Clem, 1992; Quiniou *et al*., 1998).

As a final note, management of such fish disease should be given top priority to save the freshwater aquaculture industry from huge economic loss including further epidemiological studies are required to get an insight into the role of various environment risk factors responsible for EUS. A holistic approach is necessary to understand the association of water quality parameters, pond dynamics and the outbreak of EUS disease.

Conclusion

MG identified EUS as the cause of the recent epizootic ulcerative syndrome in cultured cyprinids at farmers field at Chitwan. EUS infection was the most susceptible for *Cirrhinus mrigala* (47.3% of the 170 samples) and the least for *Labeo rohita* (12.3% of the 65 samples). The combined efficacy of application of Ciphalexin in fish feed and Kohrsolin-TH in water was found effective to control EUS infection in pond reared carps

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