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FISH BEHAVIOR AND APPEARANCE ARE KEY INDICATORS FOR EARLY EUS DISEASE DETECTION

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ABSTRACT:
Fish disease is a substantial cause of monetary loss to aquaculturists. In nature we are less aware of fish disease problems. Biological infectious agent is the primary cause of EUS. Epizootic Ulcerative Syndrome (EUS) causes severe ulceration and heavy mortalities of fish in the wild & culture ponds. Daily observation of fish behavior & feeding activity helps early detection of problems. In the present study fish behavior & appearance of the control, naturally affected & artificially infected fishes after 7, 14 & 21 days of Challenge infection was observed. Naturally affected fishes, exhibited sluggish movement, abnormal feeding, occasionally visiting to surface of water for oxygen along with red ring at several places on the body. Artificially EUS infected fishes after 7 days, also showed sluggish movement, abnormal feeding, normally stretched fins and red spots on body. After 14 days they mostly remained at the bottom, and seen coming to the surface after a long time, slow beating of the operculum, ate less food, showed vertical–diagonal movements and red spot with circular ring & collapsed fins. After 21 days they remained at the bottom in the corner of the aquaria, stopped feeding, the skin was found to be necrosed & showed some mycelia, scales were lost from the ulcerated area of the body, as compared to the normal behavior of control fishes.

Keywords: - Behaviour, Disease, EUS and Fishes.

INTRODUCTION:

Due to outbreak of fish disease, there is tremendous loss of the investment, cost of treatment and also loss of harvest, increasing the production cost. In nature we are less aware of fish disease problems because sick animals are quickly removed from the population by predators. In addition, fish are much less crowded in natural systems than in captivity.

Parasites and bacteria may be of minimal significance under natural conditions, but can cause substantial problems when animals are overpopulated causing stress under culture conditions. Daily observation of fish behaviour and feeding activity helps early detection of problems when they do occur so that a diagnosis can be made before the majority of the population becomes sick. Fishes are inevitably subjected to various kinds of stresses in farm that may lead to down regulation to immunity and as a result the outbreak of infectious diseases may occur (Prasad and Verma, 2004).

Epizootic Ulcerative Syndrome (EUS) causes severe ulceration and heavy mortalities of fish in the wild and culture ponds. This disease effected juveniles as well as adults of many species of both fresh water and brackish water fish and the extent of damage is very severe in some species. The quality of edible fish is severely reduced because the primary choice of fish lies in its protein and other constituents, which reduce considerably under EUS affected fishes. Gupta and Gupta (1981); Krishna and Rao (1982); Gur'yanova (1987); Pradhan and Pal (1990); Krishna (1993);Roberts *et al.*,(1993); Krishna (2002) have published their reports on the host biochemistry, comparison of diseased fishes and healthy fishes.

In Channa striatus species, EUS is a complex infectious condition that results in necrotic ulcerative lesions and granulomatous reactions, contributing to significant mortality. This syndrome has caused the death of approximately 92 fish species recorded in the

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wild and in commercial aquaculture systems worldwide Podeti Koteshwar Rao (2024).

The causative agent of the dreadful disease Epizootic Ulcerative Syndrome (EUS) has been baffling the scientists, since last 3 decades. It is widely suspected that a biological infectious agent is the primary cause of EUS and certain abiotic factors are responsible for creating stress to fish .The suspected biological agents are viral, bacterial, fungal and other animal parasites.

MATERIALS AND METHODS:

The present study was carried out by collecting the suspected diseased fishes from nearby EUS prone water bodies (Wadali and Malkhed lakes) with the help of fishermen and were brought to the laboratory and maintained in glass aquaria for a week-to study the type of infection. General survey of fish market was also carried out simultaneously every week in the light of infection.

Daily Examination: The healthy and diseased fishes brought to the laboratory were kept in separate aquaria having lake water and were fed and monitored daily, till acclimatization. Aquaria were monitored at least twice a day and dead and moribund fish were removed each time. The fishes with lesions were photographed and were further used for bacterial, fungal and viral examinations.

The healthy fishes weighing about 25 gm and of approximately equal length were brought to the laboratory and kept in aquarium containing 75 litre of water and were acclimatized for 15 days. They were regularly fed on pellet feed and 25% water of the aquarium was also regularly changed at an interval of 7 days to maintain the water quality. 10 fishes were kept in one aquarium each.

Experimental set up: -

The experiment was carried out on six sets of 10 fishes in each group as below:

Group I: Consisting of control (Healthy) fish (10, *Channa punctatus*).

Group II:- Consisting of Naturally EUS affected fish (10, *Channas punctatus*).

Group III:- Consisting of artificially infected fishes injected with 0.2 ml homogenate of ulcerated skin tissue from diseased *Channa punctatus* (10, *Channa punctatus*).

Group IV:- Consisting of control (Healthy) fish (10, *Clarias batrachus*).

Group V:-Consisting of Naturally EUS affected fish (10, *Clarias batrachus*).

Group VI:- Consisting of artificially infected fishes injected with 0.2 ml homogenate of ulcerated skin tissue from diseased *Clarias batrachus* (10, *Clarias batrachus*.

Behavioural study: The fishes in the aquaria were observed with respect to their movements in aquaria and the observations are given in (Table, 1).

Suspension preparation: The tissue suspension of ulcerated skin tissue was prepared in sterile fish saline (0.3%). A tissue (500 mg) homogenate was prepared by taking affected or ulcerated skin in fish saline (5ml).

Challenge infection:

Tissue suspensions of naturally EUS affected fishes were inoculated to confirm the presence of bacteria and fungi. Then 0.2 ml of this positive suspension was injected intraperitonially immediately below the skin on lateral sides of the healthy fish (Group III) and they were again released in the aquaria. The experimental fishes were sacrificed at the interval of 7 days, 14 days and 21 days for various studies. No mortality was observed during these 21 days.

OBSERVATIONS AND RESULTS:

To know the aetiology of Epizootic Ulcerative Syndrome (EUS) in fishes, a year survey of local market was carried out and it was found that the bottom dwelling fishes, *Channa punctatus* and *Clarias batrachus* were mostly EUS affected and they were brought by the fishermen to the market from two lakes, Wadali and Malkhed.

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Fish behaviour and appearance:

The swimming behaviour of the control,naturally affected and artificially infected fishes was observed and is as given in Table, 1.

Naturally EUS affected fishes when brought in the laboratory, exhibited abnormal swimming behaviour in the aquaria. They kept themselves swimming rapidly and the other time they remained in upright position at one place with mouth open.

After two three days of acclimatization they became lethargic and found staying at the bottom of aquaria with all fins collapsed. They were feeding abnormally and mostly avoiding feeding. Occassionally they used to come to surface slowly for gulping of air.

The infected *Clarias* were comparatively more sluggish than the naturally affected *Channa*. *Clarias* were found most of the time at the bottom of aquaria particularly on long sides of the aquaria instead of corners. After 10 days, the fishes appeared pale in colour. The behaviour of artificially EUS infected fishes after 7, 14 and 21 days of challenge infection is given in table, 1.

External features shown by naturally and artificially EUS infected fishes:-

The naturally affected fishes brought to the laboratory were with red spot (stage-I). After 10-12 days most of the fishes exhibited a red ring around it (stage-II) and there after the area within the ring became descaled and necrosed with puffy appearance (stage-III). Such fishes almost used to remain in the middle column of the aquaria with all their fins collapsed (Table, 2). These fishes after 30 days found to develop ulcers at several places on the body. The artificially EUS infected fishes exhibited different behavioural changes in stages (Table, 1). A red spot was developed after 7 days of challenge inoculation and later on after 12-14 days a red ring was seen with loss of scales. Then after 20-24 days, the tissue within the ring was totally degraded with muscles exposed showing puffy appearance (Fig:1 to 10).

Gross appearance of EUS affected fishes:

Gross and histopathological characterization of EUS in *Channa punctatus* and *Clarias batrachus* in the present investigation are based on 36 individuals which were artificially infected. Non-infected control fish exhibited intact skin with well formed mucus coat all over of the body surface and intact well spread fins (Fig: 1 and 2). Gross clinical signs of EUS infected *Channa* and *Clarias* were restricted to changes over the skin. There was widespread loss of the mucus coat when the fishes were removed from aquaria, rapid drying of the skin with dulling of body surface was seen. Additionally, mild diffusion, hyperemia and petechial haemorrhage, usually on the lateral body surface or at the base of the tail region was observed in all the infected fishes. They also exhibited disruption of the epidermal layer with extensive loss of scales (Fig: 3 and 4). The lesions progressed from small (0.5 to1cm) dermal ulcers with associated haemorrhaging to large necrotic ulcers, with fungal growth penetrating deep into musculature. They showed single to multiple ulcers anywhere on the body.

In artificially infected fishes, grossly, a single necrotic lesions occurred at the administration site of each fish after initial reddening. Sloughing off of necrotic tissues / scales occurred after 14 days of challenge infection (Fig: 5 to 10).

CONCLUSIONS:

A general survey of fish market at Amravati was carried out to know the intensity of EUS affected fishes coming in the market. It was observed that the fisherman brought the EUS affected fishes from nearby lakes Wadali and Malkhed. All EUS affected fishes exhibited three distinct stages during the EUS infection.

Stage – I - Red spot is seen

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Stage – II - Red ring with a white patch in the centre

Stage – III - The area of red ring became necrosed with puffy appearence with ulcer and haemorrhage. Multiple ulcers were seen on the body in advanced stage.

The healthy fishes were administered with the ulcerated tissue from the EUS affected fish and were studied after 7, 14 and 21 days of challenge infection. These fishes also showed the three stages of the disease.

Richards and Pickering (1979) also reported severe hypoproteinaemia in *Saprolegnia* infection of brown trout from spawing streams.This loss of protein may be associated with loss of protein from the extravascular fluid at sites of fungal damage (Hargens *et al.,* 1974) and may explain the rapidity with which the fungal-infected fish die. Large number of scientists are working on different aspects of EUS and its causative agents, but today also there is no concrete answer to it. Miller (1994); Kanchanakhan (1996 and 1997); Lio-Po *et al.,* (2000) suggested viral origin. Pradhan and Pal (1990) and Shariff and Subhasinghe (1992) opined bacterial origin of EUS and Roberts *et al* ., 1994) suggested fungal origin of EUS. The present results indicate the appearence of red spot at the onset of EUS with infection.

Epizootic Ulcerative Syndrome (EUS) is an ulcerative symptom of a destructive disease that attacks freshwater fish in brackish water.This disease is caused by fungal pathogens attached to the fish (Malik *et al.*,2017). Chakravorty, 2015 stated that the initial symptoms of the disease are marked by the presence of red dots on the fish's body, then within a few days, the fish infected with EUS will lose their scales and show muscles in fish, then the fish will die within a few days, also spreading the disease to the other fish in the same pool. Rachman etal (2023).

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.FAO. 2009.

Snieszko (1974) stated that an overt infectious disease occurs when a susceptible host is exposed to virulent pathogen under stress. Fishes are inevitably subjected to various kinds of stresses in farm that may lead to down regulation to immunity and as a result the outbreak of infectious diseases may occur (Prasad and Verma, 2004).

In India, traditional aquaculture has in recent years turned into a science based economic and commercial activity involving heavy inputs and therefore diseases of all kinds are now known to occur on an increasingly larger scale. Since last decade there is an increased realization of the necessity to avoid infectious agents to maintain healthy stocks to enhance productivity (Mukherjee, 2002).

Epizootic Ulcerative Syndrome (EUS) causes severe ulceration and heavy mortalities of fish in the wild and culture ponds. From an aquaculture point of view, the greatest concern is the rapid multiplication of the pathogen within the host and the danger of transfer to other individuals of the host population, which may result in an uncontrollable epizootic forms.

EUS has thus, a potential to financially decimate those who rely on fishing for income. In addition and perhaps more importantly, EUS outbreaks have threaten food security and subsequently physical health, of all those who are dependent on fish, as fish is an important source of animal protein for people in many countries of South and South-East Asia. EUS also has the potential to pose a threat to aquatic biodiversity leading to irreversible ecological damage.

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Table: 1 Behavioural aspects of Control, Naturally EUS affected and Artificially EUS infected fishes.

Control	Naturally EUS affected Fish	Artificially EUS infected fishes		
		7 days	14 days	21 days
Normal feeding habitat. Regularly coming to water surface for gulping of air. Swimming movements normal. Opercula beating and fin movement uniform.	Movement . Red ring, having whitish puffy appearance in the center.This appearance is seen at several places on the body.	Occasionally coming to surface of water for oxygen. Movements were not normal. Red spot was seen. Fins appeared with normal stretch but reddish in colour.	Remained at the bottom most of the time and seen coming to the surface of water after a long time. Beating of the operculum was considerably slowed down, Ate less food. Showed vertical – diagonal movements. Red spot with circular ring was seen at the point of administration of homogenate. Fins were collapsed.	Found resting at the bottom in the corner of the aquaria. Feeding was stopped. In the center of circular ring the skin was found to be necrosed and showed some mycelia. Scales were lost from the ulcerated area of the body .In some fishes haemorrhage was observed. Appeared pale in colour. Pectoral,dorsal as well as caudal fins were collapsed.

Table: 2, External features shown by naturally EUS affected and artificially EUS infected fishes (*Channa punctatus* and *Clarias batrachus*).

Stage I	Stage II
Red spots on the body surface were seen.No discolouration as such on the skin surface or on the skin around the red spot was seen.	The Red spots turned into lesions which were around 1 cm in diameter,They appeared to be raised and circular in shape and the body surface area showed discolouration. At this stage the shin was intact and the scales were loosely attached. **Stage III** The lesions formed showed the advanced stage of necrosis .They appeared to be circular extending deep into the skeletal musculature. Large haemarragic and necrotic ulcers were formed along with new emerging red spots seen on various parts of the body. At this stage the epidermis and scales were completely lost along with loss of dermis at the site of ulcer.

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Fig.1, Control healthy *Channa puctatus*

Fig.2, Control healthy *Clarias batracus*

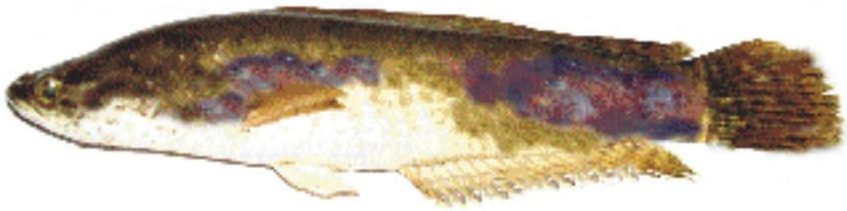

Fig.3, Naturally affected *Channa puctatus*

Fig.4, Naturally affected *Clarias batracus*

Fig.5, *Channa puctatus* showing formation of red spot after 7 days

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Fig.6, *Channa puctatus* showing formation of red spot after 14 days

Fig.7, *Channa puctatus* showing ulcer formation after 21days

Fig.8, *Clarias batracus* showing red spot formatio after 7 days

Fig.9, *Clarias batracus* showing formation of red ring after 14 days

Fig.10, *Clarias batracus* showing ulcer formation 21 days

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