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Surveillance of tilapia lake virus (TiLV) in diverse aquaculture systems of the Indian Sundarbans and adjoining regions in West Bengal, India

Snigdha Karmokar[1], Sanjoy Das[2], Leesa Priyadarsani[2], Gadadhar Dash[1] and T. Jawahar Abraham[1*]

[1]Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata - 700 094, West Bengal, India

[2]Kakdwip Research Centre, ICAR-Central Institute of Brackishwater Aquaculture, Kakdwip - 743 347, West Bengal, India

Abstract

The current study aimed to monitor the occurrence of tilapia lake virus (TiLV) in farmed tilapias in the Indian Sundarbans, an ecologically fragile world heritage site. Fifty-one samples comprising *Oreochromis niloticus* (n=41) and *O. mossambicus* (n=10) were collected from the surveyed farms, including 6 disease cases. In semi-nested PCR, 5.9 and 37.3% of the samples were positive in the 1st and 2nd step, respectively. TiLV was detected only in *O. niloticus* (46.3%) and 37.3% of the surveyed ponds. The two-step semi-nested PCR was more sensitive than the single-step SYBR green-based qPCR. The novel TiLV strains had similarities and close relations with TiLV CIFRI1 and CIFRI2 strains. Histologically, the diseased *O. niloticus* depicted typical TiLV alterations like syncytial hepatocytes. Developing and implementing good management practices and strict quarantine measures are necessary to combat the outbreak and spread of TiLV in the Indian Sundarbans.

*Correspondence e-mail:
abrahamtj1@gmail.com

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Introduction

Tilapias are native to the African continent and the Middle East, but they are cultured worldwide due to their rapid growth, ease of farming and adaptability to a wide range of environmental conditions. Recently, tilapias have emerged as the second major culturable group after the carp (Wang and Lu, 2016; FAO, 2024). The Nile tilapia, *Oreochromis niloticus*, accounts for a significant portion of tilapia production, contributing approximately 5 million t (FAO, 2024). Some of the notable commercially farmed tilapia species are Nile tilapia *O. niloticus*, Mozambique tilapia *O. mossambicus*, blue tilapia *O. aureus,* and redbreast tilapia *Tilapia rendalli* (Nicholson *et al*., 2017). *O. niloticus* is the most widely known and cultivated tilapine species worldwide (Rogers *et al*., 2006). Disease outbreaks are a constraint affecting tilapia production and the farmers' (Newaj-Fyzul *et al*., 2008). The tilapia lake virus (TiLV) of the genus *Tilapinevirus,* which contains only one species of *Tilapia tilapinevirus*, is considered a potent threat to the tilapia industry worldwide (Surachetpong, 2017). This virus has ten negative-sense RNA segments encased in a membrane-bound nucleocapsid, having spherical viral particles of about 55-100 nm diameter (Eyngor *et al*., 2014; Bacharach *et al*., 2016). TiLV outbreaks can happen in both wild and farmed tilapias. The disease has been reported in Colombia, Egypt, India, Indonesia, Israel, Malaysia, the Philippines, Peru, Tanzania, Thailand and Mexico since the initial report in Ecuador in 2012 (Al-Hussinee *et al*., 2019). In India, the TiLV was first reported in July 2016 in the North 24 Parganas District, West Bengal. In July 2017, two more cases of mortality in tilapia were observed in the South 24 Parganas District, West Bengal and the Ernakulam District, Kerala (Behera *et al*., 2018). TiLV-induced mass mortalities in 104 farms in Tamil Nadu and Pondicherry were also documented between 2017 and 2018 (Al-Hussinee *et al*., 2019).

The Sundarban, a UNESCO-declared world heritage site, is situated at the southern fringes of West Bengal, India (Fig. 1) and the neighbouring country, Bangladesh. The Sundarban mangrove forest covers an area of about 10,000 km^2, of which forests in India extend over 4,260 km^2 across the South 24 Parganas and North 24 Parganas districts (Samanta *et al*., 2021). Fisheries and aquaculture are considered integral components of the livelihood of the inhabitants of Sundarban and significantly contribute to the district's production (Ghoshal *et al*., 2019). Indian major carps in combination with exotic carps and *Oreochromis* spp. dominate freshwater aquaculture of the Sundarban (Dubey *et al*., 2016). Brackishwater farming has been a traditional practice for centuries in this region. It is confined mainly to the *bheries,* manmade impoundments in coastal wetlands, where polyculture has been practised with *Oreochromis* spp. and brackishwater fish like *Lates calcarifer*, *Liza parsia,* and *Mugil cephalus* (Ghoshal *et al*., 2019). In recent years, there have been several undocumented reports of tilapia mortality in the Indian Sundarbans. The present study on the surveillance of TiLV was, therefore, designed to understand the prevalence of this virus in and around the Indian Sundarbans and assess its genetic relatedness to other strains.

Materials and methods

Collection of fish samples

For the surveillance of TiLV, a total of 42 tilapia farms located in the Sundarbans and adjoining areas of West Bengal, India (Table 1), were surveyed for a period of 12 months from November 2020 to October 2021. Among the farms surveyed, only six farms had apparent disease conditions, while the other 36 farms did not report any disease problems. Tilapias (*O. niloticus* and *O. mossambicus*) of different sizes and different life stages were collected along with the water samples. In apparently healthy tilapia farms, randomly picked fish (n=5) were collected and euthanised following standard procedure (Behera *et al*., 2018). In farms with apparent diseased conditions, the moribund fish were collected and euthanised at the site by placing them in ice. The sampled fish were dissected carefully to collect the brain and liver tissues. The pooled liver and brain tissues were collected in 2 ml microcentrifuge tubes in an RNA preservative solution (Sample protector for RNA/DNA, Takara, Japan). The samples were transported to the laboratory on ice and stored at 4˚C for RT-PCR assay. A total of 51 sample lots were collected from 42 surveyed farms (Table 2). The fish collected from a single pond were pooled together and considered as a single lot. Among the 51 samples, 41 belonged to *O. niloticus* and the rest were *O. mossambicus*. Dissolved oxygen (DO), pH and salinity of pond water were checked on the spot using a multiparameter water testing meter (Hach, USA; Model: HQ30d). A glass thermometer was used to record the water temperature on the spot. The pond water samples were also collected in sterilised plastic bottles to examine other parameters. The alkalinity and total hardness were measured by standard titrimetric methods. Ammonia and nitrate were determined colourimetrically (Strickland and Parsons, 1972).

Total RNA isolation and PCR assay

RNA isolation from the pooled liver and brain tissues (50-100 mg) was carried out by the guanidinium thiocyanate-phenol-chloroform extraction method (Jing *et al*., 2006) and quantification was done by a Nanodrop spectrophotometer ND 2000 (Thermo Fisher Scientific, USA). The RNA samples were converted to cDNA by reverse transcription and then subjected to the semi-nested PCR and qPCR assays. The semi-nested PCR was carried out as per the protocol of the Network of Aquaculture Centres in Asia-Pacific (NACA) (Tsofack *et al*., 2017; Jansen *et al*., 2019). ME1 (5'-GTTGGGCACAAGGCATCCTA-3') and Nested Ext-1 (5'- TATGCAGTACTTTCCCTGCC-3') were the primers of the 1st step. The 2nd step used ME1 and ME2 (5'-TATCA CGTGCGTACTCGTTCAGT-3') primers. The primers used in this study were based on segment 3 of the hypothetical protein gene of the Tilapia tilapine virus (Tsofack *et al*., 2017; Jansen *et al*., 2019). The amplification program for the semi-nested PCR consisted of initial denaturation at 95°C for 2 min, followed by 25 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and

Fig. 1. Google map of the Indian Sundarban

Table 1. Details of the surveyed farms with GP locations

Farm	Location	Latitude, N	Longitude, E	Size (g)	Species
1	Frezarganj, Namkhana	21°35’11.76”	88°14’56.04”	100-250	*Oreochromis niloticus*
2	Bhutomollapur, Kakdwip	21°55’44.76”	88°11’27.96”	>500	*O. mossambicus*
3	Anandanagar, Kakdwip	21°57’28.08”	88°11’49.92”	400-500	*O. niloticus*
4	Halisahar, Kakdwip	21°53’13.92”	88°7’23.16”	100-250	*O. niloticus*
5	Paschim Darogapur, Patharpratima	21°46’4.08”	88°20’15”	100-250	*O. mossambicus*
6	Debichack, Patharpratima	21°47’57.12”	88°22’12.36”	250-500	*O. niloticus*
7	Ramnagarabaad, Patharpratima	21°52’23.52”	88°22’26.76”	100-250	*O. mossambicus*
8	Sibkalinagar, Kakdwip	21°51’56.52”	88°9’2.52”	<25	*O. mossambicus*
9	Rajnagar Srinathgram, Namkhana	21°46’19.2”	88°14’20.4”	250-500	*O. niloticus*
10	Rainagar, Jaynagar-1	22°13’37.2”	88°27’21.96”	25-100	*O. mossambicus*
11	Rainagar, Jaynagar-1	22°4’0.01”	88°25’53.9”	25-100	*O. mossambicus*
12	Rainagar, Jaynagar-1	22°13’37.2”	88°27’21.96”	25-100	*O. niloticus*
13	Dakshin Tulsighata, Jaynagar-2	22°9’23.76”	88°27’26.28”	25-100	*O. mossambicus*
14	Dakshin Tulsighata, Jaynagar-2	21°9’23.76”	88°27’26.28”	250-500	*O. niloticus*
15	Nimpith, Jaynagar-2	22°9’25.92”	88°26’23.28”	100-250	*O. niloticus*
16	Mahamaya, Mathurapur-2	22°7’13.8”	88°23’21.84”	250-500	*O. mossambicus*
17	Mahamaya, Mathurapur-2	22°4’7.32”	88°26’38.04”	25-100	*O. mossambicus*
18	Mahamaya, Mathurapur-2	22°4’7.32”	88°26’38.04”	100-250	*O. niloticus*
19	Mahamaya, Mathurapur-2	22°4’7.32”	88°26’38.04”	25-100	*O. niloticus*
20	Kharapara, Mathurapur-2	22°5’2.26”	88°25’50.66”	25-100	*O. niloticus*
21	Kautala, Mathurapur-2	21°42’15.01”	89°25’50.23”	>500	*O. niloticus*
22	Mahamaya, Mathurapur-2	22°4’2.06”	88°26’5.06”	25-100	*O. niloticus*
23	Bamunerchalk, Mathurapur-2	22°3’54.4”	88°25’39.97”	25-100	*O. niloticus*
21	Madanganj, Namkhana	21°45’9.47”	88°16’13.66”	100-250	*O. niloticus* and *O. mossambicus*
25	Baliyada, Namkhana	21°37’22.08”	88°12’15.44”	100-250	*O. niloticus*
26	Uttarchandanpiri, Namkhana	21°41’15.14”	88°23’40.02”	250-500	*O. niloticus*
27	Dakshin Chandanpiri, Namkhana	21°41’13.31”	88°23’40.02”	250-500	*O. niloticus*
28	Vivekananda Sangha, Kakdwip	21°51’44.28”	88°10’50.81”	>500	*O. niloticus*
29	Haripur, Kakdwip	21° 51’ 53.9”	88° 11’ 28.3”	25-100	*O. niloticus*
30	Buddhapur Paschim Maynapara, Kakdwip	21° 51’ 27.2”	88° 10’ 59.1”	100-250	*O. niloticus*
31	Kakdwip	21° 51’ 27.2”	88° 10’ 59.1”	100-250	*O. niloticus*
32	Haripur, Kakdwip,	21° 51’ 56.4”	88° 11’ 24.5”	25-100	*O. niloticus*
33	Kakdwip	21° 51’ 27.2”	88° 10’ 59.1”	25-100	*O. niloticus*
34	Kakdwip	21° 51’ 27.2”	88° 10’ 59.1”	< 5	*O. niloticus*
35	Ganesnagar, Namkhana	21° 47’ 39.1”	88° 14’ 39.4”	25-100	*O. niloticus*
36	Kakdwip	21° 51’ 29.8”	88° 11’ 0.5”	100-250	*O. niloticus*
37	Kakdwip	21° 51’ 29.8”	88° 11’ 0.5”	100-250	*O. niloticus*
38	Manmothopur, Kakdwip	21° 53’ 9.4”	88° 16’ 23.7”	25-100	*O. niloticus*
39	Dakshin Gobindopur	21° 48’ 59.4”	88° 21’ 47.6	5-25	*O. niloticus*
40	Sagar island	21° 39’ 9.2”	88° 8’ 32.2”	250-500	*O. niloticus*
41	Sulkuni, Hasnabad	22°32’21.48”	88°53’58.92”	250-500	*O. niloticus*
42	Srirampur, Hooghly	22°43’31.08”	88°19’6.34”	100-500	*O. niloticus*

elongation at 72°C for 30 s. Final elongation was done at 72°C for 5 min (Jansen *et al*., 2019). A TiLV-specific qPCR was also performed with Maxima SYBR green qPCR master mix (2×) (Thermofisher Scientific, USA) using ME1 and ME2 as primers. Nuclease-free water was used as a negative control. The amplification program consisted of initial denaturation at 95°C for 1 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and elongation at 72°C for 30 s (Nicholson *et al*., 2017). Melting curve analysis was carried out from an initial temperature of 55-95°C with a slow temperature increase of 0.5°C per cycle with fluorescence detection at each cycle.

Partial sequencing and phylogenetic analysis

First-step PCR amplicon obtained from two out of three 1st-step positive samples was sequenced. The target region of the primer is segment 3 of the hypothetical protein gene of the TiLV (Tsofack *et al.,* 2017; Jansen *et al.,* 2019). For the amplification for sequencing, the PCR cycling condition was almost like that of 1st step semi-nested PCR, except for the number of cycles (35) and final elongation time (20 min) to allow the synthesis of uncompleted amplicons, leading to accuracy in sequencing. Sequencing was performed using Sanger's di-deoxy chain termination method (Crossley *et al.,* 2020). Following the truncation of poorly read

sequences, 392 bases of the sequence were used to carry out BLAST analysis and phylogenetic tree construction. The DNA sequences of the two TiLV strains were compared with similar published sequences using the BLAST program available at blast.ncbi.nlm.nih.gov. The DNA sequences were aligned using the Clustal Omega multiple sequence alignment analysis tool (EMPL-EBL APIs 2019) (http://www.ebi.ac.uk/Tools/msa/clustalo/). The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA) Version 11 software (Construct/Test Maximum Parsimony tree) based on the Kimura 2-parameters model (K2P model) using the Neighbor-Joining method (Saitou and Nei, 1987).

Histopathology

The liver samples collected from six diseased cases and two cases of healthy fish were preserved separately in 10% neutral buffered formalin (NBF) for 24 h. Histopathological investigations were performed as per the standard protocol (Roberts, 2012). Briefly, the fixed tissues were dehydrated, embedded in paraffin, sectioned at 5 μm thickness, stained with haematoxylin and eosin (H&E) and examined under an advanced trinocular research microscope (Olympus, Japan, Model: CX43) using an SC180 Olympus camera 18 MP attached to the microscope.

Results

Diseased *O. niloticus* exhibited gross clinical signs such as lethargy, erratic movement, spiral swimming, scale erosion, pale gills, cuticle haemorrhages, pale liver, liquefaction of the kidney, exophthalmia, fin and tail erosion and muscle haemorrhages (Fig. 2a-f). Internal lesions were also observed in the liver, kidney, muscle and gut. Most disease outbreak cases in the surveyed farms recorded up to 80-90% morbidities. Mass mortalities to the tune of 50-70% in 60% of cases and 80-90% in 40% of cases were observed within 7-14 days of disease outbreak. Of the 51 samples, only three *O. niloticus* samples (sample numbers 32, 36, and 50) showed an amplicon of 415 bp in the 1st step semi-nested PCR, indicating heavy TiLV infection. In 2nd step, sixteen asymptomatic and three symptomatic *O. niloticus* samples showed amplicon bands. The incidence of TiLV was found to be 37.3% using nested PCR. Of the 51 samples subjected to TiLV-specific qPCR, 15 samples comprising symptomatic and asymptomatic *O. niloticus* were positive. TiLV was detected only in *O. niloticus* but not in *O. mossambicus*. TiLV was detected in 50% of diseased farms with high mortalities and 35.6% of the asymptomatic farms. The qPCR using SYBR green was found to be more sensitive than single-step PCR but slightly less sensitive than nested PCR (Tables 2 and 3).

Of the three positive samples in 1st step, two samples (Sample numbers 32 and 36) were sequenced. The deduced sequences of the novel strains, *viz.,* TiLV, KRC-Hooghly and TiLV, KRC-Namkhana, were compared with similar published sequences of TiLV using the NCBI-BLAST. The sequences of the novel strains individually showed 93-97% similarity with the segment 3 hypothetical protein genes of many TiLV isolates in the NCBI GenBank database. In the phylogenetic tree, TiLV, KRC-Hooghly and TiLV, KRC-Namkhana

Table 2. Semi-nested PCR and qPCR results on the incidence of TiLV in the surveyed farms/ponds

Species	Sample number (N=51)	Semi-nested PCR		qPCR
		1st step	2nd step	
Oreochromis niloticus (21)	1, 2, 4, 5, 7, 9*, 10, 19, 21, 23*, 24, and 38-47	-	-	-
O. niloticus (5)	13, 15, 16, 20 and 22*	-	+	-
O. niloticus (1)	37	-	-	+
O. niloticus (11)	25-31, 33, 34, 35* and 51	-	+	+
O. niloticus (3)	32, 36*, and 50*	+	+	+
O. mossambicus (10)	3, 6, 8, 11, 12, 14, 17, 18, 48 and 49	-	-	-

*: Disease cases

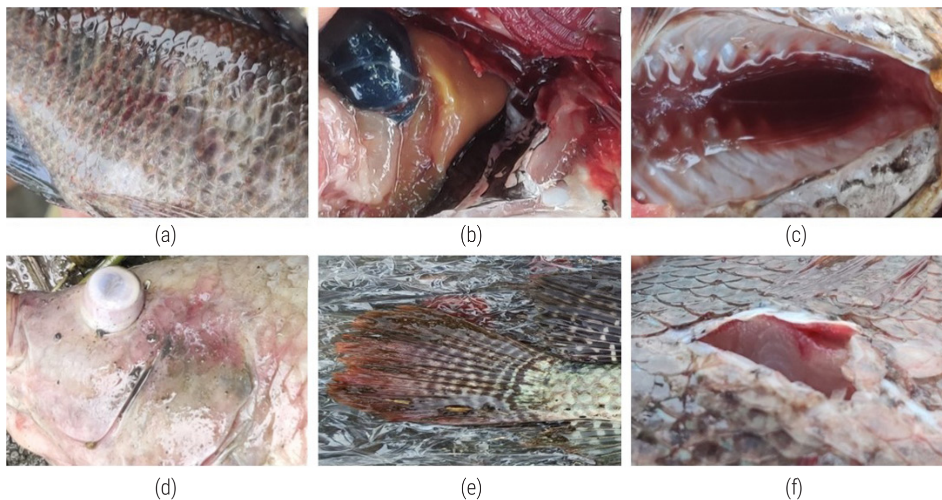

Fig. 2. Gross clinical signs observed in the diseased *O. niloticus.* (a) Cutaneous haemorrhages, (b) Pale liver, (c) Liquefaction of the kidney, (d) Exophthalmia, (e) Haemorrhages in the tail region and (f) Muscle haemorrhages

Table 3. Prevalence of TiLV in the surveyed ponds/farms culturing *O. niloticus* and *O. mossambicus*

Species, conditions and farms	Number of samples	Number positive/Number tested		
		Semi-nested PCR		qPCR
		1st step	2nd step	
Total samples	51	3/51 (5.9%)	19/51 (37.3%)	15/51 (29.4%)
Species				
Oreochromis niloticus	41	3/41 (7.31%)	19/41 (46.34%)	15/41 (36.59%)
O. mossambicus	10	0/10	0/10	0/10
Condition				
Diseased or symptomatic	6	2/6 (33.33%)	3/6 (50.00%)	3/6 (50.00%)
Non-diseased or asymptomatic	45	1/45 (2.22 %)	16/45 (35.6%)	12/45 (26.7 %)
Farms				
Freshwater farms	40	12/40 (30.00%)		12/40 (30.00%)
Brackishwater farms	11	2/11 (18.18%)		3/11 (27.27%)

strains showed >99.9% similarity. They were closely related to CIFRI1 (GenBank accession number MF502419) and CIFRI2 (MF582636) strains from West Bengal, India. Both sequences were also closely related to the KUFOS KO-M-01 (MG519779) from Kerala, India (Fig. 3). The sequences of both TiLV, KRC-Hooghly and TiLV, KRC-Namkhana strains were submitted to NCBI GenBank with accession numbers ON366580 and ON366581, respectively. Out of 6 disease cases, only 5 cases depicted typical liver pathological changes like syncytial hepatocytes. Besides, changes like loosely packed hepatocytes, severe necrosis in hepatic parenchyma, eosinophilic inclusion, pyknosis and karyorrhexis were observed (Fig. 4a-b).

Discussion

Tilapia is one of the world's most culturable farmed fish due to consistent market price and convenience in aquaculture. (FAO, 2022). However, very often, tilapia aquaculture has been affected by various diseases, including TiLV infection. (Dong *et al*., 2015; Jansen *et al*., 2019). In the present study, only 6 obvious disease cases were found, which experienced high mortalities. The observed clinical signs from the survey and the previous report descriptions (Eyngor *et al*., 2014; Nicholson *et al*., 2017; Surachetpong, 2017; Jansen *et al*., 2019) suggested a possible TiLV infection. Similarly, TiLV-induced

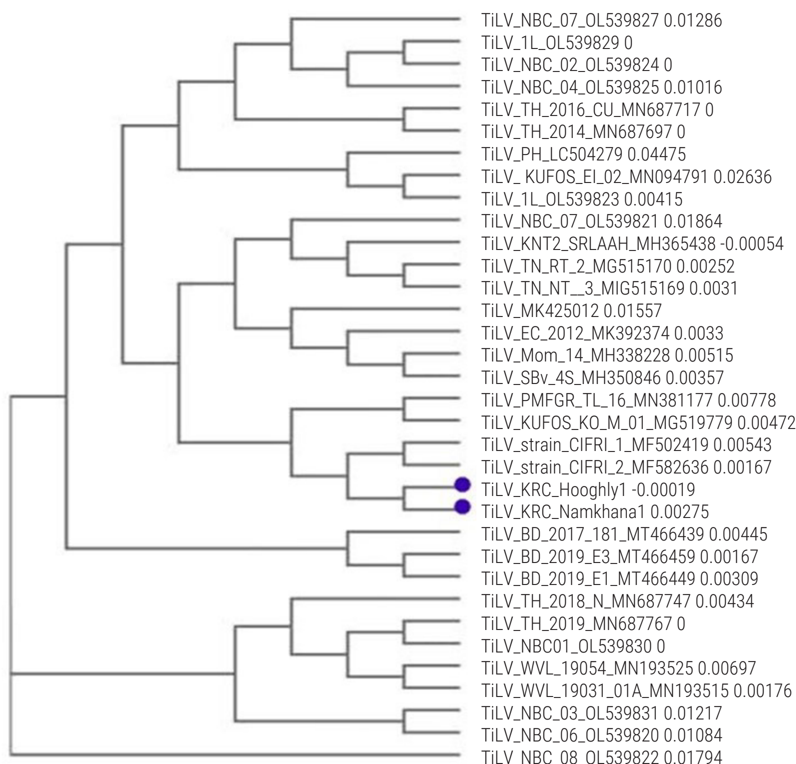

Fig. 3. Phylogenetic tree of TiLV strains of *O. niloticus* based on the hypothetical gene and segment-3 hypothetical protein gene sequences, The name of strains and the NCBI GenBank accession number are represented in the pictorial diagram

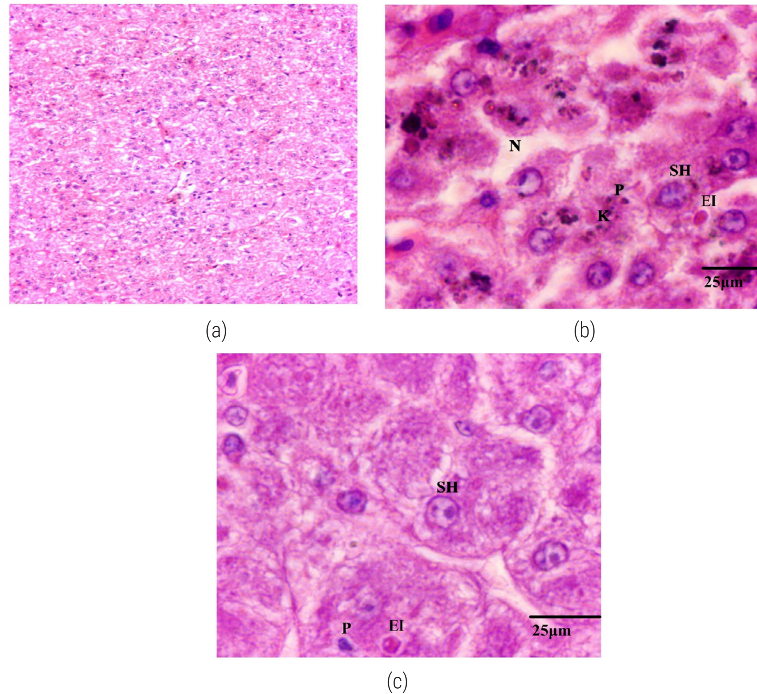

Fig. 4. (a) Control ×100 H&E staining; (b and c) TiLV-infected *O. niloticus* liver showing histopathological alterations like severe necrosis in hepatic parenchyma (N), syncytial hepatocytes (SH), eosinophilic inclusion (EI), pyknosis (P) and karyorrhexis (K) (H&E; x1000)

mass mortalities in tilapias were reported in different geographical regions. For example, above 80% of mortalities in TiLV-affected farms in Israel (Eyngor *et al*., 2014)a novel RNA virus, is described here, and procedures allowing its isolation and detection are revealed. The virus, denominated tilapia lake virus (TiLV, 10-80%) within 4-7 days of transfer to grow-out ponds in Ecuador (Ferguson *et al*., 2014) and 20-90% within the first month of grow-out cage cultures in Thailand (Surachetpong, 2017) have been documented. Interestingly, all symptomatic tilapias of the Indian Sundarban were adults and had a size of >250 g. Similarly, the report of Fathi *et al*. (2017) from Egypt indicated that the medium (>100 g) and large-sized (>250 g) *O. niloticus* experienced mass mortalities, some of which were positive for TiLV. The novel TiLV strains (KRC, TiLV-Namkhana and KRC and TiLV-Hooghly) of the present study are aboriginal, most similar to each other and closely related to CIFRI1 and CIFRI2 strains from West Bengal, India. All these TiLV strains might belong to the same hierarchy and, thus, be genetically related. These results thus suggested that the same genetically related TiLV strains are still prevailing in West Bengal and causing large-scale mortalities in cultured *O. niloticus* since the past study (Behera *et al*., 2018).

The present study confirmed the prevalence of TiLV infection in the Indian Sundarbans, a world heritage site, by semi-nested PCR and qPCR. Among 51 samples screened, the one-step qPCR yielded 15 positive results. The semi-nested PCR gave 3 and 19 positive samples in the 1st and 2nd step, respectively, which implied that the 2-step semi-nested PCR is more sensitive than the qPCR for TiLV detection. The study of Taengphu *et al*. (2020), carried out in Thailand and Bangladesh, documented that modified semi-nested PCR was rapid and more sensitive for the detection of TiLV. A comparison of TiLV-infected fish samples collected from Thailand (2013-2019) and Peru in 2018 confirmed that the semi-nested PCR was more sensitive to detecting TiLV (Taengphu *et al*., 2020). All the TiLV-positive cases of the Indian Sundarban were *O. niloticus*, indicating the greater susceptibility of this species to TiLV. The study also indicated that the occurrence of TiLV is rare in *O. mossambicus*. Further, among the 16 asymptomatic *O. niloticus* samples, 15 were TiLV positive by semi-nested PCR and one by qPCR. The size of the asymptomatic TiLV carriers was mostly adults (57.14%), comprising medium-sized (100-250 g) and large-sized (>250 g) groups and the rest (42.86%) were juveniles and sub-adults, weighing 25-80 g. In the current study, the pH (7.0-8.5) and water temperature (27.6-29.51°C) of the asymptomatic ponds were almost in the favourable range for tilapia culture (Nivelle *et al*., 2019). Also, the ammonia and nitrite levels of the asymptomatic ponds were in the range of 0.01-0.14 ppm and ≤0.01 ppm, respectively, which are well below the critical level. The favourable culture and water quality conditions probably prevented the mortalities in the PCR-positive asymptomatic cases. However, one of the asymptomatic samples (Sample no 32) showed high TiLV infection as evidenced by PCR positivity in the first step. Similarly, healthy adult tilapia reportedly had asymptomatic TiLV infection in Thailand (Senapin *et al*., 2018). The detection of TiLV was reported in several earlier studies and the incidences of asymptomatic carriers were due to the improved immune responses of the host to the virus (Aich *et al*., 2022; Kembou-Ringert *et al*., 2023).

In our study, 50% of symptomatic and 35.6% of asymptomatic cases were TiLV positive, which corroborated the observations of Debnath *et al*. (2020), where surveillance of TiLV was made in tilapia grow-out farms and hatcheries from 10 districts of Bangladesh between 2017 and 2019. Among the 11 farms that

experienced unusual mortality, eight were TiLV positive in 2017 and two out of seven tested were TiLV positive in 2019. Investigations of asymptomatic broodstock collected from 16 tilapia hatcheries revealed that samples from six hatcheries tested positive. The virus was detected in both grow-out farms experiencing unusual mortality and in asymptomatic broodstock hatcheries (Debnath *et al*., 2020), which can act as carriers to transfer the virus to their offspring. In addition, we documented TiLV-positive cases in *O. niloticus* from both freshwater and brackishwater ponds/farms. The findings of the present study corroborated the OIE report that showed TiLV infection in *O. niloticus* cultured in freshwater as well as brackishwater farms in Colombia (OIE, 2020). Also, the challenge study of Al-Hussinee *et al*. (2019) documented TiLV infection in *O. niloticus* from freshwater and brackishwater farms, where mortalities were reported in both systems. Thus, it implied that TiLV can infect both freshwater and brackishwater fish. The histopathological changes of the liver tissues also confirmed the TiLV-positive cases in the Indian Sundarban, thus providing additional constructive evidence to the molecular reports on TiLV. Out of six, five diseased cases showed histopathological alterations such as necrosis, syncytial hepatocytes, eosinophilic inclusion bodies and pyknosis, almost similar to the observations of Yamkasem *et al*. (2019). The TiLV-infected *O. niloticus* of their study showed changes like syncytial cells, severe necrosis in hepatocytes, foamy cytoplasm and pyknosis. In some cases, the histopathological observation depicted typical TiLV infection, but PCR failed to amplify the target and as a result, the molecular test yielded a negative reaction. It may be the survivours of previous TiLV exposure, which showed typical TiLV-like histopathological changes in the liver tissue without the virion at the time of sample collection. The present study highlights the occurrence of TiLV in farmed tilapia in the Indian Sundarbans, an ecologically delicate world heritage site. However, further investigations are needed with larger samples involving all the community blocks of Sundarbans.

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