CONTROL OF TiLV OUTBREAKS IN ISRAELI TILAPIA FARMS

Ra'anan Ariav, Natan Wajsbrot and Allan Heres

General background:

- Tilapia production will become one of the most important aquaculture crops in this century
- Annual global production of Tilapia is estimated at over 4.5 Million metric Tons.
- Tilapia production is estimated to increase to over 7.5 million metric tons by 2030.
- Tilapia are considered to be relatively resistant to many of the disease entities affecting other aquatic species.
- Tilapia spp. were traditionally affected by several bacterial pathogens and several species of both external and internal Parasites.
- Viral disease pathogens have not been reported in commercial Tilapia culture.

General background:

- In Summer 2009, massive losses of Tilapia were observed in several Tilapia farms in Israel.
- Mortalities were mainly observed in the Bet – Shean Valley.
- These massive mortalities of Tilapia populations were also observed in wild Tilapia populations in the Lake of Galilee in Israel.
- The losses of Tilapia occurred during the warm summer months between June and October. (26º - 29° C.)
- Very high mortality of Tilapia was observed in all sizes.

General background:

- The disease outbreak was transferred readily from one pond to the next one indicating to be highly contagious.
- Morbidity and mortality was limited to Tilapia species and its hybrids (*Oreochromis niloticus* X *O. aureus* hybrid).
- Carp (*Cyprinus carpio*) and Gray mullet (*Mugil cephalus*) cultured together with Tilapia in polyculture ponds were resilient to this new pathogen and were completely asymptomatic to the disease process.

General background:

- As such, it was clear that this disease entity was highly specific to Tilapia species, suggesting possible viral etiology.
- Tilapia that survived the initial outbreak appeared to be much more resilient than naïve Tilapia if exposed to further infections, suggesting the build – up of immunity to the causative agent of disease.

Commercial strains of Tilapia in Israel

Oreochromis sp. 'red tilapia'

"Rocky Mountain White" Tilapia
Tilapia *aurea x nilotica* hybrid

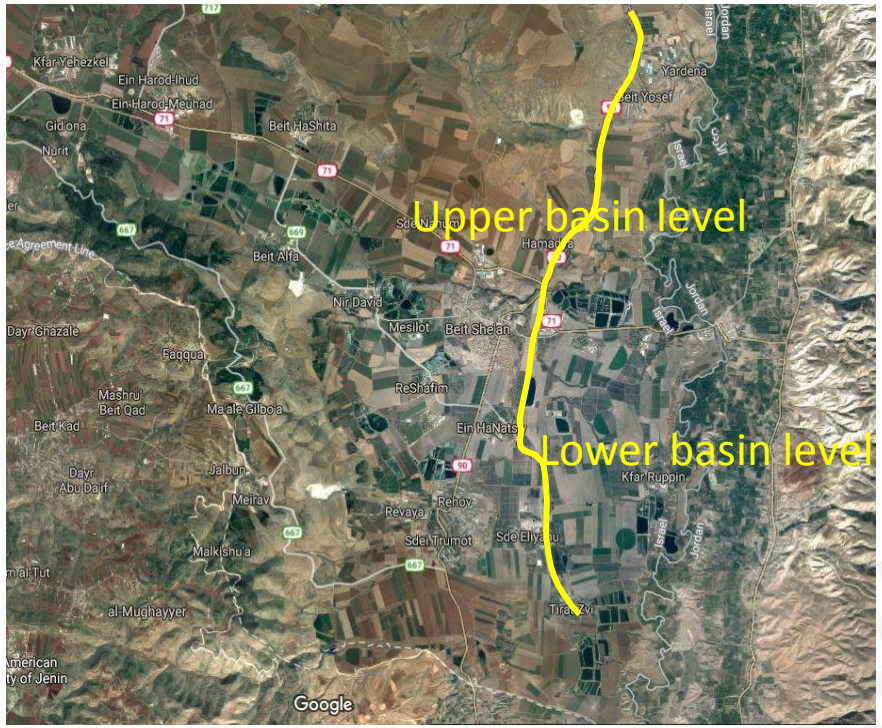
Oreochromis niloticus x O. aureus

OREOCHROMIS NILOTICUS (LINHAGEM CHITRALADA)

Tilapia zillii

Sarotherodon galilaeus "St. Peter's" fish

Geography and spreading of disease



Geography and spreading of disease

Pathological findings:

- Behavior – Anorexia and lethargy.
- Gross lesions were characterized mainly by unilateral or bilateral ocular alterations (cataract), including opacity of the lens .
- Other lesions observed in diseased Tilapia included skin erosions, deep ulceration and moderate congestion of the spleen and kidney.
- The main histologic lesions were found in the brain and included edema, focal hemorrhages in the leptomeninges, and capillary congestion in both the white and gray matter.

Cataracts, ulceration and skin erosion

Gross lesions in Tilapia affected by Tilapia Lake Virus (TiLV).

Pathological findings:

- **Tilapia were severely affected by presence of secondary Bacterial and Parasitic pathogens:**

  - Secondary Bacteriology findings:
    - *Streptococcus* spp. and *Aeromonas* spp. were commonly isolated from diseased Tilapia with TiLV.
  - Parasitic findings:
    - Diseased fishes were commonly infected with high levels of external parasites:
    - Cilliates: (*Trichodina* spp.)
    - Monogeneans: (*Gyrodactylus* spp.)
    - Digenetic Trematodes: (*Centrocestus* spp.)

Pathological findings:

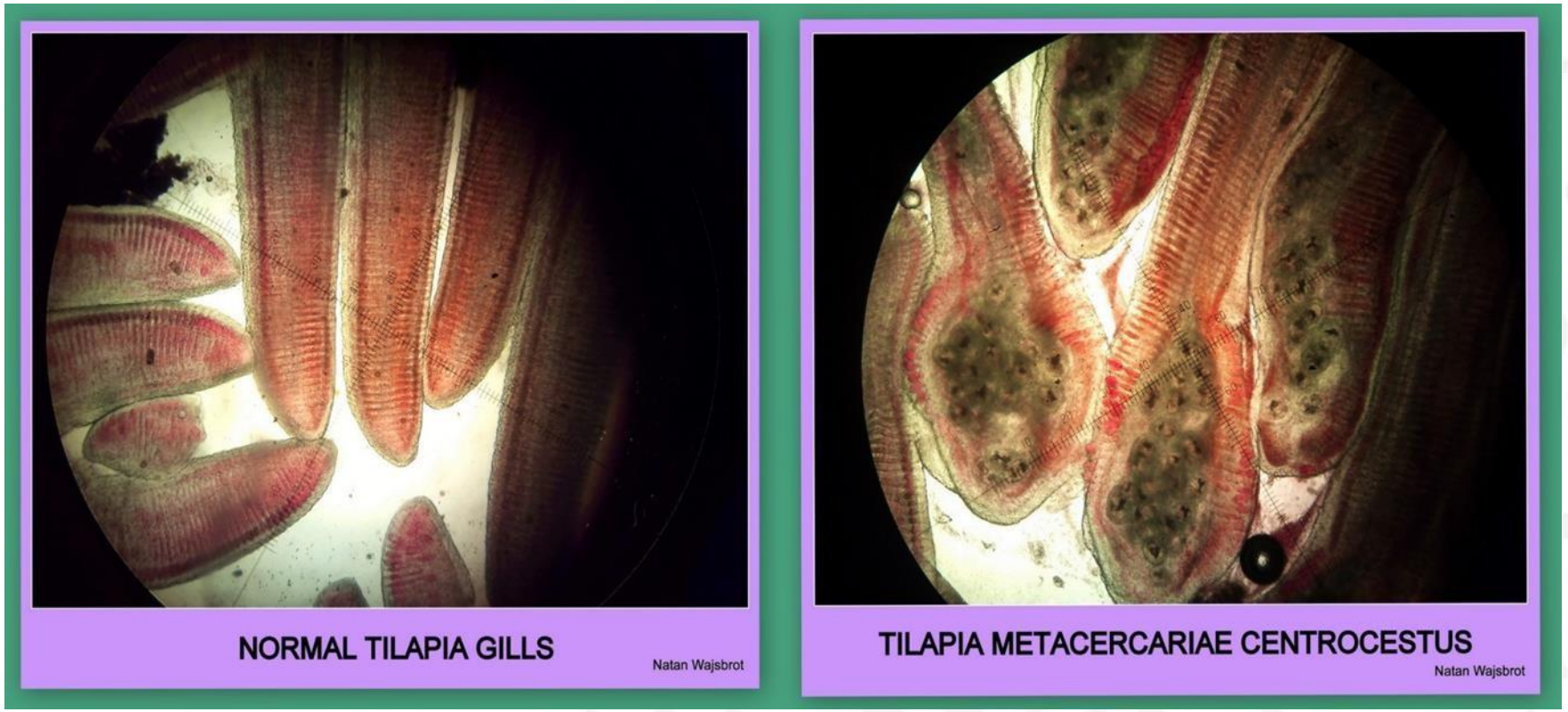
Pathological findings:

Secondary Bacterial infection

STREPTOCOCCUS SPP

AEROMONAS SPP

Pathological findings:



Very high presence of Digenetic Trematodes (*Centrocestus* spp.) during TiLV outbreaks

TiLV risk factors:

- **Direct effect:**
  - Presence of infected populations.
  - Water temperatures of 22°C to 32°C
- **Contributing factors:**
  - Dissolved oxygen/Water quality.
  - Stocking density (fish/m2)
  - Presence of Bacterial and/or Parasitic infection in the background.

Pathological findings:

Massive Tilapia mortalities during TiLV outbreaks in Israel's Bet – She'an valley

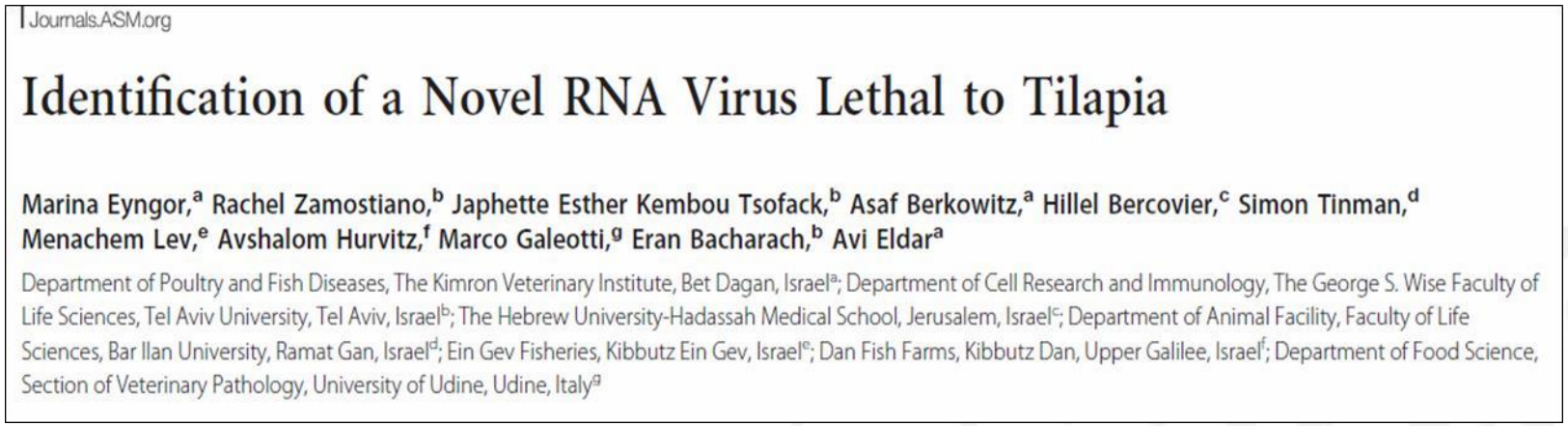
Losses in the Tilapia fish farm:

- During the first year of the outbreak (2009) overall mortality was in the range of 30% - 35%.
- Mortality was observed in all sizes of fish.
- During the second year of this outbreak, mortality of 30% - 40% was mainly observed among small naïve fingerlings, (up to 50 Grams) indicating that older fish who survived previous outbreaks were now immunized.
- This ratio and pattern of mortality has been consistent during the last 8 years.
- Today, due to massive losses in Tilapia fingerlings most Tilapia farms in Israel will produce x 2 – 3 of the number of required fingerlings in order to meet their expected goals of annual production.

Identification of Viral etiology to the disease outbreak:

- Eyngor et al. (2014) successfully isolated from diseased fish the etiological agent responsible for massive mortality in Tilapia.
- This new viral particle was grown in specific cell lines and described as enveloped icosahedral particles of 55 to 75 nm by electron microscopy.
- The virus was designated Tilapia Lake Virus (TiLV) and the disease was demonstrated by intraperitoneal injection of TiLV in naïve Tilapia populations.
- The symptoms developed in the lab were similar to natural infection including lethargy, black discoloration, skin abrasions, ocular degeneration and mortality greater than 80%.
- The target organ appeared to be the brain.
- Histologic lesions in the brain included edema, focal hemorrhages in the leptomeninges, and capillary congestion in both the white and gray matter.

Identification of Viral etiology to the disease outbreak:



Journals.ASM.org

Identification of a Novel RNA Virus Lethal to Tilapia

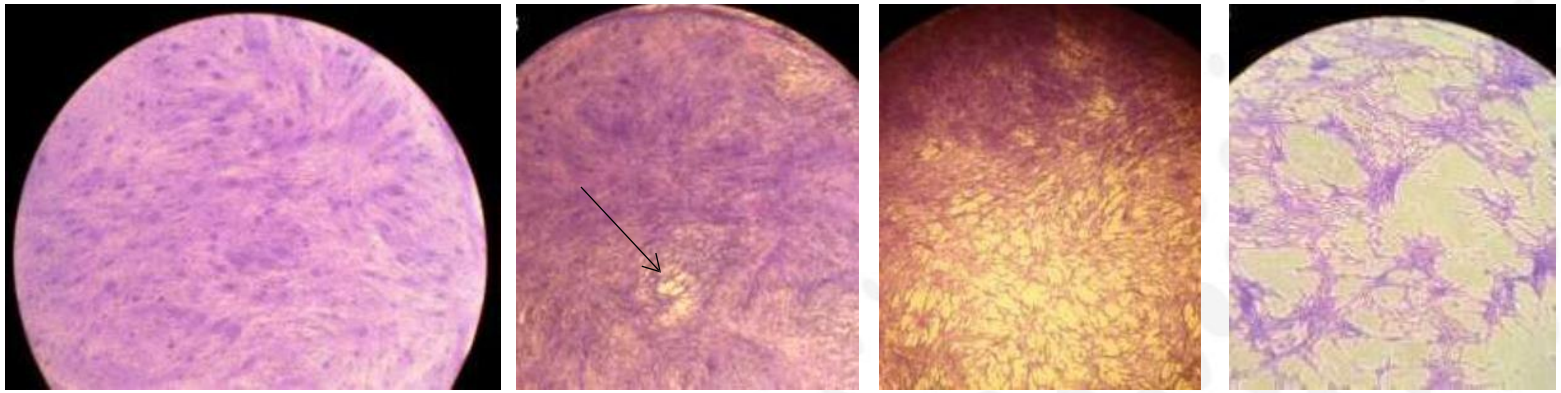
Marina Eyngor,[a] Rachel Zamostiano,[b] Japhette Esther Kembou Tsofack,[b] Asaf Berkowitz,[a] Hillel Bercovier,[c] Simon Tinman,[d] Menachem Lev,[e] Avshalom Hurvitz,[f] Marco Galeotti,[g] Eran Bacharach,[b] Avi Eldar[a]

Department of Poultry and Fish Diseases, The Kimron Veterinary Institute, Bet Dagan, Israel[a]; Department of Cell Research and Immunology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel[b]; The Hebrew University-Hadassah Medical School, Jerusalem, Israel[c]; Department of Animal Facility, Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel[d]; Ein Gev Fisheries, Kibbutz Ein Gev, Israel[e]; Dan Fish Farms, Kibbutz Dan, Upper Galilee, Israel[f]; Department of Food Science, Section of Veterinary Pathology, University of Udine, Udine, Italy[g]

- In 2016, Bacharach et al. completed the characterization of TiLV virus and revealed a novel orthomyxo-like virus and concurred to be a global threat to the Tilapia aquaculture industry.
- The researchers concluded that viral nucleic acid sequence present in lesions from the brain and the liver in diseased fish from Israel, Thailand and Ecuador respectively corresponded to TiLV.
- Complete genomic and protein sequences were described that will help TiLV detection and containment and enable possible vaccine development.

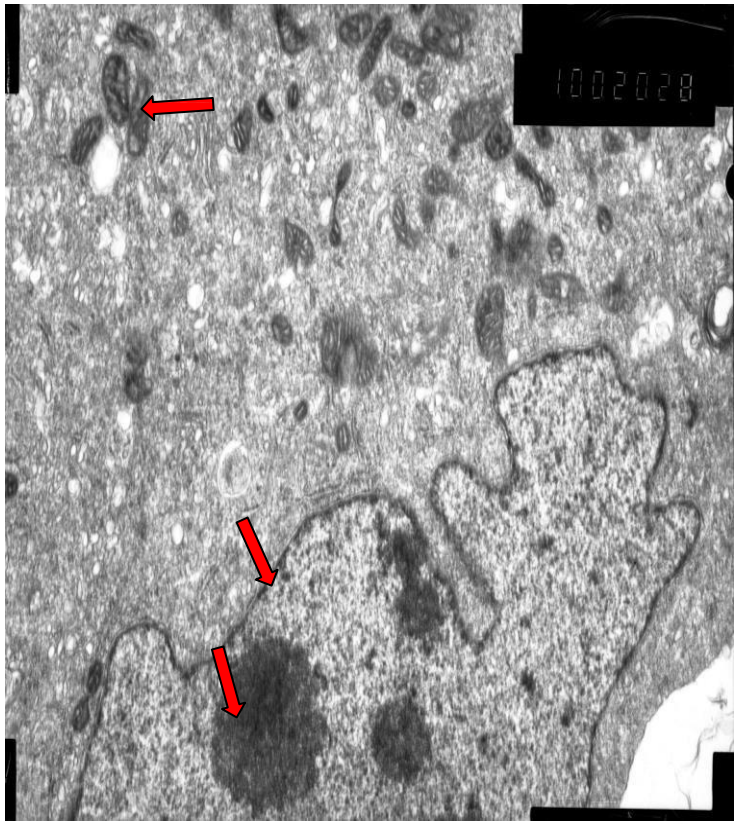
TiLV - Novel virus causing mortality in Tilapia

- **Isolation and purification of a segmented RNA virus from sick fish**

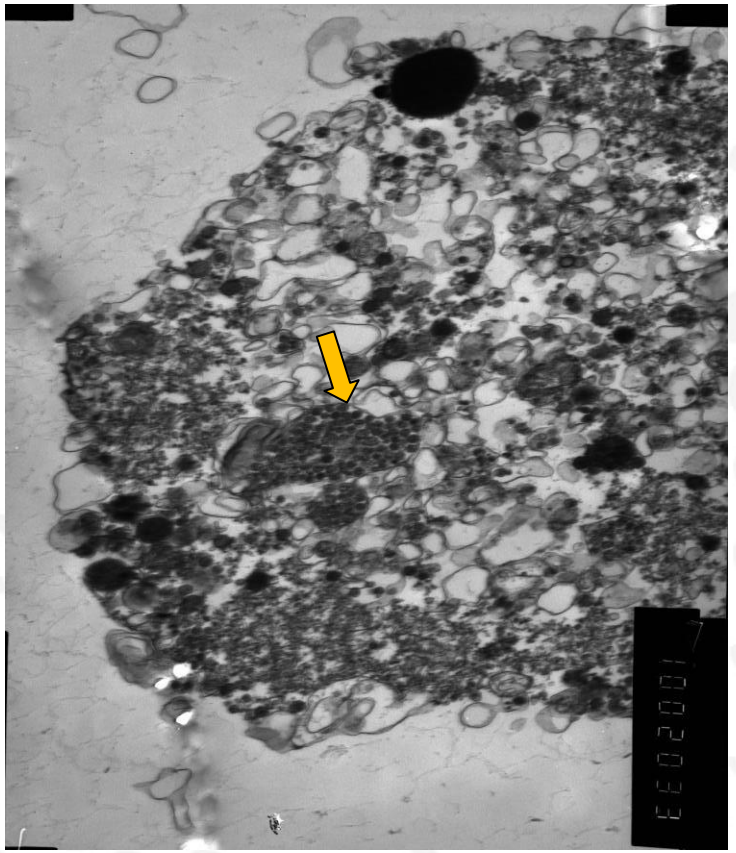


Cell culture : EM X 10,000

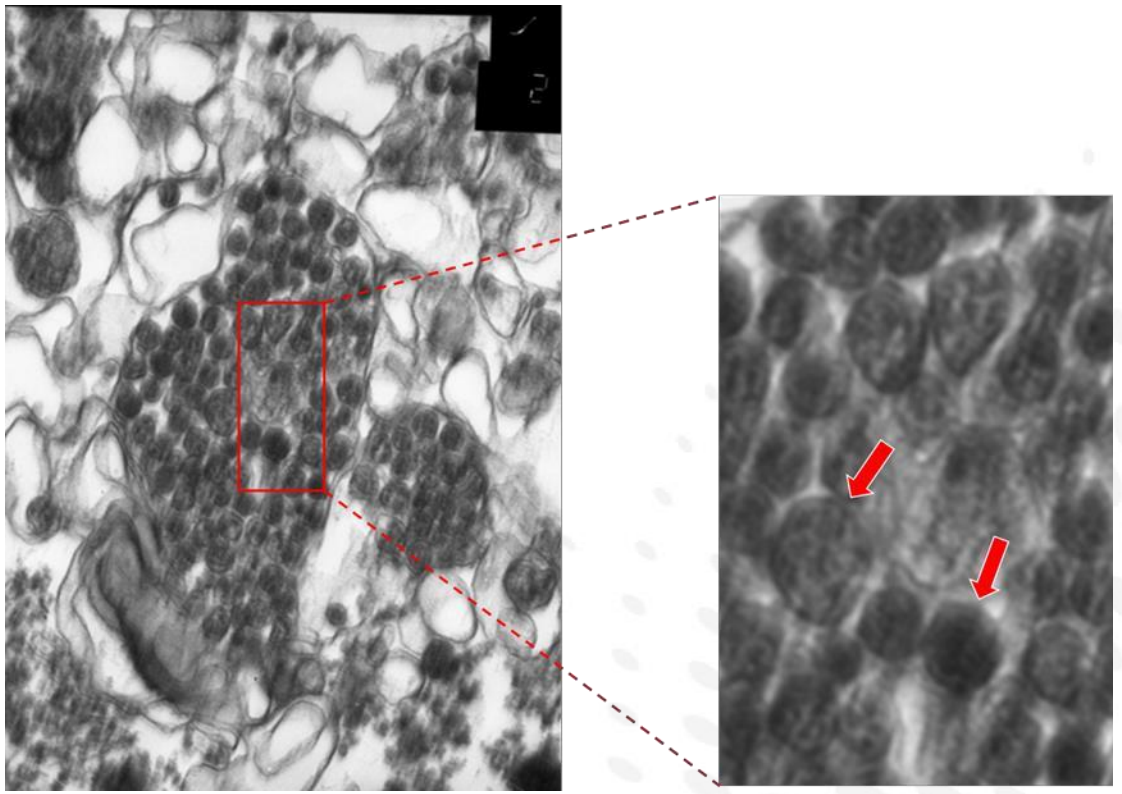
TBC – not infected



TBC – TiLV infected



Electron Microscopy of infected cells



EM of infected tilapia cells at day 5 post infection. Cells exhibit disintegration of cellular compartments and organelles. Cytoplasmic vacuoles contain an abundance of virus like structures varying in size and morphology (arrows).

Identificación de etiología viral del brote de la enfermedad:

- **Las lesiones histológicas en el cerebro incluyeron edema, hemorragias focales en las leptomeninges, y congestión capilar en materias blanca y gris.**
- **Análisis histológicos adicionales de los peces enfermos revelaron un mayor número de centros melanomacrófagos (MMCs) en el bazo y el hígado, lo que indica una enfermedad progresiva.**
- **Se diseñó un ensayo diagnóstico basado en PCR para identificar una secuencia de ácido nucleico específica de TiLV, como una potencial herramienta de control.**
- The histological lesions in the brain included edema, focal hemorrhages in the leptomeninges, and capillary congestion in white and gray matter.
- Additional histological analyzes of the diseased fish revealed a greater number of melanomacrophagous centers (MMCs) in the spleen and liver, indicating a progressive disease.
- A diagnostic assay based on PCR was designed to identify a TiLV-specific nucleic acid sequence as a potential control tool.

???

Identificación de la etiología viral del brote de la enfermedad

- **Recientemente, Bacharach *et al*. (2016) completaron la caracterización del virus TiLV y describrieron un nuevo orthomyxo-like virus y coincidieron con la aparición de una amenaza mundial para la industria de la acuicultura de Tilapia.**
- **Los investigadores concluyeron que la secuencia de ácido nucleico viral presente en las lesiones de cerebro y de hígado de peces enfermos de Israel y Ecuador respectivamente, correspondían a TiLV.**
- Recently, Bacharach et al. (2016) completed the characterization of the TiLV virus and described a new orthomyxo-like virus and coincided with the emergence of a global threat to the Tilapia aquaculture industry.
- The researchers concluded that the viral nucleic acid sequence present in the brain and liver lesions of sick fish from Israel and Ecuador respectively, corresponded to TiLV.

???

Global presence of TiLV

- Identified in Israel, causing high losses for the past 8 years
- Publication identified the virus as causing 90% mortality in Tilapia farms in Ecuador and Columbia
- Identification of Tilapia Lake Virus in Egypt in Nile tilapia affected by ‘summer mortality’ syndrome
- Outbreaks of in Thailand, Taiwan, Malaysia and India
- Africa – Tanzania and Uganda confirmed and 8 more Sub Sahara countries suspected
- FAO ALERT : “Outbreaks of Tilapia lake virus (TiLV) threaten the livelihoods and food sec millions of people dependent on tilapia farming”

5/2017 – Global TiLV alert:

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

FAO issues alert over lethal virus affecting popular tilapia fish
Though not a human health risk, Tilapia Lake Virus has large potential impact on global food security and nutrition

26 May 2017, Rome--A highly contagious disease is spreading among farmed and wild tilapia, one of the world's most important fish for human consumption.

The outbreak should be treated with concern and countries importing tilapias should take appropriate risk-management measures - intensifying diagnostics testing, enforcing health certificates, deploying quarantine measures and developing contingency plans - according to a Special Alert released today by FAO's Global Information and Early Warnings System.

Tilapia Lake Virus (TiLV) has now been reported in five countries on three continents: Colombia, Ecuador, Egypt, Israel and Thailand.

Global presence of TiLV

Short communication

Evidence of TiLV infection in tilapia hatcheries from 2012 to 2017 reveals probable global spread of the disease

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TiLV
Tilapia hatcheries

ABSTRACT

Recent outbreaks of tilapia lake virus (TiLV) in farmed tilapia in Thailand were the first indication of spread of the virus to the Southeast Asia region. Here we further investigate TiLV infection of archived and newly collected fish samples obtained from Thai hatcheries from 2012 to 2017. Fertilized eggs, yolk-sac larvae, fries, and fingerlings were tested for the TiLV using an established semi-nested RT-PCR assay. The results revealed that the majority of the tested samples were TiLV positive, including our earliest preserved samples collected in year 2012. DNA sequence analysis of representative amplified products also confirmed the presence of TiLV. Since the discovery of TiLV in 2012, over 40 countries worldwide have imported tilapia fry and fingerlings, and some may have been unaware of risk that they might be infected with TiLV. Thus, if they have not already done so, we recommend that countries that have imported tilapia for aquaculture carry out surveillance studies for its presence and also add TiLV to their import quarantine inspection list.

Global presence of TiLV

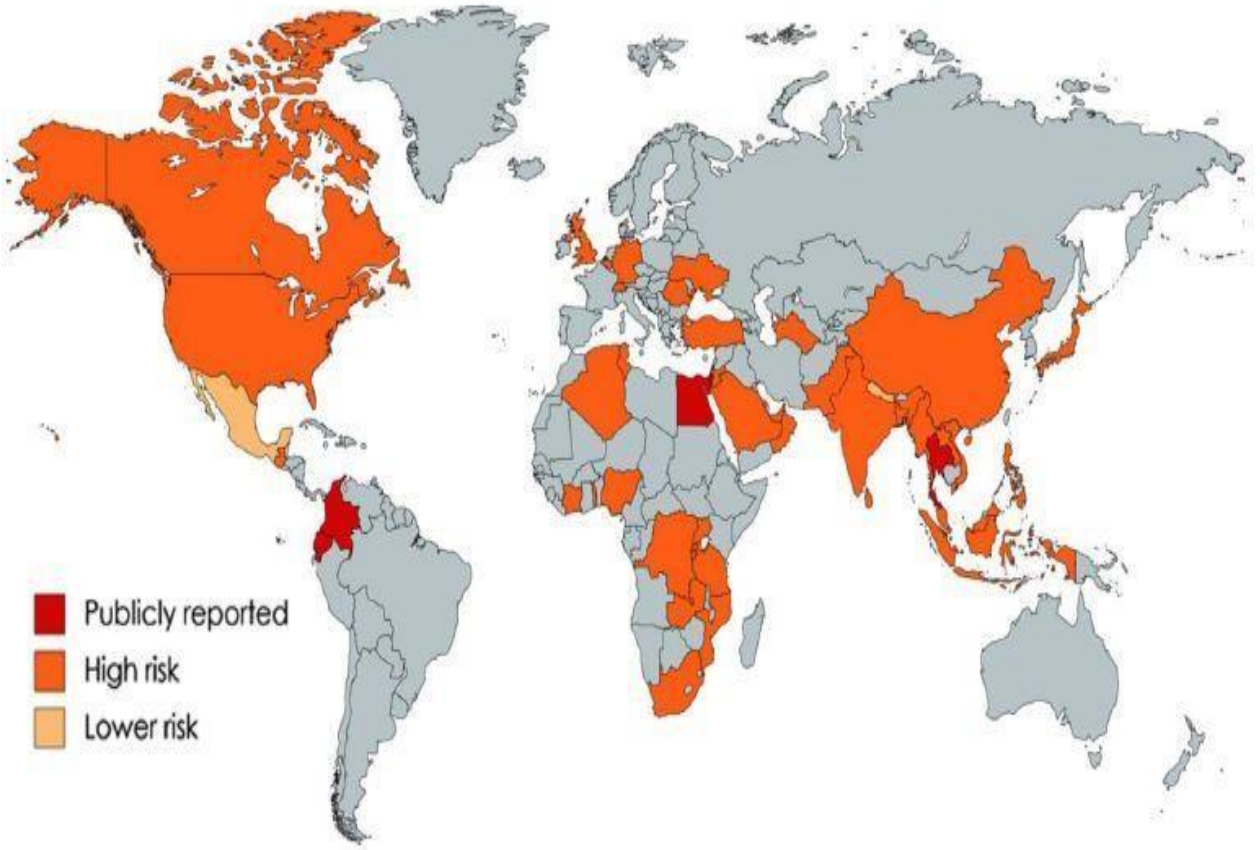
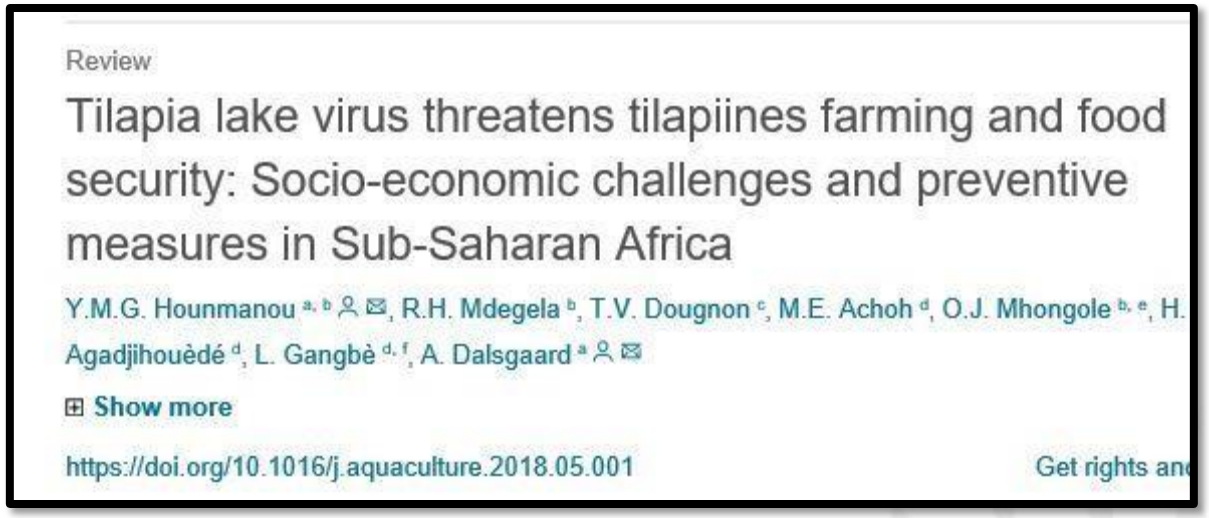


Fig. 3. The geographical distribution map of tilapia lake virus (TiLV). Red colour indicated 5 countries with firmed evidence of the presence of TiLV. Orange and light orange colors represent 40 and 3 countries with respective high risk and lower risk of TiLV spread through translocation of tilapia fry/fingerlings that may have been infected with TiLV. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Global presence of TiLV

risk of TiLV spread: Algeria, Bahrain, Bangladesh, Belgium, Burundi, Canada, China, Congo, Germany, Guatemala, India, Indonesia, Japan, Jordan, Laos, Malaysia, Mozambique, Myanmar, Nigeria, Pakistan, Philippines, Romania, Rwanda, Saudi Arabia, Singapore, South Africa, Sri Lanka, Switzerland, Tanzania, Togo, Tunisia, Turkey, Turkmenistan, Uganda, Ukraine, United Arab Emirate, United Kingdom, United States, Vietnam and Zambia. There are also 3 lower risk countries (El-Salvador, Mexico, and Nepal) that imported fish from these hatcheries before year

Tilapia lake virus threatens tilapine agriculture and food security


Review

Tilapia lake virus threatens tilapiines farming and food security: Socio-economic challenges and preventive measures in Sub-Saharan Africa

Y.M.G. Hounmanou [a, b], R.H. Mdegela [b], T.V. Dougnon [c], M.E. Achoh [d], O.J. Mhongole [b, e], H. Agadjihouèdé [d], L. Gangbè [d, f], A. Dalsgaard [a]

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- Tilapia lake virus threatens tilapine agriculture and food security: socio-economic challenges and preventive measures in sub-Saharan Africa
- Given the importance of tilapia as a source of protein in parts of the world, the losses associated with TiLV can constitute a significant risk for household income and food security.
- Socio-economic impact assessments should be encouraged to quantify the current or expected impact of the disease as a result of the infection with TiLV.

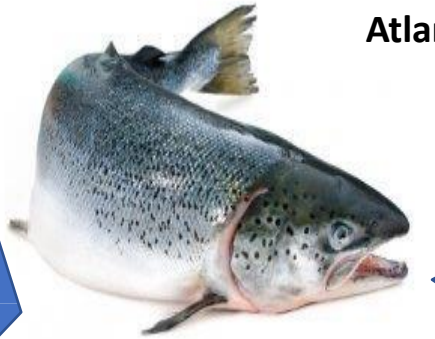
Possible mutations in Orthomyxoviridae viruses

- Infectious salmon anemia (ISA) is caused by the infectious salmon anemia virus (ISAV). ISAV, an RNA virus, is the only species of the genus "Isavirus" that belongs to the Orthomyxoviridae family and, therefore, is related to influenza viruses.

- ISA is characterized by high mortality, and natural outbreaks have only been described in Atlantic farmed salmon.

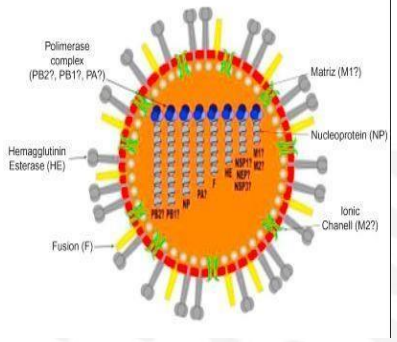
- However, ISAV has been reported in both salmonids and non-salmonid fish and the virus can, under experimental conditions, persist and replicate in other salmonids (*Salvelinus alpinus, O. mykiss, O. keta, O. kisutch*) and non-salmonids (*Clupea harengus, Gadus morhua*).

Possible mutations in the viruses Orthomyxoviridae - Infectious salmon anemia (ISA)

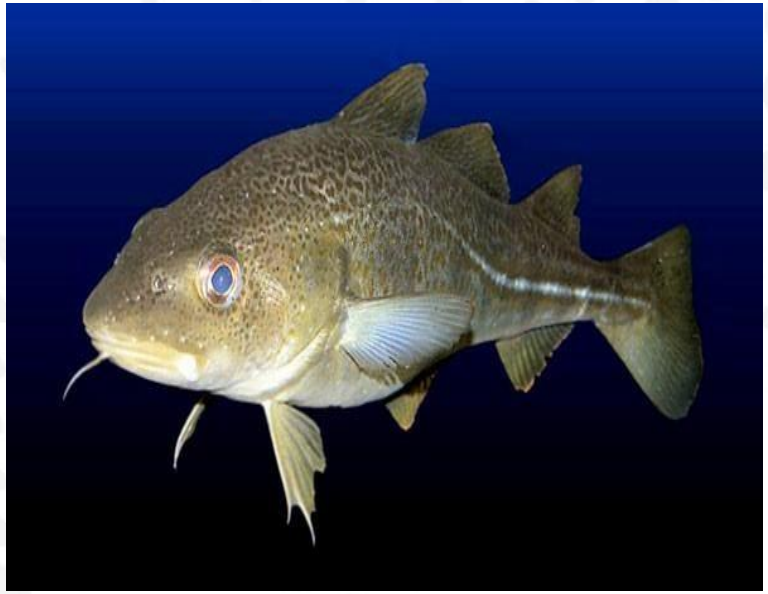


Atlantic salmon (*Salmo salar*)

Atlantic herring (*Clupea harengus*)



Atlantic cod (*Gadus morhua*)



July 2018:

DOI: 10.1111/jfd.12843

WILEY Journal of Fish Diseases

SHORT COMMUNICATION

First detection of tilapia lake virus (TiLV) in wild river carp (*Barbonymus schwanenfeldii*) at Timah Tasoh Lake, Malaysia

Azila Abdullah[1] | Rimatulhana Ramly[1] | Mohammad Syafiq Mohammad Ridzwan[1] | Fahmi Sudirwan[1] | Adnan Abas[2] | Kamisa Ahmad[1] | Munira Murni[1] | Beng Chu Kua[1]

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August 2018: Susceptibility of important species of warm-water fish to the info the tilapia lake virus (TiLV)

ELSEVIER

Aquaculture

Volume 497, 1 December 2018, Pages 462-468

Susceptibility of important warm water fish species to tilapia lake virus (TiLV) infection

Phitchaya Jaemwimol [a], Pattarasuda Rawiwan [a, b], Puntanat Tattiyapong [a, b], Pattrawut Saengnual [c], Attapon Kamlangdee [d], Win Surachetpong [a, b]

red hybrid tilapia (*Oreochromis* spp.)

giant gourami (*Osphronemus goramy*)

August 2018: Susceptibility of important species of warm-water fish to the info the tilapia lake virus (TiLV)

snakeskin gourami (*Trichogaster pectoralis*)

iridescent shark (*Pangasianodon hypophtthalmus*)

walking catfish (*Clarias macrocephalus*)

climbing perch (*Anabas testudineus*)

striped snake-head fish (Channa *striata*)

common carp (*Cyprinus*carpio)

silver barb (*Barbodes gonionotus*),

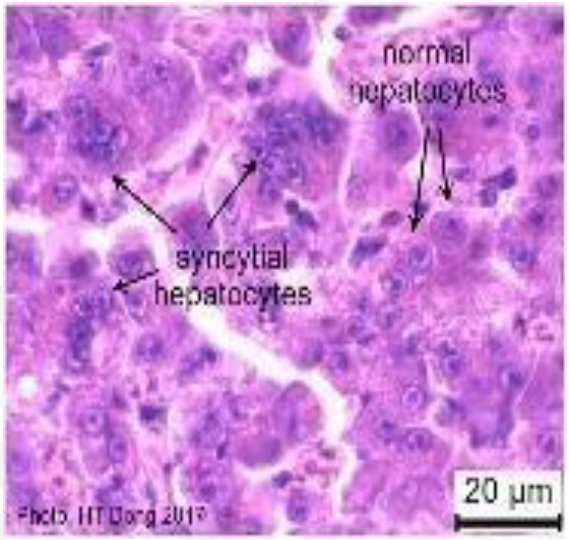
Asian sea bass (*Lates calcarifer*)

Detection of TiLV

Epidemiology and clinical symptoms

POCKIT™ Micro Series Nucleic Acid Analyzer

Histopathology



Prevention and Treatment:

- Improved management of Tilapia culture as related to health management, handling, nutrition and environment:
- Use of rapid PCR testing service for identification and monitoring of disease progress.
- Prevention of all movement of fish from infected areas:
- Prevention of live Tilapia (and other Cichlid) imports/exports:
- Transparency among Tilapia producers and well as with Government regulatory agencies:
- Supportive treatment with functional feed additives.

Prevention and Treatment:

- **Treatment: (Therapy against secondary infections)**
  - Antimicrobial therapy:
  - Antiparasitic:
- **Vaccination:**
  - Development of autogenous TiLV vaccine: (Immersion and injection)

Open professional questions:

- Does TiLV infect only tilapia?
- Can other species of non-tilapia fish act as carriers of the virus?
- Can other organisms (eg - birds and mammals, crustaceans, annelids, molluscs) act as carriers of the virus?
- Do other microorganisms and plankton work as passive carriers?
- Does the virus survive freezing and can the infectious virus be isolated from frozen whole tilapia and other frozen products (eg, gutted fish, fillets)?
- Does the virus survive in fresh products such as whole fish or filets?

Open professional questions:

- Given that the causative agent of TiLV has been described as a new orthomyxoid type virus, (to which the salmon ISA virus belongs) does TiLV share the same properties as the ISA virus?
- Is it possible to develop an efficient vaccine similar to those developed for the ISA virus?

Future considerations:

- Phibro's TiLV vaccine will be an important part of future TiLV control strategy.
- The arrival of the disease to Latin America, Africa and Asia region may cause significant economic losses to the Tilapia producers in these regions.
- It is recommended to begin an immediate international monitoring program to identify populations of Tilapia that are infected and to implement strict preventive measures among TiLV free Tilapia populations.
- It is extremely important to avoid any transportation of live fish from infected zones.
- Any future introductions of Tilapia brood fish and/or new Tilapia lines should be very carefully evaluated in view of this developing situation.

Thanks!