

Investigation of common diseases on cage reared Nile tilapia (*Oreochromis niloticus*) in Bakergonj, Barishal, Bangladesh

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Abstract. The present study was conducted to investigate common diseases from cage reared tilapia farms in Bakergonj for a duration of 10 months from August 2017 to May 2018. Fingerlings of monosex Nile tilapia with an average weight of 94.1 ± 5.66 g (mean \pm SD) were stocked in 10 floating net cages each having an area of $6.09 \text{ m} \times 3.048 \text{ m} \times 1.83 \text{ m}$ (L \times W \times H) at the densities of 24 fish/m³. Physical parameters of water (Temperature, DO and pH) were measured at the sampling spot and chemical parameters of water (nitrate, phosphate and ammonia) were analyzed in the laboratory. Both water and diseased fish were collected monthly to identify fish diseases. The ranges were: water temperature 20.4°C to 31.3°C, dissolved oxygen 5.9 to 6.7 mg/l, pH 7.1 to 8.1, phosphate 0.023 to 0.04 mg/l, ammonia 0 to 0.13 mg/l and nitrate 0 to 10.63 mg/l. In the month of September and October, nitrate concentration was high and that might be due to discharging of slaughter wastage substances in water at time of Eid-al-Adha festival. Seven genus of parasites- *Chilodonella* sp., *Trichodina* sp., *Gyrodactylus* sp., *Capillaria* sp., *Orientocreadium* sp., *Eustrongylides* sp. and *Allocreadium* sp. were identified and occurrence of relevant diseases caused by these parasites were also identified during the study period. *Trichodiniasis*, *Chilodonellosis* were main parasitic diseases. Total count of bacteria was determined and a total of five species of bacteria- *V. cholerae*, *V. parahaemolyticus* on TCBS agar, *E. aerogenes*, *S. enteritidis* and *E. coli* on VRB media were identified. Total bacterial load was determined $2.78 \pm 0.14 \times 10^5$ cfu/ml and $4.26 \pm 1.21 \times 10^6$ cfu/g in water and tilapia respectively. Enterococcosis was the main bacterial disease.

Keywords: Nile tilapia, Water quality, Parasites, Diseases

Introduction

The Nile tilapia, *Oreochromis niloticus* is currently considered to be the most important and commonly cultured species around the world and constitutes over 70% of cultured tilapia (Fitzsimmons 2004) which represent approximately 8% of total farmed fish production (FAO 2018). This fish species can also grow rapidly, require minimum oxygen, can tolerate wide range of temperature, are resistant to disease and has high yielding performance (Hussain *et al.* 1989, Shamsuddin *et al.* 2012, Ahmed *et al.* 2013). Cage culture can be practiced in any type of water body such as ponds, lakes, rivers, canals, *haors*, *baors*, *beels*, estuaries and coastal region. For cage culture low water body can be used at a minimum range and low productivity because of totally artificial feed are supplied during the culture period (Baveridge 1984). Cage aquaculture has some certain advantages over other aquaculture systems. Growing fish in existing unutilized or underutilized ponds or open water bodies like rivers and floodplains, ease of feeding, ease of stocking and harvesting, less expense associated with treating or preventing disease, easier stock management and monitoring compared with pond culture are among major beneficial point of cage culture (Mondal *et al.* 2010).

Poor water quality significantly increased the presence of various pathogenic bacteria in fish and leading to disease outbreaks (Eldar *et al.* 1995). There were financial losses of

approximately 15% of the actual production to rural fish farmers of Bangladesh due to fish diseases. This loss varied among different geographic locations of the country and mostly due to poor understanding of the farmers on disease diagnosis and health management (Faruk *et al.* 2004). Prevalence of fish disease has negative economic impact on aquaculture. The cost of disease prevention and treatment is 5 to 5.5% of total production cost (Khoi *et al.* 2008). Such loss affects the livelihood of people involved in aquaculture and the community in which they occur through reduced food availability and loss of income and employment, as well as other associated social consequences (Subashinghe *et al.* 2001). In Bangladesh, fishes have been suffering from many types of diseases such as ulcer type disease including epizootic ulcerative syndrome (EUS), bacterial haemorrhagic septicaemia, tail and fin rot, bacterial gill rot, dropsy, columnaris disease, various types of fungal and parasitic diseases (Chowdhury 1997, Faruk *et al.* 2004).

In fish, the most obvious external clinical signs are inflammation, erythema and haemorrhage of fins, skin or head, frayed or eroded fins, haemorrhaged opaque eye, open necrotic and ulcerative lesions at any location on the body, lepidorthosis of scales, and excessive mucus production. (Plumb 1994). Clinical signs and histopathology are important tools in diagnosing fish diseases. Very little works have been done on investigation of common diseases in cage reared Nile tilapia in Bangladesh and none in Barishal. Therefore, it is essential to investigate the common diseases of tilapia in cage farming in Barishal. Considering the fact, the present experiment was undertaken in net cage to examine tilapia fish disease outbreak through clinical and laboratory diagnosis.

Materials and Methods

Study area: The study area was located in Bakerganj Upazilla under Barishal district. Main Rivers are Tentulia, Barisal, Rangamatia, Khairabad, Karkhana, Char Amaddi, Pando, Pandab, and Bishkhali (Baglapedia 2015).

Cage construction and installation: Ten floating net cages each having an area of 6.09 m × 3.048 m × 1.83 m (Length × width × height) made of synthetic nylon net were installed in the river. Each net cage was tied and hanged with bamboo pole frame and covered at the top with another piece of nylon net to prevent escape of fish by jumping and bird predation. There was a small window on the top of the cage through which the fishes could be captured for sampling and the dead fish could be removed. Empty vacuum plastic drums of oil and acid were used as cage float for buoyancy of cage structure. The whole cage structure was tied with anchors by nylon rope to facilitate easy floating and moving of whole cage structure with 10 individual cages depending on water level.

Fingerlings stocking and feeding: Juvenile tilapia, initial body weight 80-100 g werestocked on 01 September, 2017at the density of 24 fingerlings/m³. The fishes were fed with floating pelleted feed (Aftab fish feed, grower, 28.10% protein) twice daily; half of the ration was given in the morning at around 8:00 am and another half in the afternoon at around 4:00 pm. Feed was applied slowly through sprinkle method on the top of the cage at the rate of 3% of body weight of stocked fish and adjusted to 2.5% and 2% towards the end of the culture period. Sampling was done monthly to adjust the amount of feed to be administered.

Study of physico-chemical parameters of water: During the study period physico-chemical parameters of water were measured monthly. Water temperature was recorded in the investigation area with the help of a portable LCD Multi-Thermometer (Model: WT-2 Jiangsu, China). Dissolved oxygen was determined by a digital DO meter (HANNA instruments, model: Lutron, DO-5509, Taipei, Taiwan). pH of water was recorded by a digital pH meter (HANNA instruments, model: HI 98107 Resita, Romania). The concentration of phosphate, nitrate and ammonia were measured in the laboratory by a spectrophotometer (Lovibond® PCspectro, model RS232, Dortmund, Germany) using a vario phosphate RGT F101 powder pillows, vario nitrate chromotropic powder pillows and 2 types ammonia tablets, respectively.

Behavioral and Clinical observation or inspection of fish: Prior to sampling of fish, fish behavior was observed carefully. The clinical signs observed included loss of appetite, listlessness, gulping at the water surface, body rubbing, loss of balance, position in water column, swimming with head up, swimming with head down etc. During each visit to the cage farms, some fish from each cage were caught using scoop net, examined for external signs and injury, infection and other abnormal conditions were recorded if present.

Investigation of fish for parasites and bacteriological study: Skin, gill, fins, eyes and scales of infected fish specimens were examined under the microscope (Optica microscopes, Italy) carefully and photographed the microscopic image. Proper attention was made during the observation of internal organs like liver, heart, kidney, spleen and intestine. Bacteriological study was conducted by following standard plate count (SPC) method (AOAC 2007). Standard plate count expressed as colony forming units per gram (cfu/g) was determined by using consecutive decimal dilution technique using spread plate method. One milliliter of each homogenate (fish and water stock solution) was transferred with a micropipette to test tube containing 9 ml of physiological salinein order to get 10⁻¹ dilution of original sample solution. Using the similar process several dilutions of 10⁻² to 10⁻⁵ was made for fish and water samples. Aliquots of 0.1 ml of the serial dilutions was pipetted out and transferred aseptically to the agar plates in triplicate using the spread plate method. The sample was then spread homogenously and carefully by sterile flamed L-shaped glass rod. The plates were incubated at 35°C for 48 hours in an inverted position. After 48 hours of incubation colonies observed on the media were counted. Plates containing 30-300 colonies were used to calculate bacterial load results, recorded as cfu per unit of sample by using following formula:

For water sample: Cf/ml = No. of colonies in Petridis × 10 × dilution factor

For fish sample:

$$\text{Cfu/g} = \frac{\text{No. of colonies in Petridis} \times 10 \times \text{dilution factor} \times \text{volume of total stock solution}}{\text{Wt. of fish sample (g)}}$$

Identification of bacteria: Bacteria were detected according to (Downes and Ito 2001, MacFaddin 1985) based on the colour of colonies and morphological characteristics of the colonies formed by specific bacteria on selective media. Statistical analysis was done by using Microsoft Excel Software 2010. Collected data were coded and entered into a data base system and analyzed.

Results and Discussion

Physico-chemical parameters of water: The physico-chemical parameters such as water temperature (°C), dissolved oxygen (mg/l), pH, phosphate (mg/l), nitrate (mg/l) and ammonia (mg/l) during the experimental period are shown in Table I.

The highest temperature was measured in the month of April (31.3°C) and the lowest temperature was recorded in the month of January (20.4°C) with a mean of $25.72 \pm 4.64^\circ\text{C}$ during the study period (Table I). Ideally, water temperature surrounding tilapia in production should be between 26°C and 28°C and within optimum range of about 23°C to 30°C. However, *O. niloticus* shows optimum food consumption and growth at temperatures ranging between 31 to 36°C (Mires 1995). FAO (1981) and DoF (2009) observed that the optimum temperature for aquatic production were 23 to 31°C and 25 to 30°C, respectively which is more or less similar to the findings of the present study. The highest value 6.7 (mg/l) of dissolved oxygen was recorded in the month of September and the lowest value 5.9 (mg/l) was recorded in the month of February. The mean value of dissolved oxygen was 6.3 ± 0.26 mg/l (Table I). For optimal fish growth, DO levels should be above 5 mg/l for warm water fish species (Boyd 1982). A sub-optimal level is very stressful for fish. DoF (1996) reported that the range of dissolved oxygen suitable for fish culture would be 5.0 to 8.0 mg/l. In the present experiment, concentration of DO in all the cages ranged between 5.9 to 6.7 mg/l which was within the favorable ranges for cage culture of tilapia. The highest pH value was determined 8.1 in the month of September and the lowest value 7.1 in the month of February with a mean of 7.6 ± 0.41 (Table I). Mean value of pH for caged tilapia seem to grow best in water that is near neutral or slightly alkaline. Lethal limit for high pH is 11 to 12 and *O. niloticus* can tolerate low pH to approximately 5 however best growth rates are achieved between 7 to 9 (Ross, 2000). But it is considered that pH value from 6.5 to 8.5 is the most suitable condition for fish culture and production. The present pH value is more or less similar to Singh (2015), Haque (2007), Kohinoor *et al.* (1999) who found the similar condition that ranged vary from 7.3 to 8.4, 6.80 to 8.27 and 7.1 to 8.3, respectively.

The highest concentration of phosphate was recorded 0.04 mg/l in the month of October and the lowest was 0.023 mg/l in the month of March. The mean value of phosphate was 0.03 ± 0.006 mg/l (Table I). Phosphate has a great negative impact on whole water body. The high concentration of phosphate produce toxicity in the water body. The range of phosphate during the study time was lower than the findings of Abedin *et al.* (2017) and Ahsan *et al.* (2009) who recorded 0.10 to 2.0 mg/l and 0.05 to 2.6 mg/l phosphate, respectively. The mean of phosphate value recorded by Ansari *et al.* (2015) was 0.257 that was higher than the values obtained in the present study. The highest value 0.13 mg/l of ammonia was recorded in September and the concentration of ammonia was zero in the month of January. The mean value of ammonia concentration was 0.05 ± 0.04 mg/l (Table I). High amount of ammonia produce toxicity in the water body and water quality deteriorated. Optimum concentrations of ammonia are estimated to be below 0.05 mg/l of caged tilapia (El-Sherif *et al.* 2008). The range of ammonia during the study time was 0 to 0.13 mg/l which was more or less similar to Rahman

(2005) and Ahmed *et al.* (2014) who found 0.01 to 0.82 mg/l and 0 to 0.14 mg/l during their investigation time. The mean value of nitrate was 2.39 ± 4.46 mg/l. The highest value was recorded 10.63 mg/l in the month of October and the concentration of nitrate was zero in the month of November, December and January (Table I). The range of nitrate was high in first two months of culture period. Nitrate produces toxicity in the water body. As a result, bacterial infection spreads over the culture system. The finding of present study was similar to the finding of Gorlach-Lira *et al.* (2013) who recorded physico-chemical parameters of the water, nutrients and presence of toxic compounds may influence the density of bacterial populations. It was identified that slaughter wastage is also account for mortality of tilapia in cages. During the present study, diseases spread frequently due to discharging of slaughter wastage substances in water at the time of Eid-al-Adha festival and the water quality deteriorated within a short time. As a result, bacterial infection outbreaks over all tilapia cage farming. The collecting information is almost similar to Escher *et al.* (1999) and who noted that the poor and non-optimum water quality may have induced weakness and stress to the fish, resulting in a greater susceptibility to bacterial infections.

Identified parasites: During the study period, seven species of parasites were identified in fish samples as shown in Table II. The parasites were found in gill, intestine and body surface.

Table II. List of different types of identified parasites

Site of infection	Category	Parasites
Body surface	Ectoparasites	<i>Chilodonella</i> sp.
		<i>Trichodina</i> sp.
Gills	Ectoparasites	<i>Gyrodactylus</i> sp.
		<i>Chilodonella</i> sp.
Intestine	Endoparasites	<i>Capillaria</i> sp.
		<i>Orientocreadium</i> sp.
		<i>Eustrongylidae</i> sp.
		<i>Allocreadium</i> sp.

The ectoparasite *Trichodina* sp. were found in the body surface and *Chilodonella* sp. found in fish gill. Walakira *et al.* (2014) also found in most of tilapia and catfish samples examined had incidences of ciliated protozoans, *Trichodina* sp. and *Ichthyobodo* sp. mainly observed on fish gill filament. The findings of the present study is more or less similar to Arguedas *et al.* (2017) who found ten parasite species: *Ichthyobodo* sp., *Apiosoma* sp., *Chilodonella* sp., *Heteropollaria* sp., *Trichodina* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Centrocestu* spp., *lasidies* and *glochidies* (two larval forms) and also to Gil and Jonathan (2013) who identified five digeneantrematode species (*Clinostomum* sp., *Euclinostomum* sp., *Erilepturus* sp., *Orientocreadium* sp., and *Opegaster* sp.) and one parasitic cyclopoid copepod species (*Lernaea* sp.) in their assessment.

Clinical symptoms of parasitic infestations: Lethargic or erratic swimming, operculum opened, scraping against walls, jumping out of water, erosion of skin, fins, ulcers and caudal fins, abnormalities and discoloration on the body surface; fishes gradually become sluggish, lose weight and become moribund. According to (Chandra 2009), the above mentioned signs and symptoms may be of trichodiniasis caused by the attack of *Trichodina* sp.

Table I. Mean (\pm SD) values of physico-chemical parameters of water in cage farm during the study period

Month Parameter	September	October	November	December	January	February	March	April	Mean (\pm SD) value
Water temp.(°C)	30.8 \pm 0.20	29 \pm 0.26	23.4 \pm 0.35	21.5 \pm 0.25	20.4 \pm 0.15	21.8 \pm 0.2	28.6 \pm 0.21	31.3 \pm 0.38	25.72 \pm 4.64
Dissolved oxygen (mg/l)	6.7 \pm 0.25	6.1 \pm 0.5	6.4 \pm 0.3	6.2 \pm 0.3	6.5 \pm 0.4	5.9 \pm 0.2	6.2 \pm 0.4	6.5 \pm 0.4	6.3 \pm 0.26
pH	8.1 \pm 0.4	7.2 \pm 0.3	7.3 \pm 0.2	7.8 \pm 0.3	7.9 \pm 0.3	7.1 \pm 0.2	7.8 \pm 0.4	7.6 \pm 0.5	7.6 \pm 0.41
Phosphate (mg/l)	0.029 \pm 0.001	0.04 \pm 0.002	0.037 \pm 0.004	0.03 \pm 0.003	0.034 \pm 0.002	0.024 \pm 0.003	0.023 \pm 0.003	0.026 \pm 0.004	0.03 \pm 0.006
Ammonia (mg/l)	0.13 \pm 0.002	0.10 \pm 0.001	0.06 \pm 0.005	0.03 \pm 0.005	0	0.02 \pm 0.006	0.01 \pm 0.003	0.02 \pm 0.005	0.05 \pm 0.04
Nitrate (mg/l)	8.41 \pm 0.88	10.63 \pm 0.68	0	0	0	0.01 \pm 0.003	0.03 \pm 0.001	0.01 \pm 0.002	2.39 \pm 4.46

Scale loses of the diseases fish, skin ulceration, smear in the skin surface reveals numerous individuals of the parasites, irregular movement, restless and sometimes the affected fish is found on the water surface. According to (Chandra 2009), the above mentioned sign and symptoms may be of chilodonellosis caused by *Chilodonella* sp.

Aerobic Plate Count (APC) of bacteria: Total bacterial load in water determine as aerobic plate count was $2.78 \pm 0.14 \times 10^5$ cfu/ml which was more or less similar to the findings of Shankar *et al.* (2009) and Banu *et al.* (2001) who determined total bacterial load in water $1.43 - 0.18 \times 10^6$ cfu/ml and 1.39×10^5 to 3.11×10^7 cfu/ml of water, respectively. Total bacterial load in tilapia was determined $4.26 \pm 1.21 \times 10^6$ cfu/g which was more or less similar to the findings of Dutta *et al.* (2018), Shankar *et al.* (2009) who determined total bacterial load in tilapia $4.0 \pm 0.56 \times 10^4$ cfu/g and 2.1×10^3 to 7.1×10^6 cfu/g, respectively.

Identification of bacteria based on different culture media: For the identification of bacteria, three selective media- Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar for *Vibrio* spp., Violet Red Bile Salts Agar (VRB) for *E. coli*, Salmonella-Shigella (SS) agar media for salmonella and shigella sp. were used to culture bacteria. Thus in the present study, a total of five species of bacteria- *Vibrio cholerae*, *V. parahaemolyticus*, *Enterobacter aerogenes*, *Salmonella enteritidis* and *E. coli* were identified on three different selective media (Table III).

Table III. List of bacteria identified on different culture media.

Media	Color of colony observed	Water	Tilapia
TCBS	Yellow	<i>Vibrio cholerae</i>	<i>Vibrio cholerae</i>
	Bluish green	<i>Vibrio parahaemolyticus</i>	<i>Vibrio parahaemolyticus</i>
SS		No colony found	No colony found
VRB	Pink to pinkish red	<i>Enterobacter aerogenes</i>	<i>Enterobacter aerogenes</i>
	Orangish yellow	<i>Salmonella enteritidis</i>	<i>Salmonella enteritidis</i>
	Pinkish red with bile	<i>E. coli</i>	<i>E. coli</i>

In the study of Jalal *et al.* (2017) *Vibrio*, *Enterobacter*, *Serratia*, and *Aeromonas* bacteria were found which is different to the present study.

Syndrome and pathology of bacterial disease: Discoloration of gill, red coloration on the fins, tails and body surfaces; bad odor from the intestine of the infected fish; abdomen swollen, lethargic swimming and lastly die; lethargic moving on the water surface and at last go under water; lesion on the body skin; hemorrhage on the dorsal anal or caudal fin. From these observed syndrome and pathology, it can be assumed that the fishes are affected by enterococcus diseases according to (Arumugam *et al.* 2017). In the present study, in case of enterococcus disease the syndrome and pathology observed were -discoloration of gill, red coloration on the fins, tails and body surfaces, bad odor from the intestine, swollen abdomen, lethargic swimming and lesion on the body skin. These symptoms are not so different from Nieto *et al.* (1995) stated in their study that the agent of this septicemia was identified previously as an *Enterococcus* like bacterium. Osman *et al.* (2017) examined enterococcus diseased fish which showed exophthalmia with accumulation of purulent and haemorrhagic fluid around eyes and ventral petechial haemorrhages. The syndromes observed by them are more or less similar to the syndromes observed in the present study.

Currently in Bangladesh, disease incidence is one of the major problems restricting tilapia farming. However, due to lack of diagnostic support and appropriate therapeutants, farmers are suffering from increasing financial losses due to diseases of these species. There is a lack of information on the pathogen associated with the diseases of farmed tilapia in Bangladesh. The present study was conducted to investigate common diseases from cage reared tilapia (*Oreochromis niloticus*) farms at Bakerganj, Barishal for a duration of 10 months from August 2017 to May 2018. A total of five species of bacteria were identified from water and tilapia samples. Seven species of parasites were identified and relevant diseases caused by these parasites occurred during the study period. The present study provided valuable information regarding tilapia fish disease problems and pathogens related to their disease occurrence. It is likely that once the farmers have a better understanding of basic fish culture and health management techniques, the likelihood of diseases occurring and their impacts will be reduced. Therefore, it is recommended that the farmers should be trained about basic fish health management strategies and there should be some support service for farmers about easy and proper diagnosis and treatment of fish diseases.

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