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Highlights

- Summer mortalities in tilapia have occured in Egypt for 3-4 years
- Epidemiological study finds 37% of tilapia farms affected
- TiLV PCR positive tilapia found on three of of the seven farms with summer mortality syndrome
- Partial sequence (255bp) shows 93% homology to published TiLV segment 3

Identification of Tilapia Lake Virus in Egypt in Nile tilapia affected by 'summer mortality' syndrome

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Abstract

Egyptian fish farms have faced unexplained mortality of tilapia during the summer months in recent years. Epidemiological surveys indicated that 37% of fish farms were affected in 2015 with an average mortality rate of 9.2% and a potential economic impact of around US$ 100 million. Despite a number of researchers and organizations investigating potential causes results so far have been inconclusive. Meanwhile recent reports emerged of the presence of a new orthomyxovirus, Tilapia Lake Virus (TiLV) in Israel, which shares a border and migrating avifauna with Egypt. Tissue samples from seven farms affected by 'summer mortality' were tested at the University of Stirling for TiLV. Samples from three of seven farms tested positive using PCR; the first time that TiLV has been identified in Egypt. Sequence analysis yielded a TiLV sequence with 93% homology to the published TiLV sequence described from Israel. More research is required to determine if TiLV is linked to 'summer mortality'.

Introduction

The Egyptian aquaculture sector is the largest producer of farmed fish in Africa (1.14 million tonnes in 2014) and the third largest global producer of farmed tilapia after China and Indonesia (FAO, 2016; Fitzsimmons, 2016). Until recently, there were no major disease concerns but over the past three to four years farmers have faced unexplained, significant mortalities of medium (>100g) and large sized pond reared Nile tilapia (*Oreochromis niloticus*) during the summer months (June-October) when water temperatures typically rise to over 25ºC. These disease outbreaks, termed 'summer mortalities', have been investigated by a number of research teams in an attempt to identify causative agents but with inconclusive results. Another study linked the opportunist pathogen *Aeromonas veronii* biovar sobria to disease outbreaks of pond reared tilapia in Egyptian fish farms but this has not been confirmed as the primary causative agent (Eissa, et al., 2015).

Meanwhile a new orthomyxovirus, termed Tilapia Lake Virus (TiLV) was isolated in Israel in 2014 after an investigation into tilapia mortalities in wild and farmed stocks and has also been associated with mortalities of farmed tilapia in Ecuador and Columbia. The full TiLV genome was described and improvements to isolation and detection methods reported. (Bacharach, et al., 2016; Eyngor, et al., 2014; Kembou Tsofack, et al., 2016).

In 2015 WorldFish conducted an epidemiological study of 'summer mortalities' on 68 fish farms in the three most important Egyptian aquaculture governorates; Kafr El Sheikh, Behera and Sharkia. The aim of this study was to identify the effect of various production factors on the incidence of disease outbreaks in Egyptian pond based aquaculture. Also, as Egypt shares a border with Israel and is on an important bird migration route passing through Israel it seemed likely that TiLV could also be

present in Egypt and might be linked to ‘summer mortalities’. Accordingly, in summer 2016 tilapia from Egyptian farms affected by ‘summer mortalities’ were sampled and analysed for TiLV.

Materials and Methods

Epidemiological Study

The study was conducted on 68 randomly selected fish farms from the WorldFish database in the governorates of Kafr el Sheikh (32 farms), Behera (15 farms), and Sharkia (21) farms. These areas are responsible for around 85% of Egyptian aquaculture production (GAFRD, 2016). A structured questionnaire and monthly field visits by WorldFish staff recorded fish mortalities over the growing season. Data were also collected on; farm size, stocking density, production systems, and economic impact of losses due to ‘summer mortalities’.

Sampling for TiLV

After consultation with the Egyptian authorities, liver, brain, kidney and spleen tissues from moribund fish from 7 ‘summer mortality’ affected farms (3 fish per farm) were sent to the Institute of Aquaculture at Stirling University to test for TiLV. Tissue samples from healthy fish from 4 non-affected farms were also sent as controls. Samples for PCR assay were transported in RNA preservation reagent while viral transport medium and sterile tubes, kept chilled, were used to transport samples for virus isolation.

Tissues from all sampled fish were fixed in neutral buffered formalin for histological analysis. Tissue homogenates of these samples were also inoculated onto BF-2, EPC

and CHSE-214 cell lines and RNA was extracted from tissue samples using TRI-reagent and tested by RT-PCR for segment 3 as described (Eyngor et al, 2014) .

Results

Epidemiological study

Out of 68 randomly selected fish farms, 37% (25 farms) were affected by 'summer mortalities" with an average mortality rate of 9.2% (range 5-15%). Incidence rates were significantly higher in farms with higher stocking densities and in large farms compared to smaller farms (Tab. 1). Many farms grow mullet (*Mugil cephalus* L and *Liza ramada* Risso, 1827) in co-cultivation with tilapia (85% tilapia: 15% mullet) because of the higher prices obtained for these species. Notably only 3 / 38 farms practicing monoculture of tilapia were affected whereas 22 / 30 farms practicing tilapia / mullet polyculture were affected. Further studies are required to investigate the possible role of mullet (which appear to be resistant to summer mortalities) in disease transmission (Tab 1). The overall impact of summer mortalities in 2015 was estimated at 98,000 tonnes of lost production (US$ 100 million).

Analysis of clinical samples

the following pooled tissue samples (n=3, brain = B, kidney = K, liver =L, spleen = S) of three farms tested positive: L and S samples from farm 1, S from farm 2 and B, K, L, S from farm 3 - a first time detection of TiLV in this laboratory and in tilapia from Egypt. Histologic lesions in tissues of the PCR positive fish included multiple foci of gliosis, encephalitis and mild perivascular cuffing by lymphocytes in the brain; mild chronic meningitis; mild perivascular and multifocal, chronic hepatitis and moderate, multifocal interstitial haemorrhage in the kidney (Fig. 1.). The epidermis was

moderately disrupted with superficial haemorrhage, inflammation, and oedema. In comparison, unaffected, PCR negative fish from uninfected negative control farms showed very mild perivascular hepatitis and occasional encysted trematodes within the gut wall. Moderate diffuse congestion/haemorrhage of the spleen was common to both PCR positive and PCR negative fish.

Cycle sequencing of the PCR amplificates yielded a TiLV sequence with 93% homology to the published TiLV sequence (Fig. 2.). We did not isolate the virus.

Discussion

Clearly 'summer mortalities' of tilapia are having a significant impact on Egyptian aquaculture. Anecdotal evidence suggests that the incidence is increasing year by year although this survey is the first to provide credible figures on incidence and mortality rates. In the current study, larger farms, higher stocking densities and co-cultivation of tilapia with mullet appear to be contributory factors in the observed incidence of 'summer mortalities' in Egyptian fish farms but further studies are required.

Now that a virus with high homology to TiLV has been identified in Egypt in tilapia affected by 'summer mortality', further research including epidemiological and experimental infection studies are needed to determine if it is the primary cause. If so, rapid action can be taken to control the spread of the disease including immediate improvements to aquaculture biosecurity practices and in the longer term, vaccines and breeding TiLV resilient strains of tilapia.

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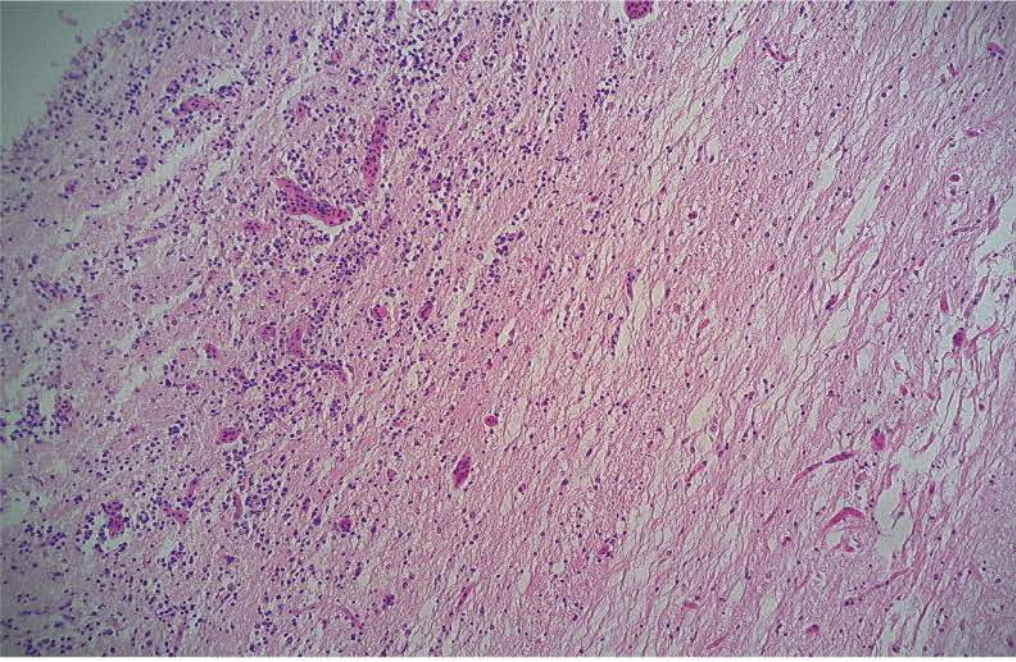
Table 1
Farm management factors affecting incidence of summer mortalities

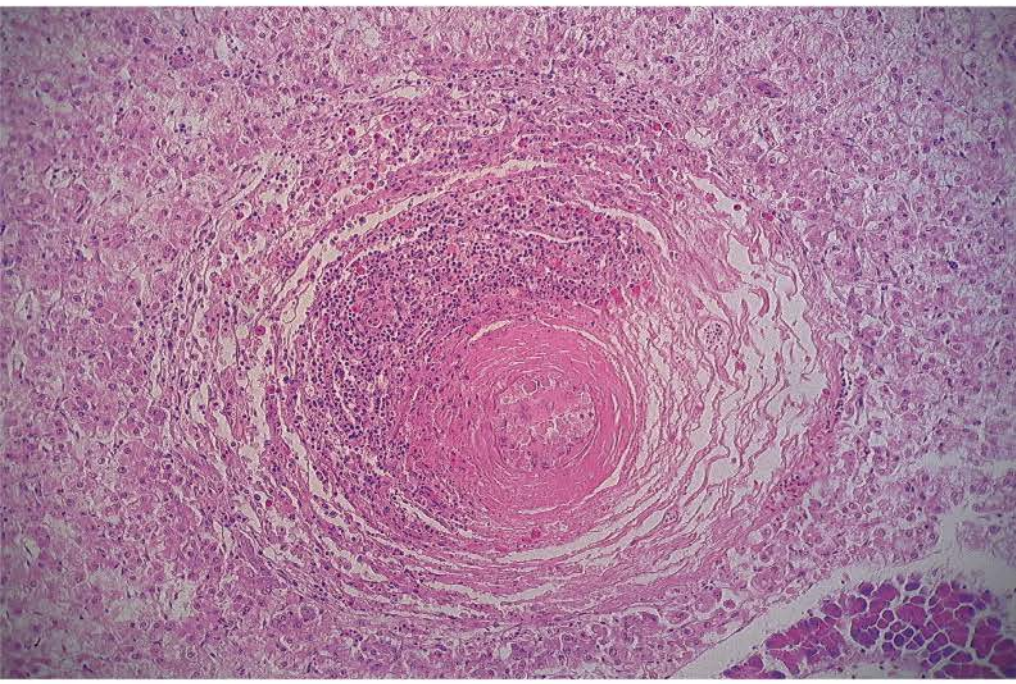
Management factor	**Affected farms**	**Un-affected farms**	**P-value**
Mean stocking density (1000 fish.ha^{-1})	51.45	33.98	0.050
Average farm size (ha)	13.44	7.00	0.043
Farms practicing tilapia monoculture (no)	3	35	0.0001
Farms practicing tilapia-mullet polyculture (no)	22	8	0.015

Stocking densities and average farm sizes were higher in 'summer mortality' affected farms than in un-affected farms while farms practicing polyculture were more likely to be affected than farms practicing monoculture of tilapia

Figures

Fig. 1. Histological findings in PCR positive tilapia (HE staining, × 200). Upper panel: Focally extensive chronic encephalitis – brain stem. Middle panel: Chronic perivascular hepatitis centred around vasculitis. Lower panel: Renal interstitial haemorrhage. All: Haematoxylin and eosin stain, magnification x200.





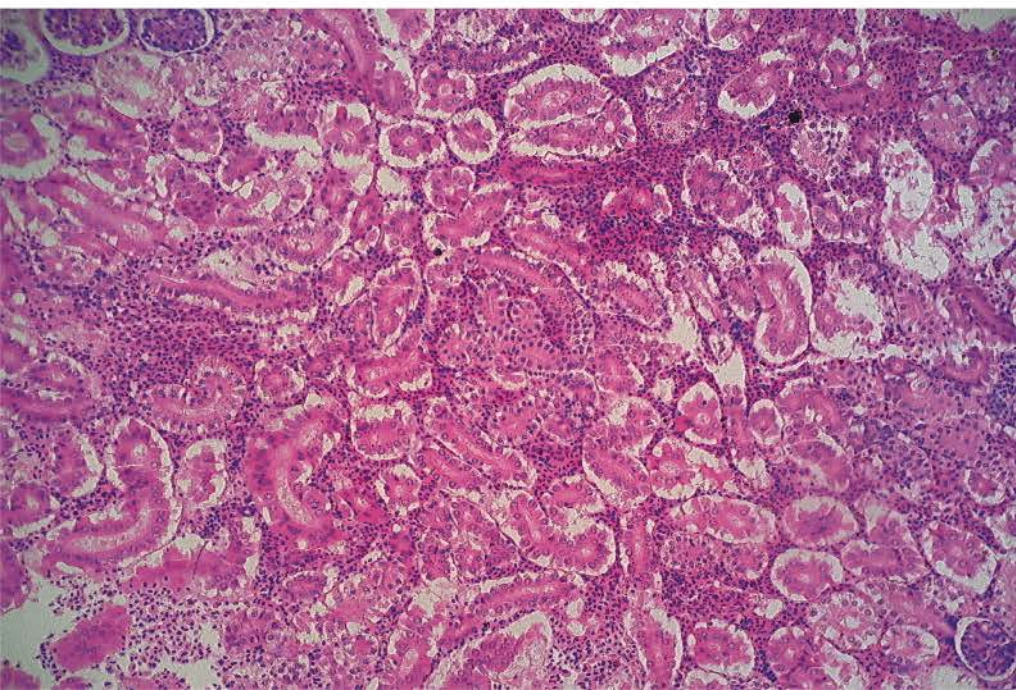
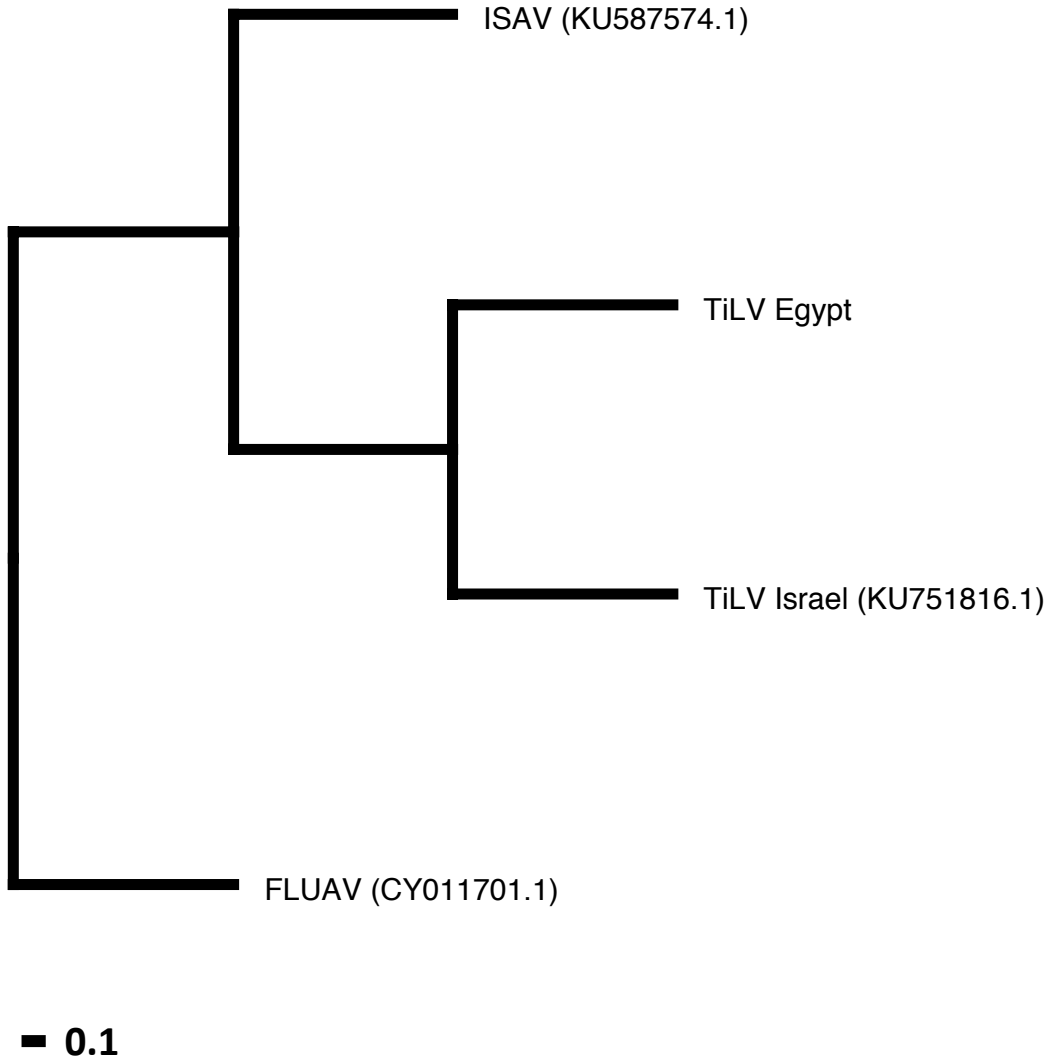


Fig. 2. Weighted dendrogram of a 255 nucleotide PCR fragment of TilV segment 3, aligned with ClustalW, modelled with RaxML. ISAV = Infectious salmon anaemia virus, FLUAV = Influenza A virus.


ISAV (KU587574.1)
TiLV Egypt
TiLV Israel (KU751816.1)
FLUAV (CY011701.1)
0.1

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