REVIEWS IN Aquaculture

Reviews in Aquaculture, 1–15 doi: 10.1111/raq.12254

Tilapia lake virus: a threat to the global tilapia industry?

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Received 24 January 2018; accepted 10 April 2018.

Abstract

Tilapia lake virus (TiLV) is a recently described virus affecting wild and farmed tilapines. At present, it has been reported on three continents (Asia, Africa and South America) and the number of countries where the agent has been detected is likely to increase rapidly as a result of increased awareness, surveillance and availability of diagnostic methods. Any lack of openness regarding the TiLV status of a translocating live tilapia population destined for aquaculture may inadvertently contribute to the spread of the agent. Currently, there is no cure for viral diseases in aquaculture and while vaccines and selective breeding have proved successful in reducing the severity of some viral diseases, there are currently severe knowledge gaps relating to TiLV and no effective, affordable vaccines are yet available. This paper summarizes the published scientific information on TiLV and highlights important issues relating to its diagnosis, mitigation and control measures. While there have been no scientific studies on the socio-economic impact of TiLV, it may pose a significant threat particularly to small-scale fish farmers' livelihoods and wild tilapine populations if left uncontrolled. To aid disease investigations, the authors propose case definitions for suspected and confirmed cases of TiLV infections.

Key words: review, syncytial hepatitis, tilapia, Tilapia lake virus.

Introduction

Tilapia (*Oreochromis* sp.) is farmed on several continents, with production ranging from extensive backyard ponds to large, commercial operations. According to the Food and Agriculture Organization of the United Nations (FAO), the global production of tilapia was estimated at 6.4 million tons (MT) in 2015, with the top three producers being the People's Republic of China (1.78 MT), Indonesia (1.12 MT) and Egypt (0.88 MT) (FAO 2017a). Bangladesh, Vietnam and the Philippines are other leading producers (FAO 2017a).

Amongst the advantages of farming tilapia is its general hardiness, adaptability to various production systems and rapid growth, with advances in genetic selection and targeted breeding having further improved these characteristics (Ponzoni *et al.* 2011; e.g. FAO 2017b). Amongst important disease challenges are Streptococcus infections, which affect production worldwide and can occur in most production systems. Clinical signs of streptococcal infection may include skin haemorrhages, ocular alterations, ascites and abnormal behaviour (Amal & Zamri-Saad 2011; Suwannasang *et al.* 2014). In 1997, it was estimated that the yearly economic loss due to infection with Streptococcus was in the order of $150 million (Shoemaker & Klesius 1997). Apart from Streptococcosis, there are several common infectious diseases in farmed tilapia. Columnaris caused by *Flavobacterium columnare* often shows clinical signs of necrotic gills, fin rot, skin erosion or necrotic muscle (Figueiredo *et al.* 2005; Dong *et al.* 2015a). Francisellosis caused by *Francisella noatunensis* subsp. *orientalis* and Edwardsiellosis caused by *Edwardsiella ictaluri* produce clinical signs of visceral white spots in internal organs (Soto *et al.* 2009, 2012; Nguyen *et al.* 2016). Haemorrhagic septicaemia caused by motile aeromonads (*Aeromonas hydrophila*, *A. sobria*, *A. veronii* and *A. jandaei*) may present clinical signs of haemorrhage, exophthalmia and ascites (Li & Cai 2011; Dong *et al.* 2015b, 2017d) and mixed clinical signs of complicated multiple infections (Dong *et al.* 2015b; Assis *et al.* 2017). A vast array of viruses have been reported to affect cultured finfish (Crane & Hyatt 2011; Zhang & Gui 2015). In tilapia, several viral infections were

occasionally reported in tilapia fry including betanodavirus and tilapia larvae encephalitis virus (TELV) which present with neurological signs of erratic swimming or whirling syndrome (Shlapobersky *et al.* 2010; Keawcharoen *et al.* 2015); infectious spleen and kidney necrosis virus (ISKNV) associated with gross signs of lethargy, gill pallor and distension of the coelomic cavity (Subramaniam *et al.* 2016).

In the late 2000s, there was a large reduction in the annual wild catch of the Israeli Sea of Galilee's main edible fish, *S. galilaeus*, from 316 metric tons in 2005 to a low of 8 metric tons in 2009 (Eyngor *et al.* 2014). At the same time (2009), large losses of farmed tilapia were recorded throughout Israel (Eyngor *et al.* 2014). A novel RNA virus was subsequently identified and termed tilapia lake virus (TiLV) (Eyngor *et al.* 2014). Subsequent to the Israeli publication, scientific publications have reported identification of TiLV from samples collected in Colombia (Kembou Tsofack *et al.* 2017), Ecuador (Ferguson *et al.* 2014; Bacharach *et al.* 2016a), Egypt (Fathi *et al.* 2017; Nicholson *et al.* 2017), India (Behera *et al.* 2018), Indonesia (Koesharyani *et al.* 2018), Malaysia (Amal *et al.* 2018) and Thailand (Dong *et al.* 2017a; Surachetpong *et al.* 2017). In May 2017, the FAO released a Global Information and Early Warning System (GIEWs) special alert 338 on TiLV (FAO 2017c) and the World Organization for Animal Health (OIE) published a TiLV technical disease card (OIE 2017a). TiLV is currently not listed by the OIE, but there is ongoing work evaluating whether listing should take place. However, TiLV is listed for reporting under the NACA regional Quarterly Aquatic Animal Diseases (QAAD) reporting system for the Asia-Pacific. Subsequent to the OIE publication, six countries/territories have submitted notification to the OIE of TiLV presence, namely Chinese Taipei (OIE 2017b), Israel (OIE 2017c), Thailand (OIE 2017d), Malaysia (OIE 2017e), Peru (OIE 2018) and the Philippines (OIE 2017f). While the OIE terms the disease associated with TiLV as tilapia lake virus disease (OIE 2017a), other names such as syncytial hepatitis of tilapia (SHT) (Ferguson *et al.* 2014) and 1-month mortality syndrome (Tattiyapong *et al.* 2017) have been used in scientific papers. TiLV has been identified in samples from farms experiencing summer mortalities in Egypt (Fathi *et al.* 2017); however, the direct association with the virus and the summer mortality events has not yet been determined.

In addition to scientific papers and OIE notification documents, several other non-scientific documents, such as a Network of Aquaculture Centres in Asia-Pacific (NACA) disease advisory (NACA 2017) and a CGIAR Research Program on Fish Agri-food Systems factsheet (CGIAR 2017), have been published in relation to this emerging disease problem in an effort to notify relevant stakeholders. This review summarizes the currently available scientific information on TiLV and highlights important research gaps and issues relating to prevention and control of the associated disease. Some additional information currently only available in grey literature and through personal communication has also been included for the sake of completeness.

Aetiological agent

Viral properties

The virus has been described as a novel enveloped, negative-sense, single-stranded RNA virus with 10 segments encoding 10 proteins (Eyngor *et al.* 2014; Bacharach *et al.* 2016a; Surachetpong *et al.* 2017) and a diameter between 55 and 100 nm (Ferguson *et al.* 2014; Eyngor *et al.* 2014; del-Pozo *et al.* 2017; Surachetpong *et al.* 2017; Fig. 1). All 10 segments contain an open reading frame (ORF), with the largest segment, segment 1, containing an open reading frame with weak sequence homology to the influenza C virus PB1 subunit (~17% amino acid identity, 37% segment coverage) (Bacharach *et al.* 2016a). The remaining

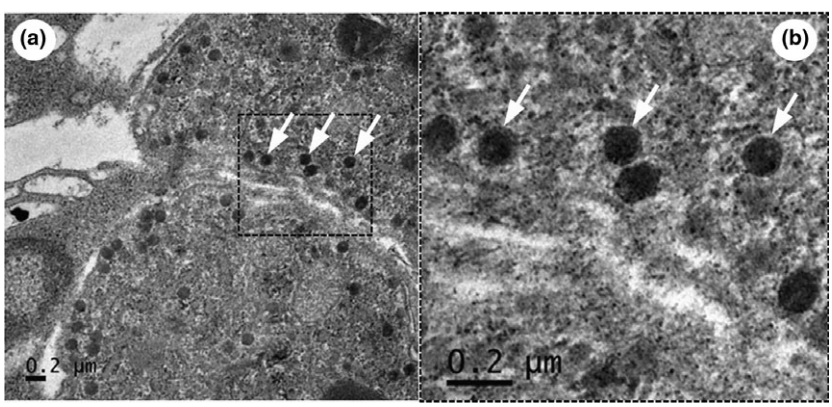

Figure 1 Transmission electron micrographs of TiLV-infected fish liver tissue showing cytoplasmic viral particles (white arrows) at low (a) and high (b) magnification. The electron micrograph in (b) is a magnification of the box outlined in black in (a), and the same 3 example virions (80–90 nm diameter) are indicated with white arrows in both electron micrographs (Images by H.T. Dong).

segments show no homology to other known viruses (Eyngor *et al.* 2014; Bacharach *et al.* 2016a); however, the conserved complimentary sequences at the 5′ and 3′ termini are similar to the genome organization found in orthomyxoviruses (Bacharach *et al.* 2016a). A taxonomic proposal has been submitted to the International Committee on Taxonomy of Viruses (ICTV) for a new, unassigned genus *Tilapinevirus* that include the new species *Tilapia tilapinevirus* (Bacharach *et al.* 2016b).

Viral particles have been found to be sensitive to organic solvents (ether and chloroform) due to their lipid membrane (Eyngor *et al.* 2014). Duration of survival outside the host has not been determined; however, horizontal, waterborne spread has been demonstrated under experimental conditions (Eyngor *et al.* 2014).

Results from *in situ* hybridization (ISH) indicate that TiLV replication and transcription occurs at sites of pathology (i.e. the liver in samples with liver lesions and the central nervous system in samples with central nervous system lesions) (Bacharach *et al.* 2016a). In samples collected from Thailand, ISH yielded positive signals in multiple organs (liver, kidney, brain, gills, spleen and muscle connective tissue), with the strongest signals found in liver, kidney and gills (Dong *et al.* 2017a). In samples originating from Ecuador, a viral predilection to liver and gastrointestinal tract was suggested, with an apparent tropism for hepatic epithelium (del-Pozo *et al.* 2017). Analyses of samples originating from the Tanzanian and Ugandan parts of Lake Victoria found TiLV RNA prevalence to be highest in the spleen, followed by the head kidney, heart and liver (Mugimba *et al.* 2018). No brain samples were found to be TiLV-positive; however, only two of the 17 fish where the brain was sampled tested positive for TiLV by another tissue (Mugimba *et al.* 2018).

Genetic variation

Currently, TiLV sequences from samples originating from Ecuador, Egypt, India, Israel, Malaysia, Tanzania (Lake Victoria), Thailand, Uganda (Lake Victoria) are available in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). The majority of sequences are from segment 1; however, there are currently two whole-genome sequences available, one from Israel (Bacharach *et al.* 2016a) and one from Thailand (Surachetpong *et al.* 2017).

The reported nucleotide identity between the Israeli TiLV (prototype strain) and isolates originating from South America, Africa and Asia has been listed in Table 1. From samples originating in Israel, available sequences include KJ605629 (clone 7450, ORF) (Eyngor *et al.* 2014) and KU751814 to KU751823 (whole genome, segments 1–10) (Bacharach *et al.* 2016a) and KU552132 (Tal *et al.* 2016).

Table 1 Overview of available TiLV sequences in GenBank and the percentage nucleotide identity found between sequences originating from Israel and sequences from other countries and territories

Source (non-Israeli sources)	GenBank accession no.	Identity to TiLV from Israel (prototype strain)		References
		GenBank accession no. of Israeli TiLV	% nt identity	
Chinese Taipei	Not available	Segment 3 (Accession number not specified)	93%	OIE (2017b)
Ecuador	Not available	Full genome sequences KU751814–KU751823	97.2–99.0%	Bacharach *et al.* (2016a)
Ecuador	Not available	KJ605629 (ORF)	98% to 100%	del-Pozo *et al.* (2017)
Egypt	Not available	KU751816 (segment 3)	93%	Fathi *et al.* (2017)
Egypt	KY817381–KY817390	Segments 3, 4 and 9 (Accession numbers not specified)	93%	Nicholson *et al.* (2017)
India	MF502419, MF574205 and MF582636	KJ605629 (segment 3)	96.4–97.2%	Behera *et al.* (2018)
Indonesia	Not available	KU751816 and KJ605629 (segment 3)	97%	Koesharyani *et al.* (2018)
Malaysia	MF685337	KU751822 (segment 9)	97%	Amal *et al.* (2018)
Philippines	Not available	Segment 3 (Accession number not specified)	94–95%	OIE (2017f)
Tanzania (Lake Victoria)	MF526980–MF526996	KU552132 (contig 7 = segment 2) KU751815 (= NC029921, segment 2)	Not given†	Mugimba *et al.* (2018)
Thailand	KY615742	KU751814 (segment 1)	96.3–97.5%	Dong *et al.* (2017a)
Thailand	KY615743	KU751818 (segment 5)		
Thailand	KY615744 to KY615745	KU751822 (segment 9)		
Thailand	KX631921 KX631930–KX631936	Full genome sequences KU751814–KU751823	95.6–99.1%	Surachetpong *et al.* (2017)
Uganda (Lake Victoria)	MF536423–MF536432	KU552132 (contig 7 = segment 2) KU751815 (= NC029921,segment 2)	Not given†	Mugimba *et al.* (2018)

†Authors state that sequences were 'identical with' or 'closely related to' the Israeli sequences.

Host factors

Susceptible species

Affected farmed species include hybrid tilapia (*Oreochromis niloticus* × *O. aureus* hybrids) in Israel (Eyngor *et al.* 2014); Nile tilapia (*O. niloticus*) in Ecuador (Ferguson *et al.* 2014), Egypt (Fathi *et al.* 2017), India (Behera *et al.* 2018), Indonesia (Koesharyani *et al.* 2018), Thailand (Dong *et al.* 2017a; Surachetpong *et al.* 2017) and Uganda (Mugimba *et al.* 2018); red tilapia (*Oreochromis* sp.) in Thailand (Dong *et al.* 2017a; Surachetpong *et al.* 2017) and red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) in Malaysia (Amal *et al.* 2018).

A range of wild tilapines (including *Sarotherodon galilaeus*, *Tilapiazilli*, *Oreochromis aureus*, and *Tristamellasimonis intermedia*) from the Sea of Galilee have tested positive for TiLV in Israel (Eyngor *et al.* 2014). In Malaysia, wild black tilapia (*Oreochromis* sp.) has been reported affected (OIE 2017e), while wild Nile tilapia tested positive in Lake Victoria (Tanzania and Uganda) (Mugimba *et al.* 2018) and in Peru (OIE 2018). Upon testing in Malaysia, Tinfoil barb (*Puntius schwanenfeldii*) was found to be TiLV-positive; however, the significance of this remains yet to be determined (Azila Abdullah, personal communication).

Cocultivated grey mullet (*Mugil cephalus*) and carp (*Cyprinus carpio*) have not shown mortality during disease outbreaks in Israel (Eyngor *et al.* 2014). Similarly, cocultivated grey mullet and thin-lipped mullet (*Liza ramada*) were found to be unaffected during Egyptian outbreaks (Fathi *et al.* 2017) and cocultivated Indian Major Carps (rohu (*Labeo rohita*), catla (*Catla Catla*), mrigal (*Cirrhinus mrigala*)), milk fish (*Chanos chanos*) and pearl spot (*Etroplus suratensis*) were unaffected in India (Behera *et al.* 2018).

Susceptible life stages

In Israel, mortalities have been observed over a wide weight range (Eyngor *et al.* 2014). Fingerlings and juveniles (up to 80 g) have been affected in Ecuador (Ferguson *et al.* 2014), India (Behera *et al.* 2018), Malaysia (Amal *et al.* 2018) and Thailand (Dong *et al.* 2017a; Surachetpong *et al.* 2017). In Egypt, medium- (>100 g) and large-sized fish have been affected by summer mortality, some of which have tested positive for TiLV (Fathi *et al.* 2017). Both juvenile and adult tilapia have been reported affected in Peru (OIE 2018). Early developmental stages of tilapia (fertilized eggs, yolk-sac fish and fry) have also tested positive for TiLV (Dong *et al.* 2017b). Subclinical TiLV infection has been reported from Thailand in clinically healthy adults (two of two tested) and fingerlings (nine of 19 tested), with no clinical disease reported to have been observed 1 month after sampling was completed (Senapin *et al.* 2018).

Clinical signs and diagnostics

Clinical signs and gross pathology

The reported clinical signs and gross pathological lesions associated with TiLV infections are somewhat variable, depending on geographical origin. Clinical signs include lethargy, ocular alterations, skin erosions and discoloration (darkening) in farmed tilapia in Israel (Eyngor *et al.* 2014) while lethargy, skin erosions and ocular lesions were reported from cases in wild tilapines (OIE 2017c). The case in Ecuador presented with exophthalmia, discoloration (darkening), abdominal distension, scale protrusion and gill pallor (Ferguson *et al.* 2014), while ulcers and exophthalmia have been reported from Peru (OIE 2018). In Thailand, loss of appetite, lethargy, abnormal behaviour (e.g. swimming at the surface, stop schooling), pallor, anaemia, exophthalmia, abdominal swelling and skin congestion and erosion have been reported (Dong *et al.* 2017a; Surachetpong *et al.* 2017). Brain congestion and paleness of the gills and liver have additionally been observed (Surachetpong *et al.* 2017). In the laboratory, naturally infected Thai Nile tilapia fingerlings showed darkening and some moribund fish exhibited scale protrusion prior to death (Dong, personal observation). From India, clinical signs in naturally infected fish were skin erosions and loss of scales while experimentally infected fish exhibited exophthalmia, swollen abdomen and scale protrusion (Behera *et al.* 2018). Clinical signs reported from the Philippines include abdominal swelling and bulging of the eyes (OIE 2017f). In Egyptian farms experiencing 'summer mortality', affected fish showed haemorrhagic patches, detached scales, open wounds, dark discoloration and fin rot, with some of these fish testing positive for TiLV, with or without coinfection by *Aeromonas* spp. (Nicholson *et al.* 2017). No case description was provided for the fish from which the Colombian samples originated (Kembou Tsofack *et al.* 2017). A range of clinical signs representative of TiLV infection is shown in Figure 2. Based on the available information, it seems that a complete list of pathognomonic signs, to allow a reliable diagnosis based on clinical signs alone, is not currently feasible.

Histopathology

There appear to be some geographical and individual variations in the histopathological lesions associated with TiLV infections. Observed lesions in affected fish in Israel include congestion of internal organs (kidney and brain), foci of gliosis and perivascular cuffing in the brain cortex and ocular lesions (endophthalmitis and cataractous changes of the lens) (Eyngor *et al.* 2014). In affected fish from Egypt, histopathological findings included gliosis, encephalitis and mild perivascular cuffing in the brain, multifocal chronic

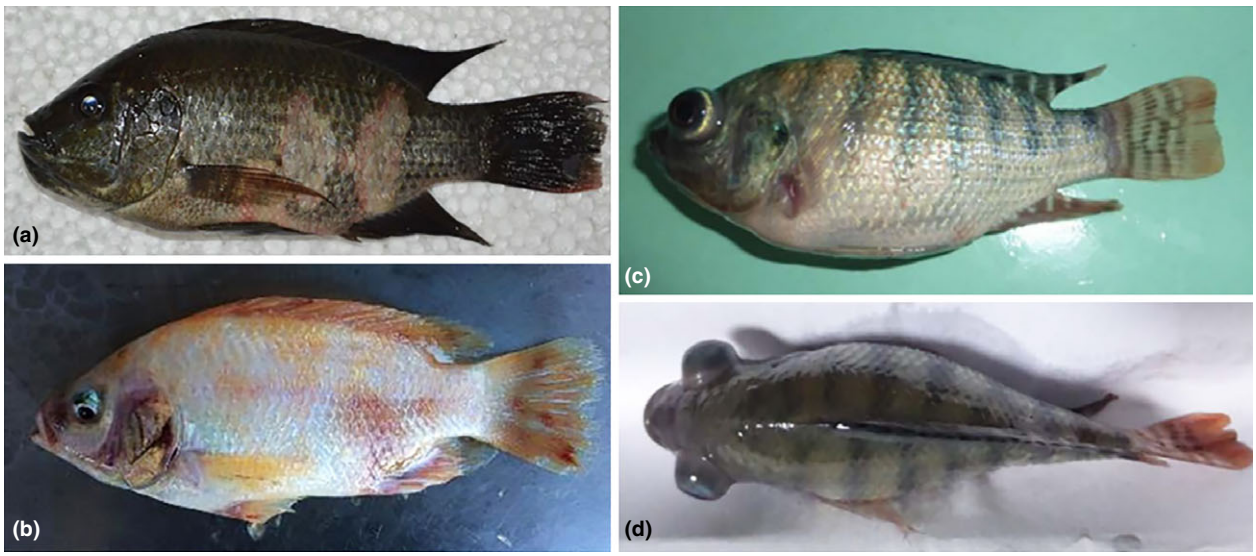

Figure 2 Clinical signs of representative TiLV-infected Nile tilapia and red tilapia: naturally diseased Nile tilapia showing discolouration, loss of scales and skin erosion (a), naturally diseased red tilapia showing skin haemorrhages (b), experimentally diseased Nile tilapia showing exophthalmia, abdominal swelling and scale protrusion (c and d). Images (a), (c) and (d) are reprinted from Aquaculture, Volume 484, Behera *et al.*, Emergence of tilapia lake virus associated with mortalities of farmed Nile tilapia *Oreochromis niloticus* (Linnaeus 1758) in India, pages 168–174, Copyright (2018), with permission from Elsevier. Image C provided by H. T. Dong (taken in conjunction with the outbreak described in Dong *et al.* 2017a.).

hepatitis and multifocal interstitial haemorrhage in the kidney (Fathi *et al.* 2017). The case in Ecuador showed hepatocyte necrosis and syncytial cell formation, necrosis of gastric glands and diffuse congestion in multiple tissues (Ferguson *et al.* 2014). Syncytial hepatitis was also reported in samples from Colombia (Kembou Tsofack *et al.* 2017), India (Behera *et al.* 2018), Malaysia (Amal *et al.* 2018) and Thailand (Dong *et al.* 2017a). In Indian cases, syncytial cell formation was observed in the liver of both naturally and experimentally infected fish and occasionally observed in the brain of experimental fish (Behera *et al.* 2018). Additional observations from Thailand include aggregation of lymphocytes and perivascular cuffing in brain tissue (Surachetpong *et al.* 2017). The presence of eosinophilic intracytoplasmic inclusions has been described with both natural infections (Ferguson *et al.* 2014) and in experimentally infected fish (Tattiyapong *et al.* 2017).

A degree of histopathological variation in fish from a single farm has been observed in Thailand (Fig. 3). While syncytial hepatitis and foamy cytoplasm were observed in the liver of the majority of tested fish, all naturally infected fish showed severe pancreatic necrosis and some occasionally exhibited the presence of intracytoplasmic inclusion bodies in the hepatocytes. Inflammation with severe infiltration of lymphocytes was observed in some areas of the kidney tubules and brain where syncytial cells were located in the centre of areas of inflammation (Dong, personal observation). Currently available information suggests syncytial hepatitis to be the most common histopathological feature found in TiLV outbreaks. While it was not reported from outbreaks in the earliest report from Israel (Eyngor *et al.* 2014), syncytial hepatitis was described in a later study from the same research group (Bacharach *et al.* 2016a).

Cell culture

Experiments have shown multiple cell lines to be suitable for TiLV cell culture (Eyngor *et al.* 2014; Kembou Tsofack *et al.* 2017).

The E-11 cell line was found to show visible cytopathic effect (CPE) 5–7 days after inoculation, with cytoplasmic vacuoles and plaque formation followed by disintegration of cell monolayer nine to 10 days after inoculation (Eyngor *et al.* 2014; Tattiyapong *et al.* 2017). Cell lines of primary tilapia brain cells showed swollen, rounded, granulated cells 10–12 days after inoculation, with monolayer detachment 14–19 days after inoculation (Eyngor *et al.* 2014). E-11 cells at 25°C have been reported to provide optimal conditions for TiLV replication (Kembou Tsofack *et al.* 2017). The OmB and TmB cell lines derived from *O. mossambicus* showed similar sensitivities to TiLV infection as the E-11 cell line; however, the E-11 cultures were deemed superior due to the clear and rapid CPE development (Kembou Tsofack *et al.* 2017). OmB has been suggested as a useful cell line for endpoint dilution (TCID50) assays and both OmB and TmB may reportedly be useful for generating pure

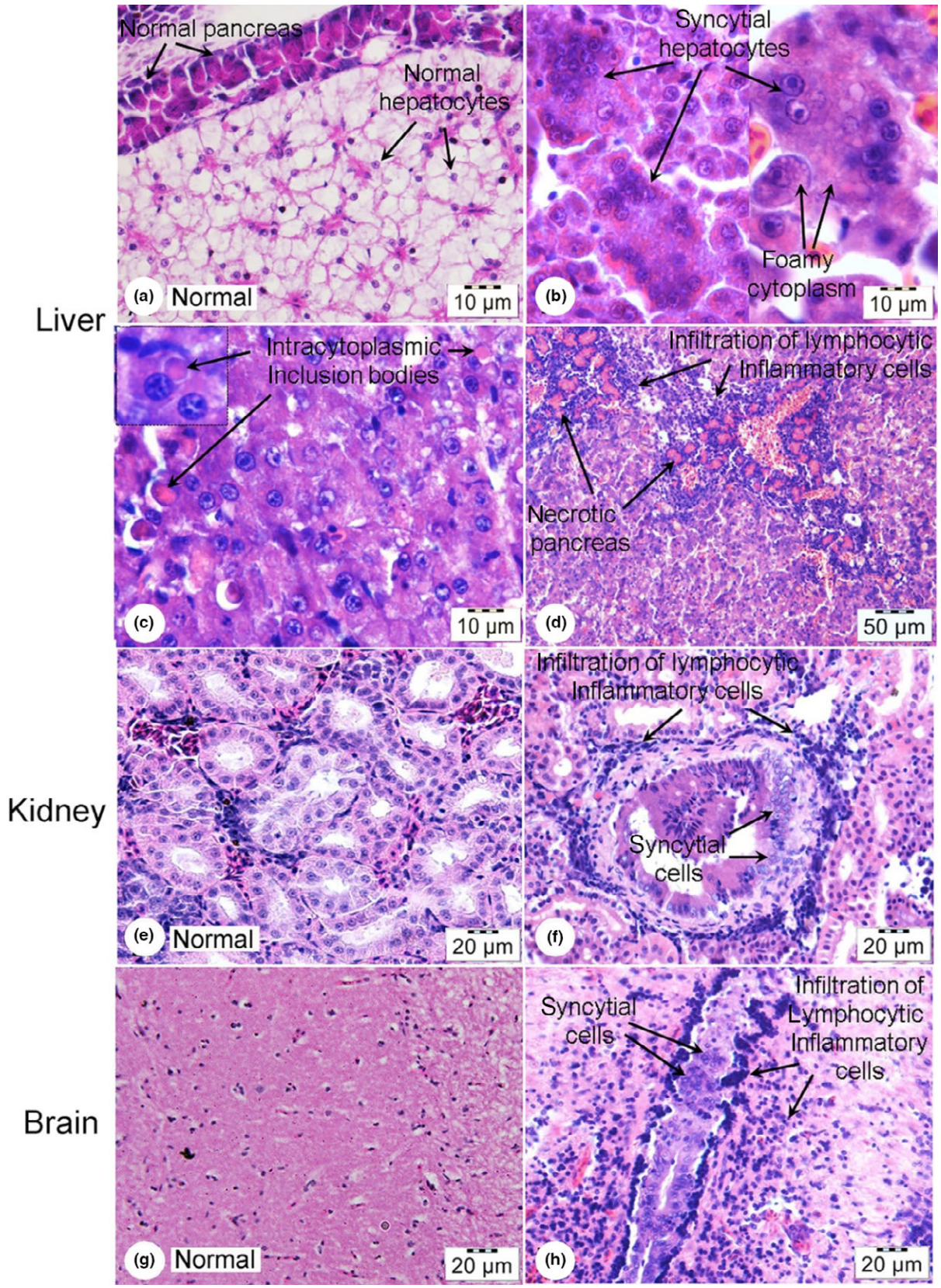

Figure 3 Photomicrographs of haematoxylin and eosin-stained sections of tissue from the liver, kidney and brain of normal fish (a, e, g) and TiLV-infected fish (b–d, f, h). The infected liver tissue showed syncytial hepatocytes and foamy cytoplasm (b), intracytoplasmic inclusion bodies (c) and inflammation with pancreatic necrosis (d). Kidney tissue showed syncytial cells and severe infiltration of inflammatory lymphocytes (f). Brain tissue also showed syncytial cells and severe infiltration of inflammatory lymphocytes (h) (Images by H.T. Dong.)

CCAAATATTACCCCTTAATCCTTAATAGACCGTTAACTTTCTTTTGAAATGGACTCGCGGTTTGCACAGCT
AACTGGGGTTTTCTGTGACGATTTCACTTATAGCGAAGGGAGCCGAAGGTTCCTAAGTTCTTACAGTACAG
TAGAGAGACGTCCAGGAGTCCCCGTAGAGGGTGACTGTTATGACTGTTTGAAGAATAAGTGGATTGCCTTT
GAGCTGGAAGGCCAGCCGCGGAAATTTCCAAAGGCAACAGTTCGTTGCATTTTGAACAATGATGCTACATA
CGTTTGCTCTGAGCAAGAGTACCAGCAGATTTGTAAGGTACAATTCAAGGATTATTTGGAGATCGACGGGG
TTGTTAAAGTTGGGCACAAGGCATCCTACGATGCTGAGCTAAGGGAACGGCTATTGGAACTACCACATCCA
AAGAGTGGCCCGAAGCCTCGTATTGAGTGGGTGGCACCACCCAGACTTGCGGACATATCCAAGGAAACAGC
TGAGCTAAAGAGGCAATATGGATTCTTCGAGTGCTCAAAGTTCCTCGCCTGCGGTGAGGAGTGTGGTCTTG
ACCAAGAGGCAAGAGAACTTATACTGAACGAGTACGCACGTGATAGAGAATTTGAGTTCCGCAATGGAGGG
TGGATACAAAGGTATACAGTTGCTTCTCAYAAGCCTGCTACACAGAAGATATTACCTCTACCGGCTAGTGC
YCCACTTGCTCGTGAGCTTTTGATGTTGATTGCTAGAAGCACAACTCAGGCAGGGAAAGTACTGCATAGCG
ATAATACCAGCATACTAGCTGTACCGGTCATGCGCGACTCTGGAAAGCACAGTAAAAGGAGACCAACCGCC
TCCACTCACCACTTAGTTGTAGGTCTAAGTAAACCTGGYTGTGAACACGATTTTGAGTTTGACGGGTACAG
GGCAGCTGTGCATGTGATGCACCTAGATCCCAAGCAATCGGCTAATATAGGGGAGCAAGACTTTGTGAGTA
CCCGAGAAATTTACAAGCTGGATATGTTGGAACTACCTCCCATAAGTAGGAAGGGTGATCTGGACAGAGCT
AGTGGTCTTGAGACAAGATGGGACGTCATCTTACTTCTGGAATGCCTCGACTCTACAAGGGTTAGCCAAGC
AGTGGCTCAACATTTTAATAGGCACCGGCTAGCACTTAGCGTCTGTAAGGACGAGTTCAGGAAAGGCTACC
AGCTGGCTTCTGAGATAAGGGGTACAATACCCTTAAGCTCACTTTATTATTCACTTTGTGCAGTAAGATTG
CGGATGACAGTACACCCATTTGCGAGATGAATCGCTTTCGACGCCTTCGCTAAAGGTTACGACGTTCTAATA
GAGGATTATGGGAAAAATTTGC

Figure 4 Coding strand sequence of TiLV genome segment 3 (KU751816). Putative start and stop codons are enclosed in boxes. The shaded sequences represent positions of primers used in nested PCR (from left to right; Nested ext-2, ME1, 7450/150R/ME2 and Nested ext-1) published by Eyngor *et al.* (2014) and Kembou Tsofack *et al.* (2017). The two inner primers were also employed for SYBR green-based RT-qPCR (Kembou Tsofack *et al.* 2017). Nested ext-2 primer indicated by shaded underlined sequence was omitted in the semi-nested RT-PCR method (Dong *et al.* 2017a). Double underlines indicate the sites that were used to design primers for a recent SYBR green-based RT-qPCR protocol (Tattiyapong *et al.* 2018).

TiLV strains as they are snakehead reovirus-free (Kembou Tsofack *et al.* 2017). Pooling of two to three samples of brain tissue has yielded positive TiLV culture results (Kembou Tsofack *et al.* 2017).

More recently, the CFF cell line, originating from *Pristolepis fasciatus*, has been used for viral propagation in India, which exhibited CPE at day 3 postinoculation and caused severe cell detachment at days 6–7 postinfection (Behera *et al.* 2018). Most recently, two tilapia-derived cell lines, OnlB from brain and OnlL from liver, has been shown to be highly permissive for propagating TiLV (Swaminathan *et al.* 2018). Other cell lines (CHSE-214, BF-2, BB, EPC, KF-1, RTG-2 and FHM) have been reported to show inconsistent CPE with TiLV (Eyngor *et al.* 2014). No CPE was reported in CCKF, RTF, PSF, HBF and FtGF cells lines (Swaminathan *et al.* 2018).

Molecular methods

Several polymerase chain reaction (PCR)-based methods for TiLV detection have been described in the literature. Initially, a reverse transcriptase-PCR (RT-PCR) method for TiLV detection was published together with information on TiLV-specific primers targeting segment 3 (Eyngor *et al.* 2014). This was followed by the publication of a nested RT-PCR assay using the same primer sets with detailed conditions enabling detection down to seven copies of TiLV, 10 000 times more sensitive than the single RT-PCR (limit detection of ~70 000 copies) (Kembou Tsofack *et al.* 2017). The nested RT-PCR assay was found to detect TiLV in both fresh and preserved (RNAlater; QIAGEN) samples from diseased fish, and identified TiLV RNA in samples from diseased fish from Israel, Ecuador and Colombia (Kembou Tsofack *et al.* 2017). A SYBR green-based qPCR method using nested primers (ME1 & 7450/150R/ME2) gained a limit detection of 70 copies (Kembou Tsofack *et al.* 2017). Subsequently, an alternative semi-nested RT-PCR method has been described where the primer Nested ext-2 was omitted to reduce the risk of false-positive detections (Dong *et al.* 2017a). This protocol had a detection limit of 7.5 copies (Dong *et al.* 2017a) and was able to detect TiLV from clinically healthy fish (Senapin *et al.* 2018). Pooling of between two to five samples has been reported to yield successful agent identification (Dong *et al.* 2017a; Fathi *et al.* 2017; Kembou Tsofack *et al.* 2017; Surachetpong *et al.* 2017). Recently, a newly SYBR green-based reverse transcription quantitative PCR (RT-qPCR) method targeting the same genome segment 3 was developed for detection of TiLV from clinical samples with a reported sensitivity of two copies/μL (Tattiyapong *et al.* 2018). The locations

of the published primers are shown in Figure 4. None of the currently available PCR methods have been fully validated. Until validations have been performed and published current detection methods should be combined with sequencing of representative PCR products for agent confirmation.

An *in situ* hybridization (ISH) method has been described and has been used to reveal TiLV tissue tropism (Bacharach *et al.* 2016a; Dong *et al.* 2017a). ISH revealed that mRNA of the virus was detected in both the nucleus and the cytoplasm of the infected cells (Bacharach *et al.* 2016a). It should be noted that ISH using a DIG-labelled probe derived from the partial genome segment 3 of TiLV (415 bp) allowed detection of TiLV from different organs of naturally TiLV-infected fish which tested positive in the first step RT-PCR (Dong *et al.* 2017a). However, no detectable positive signal by ISH was found for those samples that tested positive for TiLV in the second step RT-PCR, suggesting that the ISH method by Dong *et al.* (2017a) may be suitable for heavily infected samples only (Dong, personal observation).

Serology

There are no publications relating to the use of serology in the context of TiLV diagnostics. Ferguson *et al.* (2014) observed a reduced packed cell volume (16% versus the normal 48–50%) in the TiLV case in Ecuador, together with an increased number of immature erythrocytes in blood smears.

Nonlethal sampling

A comparative study of mucus and liver samples collected from 35 randomly selected moribund and healthy adult red tilapia revealed identical TiLV status for both tissues when analysed by the RT-qPCR method described by Tattiyapong *et al.* (2018) (Liamnimitr *et al.* 2018). Twenty-one of 35 samples tested positive for TiLV, with the Ct values for the two tissue types noted to be relatively close (Liamnimitr *et al.* 2018), although no statistical evaluation was reported. TiLV-infected mucus inoculated onto E-11 cells showed typical CPE after 3–7 days after infection (Liamnimitr *et al.* 2018). In an infection trail, TiLV RNA was detected in cohabitant fish mucus one to 12 days postinfection (dpi) (comparative values for liver and intestines being two to 14 dpi and five to 12 dpi, respectively, with no TiLV was detected in faeces) (Liamnimitr *et al.* 2018).

Epidemiology

Geographical distribution

Based on information in scientific publications, OIE notifications and sequence information available in Genbank, TiLV appears to be present on the three continents with the largest tilapia production, namely Africa (Egypt (e.g. Fathi *et al.* 2017), Tanzania (Mugimba *et al.* 2018) and Uganda (Mugimba *et al.* 2018)), Asia (Chinese Taipei (OIE 2017b), India (Behera *et al.* 2018), Indonesia (Koesharyani *et al.* 2018), Israel (e.g. Eyngor *et al.* 2014; OIE 2017c), Thailand (e.g. Dong *et al.* 2017a; OIE 2017d), Malaysia (OIE 2017e; Amal *et al.* 2018) and the Philippines (OIE 2017f) and South America (Colombia (Kembou Tsofack *et al.* 2017), Ecuador (e.g. Bacharach *et al.* 2016a) and Peru (OIE 2018)).

The majority of countries have reported a limited number of TiLV detections and/or disease outbreaks so far. The exception is Israel where TiLV has been found more widespread, ranging from wild tilapia stocks in the Sea of Galilee to farmed stocks in all the major aquaculture areas (coastal shore, Jordan Valley and Upper and Lower Galilee) (Eyngor *et al.* 2014). In Egypt, 37% of randomly selected fish farms in the major aquaculture areas (Kafr el Sheikh, Behera, Sharkia) were affected by summer mortalities when sampled in 2015 (Fathi *et al.* 2017). Amongst sampled farms that had experienced 'summer mortalities', four of eight farms sampled in 2015 (Nicholson *et al.* 2017) and three of seven farms sampled in 2016 (Fathi *et al.* 2017) were found to be TiLV-positive.

Although TiLV became known to science in 2014, the virus was suspected to be responsible for massive mortalities of tilapia in Israel and Ecuador since 2008–2009 (Eyngor *et al.* 2014; Bacharach *et al.* 2016a; FAO 2017c). Similarly, TiLV was reported in Thailand in early 2017 (Dong *et al.* 2017a; Surachetpong *et al.* 2017). However, samples collected in 2015 and 2016 (Surachetpong *et al.* 2017), and archived samples from unexplained mortalities events between 2012 and 2016 (Dong *et al.* 2017b), have tested positive for TiLV. A SHT histopathological feature resembling TiLV infection was observed in the experimental fish used for a student thesis (MSc) at Chulalongkorn University in 2014 (Weerapornprasit *et al.* 2014), possibly representing an earliest known case of SHT in Thailand (Nopadon Pirarat, personal communication). Retrospective investigation of available archived samples in other fish health laboratories may shed further light on origins and distribution of TiLV (Dong *et al.* 2017b).

Mortality in natural and experimental outbreaks

High levels of mortality have been reported in association with TiLV infections on all three continents. Mortality levels of above 80% have been observed in affected farmed populations in Israel, while no such level of mass mortality has been reported in wild stocks from which positive samples have been obtained (Eyngor *et al.* 2014). In Thailand, mortality levels between 20% and 90% have been reported, with mortality usually seen within the first month after transfer to grow-out cages (Dong *et al.* 2017a;

Surachetpong *et al.* 2017) and peak mortality rates observed within 2 weeks of onset of mortality (Surachetpong *et al.* 2017). Similarly, a case in Ecuador showed onset of mortality from 4 to 7 days posttransfer to on-growing ponds, with mortality ranging from a low level of 10–20% to a high level of 80%, depending on the fish strain (Ferguson *et al.* 2014). In India, outbreaks of TiLV associated with 80–90% mortality (Behera *et al.* 2018). In contrast, the average mortality level at farms experiencing 'summer mortality' in Egypt was found to be 9.2% (range 5–15%), with the mortality level attributable to TiLV infection currently unknown (Fathi *et al.* 2017). Similarly, several cases of natural TiLV infection have been found to be associated with relatively low levels of mortality (6.4% in Chinese Taipei and 0.71% and 15% in wild and farmed tilapia, respectively, in Malaysia) (OIE 2017b,e). Subclinical infections in both adults and fingerlings have also been reported in Thailand (Senapin *et al.* 2018), and variations in mortality have been reported in farms with different species and production form combinations in Thailand, ranging from around 20% in farms with mixed stocking of red tilapia and Nile tilapia in earthen pond in Phetchaburi province to around 90% in farms with Nile tilapia in Pathum Thani province and farms with red tilapia in open floating cages in Chai Nat province (Dong *et al.* 2017a). A significant strain differences in mortality have been observed in Ecuador where the Chitralada strain was found to have significantly higher mortality than GMT and GIFT strains (Ferguson *et al.* 2014; Kabuusu *et al.* 2018).

Successful viral propagation has allowed the conduction of TiLV infection experiments by several research groups. In Israel, experimental infection of Nile tilapia juveniles (30–35 g, strain Chitralada) by intraperitoneal (IP) injection resulted in 75–85% mortality within 10 days, with a similar mortality pattern observed in a cohabitation experiment (Eyngor *et al.* 2014). In Thailand, recorded mortality levels of red tilapa and Nile tilapia juveniles (~30 g) within 12 dpi were 66% and 88%, respectively (Tattiyapong *et al.* 2017), with a second cohabitation trail showing a cumulative mortality level of 55.7% for cohabitant fish (Liamnimitr *et al.* 2018). A cumulative mortality of 100% by day seven postinfection was observed in an infection trail using IP injection of 12–15 g tilapia (Behera *et al.* 2018). Eyngor *et al.* (2014) noted that fish surviving disease outbreaks have been found to be resistant to subsequent outbreaks, which indicate a host immune response against primary infection suggesting that vaccination may be an appropriate approach for disease control.

Risk factors

A study of production-level risk factors for the presence and severity of SHT in Ecuador assessed tilapia strain, stocking density, fry weight at transfer, weather pattern, water temperature, dissolved oxygen, the number of days spent preparing the pregrow-out pond, month of transfer to pregrow-out pond, daily feeding rate, number of pond production cycles per year, year of stocking and mortality rate per production cycle (Kabuusu *et al.* 2018). It was found that infected populations showed about five times higher mortality levels than uninfected populations (RR = 4.8, 95% CI 2.9–7.9), with tilapia of the Chitralada strain showing twice as high mortality as GMT and GIFT strains (RR = 2.1, 95% CI 1.8–2.4) (Kabuusu *et al.* 2018). Excess mortality was significantly associated with dissolved oxygen, stocking density (fish/m2), number of pond production cycles per year (Kabuusu *et al.* 2018). In Egypt, large farm size, high stocking densities and tilapia-mullet polyculture have been identified as risk factors for TiLV outbreaks (Fathi *et al.* 2017).

In Ecuador, increased water temperature (°C) and increased fry weight at transfer (g) were protective factors for both excess mortality and severe SHT (defined as very high excess mortality), and no association was found between season (wet/dry) and the presence and severity of SHT (Kabuusu *et al.* 2018). Clinical outbreaks have been reported during the hot season, namely May to October (at water temperatures of 22°C to 32°C) in Israel (Eyngor *et al.* 2014), June to October (≥25°C) in Egypt (Fathi *et al.* 2017) and May to November (25°C to 27°C) in Ecuador (Ferguson *et al.* 2014). Some of the samples yielding positive TiLV detection in Thailand were collected in the months between October and May (Surachetpong *et al.* 2017). Affected fingerlings in Ecuador were commonly detected within 4–7 days posttransfer to grow-out ponds (Ferguson *et al.* 2014), with tilapia strain being found to be a risk factor for high mortality (Ferguson *et al.* 2014; Kabuusu *et al.* 2018). In Thailand, variations in mortality have been observed with different species and production form combinations (Dong *et al.* 2017a).

Coinfections

It has previously been proposed that multiple infections outweigh single infection during disease outbreaks in farmed tilapia (Dong *et al.* 2015b). In the case of TiLV, reported coinfections in TiLV-positive fish from Thailand included bacteria (Flavobacterium, Aeromonas and Streptococcus), external monogenean parasites (Gyrodactylus and Dactylogyrus) and ciliated protozoa (Trichodina) (Surachetpong *et al.* 2017). Several of the TiLV-positive fish from Egypt in 2015 were reported to have a coinfection of one or more Aeromonas spp. (*A. veronii*, *A. ichthiosmia*, *A. enteropelogenes* and *A. hydrophilia*) (Nicholson *et al.* 2017). A case of coinfection between *A. veronii* and TiLV in juvenile hybrid red tilapia (*O. niloticus* × *O. mossambicus*)

has been reported in Malaysia, which resulted in a mortality rate of approximately 25% (Amal *et al.* 2018). Examination of 20 diseased fish revealed an infection rate of 20% and 50% for TiLV and *A. veronii*, respectively (Amal *et al.* 2018). In a case of TiLV infection in red tilapia juveniles, it was observed that while all clinically diseased fish tested positive for TiLV, 50% of the examined fish were also infected by an unknown microsporidian-like organism in their muscle (Dong, personal observation). The relative importance of TiLV and any coinfections in terms of clinical severity, mortality, incubation time and so on has not been determined.

Socio-economic impact

No estimate has been published on the socio-economic impact of TiLV in a national or global context. High levels of mortality have been reported from field cases on all three continents (Eyngor *et al.* 2014; Ferguson *et al.* 2014; Dong *et al.* 2017a; Surachetpong *et al.* 2017; Behera *et al.* 2018) suggesting that the impact may be significant. However, relatively lower mortality levels have been reported in some field cases (Fathi *et al.* 2017; OIE 2017b,e) and subclinical infections have been described (Senapin *et al.* 2018) and the reasons for these differences are yet unknown. Estimates from Egypt indicate a production loss of 98 000 metric tons, at a value of around USD 100 million, due to the 'summer mortality' syndrome in 2015 (Fathi *et al.* 2017), of which TiLV may play a part.

The impact on wild stocks may also be highly significant, both in economic terms and in relation to biodiversity- and ecological effects. In the Sea of Galilee in Israel, the annual wild catch figures for the main edible fish in the lake, *S. galilaeus*, were reported to have decreased from 316 metric tons in 2005 to 8 metric tons in 2009, with a subsequent increase to 160 metric tons in 2013 and 140 metric tons in 2014 (Eyngor *et al.* 2014). The contribution of TiLV infection to this decline has not been determined; however, TiLV has been identified in samples from several wild tilapines, including S. Galileaus (Eyngor *et al.* 2014).

Discussion

Knowledge of the geographical distribution of TiLV has rapidly increased with the heightened awareness of this new agent affecting tilapia. The increase in the number of countries detecting TiLV in their tilapia populations and the detection of TiLV from archived samples suggest that TiLV has been present as a hidden pathogen for several years. International trade of tilapia has taken place for more than 50 years with a resultant global distribution only exceeded by common carp. Tilapia is native to Africa, with movements having taken place since 1944, and it is currently present in over 90 countries (De Silva *et al.* 2004). Beginning with the programme in 1988, GIFT represents the first systematic collection and transfer of Nile tilapia germplasm from Africa to South-East Asia (Gupta & Acosta 2004; Acosta & Gupta 2010); however, tilapia was widely moved around also prior to this programme. As a result, international trade may have been circulating the agent worldwide through movement of live fish for aquaculture in the absence of knowledge of the existence of an associated risk. The observation of subclinical infections increases the risk of such transfers having occurred. It is speculated that over 40 countries may have a theoretical risk of inadvertent TiLV introduction due to trade and suggest the importance of initiation of surveillance activities in these countries (Dong *et al.* 2017b,c). Similarly, extensive trade of ornamental cichlids could theoretically pose a threat for the spread of TiLV although no TiLV has been reported in ornamental cichlids to date. Emergence and spread of viral diseases in farmed shrimp and its association with global movement of live shrimp are well documented (Walker & Mohan 2009). Taking cues from shrimp, it would be worth exploring if a massive ecological shift and changes in the trade patterns that have accompanied the establishment and growth of tilapia farming industry has anything to do with the emergence of TiLV.

As the agent was new to science until first published in 2014 and no easily available diagnostic tests for its detection was available, it is only recently that competent authorities, scientists and other stakeholders could initiate the work of unravelling the true distribution of TiLV. With a multicontinent presence of TiLV, there is a need for regional capacity building within all stakeholder groups and participatory approaches at all stakeholder levels should be encouraged. According to the FAO, multiple countries have initiated official screening and surveillance programmes (FAO 2017c) and expansion of such activities in other at-risk countries should be encouraged. The FAO has also published recommended biosecurity measures that countries need to follow when translocating live tilapias, for countries found positive for TiLV and for countries with an unknown TiLV status (FAO 2017c). International collaboration on such screening/surveillance efforts may expedite knowledge generation while local diagnostic capacity building is being undertaken. Conducting TiLV import risk analysis should be encouraged in countries with significant tilapia production where TiLV has not been detected. The current uncertainty regarding the distribution of TiLV both geographically (continents and countries), and more specifically within the various sectors of the affected tilapia industries, represents large challenges for designing and conducting cost-effective surveillance programmes. Added to this challenge is the need for the development of sufficient diagnostic capacity specific to TiLV and the

development of a common understanding amongst all stakeholders. Collaborative programmes between the private sector and relevant governments should be promoted to limit the impact of TiLV and the associated disease. The potential to use a nonlethal sampling for initial screening may significantly aid farmer compliance for investigation for TiLV. Nonlethal sampling methods are not new to aquatic disease investigations. A nonlethal sampling technique (pectoral fin) has for example been described for infectious pancreatic necrosis (IPN) in salmonids (Bowers *et al.* 2008), and studies on ISAV have shown early replication in several mucosal tissues including gills, pectoral fin, skin and gastrointestinal tract (Aamelfot *et al.* 2015) supporting the feasibility of using mucosal samples for initial detection of TiLV.

The importance of an appropriate case definition, despite the presence of some knowledge gaps, has been discussed by for example Baldock *et al.* (2005). While Koch's postulates have been fulfilled for TiLV by independent studies (Eyngor *et al.* 2014; Tattiyapong *et al.* 2017; Behera *et al.* 2018), the associated clinical signs and histopathological changes appear to reflect a degree of geographical and individual variations. As a result, no scientifically sound set of pathognomonic signs has been defined for the disease associated with TiLV infection. Despite this fact, the authors would like to suggest some temporary case definitions for TiLV infection until definite case definitions can be proposed. Although bound to be inaccurate in some cases, it may serve as a guideline to aid disease investigations, particularly in areas where TiLV has not yet been confirmed or reported. In the event of listing by the OIE, it will be 'infection by TiLV' that will be listed and not the resultant clinical disease. As a result, the suggested case definitions are reflecting this and are also referring to the group of animals (e.g. pond-level), and all life stages of tilapia should be considered at risk and susceptible. A suspected case of TiLV infection may have one or more of the following characteristics: (i) A pond/cage of tilapia fingerlings or juveniles (<80 g) with increased abnormal mortality during early period of cultivation (1–4 weeks after stocking) in the absence of obvious noninfectious causes or (ii) A pond/cage of tilapia subadults/adults with increased abnormal mortality in the absence of obvious noninfectious causes or (iii) A pond/cage where the tilapia show one or more of the following clinical signs: behavioural changes, exophthalmia or other ocular lesions, skin erosions, discolouration, skin haemorrhage, scale protrusion and/or abdominal swelling or (iv) A pond/cage where at least one tested tilapia show histopathological feature of syncytial hepatitis. A confirmed case of TiLV infection has a positive PCR analysis for TiLV with subsequent sequencing of the representative PCR product showing TiLV presence.

At present, none of the currently available PCR methods have been fully validated. While there has been some data published on the analytical- and diagnostic sensitivities and specificities, none of the methods have been fully validated in both healthy and diseased populations and no information on optimal tissues and the appropriateness of pooling has been made available. Therefore, it seems prudent to encourage the combination of the currently available PCR detection methods with sequencing of representative PCR products for agent confirmation until the diagnostic tests have been sufficiently validated. The development of new or improved diagnostic methods for TiLV (e.g. enzyme-linked immune sorbent assay (ELISA), rapid antigen strip test, recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP)) should be encouraged. As tilapia is frequently farmed by lower income farmers, there is a need for low cost, accurate diagnostic methods that does not require extensive laboratory facilities.

There is little scientific knowledge available regarding important epidemiological aspects of TiLV. For example, the reasons for the large variations in observed mortality, which may be related to genetic variation in the virus, differing host susceptibility, environmental factors, coinfections or a combination of these, need to be elucidated. Further knowledge on viral properties such as survival of TiLV outside the host (in water, on fomites, in fresh/frozen products), risk factors for disease outbreaks and the presence of any nontilapine hosts/carrier species need to be further investigated. The survival of viral particles in general fish commodities has been widely discussed, but little scientific information is available. From Norway, it has been observed that infectious viral particles of infectious salmon anaemia (ISA) virus have been cultured after more than 20 years in −80°C (Knut Falk, personal communication); however, its relevance for agent spread in the field remains undetermined and may be questionable. Due to varying factors, such as production methods and fish genetics, the possibility of a variation in risk factors between different countries/regions should not be overlooked. Given the long-term efforts that have been invested in producing genetically improved strains of tilapia, susceptibilities for TiLV need to be thoroughly investigated under field conditions. Similarly, there is an urgent need to determine the potential for vertical transmission of TiLV, as well as further investigations of the frequency and duration of subclinical cases. Both descriptive, observational and experimental studies should be conducted to address such knowledge gaps. While it has been observed that fish surviving the initial outbreak are immune to subsequent outbreaks (Eyngor *et al.* 2014), further investigations should be conducted before such fish are automatically assumed to be TiLV-resistant and used as broodstock.

To minimize the impact of TiLV in affected countries and reduce the risk of further spread, implementation of good biosecurity practices, combined with the introduction of intervention and containment programmes, should be conducted. While stringent biosecurity may be impossible to achieve in the field in many tilapia operations, achieving the highest possible biosecurity standards for the operation in question should be sought. The importance of biosecurity measures needs to be promoted by competent authorities as there is currently no cure for viral diseases in aquaculture. The combination of biosecurity measures, breeding of fish with improved genetic resistance and vaccination have proven useful in reducing the number of viral disease outbreaks in some cases (e.g. IPN in Norwegian salmonid production (Hjeltnes *et al.* 2017). A patent for a TiLV-vaccine has been filed in the United States (Anonymous, 2017), and vaccination against TiLV may be a reality in the future. However, it remains to be seen whether such a vaccine will be sufficiently cost-effective and easily administered to allow a widespread dissemination and use in a large proportion of the major tilapia-producing countries. It is a general concern that vaccinated fish may test positive by PCR analysis for the agent against which they have been vaccinated. This may be of particular concern in cases where there has been an intraperitoneal vaccination with subsequent sampling of abdominal tissues for diagnostic testing. Any proportion of false positives due to vaccine residues is likely to be of special concern for tilapia exporting countries, particularly in the event of TiLV listing by the OIE and where there is no method to differentiate the vaccine strain and wild strain (e.g. through sequencing). Depending on the efficacy of the vaccine, there may be an additional risk that vaccinated fish may become subclinically infected and contributing to the inadvertent spread of the agent. Such issues need to be considered prior to the instigation of mass vaccination programmes. It may be relevant to consider whether the use of autogenous inactivated vaccines (single or polyvalent), with or without adjuvants, should be promoted at large in countries and areas where other approaches are unrealistic.

Current information suggests that stress (e.g. transportation and stocking of fingerlings and juveniles) may be an important factor for the development of clinical outbreaks, and management practices should aim to minimize the effect of transportation and handling. Good management practices (GMPs) of rapid removal of moribund and dead fish should be encouraged together with the safe disposal of removed fish. The technology for producing specific pathogen-free (SPF) stocks is available and it would be possible to establish SPF broodstock also for TiLV. While requiring a highly sensitive, nonlethal sampling methods for screening and selection of SPF broodstock and offspring, the method could provide TiLV-free stocks to reduce the spread of TiLV. However, the advantage of stocking such SPF fish would be removed if the water body to which they are transferred are inadequately cleaned and disinfected between production cycles or there are a lack biosecurity measures to prevent the introduction of TiLV after stocking. While an SPF programme, combined with early immersion/oral vaccination prior to stocking, would be an ideal approach for effective prevention of not only TiLV but also other major diseases in farmed tilapia, it may be unrealistic for the majority of small-scale tilapia producers.

While the initial proposal was to classify this virus as an Orthomyxo-like virus based on the genome arrangement (Bacharach *et al.* 2016a), the fact that only segment 1 shows a weak homology to PB of influenza virus is not fully supporting this classification. The frequently observed histopathological feature of SHT has never been reported related to Orthomyxovirus but do occur in outbreaks caused by Paramyxovirus, a nonsegmented RNA virus (Phillips *et al.* 1991; Sussman *et al.* 1994). Thus, in terms of genomic characterization, the proposed *Tilapia Tilapinevirus*, in a new genus *Tilapinesvirus* as proposed by Bacharach *et al.* (2016b), seems more appropriate. There are, however, important aspects in terms of taxonomy, genome and protein functions, receptors and so on that needs to be studied. For ISA, both virulent and avirulent strains of the virus have been identified (Christiansen *et al.* 2011; Cottet *et al.* 2011) and alterations in virulence and cell tropism have been observed in the field (Christiansen *et al.* 2017). Whether such variations exist in other aquatic viruses such as TiLV remains to be determined and is of importance in terms of surveillance and control efforts.

Given the importance of tilapia as a protein source in parts of the world, TiLV-associated losses may constitute a significant risk to household incomes and food security. Socio-economic impact assessments should be encouraged to quantify the current or expected impact of disease as a result of infection with TiLV. It seems impossible to expand tilapia farming with the widely held belief that tilapia, whether of nonimproved- or improved strains, is resistant to diseases. Basic principles of health management and biosecurity should be considered at all levels to ensure sustainability of the tilapia industry. There must be focused attention to the development and implementation of practical, affordable and effective BMPs to reduce disease and environmental impacts for small-holder farmers. Effective disease management is a shared responsibility and international cooperation and productive alliances of governments, industry and the community will be required to accomplish this goal. Future disease control programmes should harness the potential of information technology tools (e.g. data mining, machine learning, image recognition, GIS mapping and predictive modelling) combined with novel surveillance and diagnostic technologies to

accomplish provision of real-time solutions and early warning systems to a diverse array of stakeholders (e.g. farmers, researchers, policymakers). Only then will the impact of serious aquatic animal diseases on aquaculture production, livelihoods and trade be minimized.

In summary, TiLV is a newly emerging viral pathogen that poses a potential threat to the global tilapia industry. As current knowledge is still limited, there are several important knowledge gaps that remain to be filled. To limit the negative impact and to prevent further spread of the virus, combined approaches are required. National- and international biosecurity efforts, effective BMPs, capacity building and widespread collaboration between international and national stakeholders must be prioritised. Such strategies will aid the management and control efforts aimed at tackling TiLV while simultaneously aiding preparedness for rapid response to other emerging diseases in the future.

Acknowledgement

This work was undertaken as part of the CGIAR Research Program on Fish Agri-food Systems (FISH) in collaboration with King Mongkut's University of Technology Thonburi (KMUTT) and the Norwegian Veterinary Institute. CV Mohan was supported by the CGIAR Research Program on Fish Agri-Food Systems (FISH). The authors declare no conflict of interest.

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