Genetic diversity and distribution of Tilapia lake virus in fish polyculture system in Bangladesh

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จุฬาลงกรณ์มหาวิทยาลัย
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          THONTIRAVONG)

พาร์โท พรานิม เตปนัท : ความหลากหลายทางพันธุกรรมและการกระจายของไวรัสทิลาเปียเลคในระบบการเพาะเลี้ยงปลาแบบหลากหลายชนิดในประเทศบังคลาเทศ. (Genetic diversity and distribution of Tilapia lake virus in fish polyculture system in Bangladesh) อ.ที่ปรึกษาหลัก : รศ. ศ. ดร.ชาญณรงค์ รอดคำ, อ.ที่ปรึกษาร่วม : ผศ. ศ. ดร.ฮา ทานห์ ดง

ปลานิลเป็นปลาเศรษฐกิจที่สำคัญของโลกเป็นลำดับที่สองรองมาจากปลาใน ประเทศบังคลาเทศถือเป็นประเทศผู้ผลิตปลานิลมากเป็นอันดับที่สี่ของโลกและมีการเพาะเลี้ยงปลานิลในระบบการเพาะเลี้ยงปลาแบบหลากหลายชนิด มีงานวิจัยเพียงไม่กี่งานที่ศึกษาเกี่ยวกับปัจจัยที่มีความเกี่ยวข้องกับอัตราการตายและความเสียหายทางเศรษฐกิจที่เกิดขึ้นจากอัตราการตายของปลาเพาะเลี้ยงในประเทศบังคลาเทศเนื่องจากความยุ่ง ยากในการศึกษาวิจัย ไวรัสทิลาเปียเลค (TiLV) คือเชื้อไวรัสอุบัติใหม่ในวงการเลี้ยงสัตว์น้ำซึ่งมีรายงานการก่อความเสียหายต่อฟาร์มปลานิลใน 16 ประเทศ นับจากรายงานการแจ้งเตือนในปี ค.ศ. 2017 ว่าไวรัสทิลาเปียเลคอาจมีการแพร่ระบาดอย่างกว้างขวางเราได้ทำการสำรวจฟาร์มปลานิล 565 ฟาร์มใน 15 จังหวัดที่เป็นจังหวัดสำคัญในการผลิตปลานิลในประเทศบังคลาเทศโดยการใช้ online tilapia epidemiology และ health economics survey tool วิเคราะห์และหลังจากนั้นเราได้ทำโปรแกรมการสำรวจโรคติดเชื้อไวรัสทิลาเปียเลคโดยเฉพาะในฟาร์มปลานิลขุนและปลานิลอนุบาลใน 10 จังหวัดของประเทศบังคลาเทศตั้งแต่ปี ค.ศ. 2017-2019 จากการสำรวจพบว่ามีการตายโดยไม่ทราบสาเหตุของปลาชนิดอื่น ๆ ที่ไม่ใช่ปลานิลเกิดขึ้นในการเลี้ยงปลาเหล่านั้นร่วมกับปลานิลที่มีการติดเชื้อไวรัสทิลาเปียเลค ด้วยเหตุนี้เองจึงทำให้เราสนใจที่จะศึกษาว่าปลาชนิดอื่น ๆ ที่ถูกเลี้ยงรวมกับปลานิลที่มีการติดเชื้อไวรัสทิลาเปียเลคจะให้ผลการตรวจพบเชื้อไวรัสทิลาเปียเลคด้วยหรือไม่และจะเป็นปลาชนิดที่ไวต่อการติดเชื้อไวรัสทิลาเปียเลคด้วยหรือไม่ในสภาพการท ดลองในห้องปฏิบัติการ การสำรวจได้รายงานปัจจัยต่างๆ ที่พบได้แก่ ปัจจัยทางภูมิศาสตร์ คุณลักษณะของเกษตรกรผู้เลี้ยงปลา คุณลักษณะของฟาร์ม ปัจจัยความหนาแน่นในการเลี้ยง มาตรการความปลอดภัยทางชีวภาพ ลักษณะและระดับการตายทั่วไปและการตายโดยไม่ทราบสาเหตุ พบว่าร้อยละ 18.2 ของฟาร์มที่ทำการสำรวจทั้งหมดได้รับการรายงานว่าพบการตายโดยไม่ทราบสาเหตุของปลาด้วยระดับการตายร้อยละ 23.2 พบว่า ขนาดของฟาร์ม การตายโดยทั่วไป ความกังวลของเกษตรกรกับการตายทั่วไป และการรักษาด้วยยาปฏิชีวนะ มีความสัมพันธ์อย่างมีนัยสำคัญกับการเพิ่มขึ้นของ odds ในการรายงานการตายแบบไม่ทราบสาเหตุใน multivariable model และพบว่าแหล่งเขตของฟาร์ม แหล่งน้ำ ความถี่ในการเก็บปลาที่ตายแล้วทิ้ง และการรักษาด้วยยาปฏิชีวนะ ก็มีความสัมพันธ์อย่างมีนัยสำคัญเช่นกันกับระดับการตายโดยไม่ทราบสาเหตุเมื่อวิเคราะห์ด้วยสถิติขั้นพื้นฐาน ในขณะที่แหล่งน้ำและความถี่ในการเก็บปลาตายทิ้งยังคงมีความสัมพันธ์อย่างมีนัยสำคัญกับระดับการตายโดยไม่ทราบสาเหตุเช่นกันเมื่อวิเคราะห์ด้วย multivariable model จากการตายโดยทั่วไปและการตายโดยไม่ทราบสาเหตุในปลานิล สามารถคำนวณ total hidden loss คร่าว ๆ ได้เท่ากับ 875.7 million USD เมื่อต้องพิจารณาถึงการตายโดยไม่ทราบสาเหตุเราจึงเก็บตัวอย่างปลาภายใต้โปรแกรมการสำรวจหาเชื้อไวรัสทิลาเปียเลคด้วยวิธี PCR และพบว่ามีตัวอย่างปลาจากฟาร์มที่ให้ผลบวกจำนวน 8 ฟาร์มจากฟาร์มที่สำรวจทั้งหมด 11 ฟาร์มในปี ค.ศ. 2017 และ 2 ฟาร์ม จากทั้งหมด 7 ฟาร์ม ในปี ค.ศ. 2019 ก็ให้ผลบวกเช่นกัน การสำรวจหาเชื้อไวรัสทิลาเปียเลคด้วย PCR ในปลานิลพ่อแม่พันธุ์ที่ไม่มีอาการของโรคจากฟาร์มจำนวน 16 ฟาร์ม พบว่ามี 6 ฟาร์มที่ให้ผลบวก ผลการทดสอบถูกยืนยันด้วยการวิเคราะห์ทางจุลพยาธิวิทยาซึ่งถือเป็นวิธีทางเลือกในการวินิจฉัยการติดเชื้อไวรัสทิลาเปียเลค นอกจากนี้เราได้ทำการวิเคราะห์ complete genomes ของไวรัสทิลาเปียเลคจากปลาติดเชื้อ 1 ตัวอย่างในปี ค.ศ. 2017 และ อีก 2 ตัวอย่างในปี ค.ศ. 2019 จากการทำ Phylogenetic analyses พบว่าเชื้อไวรัสทิลาเปียเลคสายพันธุ์จากประเทศบังคลาเทศอยู่ในกลุ่มเดียวกับไวรัสทิลาเปียเลคสายพันธุ์จากประเทศไทย แสดงให้เห็นถึงความสัมพันธ์อย่างใกล้ชิดทางพันธุกรรมของไวรัส นอกจากนี้เราได้ทดสอบตัวอย่างปลานิล จำนวน 183 ตัวอย่างที่เก็บจากฟาร์มเพาะเลี้ยงปลาแบบหลากหลายชนิด ใน 6 จังหวัดทั่วประเทศบังคลาเทศ พบว่าร้อยละ 20 ของตัวอย่างทั้งหมดให้ผลบวกต่อเชื้อไวรัสทิลาเปียเลคด้วยวิธี PCR ในขณะที่ปลาชนิดอื่น อีก 15 ชนิด และสัตว์ไม่มีกระดูกสันหลัง เช่นแมลงหรือกุ้ง ที่มีความเข้าใจกันว่าอาจเป็นพาหะของไวรัสทิลาเปียเลค ให้ผลการทดสอบเป็นลบต่อไวรัสทิลาเปียเลคทั้งหมด ผลจากการทดลองนำเชื้อไปทำให้ปลา 6 ชนิดติดเชื้อในห้องปฏิบัติการพบว่ามีเพียงปลานิลเท่านั้นที่ติดเชื้อ แสดงอาการเฉพาะของโรค และมีอัตราการตายร้อยละ 70 ภายใน 12 วันภายหลังการให้เชื้อ ส่วนปลาใน 4 สปีชีส์และปลาดุกไม่มีพยาการใดๆ ภายหลังการให้เชื้อไวรัสทิลาเปียเลค ปลานิลทดลองที่ถูกนำมาให้เชื้อได้รับการยืนยันว่ามีการติดเชื้อไวรัสทิลาเปียเลคจริงด้วยวิธี RT-qPCR ในขณะที่ปลาชนิดอื่นๆ ให้ผลลบ โดยสรุป การศึกษาภาคสนามและในห้องปฏิบัติการในครั้งนี้แสดงให้เห็นว่าปลาชนิดอื่น ๆ ที่เลี้ยงร่วมกับปลานิลในระบบการเพาะเลี้ยงปลาแบบหลากหลายชนิดไม่ไวต่อการติดเชื้อไวรัสทิลาเปียเลค และเพื่อเป็นการลดการแพร่ระบาดและความรุนแรงจากโรคของเชื้อไวรัสทิลาเปียเลค เราได้แนะนำว่ากิจกรรมการสำรวจหาเชื้อไวรัสทิลาเปียเลคควรกระทำอย่างต่อเนื่องเพื่อตรวจสอบแหล่งที่ปนเปื้อนเชื้อไวรัส แม้ว่าจากข้อมูลของเราแสดงให้เห็นว่าเชื้อไวรัสทิลาเปียเลคมีความจำเพาะกับปลานิลค่อน ข้างมากแต่การทำการสำรวจหาเชื้อไวรัสทิลาเปียเลคในปลาชนิดอื่นๆ ที่มักจะนำมาเลี้ยงในระบบการเพาะเลี้ยงปลาแบบหลากหลายชนิดก็ควรจะกระทำต่อไปเป็นการเตรียมพร้อมรับมือเมื่อเกิดเหตุการณ์ใหม่ที่เชื้อไวรัสทิลาเปียเลคสามารถกลายพันธุ์หรือสามาร ถปรับตัวให้เข้ากับโฮสต์ชนิดใหม่ๆ ได้

สาขาวิชา	วิทยาศาสตร์ทางการสัตวแพทย์และเทคโนโลยี	ลายมือชื่อนิสิต ....
ปีการศึกษา	2564	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

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KEYWORD: Epidemiology, Polyculture, Genetic diversity, Distribution dynamics, Tilapia Lake Virus

Partho Pratim Debnath : Genetic diversity and distribution of Tilapia lake virus in fish polyculture system in Bangladesh. Advisor: Assoc. Prof. Dr. CHANNARONG RODKHUM Co-advisor: Asst. Prof. Dr. Ha Thanh Dong

Tilapia is the world's second most important farmed fish species, following carp. Bangladesh is the world's fourth-largest tilapia producer and has largely adopted fish polyculture. Few research on factors associated with mortality and economic losses following such deaths have been undertaken in Bangladesh due of its perceived hardiness. Tilapia lake virus (TiLV) is an emerging pathogen in aquaculture, reportedly affecting farmed tilapia in 16 countries across multiple continents. Following an early warning in 2017 that TiLV could be widespread, we surveyed 565 tilapia farms in 15 of Bangladesh's most important tilapia-producing districts using online tilapia epidemiology and health economics survey tool, followed by a surveillance program on tilapia grow-out farms and hatcheries in 10 districts of Bangladesh in 2017 and 2019. Furthermore, several unusual mortalities observed in species co-cultivated with TiLV-infected tilapia prompted us to examine whether any of the co-cultivated species would test positive for TiLV and whether they were susceptible to TiLV infection in controlled laboratory experiments. The survey examined a range of factors, including geographical factors, farmer characteristics, farm characteristics, stocking factors, biosecurity measures, and baseline – and unusual mortality levels and characteristics. A total of 18.2 % of farms reported having experienced unusual mortality, with an average mortality level of 23.2 %. Farm size, baseline mortality level, farmer concern about baseline mortality, and antibiotic treatment were all significantly associated with increased odds of reporting unusual mortality in a multivariable model. Similarly, in basic statistics, farming region, water source, dead fish removal frequency, and antibiotic treatment were all found to be significantly associated with the level of unusual mortality, where water source and dead fish removal frequency remained significant in the multivariable model. Based on the baseline and unusual mortality in tilapia, a total hidden loss of 875.7 million USD was estimated. Considering this unusual mortality, biological sampling was done through surveillance study where eight out of 11 farms tested positive for TiLV in 2017, and two out of seven tested positive in 2019. Investigation of asymptomatic broodstock collected from 16 tilapia hatcheries revealed that six hatcheries tested positive for TiLV. Test result was confirmed through histopathology as an alternate method. We recovered three complete genomes of TiLV from infected fish, one from 2017 and two from 2019. Phylogenetic analyses based on both the concatenated coding sequences of 10 segments and only segment 1 consistently revealed that Bangladeshi TiLV isolates formed a unique cluster within Thai clade, suggesting a close genetic relation. Additionally, using 183 samples obtained from 15 polyculture farms in six districts across Bangladesh, we determined that 20% of the farms tested positive for TiLV in tilapia, while 15 co-cultivated fish species and seven other invertebrates (e.g., insects and crustaceans) were considered potential carriers all tested negative. Of the six representative fish species experimentally infected with TiLV, only Nile tilapia showed the typical clinical signs of the disease, with 70% mortality within 12 days. By contrast, four carp species and one catfish species challenged with TiLV showed no signs of TiLV infection. Challenged tilapia were confirmed as TiLV-positive by RT-qPCR, while challenged carp and walking catfish all tested negative. Overall, our field and laboratory findings indicate that species used in polycultures are not susceptible to TiLV. To reduce the nationwide spread and severity of TiLV infection, we propose that TiLV-targeted surveillance activities continue to detect contaminated sources. Although current evidence suggests that TiLV is likely host-specific to tilapia, targeted surveillance for TiLV in other fish species in polyculture systems should continue, in order to prepare for a possible future scenario where TiLV mutates and/or adapts to the new host(s).

จุฬาลงกรณ์มหาวิทยาลัย

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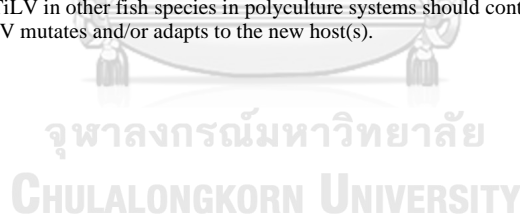
Field of Study:	Veterinary Science and technology	Student's Signature ....
Academic Year:	2021	Advisor's Signature ....
		Co-advisor's Signature ....

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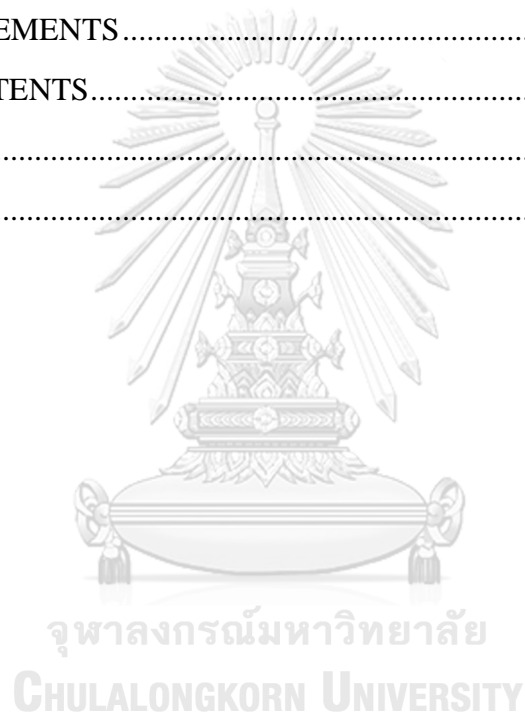
Chapter 1

Inroduction

1.1 Importance and rationale of the research

The aquaculture sector is becoming increasingly essential for global food security, as well as a powerful tool for poverty reduction (FAO, 2014; FAO, 2017a; FAO, 2016). Millions of people across the world rely on fish and fishery-related activities for food, nutrition, income, and livelihood. In 2014, over 57 million people were employed in fisheries or aquaculture throughout the world (FAO, 2016). Fish is an important and considerable source of animal protein (Roos et al., 2007; FAO/WHO., 2011; Kawarazuka, 2011; Beveridge et al., 2013; Thilsted et al., 2016). In 2018, the global population was 7.6 billion people; it is expected to reach 8.5 billion by 2030, 9.7 billion by 2050, and 11 billion by 2100, implying that global food production will need to grow by 50% to feed the whole globe (DESA, 2019). Aquaculture is seen as the next, and maybe final, large-scale animal protein-producing sector to limit livestock land growth and reduce overfishing at sea (FAO, 2014).

Tilapia is the common name for a kind of cichlid fish that may be found in freshwater streams, ponds, rivers, and lakes, as well as brackish water. Tilapias, once thought to be an invasive species, are increasingly becoming more important in aquaculture (Wang et al., 2016). Tilapia, which comprises more than 100 species, is the second-most important group of farmed fish globally, after carp, and is regarded one of the most important fish species to fulfil the growing global need for protein, vitamin, and mineral sources. (Ng and Romano, 2013; Amal et al., 2018). Tilapia is farmed in over 135 countries, with global production estimated at 6.5 million metric tons (MMT) (FAO, 2017). In 2015, the top four producers were China (1.78 MMT), Indonesia (1.11 MMT), Egypt (0.88 MMT) and Bangladesh (0.32 MMT) (FAO, 2017).

Bangladesh is the fifth-largest producer of aquaculture in the world (FAO, 2017), with a total annual fisheries production of 4.38 MMT, with aquaculture accounting for 56.44 percent (DoF, 2018). The fisheries sector generates a substantial quantity of foreign exchange for the country through exporting fish, shrimp, and other aquatic

animal products. Bangladesh gained USD 513 million through the export of roughly 68,940 MT of fish and fisheries products in 2017–2018 (DoF, 2018). The aquaculture sector has grown at an annual rate of 5.43 percent on average during the previous ten years (DoF, 2018). Furthermore, tilapia accounts for 10.62 percent of total output and ranks second in the country, after channel catfish (DoF, 2018). The main advantages of tilapia cultivation are its general hardiness, adaptability to various production systems, and rapid growth, as well as advances in genetic determination and focused breeding, which have increased these qualities (Ponzoni et al., 2011). Conversely, the intensification of tilapia farming and the increase in the number of tilapia farms has resulted in an increase in the incidence of infectious diseases (Carvalho-Castro et al., 2010; Figueiredo et al., 2012; Leal et al., 2019).

Bacteria (54.9 %) are the most prevalent cause of infectious diseases in aquaculture, followed by viruses (22.6 %), parasites (19.4 %), and fungi (3.1%) (McLoughlin, 2006; Kibenge et al., 2012). Major common bacterial diseases reported in farmed tilapia are Streptococcosis, caused by *Streptococcus* sp.(Amal and Zamri-Saad, 2011; Suwannasang et al., 2014), Columnaris caused by *Flavobacterium columnare* (Figueiredo et al., 2005; Dong et al., 2015a), Francisellosis caused by *Francisella noatunensis* subsp. *Orientalis and* Edwardsiellosis caused by *Edwardsiella ictaluri* (Soto et al., 2009b; Soto et al., 2012c; Nguyen et al., 2016) and Haemorrhagic septicaemia caused by motile aeromonads (*Aeromonas hydrophila, A. sobria, A. veronii and A. jandaei*) (Li and Cai, 2011a; Dong et al., 2015b; Dong et al., 2017a). Simultaneously most common reported viral diseases in tilapia are betanodavirus, Tilapia Larvae Encephalitis Virus (TLEV), Infectious Spleen and Kidney Nacrosis Virus (ISKNV) (Shlapobersky et al., 2010; Keawcharoen et al., 2015; Subramaniam et al., 2016). Shortly after the first report of a novel disease among tilapia in Ecuador (Ferguson et al., 2014), tilapia lake virus (TiLV) was discovered as a newly emerging virus that caused mass die-offs in tilapia in Israel (Eyngor et al., 2014). Molecular analyses indicated that the same virus, TiLV, was the causative agent of these unusual mortality events in both Ecuador and Israel (Bacharach et al., 2016a; Del-Pozo et al., 2016).

In early 2017, in response to the rapid spread of TiLV, several international organizations issued a disease advisory (NACA, 2017), a global special alert (FAO, 2017a), a factsheet (CGIAR, 2017) and a pathogen information sheet (OIE, 2017a). At the time, it was expected that TiLV would have been spread through the translocation of live tilapia for aquaculture in over 40 countries, including Bangladesh (Dong et al., 2017c). The scientific community urged tilapia-producing countries to quickly investigate unusual mortality events and initiate TiLV-targeted surveillance to prevent its spread and the resulting negative consequences. Bangladesh is one of leading producer of Tilapia, which has imported tilapia broodstock from Malaysia, Thailand and the Philippines and increasing the chance of viral introduction. Again, production of Tilapia is mainly coming from pond culture system followed by polyculture of tilapia.

This project aims to investigate major risk factors associated with mortalities and economic impact of tilapia diseases, the distribution of TiLV in polyculture systems, to assess the transmission of TiLV in tilapia farms and hatcheries and to understand the genetic diversity and molecular epidemiology of TiLV.

1.2 Research Questions

> ➢ What is the diseases status of tilapia in tilapia polyculture farming, how this disease relates with TiLV and is there any economic impact of tilapia diseases as well as TiLV in Bangladesh?

> ➢ Is TiLV, one of the key pathogens for tilapia diseases in Bangladesh?

> ➢ What extent TiLV can be distributed within polyculture farming systems in Bangladesh?

> ➢ Is there any genetic diversity of TiLV isolated from tilapia or other co-cultivated species in polyculture systems in Bangladesh?

1.3 Objective of the research project

> ➢ To get an overview of tilapia diseases in polyculture systems in Bangladesh

> ➢ To investigate prevalence (cross sectional epidemiological survey) and genetic diversity (comparative genomics) of TiLV from Bangladesh

➢ To investigate natural distribution of TiLV and susceptibilities of non-tilapia species to TiLV in fish polyculture system in Bangladesh.

1.4 Conceptual Framework:

Genetic diversity and Distribution of Tilapia lake virus in fish polyculture system

Distribution of TiLV

Epidemiology survey for polyculture farms

Investigate Tilapia hatchery for TiLV

Diseases in Tilapia

Diseases in tilapia along with non Tilapia species

Investigate farms for TiLV in tilapia and other co-cultivated species

Representative sample for genetic analysis

Information generate on natural distribution and genetic diversity of TiLV for restrict TiLV infection in tilapia and non-Tilapia species in fish polyculture system

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Chapter 2

Literature review

2.1 Importance of tilapia culture and infectious diseases:

Capture fisheries productivity has declined, and they are no longer deemed capable of providing the supply of fishery products required to meet increasing global demand (Subasinghe et al., 2009). Tilapia is a fish that is farmed in over 135 countries, with a global production of 6.5 million metric tons (MMT) (FAO, 2017). Tilapias are native to the Middle East and Africa, yet fascinatingly, the vast majority of tilapia produced (98 percent) comes from outside of their original habitats (Shelton, 2002). Tilapia has been considered the 21st century's most important aquaculture species (Shelton, 2002). Tilapia is the generic name for over a hundred different species of cichlid fish. Tilapia are freshwater fish that live in shallow streams, ponds, rivers, and lakes, with brackish water being less prevalent. Currently, all commercially significant tilapia outside of Africa belong to the genus Oreochromis, and over 90% of all commercially farmed tilapia outside of Africa are Nile tilapia. Less frequently farmed species are Blue tilapia (*O. aureus*), Mozambique tilapia (*O. mossambicus*) and the Zanzibar tilapia (*O. urolepis hornorum*). They are probably the most profitable and widely traded freshwater food fish in the world. Because of its simple culture methods and marketing, tilapia culture has grown in popularity over the last thirty years.

Fish farming systems considering two or more species together while species are differing in habitats, feeding behavior and ecological requirements to increase production from the same pond is called polyculture (Zimmermann and New, 2000). Polyculture is also known as multi-trophic aquaculture, co-culture or integrated aquaculture (Bunting, 2008). There are different combination of tilapia polyculture has been followed in Bangladesh such as tilapia with Indian major carps, tilapia with catfish along with other native species (Azad et al., 2004). The foremost aquaculture production systems in Bangladesh are extensive, semi-intensive, and small-scale pond-based polyculture systems (Belton and Azad, 2012). Polyculture farming systems in Bangladesh can produce 5–10 large fish species together; typically, tilapia, carp, and catfish species are included (Castine et al., 2017).

Production of tilapia had increased from 3,165,000 tons to 5,377,000 tons by 2010 to 2016 rationalized 10 percent of fresh water fish production through aquaculture globally (FAO, 2018). Bangladesh ranked 3rd in inland open water capture production and 5th in world aquaculture production. Currently Bangladesh ranks 4th in tilapia production in the world and 3rd in Asia (FAO, 2018). Conversely, the intensification of culture technique and gradually increasing the area and production of tilapia and diversified species combination for tilapia polyculture led to encounter in an increase in diseases incidence and severity of disease agents in tilapia.

Tilapia are infected by a variety of internal and external parasites. Several factors influence the occurrence and severity of a parasitic infection, including the number of parasites infesting the fish, culture systems and fish species, sex, size, and health state (El-Sayed, 2019). In both hatcheries and rearing facilities, protozoan parasites have been documented to cause severe mortality in wild and farmed tilapia (El-Sayed, 2019). Tilapia have been shown to carry three types of parasite protozoa: ciliates, flagellates, and sporozoa (Tonguthai and Chinabut, 1997). Ectoparasitic infection by ciliated protozoans, such as Trichodina sp. and Ichthyophthirius multifiliis, has been extensively studied and characterized in tilapia (Shoemaker et al., 2000; Pantoja MF et al., 2012). Trichodiniosis, Ichthyophthiriasis/white spot disease, Chilodonellosis, Ichthyobodoosis, Myxosporidia etc. are the most reported protozoan diseases in tilapia(Lightner et al., 1988; Bondad-Reantaso and Arthur, 1989; Okaeme and Okojie, 1989; Brock et al., 1993; Tonguthai and Chinabut, 1997; de Ocampo and Camberos, 1998; Lua et al., 1999; Gbankoto et al., 2001; Eissa, 2002; Abdel-Ghaffar et al., 2008; El-Dien and Abdel-Gaber, 2009; Akoll et al., 2012a; El-Gayar and Aly, 2013; Valladão et al., 2013; Abdel-Baki et al., 2014; Abdel-Baki et al., 2015a; Abdel-Baki et al., 2015b; Valladão et al., 2016; Taghreed Ibrahim, 2020). Monogenesis is induced by monogenetic trematodes such as Dactylogyridae (Gill Flukes), Cichlidogyrides (only Tilapia Gill Flukes), and Gyrodactylidae (only Tilapia Gill Flukes) (Skin Flukes) (Taghreed Ibrahim, 2020). Digenetic trematodes can be a major concern in tilapia farming, causing significant losses in fingerling and juvenile fish (El-Sayed, 2020). Clinostomum and Euclinostomum are two genuses of digenetic trematodes reported to affect tilapia. Clinostomum tilapiae and C. complanatum have been detected in the intestines of Nile tilapia and mango tilapia (Sarotherodon galilaeus)

(Ukoli, 1966). Many species of nematodes, cestodes, and acanthocephalans have been reported in both wild and cultivated tilapia, but little is known about their parasitic relevance (Fryer and Iles, 1972; Scott, 1977; Ramadan, 1991; Aloo et al., 1995; Omoregie et al., 1995). There have been reports of tilapia diseases induced by these parasites, including diphyllobothriosis, heart worm disease, and cichlid acanthocephaliasis (Eissa, 2002). The copepods Ergasilus spp., Lernaea spp., Caligus spp., and Lamperoglena spp., the branchiurans Argulus spp. and Dolops spp., and the isopod Alitropus typus are parasitic crustaceans that frequently infest wild and farmed tilapia (Douëllou and Erlwanger, 1994). Many of these parasites pose serious health risks to cultured tilapia, resulting in significant losses for tilapia producers (Douëllou and Erlwanger, 1994).

Tilapia are susceptible to a variety of bacterial infections such as streptococcosis (Suanyuk et al., 2008; Anshary et al., 2014; Costa et al., 2014; Kayansamruaj et al., 2014); francisellosis (Soto et al., 2009a; Nguyen et al., 2015; Lin et al., 2016); edwardsiellosis (Clavijo et al., 2002; Soto et al., 2012b); and bacterial haemorrhagic septicaemia (Huys et al., 2005; Nhung et al., 2007; Li and Cai, 2011b; Soto-Rodriguez et al., 2013; Dong et al., 2015b; Eissa et al., 2015; Austin and Austin, 2016). Among them, streptococcosis is one of the most common bacterial infections in tilapia, which led to USD40 million in economic losses in China in 2011 (Chen et al., 2012). Numerous other bacterial infections, such as columnaris, francisellosis, and edwardsiellosis, have been reported as the most prevalent emerging diseases in the tilapia industry, causing severe infection in fry and fingerling stages (Soto et al., 2009a; Soto et al., 2012b; Soto et al., 2012d; Situmorang et al., 2014; Dong et al., 2015a; Dong et al., 2016; Dong et al., 2019; Hai et al., 2020). The novel bacterial disease hahellosis, caused by Hahella chejuensis, reportedly affects eggs and leads to red egg syndrome prior to hatching (Senapin et al., 2016). Several stressors, such as fluctuating water temperature, pH, and salinity, low levels of dissolve oxygen, increasing levels of ammonia, higher stocking density, improper fish handling, and poor management may increase the risk of bacterial disease outbreaks in tilapia populations (Hedrick et al., 1987; Bragg et al., 1990; Smith, 1997; Mauel et al., 2003; Soto et al., 2012a; Stratev and Odeyemi, 2016).

Fungal infections are classified as 'secondary diseases' because they thrive in necrotic tissues linked with injuries, bacterial or parasite lesions, dead and rotting eggs, and inadequate culture conditions (Pillay, 1990). A variety of fungal species have been identified from wild and farmed tilapia, with the following fungal infections being the most common and well-documented (El-Sharouny and Badran, 1995; Okaemae and Olufemi, 1997). Saprolegniasis is a tilapia disease caused by Saprolegnia ubiquitous, which is a water mold oomycete (Goodwin, 2012) and appears as cottony white, gray, brown, red, or greenish masses (Okaemae and Olufemi, 1997). Branchiomycosis (gill rot) is another fungal infection in tilapia that affects the gills and is caused by two oomycetes: B. sanguinis and B. demigrans (Goodwin, 2012). Infection of farmed Nile tilapia with Branchiomyces spp. has been recorded in both Israel (Paperna and Di Cave, 2001) and Egypt (Khalil et al., 2015). Branchiomyces spp. infection is sometimes referred to as 'bad-management disease,' since it thrives in low-quality water with large amounts of organic debris. Another fungal disease, Ichthyophoniasis which is one of the most economically and environmentally devastating diseases that affects Nile tilapia in aquaculture (El-Ghany and El-Ashram, 2008; Yokota et al., 2008). Aspergillomycosis is a fungal infection in tilapia caused by Aspergillus niger (Taghreed Ibrahim, 2020).

Over the past few years, there have been several reports of viral infections significantly impacting tilapia production. Nine viral diseases have been reported in tilapia, to date, including six DNA viruses namely Tilapia parvovirus (TiPV), infectious spleen and kidney necrosis virus (ISKNV), tilapia larvae encephalitis virus (TLEV), Ranavirus, Iridovirus-like agent, Lymphocystis disease virus (LCDV) and three RNA viruses namely Infectious pancreatic necrosis virus (IPNV), Nervous necrosis virus (NNV) and tilapia lake virus (TiLV). Tilapia parvovirus (TiPV), reported on tilapia farms in China (Liu et al., 2020) and Thailand (Yamkasem et al., 2021), is the most recently discovered viral disease in tilapia. However, tilapia lake virus (TiLV) has had the most significant impact on the tilapia industry. TiLV was initially discovered in Israel (Eyngor et al., 2014) in 2014 and has now spread to 16 nations across four continents (Surachetpong et al., 2020) and was revealed as a newly emerging virus that caused mass die-offs in tilapia in Israel (Eyngor et al., 2014).

2.2 Tilapia Lake Virus Disease (TILVD)

Causative agent:

Through viral isolation and identification, the causative agent for TiLVD was identified as tilapia lake virus (TiLV), is an enveloped, negative sense, single-stranded RNA virus that contains 10 genome segments, with a total genome size of 10.323 kb, ranging from 465 to 1,641 bp for each of the 10 segments (Eyngor et al., 2014; Bacharach et al., 2016b). Among the 10 segments only the 1st segment (largest segment) of the virus has very weak similarities with influenza C virus (Bacharach et al., 2016a). In addition 1st segment is consists with major polymerase motifs and subject to encode the polymerase of TiLV (Bacharach et al., 2016a). There was no homology identified with any other viruses for the rest of the 9 segments but at the terminal, existence of complementary sequences and protein identification from the infected cell extractions which associated with the ORFs carried by the viruses provided representative evident of TiLV gene segments (Bacharach et al., 2016a). The virus was initially classified as a novel *Orthomyxo*-like virus and is now officially classified as *Tilapia tilapinevirus*, the only species in the genus *Tilapinevirus*, under the new family *Amnoonviridae* (Bacharach et al., 2019). TiLV is recognized as a significant infectious agent that may threaten the development of the global tilapia industry (Bacharach et al., 2016a; Jansen et al., 2018). TiLV outbreaks purportedly caused mortality in the range of 20% to 90% (Surachetpong et al., 2017; Dong et al., 2017b; Jansen et al., 2018).

Gross signs and pathology:

Depending on their geographical origin, the reported clinical and gross pathological signs of TiLV infection vary to some point. According to Israel's first report in 2014, mainly ocular modifications, including opacity of the lens, characterized major pathological findings such as gross lesions. In advanced circumstances, the lenses have been broken with endophthalmitis and the eyeball swells, the shrinkage of the eye and eye loss. Other damage was skin erosion, leptomening hemorrhages (Eyngor et al., 2014) . Exophthalmia, discoloration (Eyngor et al., 2014), abdominal distension, protruding scaling, progressively emaciated look, watery and colorless fluid in the abdominal cavity and gill pallor were presented in the case in Ecuador (Ferguson et

al., 2014). In Thailand there have been reports of loss of appetite, lethargy, abnormal comportability (e.g. surface swimming, stopping school), pallor, anemia, exophthalmia, skin swelling and erosion (Surachetpong et al., 2017; Tattiyapong et al., 2017; Dong et al., 2017b). Additionally, brain congestion and gill paleness were observed.[4] From India there were clinical signs of skin erosion and a loss of scales in naturally infected fish, while experimentally infected fish had exophthalmia and swollen abdomen (Behera et al., 2018). The clinical signs reported in the Philippines include abdominal swelling and bulging eyes; the diseases and exophthalmia have been reported in Peru (OIE, 2017a; Pulido et al., 2019). The affected fish have shown haemorrhea patches, uncontaminated scales, open wounds, dark colors and fine red on farms in Egypt, some of which have been positively tested by TiLV for coinfection with or without Aeromonas spp.(Nicholson et al., 2017). Based on the information available, a complete list of pathognomonic signs appears currently impossible to make a reliable diagnosis based on clinical signs alone.

In the histopathological lesions with TiLV infections there appear to be some geographical and individual variations. Currently present, the information indicates that TiLV outbreaks have syncytial hepatitis as the most common histopathology. Although outbreaks were not reported in the earliest Israeli report (Eyngor et al., 2014), a later study of the same research group described syncytial hepatitis (Bacharach et al., 2016a). Major pathological changes in TiLV affected liver presence of syncytial giant cell(s) or multinucleated giant cells, followed by Intracytoplasmic inclusion bodies (eosinophilic inclusion or droplets of lipoprotein), reduction in fat storage, disassociation of hepatocytes, necrotic pancreases and lymphocyte infiltrations, hemorrhoea, cellular necrosis, pyknosis and karyorrhexis, cytoplasms foaming and multifocal chronic hepatitis are present. In kidney, lymphocytes aggregation, pyknesis and karyorrhexis, increasing number of centers for melano-macrophages. Sometimes Syncytia-like has been seen in spleen, the degeneration of asplenic cells in splenic ellipses, pyknosis and karyorrhexis, the presence of macrophages loaded with debris and an increasing number of mellanomacrophage centers. In brain, severe inflammation has been observed occasionally with massive lymphocyte infiltration, Encephalitis, perivascular fusing, Blood congestion or hemorrhage and in gills lymphozytic inflammatory cells infiltration, pycnosis and

caryorrhexis, presence of macrophages loaded with debris (Ferguson et al., 2014; Bacharach et al., 2016a; Dong et al., 2017b; Amal et al., 2018; Behera et al., 2018).

Host Factor:

In case of TiLV, susceptible host species was identified as affected farmed species include hybrid tilapia (*Oreochromis niloticus* X *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. I et al., 2018), Thailand (Surachetpong et al., 2017; Dong et al., 2017b) and Uganda (Mugimba et al., 2019); red tilapia (*Oreochromis* sp.) in Thailand (Surachetpong et al., 2017; Dong et al., 2017b) and hybrid red tilapia (*Oreochromis niloticus* X *O. mossambicus*) in Malaysia (Amal et al., 2018). Wild tilapine has been found to be positive for TiLV amongst Sarotherodon galilaeus, *Tilapia zilli*, *Oreochromis aureus* and *Tristamellasimonis intermedia* of the Sea of Galilee in Israel, Wild black tilapia (*Oreochromis* sp.) in the province Malaysia, Wild nile tilapia in Lake Victoria in the state of Uganda and Peru (Eyngor et al., 2014; OIE, 2017b; Mugimba et al., 2018; OIE, 2018). In Israel there was no death in co-cultivated gray mullet (*Mugil cefhalus*) and carp (*Cyprinus carpio*) during outbreaks of disease (Eyngor et al., 2014). Similarly, during Egyptian outbreaks, the co-cultivated gray mullet and thin-lipped mullet (*Liza ramada*) have been found to not have been affected (Fathi et al., 2017), and In India, co-cultivated Carps mainly rohu (*Labeo rohita*), catla (*Catla Catla*), mrigal, milk fish (*Chanos chanos*), and pearl spot (*Etroplus suratensis*) had not been affected by TiLV (Behera et al., 2018). In addition to tilapia, giant gourami (Osphronemus goramy) naturally infected with TiLV have been found (Chiamkunakorn et al., 2019) and also shown to be susceptible to TiLV in an experimental challenge study (Jaemwimol et al., 2018). TiLV has also been identified in wild tinfoil barb (Barbonymus schwanenfeldii) in Malaysia (Abdullah et al., 2018) as well as in farmed barramundi (Lates calcarifer) in Thailand (Piamsomboon and Wongtavatchai, 2021). Again Zebrafish (*Danio rerio*) are also susceptible to TiLV, according to laboratory challenge trials (Rakus et al., 2020; Widziolek et al., 2021). Tilapia susceptible phases in life were reported to have been affected by TiLV, with deaths over a wide band of weights observed in Israel (Eyngor et al., 2014) and in

Ecuador (Ferguson et al., 2014), India (Behera et al., 2018), Malaysia (Amal et al., 2018) and Thailand (Surachetpong et al., 2017; Dong et al., 2017b). Fingerlings and young tilapia (up to 80 g) was reported to be affected in Israel(Eyngor et al., 2014). 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), initial stages of development of tilapia, also tested positive for TiLV (fertilized eggs, yolk-sac fish and fry) (Dong et al., 2017c).

Genome characterization and genetic diversity:

The viral shape and size are comparable to members of the Orthomyxoviridae family, and it was previously classified as an orthomyxovirus-like virus, but its genomic sequence showed minimal similarity to orthomyxoviruses. TiLV was eventually classified as a new species, Tilapia tilapinevirus, and placed in the Amnoonviridae family of the Articulavirales order (ICTV, 2020). The TiLV genome has conserved complementary sequences at the 5' and 3' termini, which is a standard genomic organization seen in other orthomyxoviruses. Among 10 genome segments of TiLV, most of the segments have no homology to other recognized viruses, with the exception of segment 1, which has a weak sequence homology to the RNA-dependent RNA polymerase (RdRp) subunit of influenza C virus PB1 (Bacharach et al., 2016b). From these 10 TiLV gene fragments, researchers discovered 14 functional genes responsible for the development of 14 viral proteins (Acharya et al., 2019).

Eyngor et al. (2014) obtained the TiL-4-2011 isolate in Israel in 2011. It is the earliest defined isolate among those investigated. This isolate is used as a reference genome to compare 19 other whole genome sequenced isolates from Israel (2012), Ecuador (2012), Thailand (2014–2019), Bangladesh (2 isolates in 2017; 2 isolates in 2019), Peru (2018), and the United States of America (2 isolates from an Idaho farm outbreak in 2019). Thawornwattana et al. (2020) used Bayesian phylogenetic analysis to estimate TiLV evolution rates of 1.81–3.47 x 10^{-3} substitutions per site per year based on the complete genome sequences of 17 isolates from six countries. Consistent with alignment analyses, sequence identity ranged from 93 percent to 100 percent in these TiLV isolates (Jansen et al., 2019). Furthermore, 96-100% sequence identity

was showed with TiLV segment 1 from Ecuador, Thailand, and India to the original strain from Israel (Bacharach et al., 2016b; Surachetpong et al., 2017; Dong et al., 2017c; Behera et al., 2018). The nucleotide sequence similarity of segment 3 of the virus from Egypt and Thailand against the Israel isolate was 93 % and 98 %, respectively (Fathi et al., 2017; Surachetpong et al., 2017; Dong et al., 2017c). Another study revealed, nucleotide sequences of segment 2 of TiLV from Tanzania and Uganda, isolated from Lake Victoria, were found to have a similarity of more than 99.8% and were clustered with strains from Israel and Thailand by phylogenetic analysis, implying that the virus from Lake Victoria may have a shared origin with the virus from Thailand and Israel (Mugimba et al., 2019).

Based on multilocus sequence phylogenetic analysis (MLSA) of 8305 nucleotides from five TiLV genomes, two genetic clades of TiLV (Israeli and Thai clades) were suggested by Pulido et al., 2019. Since the number of complete TiLV genomes available in the GenBank database is small, one recent study used the open reading frame (ORF) of segment 1 PB1 gene (1560 nucleotides) from 21 TiLV isolates from Israel, Peru, Ecuador, and Thailand to investigate TiLV genetic diversity and study revealed that Phylogenetic tree inferred from the segment 1 dataset suggests three distinct clades (Israeli-2011, Israeli-2012, and Thai clades), despite low bootstrap values (Taengphu et al., 2020).

Reassortment of TiLV was also discovered in three isolates using whole genome phylogenetic analysis: Israel isolate Til-4-2011 contains EC-2012's segments 5 and 6; TH-2018-K consists of BD2017's segment 5; TH-2016-CN contains the segments 1–4 from an unknown TiLV isolate (Chaput et al., 2020; Thawornwattana et al., 2021). Reassortment occurs when two (or more) strains co-infect a host cell, resulting in shuffling viral segments in the offspring viruses; this occurrence may increase TiLV genetic diversity.

Epidemiology:

TiLV was first reported in 2014 through the investigation of sharp decline of wild catch of tilapia from the Sea of Galilee and it was described a new RNA virus was accountable for this massive mortality (Eyngor et al., 2014). At the same time massive mortality in farm tilapia was described in Ecuador where syncytial hepatitis was

described as histopathological lesions and later it was confirmed as the same virus as identified in Israel (Bacharach et al., 2016b; Del-Pozo et al., 2016). In 2015, Egypt described this disease as "summer mortality syndrome" of tilapia (Fathi et al., 2017). But this disease was taken attention while in 2017, Thailand identified TiLV for the causative agent of huge mortality in farmed tilapia (Surachetpong et al., 2017; Dong et al., 2017c).

To date, TiLV has been detected across Asia, Africa, and North and South America in 16 tilapia producing countries: Ecuador, Israel, Colombia, Thailand, Uganda, the United Republic of Tanzania, Egypt, India, Indonesia, Chinese Taipei, the Philippines, Malaysia, Peru, Mexico, United States and Bangladesh (Jansen et al., 2018; FAO, 2019).

TiLV can transmitted through both horizontal and vertical pathway. TiLV can successfully spread through cohabitation which has been successfully confirmed through several laboratory experiment (Eyngor et al., 2014; Liamnimitr et al., 2018). Similarly TiLV was identified from feces and contaminated water from laboratory experiment (Pierezan et al., 2019). Again vertical transmission was described in latest studies (Yamkasem J, 2019; Ha Thanh Dong et al., 2020).

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2.4 References:

Abdel-Baki AA, Abdel-Haleem HM, Sakran T, Zayed E, Ibrahim KE and Al-Quraishy S 2015b. Two Myxobolus spp. infecting the kidney of Nile tilapia (Oreochromis niloticus) in the River Nile at Beni-Suef governorate, Egypt, and the associated renal changes. Parasitological Research. 114: 1107-1112.

Abdel-Baki AA, Gewik MM and Al-Quraishy S 2014. First records of Ambiphrya and Vorticella spp. (Protozoa, Ciliophora) in cultured Nile tilapia (Oreochromis niloticus) in the Central region of Saudi Arabia. Saudi Journal of Biological Sciences. 21.

Abdel-Baki AA, Zayed E, Sakran T and Al-Quraishy S 2015a. A new record of Myxobolus brachysporus and M. israelensis in the tilapia (*Oreochromis niloticus*) collected from the Nile River, Egypt. Saudi Journal of Biological Sciences. 22: 539-542.

Abdel-Ghaffar F, El-Toukhy A, Al-Quraishy S, Al-Rasheid K, Abdel-Baki AS, Hegazy A and Bashtar A 2008. Five new myxosporean species (Myxozoa: Myxosporea) infecting the Nile tilapia Oreochromis niloticus in Bahr shebin, Nile tributary, Nile Delta, Egypt. Parasitological Research. 103: 1197-1205.

Abdullah A, Ramly R, Mohammad Ridzwan MS, Sudirwan F, Abas A, Ahmad K, Murni M and Kua BC 2018. First detection of tilapia lake virus (TiLV) in wild river carp (Barbonymus schwanenfeldii) at Timah Tasoh Lake, Malaysia. J Fish Dis. 41(9): 1459-1462.

Acharya V, Chakraborty HJ, Rout AK, Balabantaray S, Behera BK and Das BK 2019. Structural Characterization of Open Reading Frame-Encoded Functional Genes from Tilapia Lake Virus (TiLV). Mol Biotechnol. 61(12): 945-957.

Akoll P, Konecny R, Mwanja WW, Nattabi JK, Agoe C and Schiemer F 2012a. Parasite fauna of farmed Nile tilapia (*Oreochromis niloticus*) and African catfish (Clarias gariepinus) in Uganda. Parsitological Research. 110: 315-323.

Aloo P, Okelo RO and Ntiba MJ 1995. Helminth parasites of Oreochromis leucostictus (Trewavas) in Lake Naivasha, Kenya. *in* Pan African Fisheries

Congress on Sustainable Development of Fisheries in Africa. Vol. Abstract No. 172, FSA, Nairobi, Kenya.

Amal MNA, Koh CB, Nurliyana M, Suhaiba M, Nor-Amalina Z, Santha S, Diyana-Nadhirah KP, Yusof MT, Ina-Salwany MY and Zamri-Saad M 2018. A case of natural co-infection of Tilapia Lake Virus and Aeromonas veronii in a Malaysian red hybrid tilapia (Oreochromis niloticus×O. mossambicus) farm experiencing high mortality. Aquaculture. 485: 12-16.

Amal MNA and Zamri-Saad M 2011. Streptococcosis in tilapia (Oreochromis niloticus): a review. : . Pertanika Journal of Tropical Agricultural Science. 34: 195–206.

Anshary H, Kurniawan RA, Sriwulan S, Ramli R and Baxa DV 2014. Isolation and molecular identification of the etiological agents of streptococcosis in Nile tilapia (Oreochromis niloticus) cultured in net cages in Lake Sentani, Papua, Indonesia. SpringerPlus. 3(1).

Austin B and Austin DA 2016. Bacterial fish pathogens: Disease of farmed and wild fish, sixth edition. In: Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, Sixth Edition. 1-732.

Azad MAK, Rahman MM, Rahman Z, Kader MA, Haque MM and Alam MJ 2004. Polyculture of Carp, Tilapia and Pangas Using Low Cost Inputs. Pakistan Journal of Biological Sciences. 7: 1918-1926.

Bacharach E, Mishra N, Briese T, Eldar A, Lipkin WI and Kuhn JH 2019. Create one (1) new genusincluding one (1) new species. International Committee on Taxonomy of Viruses (ICTV).

Bacharach E, Mishra N, Briese T, Zody MC, Kembou JET, Zamostiano R, Berkowitz A, Ng J, Nitido A, Corvelo A, Toussaint NC, Abel Nielsen SC, Hornig M, Del-Pozo J, Bloom T, Ferguson H, Eldar A and Lipkin WI 2016a. Characterization of a novel Orthomyxo-like virus causing mass die-offs of tilapia. MBio. 7: pp. e00431-00416.

Bacharach E, Mishra N, Briese T, Zody MC, Tsofack JEK, Zamostiano R, Berkowitz A, Ng J, Nitido A, Corvelo A, Toussaint NC, Abel Nielsen SC, Hornig M, del Pozo J, Bloom T, Ferguson H, Eldar A and Lipkin WI 2016b. Characterization of a novel orthomyxo-like virus causing mass die-offs of Tilapia. mBio. 7(2).

Behera BK, Pradhan PK, Swaminathan TR, Sood N, Paria P, Das A, Verma DK, Kumar R, Yadav MK, Dev AK, Parida PK, Das BK, Lal KK and Jena JK 2018. Emergence of Tilapia Lake Virus associated with mortalities of farmed Nile Tilapia Oreochromis niloticus (Linnaeus 1758) in India. Aquaculture. 484: 168-174.

Belton B and Azad A 2012. The Characteristics and Status of Pond Aquaculture in Bangladesh. Aquaculture. 358–359: 196–204.

Beveridge MCM, Thilsted SH, Phillips MJ, Metian M, Troell M and Hall SJ 2013. Meeting the food and nutrition needs of the poor: The role of fish and the opportunities and challenges emerging from the rise of aquaculture. Journal of Fish Biology. 83(4): 1067–1084.

Bondad-Reantaso MG and Arthur JR 1989. Trichodinids (Protozoa: Ciliophora: Peritrichida) of Nile tilapia (Oreochromis niloticus) in the Philippines. Asian Fisheries Science. 3: 27-44.

Bragg RR, Huchzermeyer HF and Hanisch MA 1990. Mycobacterium fortuitum isolated from three species of fish in South Africa. Onderstepoort Journal of Veterinary Research. 57(1): 101-102.

Brock JA, LeaMaster BR and Lee CS 1993. An overview of pathogens and diseases in marine finfish hatcheries in Hawaii with comments on strategies for health management and disease prevention. In: Proceedings of Finfish Hatchery in Asia'91. C. S. Lee, M.S. Su, and I.C. Liao (eds). TFRI, Keelung, Taiwan: Tungkang Marine Laboratory. 221-238.

Bunting SW 2008. Horizontally integrated aquaculture development: exploring consensus on constraints and opportunities with a stakeholder Delphi. Aquaculture International 16: 153–169.

Carvalho-Castro GA, Lopes CO, Leal CAG, Cardoso PG, Leite RC and Figueiredo HCP 2010. Detection of type III secretion system genes in Aeromonas hydrophila and their relationship with virulence in Nile tilapia. Veterinary Microbiology. 144(3): 371–376.

Castine SA, Bogard JR, Barman BK, Karim M, Hossain MM, Kunda M, Haque ABMM, Phillips MJ and Thilsted SH 2017. Homestead pond polyculture can improve access to nutritious small fish. Food Security. 9: 785–801.

CGIAR 2017. Tilapia lake virus (TiLV): What to know and do? Penang, Malaysia: CGIAR Research Program on Fish Agri-food Systems. Factsheet: FISH-2017-03 https://hdl.handle.net/20.500.12348/115.

Chaput DL, Bass D, Alam MM, Al Hasan N, Stentiford GD, van Aerle R, Moore K, Bignell JP, Haque MM and Tyler CR 2020. The Segment Matters: Probable Reassortment of Tilapia Lake Virus (TiLV) Complicates Phylogenetic Analysis and Inference of Geographical Origin of New Isolate from Bangladesh. Viruses 12, (doi:10.3390/v12030258): 258.

Chen M, Li LP, Wang R, Liang WW, Huang Y, Li J, Lei AY, Huang WY and Gan X 2012. PCR detection and PFGE genotype analyses of streptococcal clinical isolates from tilapia in China. Veterinary Microbiology. 159(3-4): 526-530.

Chiamkunakorn C, Machimbirike VI, Senapin S, Khunrae P, Dong HT and Rattanarojpong T 2019. Blood and liver biopsy for the non-destructive screening of tilapia lake virus. J Fish Dis. 42: 1629–1636.

Clavijo AM, Conroy G, Conroy DA, Santander J and Aponte F 2002. First report of Edwardsiella tarda from tilapias in Venezuela. Bulletin of the European Association of Fish Pathologists. 22(4): 280-282.

Costa FAA, Leal CAG, Leite RC and Figueiredo HCP 2014. Genotyping of Streptococcus dysgalactiae strains isolated from Nile tilapia, Oreochromis niloticus (L.). Journal of Fish Diseases. 37(5): 463-469.

de Ocampo AA and Camberos LO 1998. Histopatologica de la respuesta de la tilapia (Oreochromis sp) a una infeccion mixta por myxosporidios Estudio en un caso natural. Veterinaria México 29: 213-216.

Del-Pozo J, Mishra N, Kabuusu R, Cheetham S, Eldar A, Bacharach E, Lipkin WI and Ferguson HW 2016. Syncytial Hepatitis of Tilapia (Oreochromis niloticus L.) is Associated With Orthomyxovirus-Like Virions in Hepatocytes. Veterinary Pathology. 54(1): 164-170.

DESA U 2019. World Population Prospects 2019: Highlights. . New York (US): United Nations Department for Economic and Social Affairs. 2019: 1-46.

DoF 2018. Yearbook of Fisheries Statistics of Bangladesh 2017-18. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh : Director General, DoF, 2018. Volume 34 : p. 129. Vol. 34. DoF. 2017. Yearbook of Fisheries Statistics of Bangladesh 2016-17. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh : Director General, DoF, 2017. Volume 34 : p. 129 ed.

Dong HT, Ataguba GA, Khunrae P, Rattanarojpong T and Senapin S 2017c. Evidence of TiLV infection in tilapia hatcheries from 2012 to 2017 reveals probable global spread of the disease. Aquaculture. 479: 579-583.

Dong HT, LaFrentz B, Pirarat N and Rodkhum C 2015a. Phenotypic characterization and genetic diversity of Flavobacterium columnare isolated from red tilapia, Oreochromis sp., in Thailand. Journal of Fish Diseases. 38(10): 901-913.

Dong HT, LaFrentz B, Pirarat N and Rodkhum C 2015a. Phenotypic characterization and genetic diversity of Flavobacterium columnare isolated from red tilapia, Oreochromis sp., in Thailand. Journal of Fish Diseases. 38: 901–913.

Dong HT, Nguyen VV, Le H. D., Sangsuriya P, Jitrakorn S, Saksmerprome V, Senapin S and Rodkhum C 2015b. Naturally concurrent infections of bacterial and viral pathogens in disease outbreaks in cultured Nile tilapia (Oreochromis niloticus) farms. Aquaculture. 448: 427-435.

Dong HT, Nguyen VV, Le HD, Sangsuriya P, Jitrakorn S, Saksmerprome V, Senapin S and Rodkhum C 2015b. Naturally concurrent infections of bacterial and viral pathogens in disease outbreaks in cultured Nile tilapia (Oreochromis niloticus) farms. Aquaculture. 448: 427-435.

Dong HT, Senapin S, Jeamkunakorn C, Nguyen VV, Nguyen NT, Rodkhum C, Khunrae P and Rattanarojpong T 2019. Natural occurrence of edwardsiellosis caused by Edwardsiella ictaluri in farmed hybrid red tilapia (Oreochromis sp.) in Southeast Asia. Aquaculture. 499: 17-23.

Dong HT, Senapin S, Lafrentz B and Rodkhum C 2016. Virulence assay of rhizoid and non-rhizoid morphotypes of Flavobacterium columnare in red tilapia, Oreochromis sp., fry. Journal of Fish Diseases. 39(6): 649-655.

Dong HT, Siriroob S, Meemetta W, Santimanawong W, Gangnonngiw W, Pirarat N, Khunrae P, Rattanarojpong T, Vanichviriyakit R and Senapin S 2017b. Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. Aquaculture. 476: 111-118.

Dong HT, Techatanakitarnan C., Jindakittikul P., Thaiprayoon A., Taengphu S, Charoensapsri W., Khunrae P., Rattanarojpong T. and S. S 2017a. Aeromonas jandaei and Aeromonas veronii caused disease and mortality in Nile tilapia (Oreochromis niloticus). Journal of Fish Diseases. 40: 1395–1403.

Douëllou L and Erlwanger KH 1994. Crustacean parasites of fishes in Lake Kariba, Zimbabwe, preliminary results. Hydrobiologia. 287(3): 233-242.

Eissa IAM 2002. Parasitic Fish Diseases in Egypt. 1st Edn., . In: Dar El-Nahda El-Arabia Publishing, Cairo, Egypt.

Eissa IAM, Maather E, Mona S, Desuky E, Mona Z and Bakry M 2015. Aeromonas veronii sobria a causative agent of mass mortalities in cultured nile tilapia in-Sharkia governorate, Egypt. Life Sci. J. 12(5): 90-97.

El-Dien AH and Abdel-Gaber RA 2009. Ichthyophthirius multifiliis infection induces apoptosis in different species of tilapia. Journal of the Egyptian Society of Parasitology. 39: 665-678.

El-Gayar K and Aly SM 2013. Studies on some protozoan parasites and encysted metacercarial infection of freshwater fishes in Egypt. Egyptian Veterinary Medical Society of Parasitology Journal (EVMSPJ). 9: 31-43.

El-Ghany NAA and El-Ashram AMMM 2008. Diagnosis of ichthyophoniasis in oreochromis niloticus in egypt by polymerase chain reaction (pcr. International Symposium on Tilapia in Aquaculture. 1307-1328.

El-Sayed A-FM 2020. Chapter 9 - Stress and diseases, Editor(s): Abdel-Fattah M. El-Sayed, Tilapia Culture (Second Edition). Academic Press. 205-243.

El-Sayed AFM 2019. Tilapia culture: Second edition. In: Tilapia Culture: Second Edition. 1-348.

El-Sharouny HM and Badran RAM 1995. Experimental transmission and pathogenicity of some zoosporic fungi to Tilapia fish. Mycopathologia. 132(2): 95-103.

Eyngor M, Zamostiano R, Tsofack JEK, Berkowitz A, Bercovier H, Tinman S, Lev M, Hurvitz A, Galeotti M, Bacharach E and Eldar A 2014. Eldar Identification of novel RNA virus lethal to tilapia , ,. *J. Clin. Microbiol.* . JCM. 52 (2014): pp. 4137-4146.

FAO 2014. Sustainable fisheries and aquaculture for food security and nutrition. A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security. Retrieved from Rome: http://www.fao.org/3/a-i3844e.pdf.

FAO 2017. Food and Agriculture Organization of the United Nations. 2017. Global aquaculture production. http://www.fao.org/fshery/statistics/global-production/en.

FAO 2017a. Outbreaks of Tilapia lake virus (TiLV) threaten the livelihoods and food security of millions of people dependent on tilapia farmingGIEWS Special Alert No: 338 - Global, Food and Agriculture Organization of the United Nations (FAO) http://www.fao.org/giews/.

FAO 2018. The State of World Fisheries and Aquaculture 2018. FAO, Rome, Italy.

FAO 2019. Food and Agriculture Organization of the United Nations. Aquaculture Newsletter. FAO, 60th. Retrieved from www.fao.org/fi shery/publications/fan.

FAO 2016. The state of world fisheries and aquaculture, 2016. Contributing to food security and nutrition for all. Retrieved from Rome: http://www.fao.org/3/a-i5555e.pdf.

FAO/WHO. 2011. Report of the joint FAO/WHO expert consultation on the risks and benefits of fish consumption. Retrieved from Rome: http://www.fao.org/3/ba013 6e/ba0136e00.pdf.

Fathi M, Dickson C, Dickson M, Leschen W, Baily J, Muir F, Ulrich K and Weidmann M 2017. Identification of Tilapia Lake Virus in Egypt in Nile tilapia affected by 'summer mortality' syndrome. Aquaculture. 473: 430-432.

Ferguson HW, Kabuusu R, Beltran S, Reyes E, Lince JA and Del Pozo J 2014. Syncytial hepatitis of farmed tilapia, Oreochromis niloticus (L.): A case report. Journal of Fish Diseases. 37(6): 583–589.

Figueiredo HC, Klesius PH, Arias CR, Evans J, Shoemaker CA, Pereira DJJ and Peixoto MTD 2005. Isolation and characterization of strains of Flavobacterium columnare from Brazil. Journal of Fish Diseases. 28: 199–204.

Figueiredo HC, Netto LN, Leal CA, Pereira UP and Mian GF 2012. Streptococcus iniae outbreaks in Brazilian Nile tilapia (Oreochromis niloticus L:) farms. Brazilian Journal of Microbiology. 43(2): 576–580.

Fryer G and Iles TD 1972. The Cichlid Fishes of the Great Lakes of Africa.

Gbankoto A, Pampoulie C, Marques A and Sakiti GN 2001. Occurrence of myxosporean parasites in the gills of two tilapia species from Lake Nokoué (Bénin, West Africa): effect of host size and sex, and seasonal patterns of infection. Diseases of Aquatic Organisms. 44: 217-222.

Goodwin AE 2012. 4.2.1 Branchiomycosis. Handbook of Diagnostic Techniques. 1-8.

Ha Thanh Dong, Senapin S, Gangnonngiw W, Nguyen VV, Rodkhum C, Debnath PP, Delamare-Deboutteville J and Mohan CV 2020. Experimental infection

reveals transmission of tilapia lake virus (TiLV) from tilapia broodstock to their reproductive organs and fertilized eggs. Aquaculture 515

Hai LD, Chockmangmeepisan P, Sakulworakan R, Dong HT, Kayansamruaj P, Rung-Ruangkijkrai T, Pirarat N and Rodkhum C 2020. Virulence properties and pathogenicity of Flavobacterium columnare in hybrid red tilapia (Oreochromis sp.). Thai Journal of Veterinary Medicine. 50(1): 103-108.

Hedrick RP, McDowell T and Groff J 1987. Mycobacteriosis in cultured striped bass from California. Journal of wildlife diseases. 23(3): 391-395.

Huys G, Cnockaert M and Swings J 2005. Aeromonas culicicola Pidiyar et al. 2002 is a later subjective synonym of Aeromonas veronii Hickman-Brenner et al. 1987. Systematic and Applied Microbiology. 28(7): 604-609.

ICTV 2020. **International Committee on Taxonomy of Viruses (ICTV)** 2020. https://talk.ictvonline.org/taxonomy/

Jaemwimol P, Rawiwan P, Tattiyapong P, Saengnual P, Kamlangdee A and Surachetpong W 2018. Susceptibility of important warm water fish species to tilapia lake virus (TiLV) infection. Aquaculture. 497: 462-468.

Jansen MD, Dong HT and Mohan CV 2018. Tilapia lake virus: a threat to the global tilapia industry? Reviews in Aquaculture.

Kawarazuka N, & Bene, C. 2011. The potential role of small fish species in improving micronutrient deficiencies in developing countries: Building evidence. Public Health Nutrition. 14(11): 1927–1938.

Kayansamruaj P, Pirarat N, Hirono I and Rodkhum C 2014. Increasing of temperature induces pathogenicity of Streptococcus agalactiae and the up-regulation of inflammatory related genes in infected Nile tilapia (Oreochromis niloticus). Veterinary Microbiology. 172(1-2): 265-271.

Keawcharoen J, Techangamsuwan S, Ponpornpisit A, Lombardini ED, Patchimasiri T and Pirarat N 2015. Genetic characterization of a betanodavirus isolated from a clinical disease outbreak in farm-raised tilapia Oreochromis niloticus (L.) in Thailand. Journal of Fish Diseases. 38: 49–54.

Khalil RH, Saad TT, Abo Selema TAM and Abdel-Latif HMR 2015. Branchiomyces Demigrans Infection in Farm Reared Common Carp (Cyprinus carpio L.) and Nile Tilapia (Oreochromis niloticus) at Different Localities in Egypt, with special Emphasis to the Role of Environmental Stress Factors. International Journalof Innovative Studies in Aquatic Biology and Fisheries. 1(1): 15-23.

Kibenge FS, Godoy MG, Fast M, Workenhe S and Kibenge MJ 2012. Countermeasures against viral diseases of farmed fish. Antiviral Research. 95(3): 257–281.

Koesharyani. I, Gardenia. L, Widowati. Z, Khumaira. K and D R 2018. Studi kasus infeksi Tilapia lake virus (TiLV) pada ikan nila (Oreochromis niloticus). Jurnal Riset Akuakultur. (13): pp. 85-92.

Leal CAG, Queiroz GA, Pereira FL, Tavares GC and Figueiredo HCP 2019. Streptococcus agalactiae Sequence Type 283 in Farmed Fish. Brazil. Emerging Infectious Diseases. 25(4): 776–779.

Li Y and Cai SH 2011a. Identification and pathogenicity of Aeromonas sobria on tail-rot disease in juvenile tilapia Oreochromis niloticus. Current Microbiology. 62: 623–627.

Li Y and Cai SH 2011b. Identification and pathogenicity of Aeromonas sobria on Tail-rot disease in Juvenile Tilapia Oreochromis niloticus. Current Microbiology. 62(2): 623-627.

Liamnimitr P, Thammatorn W, U-thoomporn S, Tattiyapong P and Surachetpong W 2018. Non-lethal sampling for tilapia lake virus detection by RT-qPCR and cell culture. Aquaculture. 486: 75–80.

Lightner D, Redman R, Mohney L, Dickenson G and Fitzsimmons K 1988. Major diseases encountered in controlled environment culture of tilapias in fresh- and brackishwater over a three-year period in Arizona. In: Proceedings of the Second International Symposium on Tilapia in Aquaculture. R.S.V. Pullin, T. Bhukaswan, K. Tonguthai, and J.L. Maclean (eds). Manila, Philippines: Department of Fisheries, Thailand, and ICLARM, Manila, Philippines. 111-116.

Lin Q, Li N, Fu X, Hu Q, Chang O, Liu L, Zhang D, Wang G, San G and Wu S 2016. An outbreak of granulomatous inflammation associated with Francisella noatunensis subsp. orientalis in farmed tilapia (Oreochromis niloticus × O. aureus) in China. Chinese Journal of Oceanology and Limnology. 34(3): 460-466.

Liu W, Zhang Y, Ma J, Jiang N, Fan Y, Zhou Y, Cain K, Yi M, Jia K, Wen H, Liu W, Guan W and Zeng L 2020. Determination of a novel parvovirus pathogen associated with massive mortality in adult tilapia. PLoS Pathogens. 16(9).

Lua DT, Te BQ and Thanh NV 1999. A study on parasites in different stages of three strains of cultured Nile tilapia (*Oreochromis niloticus*): Thai strain, GIFT strain and Viet strain in North Vietnam. *in* Fourth Symposium on Diseases in Asian Aquaculture: Aquatic Animal Health for Sustainability. Cebu City, Philippines.

Mauel MJ, Miller DL, Frazier K, Liggett AD, Styer L, Montgomery-Brock D and Brock J 2003. Characterization of a piscirickettsiosis-like disease in Hawaiian tilapia. Diseases of Aquatic Organisms. 53(3): 249-255.

McLoughlin M 2006. Fish vaccination – A brief overview. https://www.hpra.ie/docs/default-source/default-document-library/fish-vaccine-overview.pdf?sfvrsn=0 (Published January 25, 2006; Consulted February 27, 2012).

Mugimba KK, Chengula AA, Wamala S, Mwega ED, Kasanga CJ, Byarugaba DK, Mdegela RH, Tal S, Bornstein B, Dishon A, Mutoloki S, David L, Evensen O and Munang'andu HM 2018. Detection of tilapia lake virus (TiLV) infection by PCR in farmed and wild Nile tilapia (Oreochromis niloticus) from Lake Victoria. J Fish Dis.

Mugimba KK, Chengula AA, Wamala S, Mwega ED, Kasanga CJ, Byarugaba DK, Mdegela RH, Tal S, Bornstein B, Dishon A, Mutoloki S, David L, Evensen O and Munang'andu HM 2019. Detection of tilapia lake virus (TiLV) infection by PCR in farmed and wild Nile tilapia (Oreochromis niloticus) from Lake Victoria. J Fish Dis.

NACA 2017. Tilapia Lake Virus (TiLV) – an Emerging Threat to Farmed Tilapia in the Asia-Pacific Region. Network of Aquaculture Centres in Asia-Pacific.

Nguyen VV, Dong HT, Senapin S, Pirarat N and Rodkhum C 2015. Francisella noatunensis subsp. Orientalis, an emerging bacterial pathogen affecting cultured red tilapia (Oreochromis sp.) in Thailand. Autonomous Robots. 1-6.

Nguyen VV, Dong HT, Senapin S, Pirarat N and Rodkhum C 2016. Francisella noatunensis subsp. orientalis, an emergingvbacterial pathogen affecting cultured red tilapia (Oreochromis sp.) in Thailand. Aquaculture Research. 47: 3697–3702.

Nhung PH, Hata H, Ohkusu K, Noda M, Shah MM, Goto K and Ezaki T 2007. Use of the novel phylogenetic marker dnaJ and DNA-DNA hybridization to clarify interrelationships within the genus Aeromonas. International Journal of Systematic and Evolutionary Microbiology. 57(6): 1232-1237.

Nicholson P, Fathi MA, Fischer A, Mohan C, Schieck E, Mishra N, Heinimann A, Frey J, Wieland B and Jores J 2017. Detection of Tilapia Lake Virus in Egyptian fish farms experiencing high mortalities in 2015. J Fish Dis. 40(12): 1925-1928.

OIE 2017a. Disease notification report 25278, 23/11/2017. [https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&repo rtid=25278].

OIE 2017b. Tilapia Lake Virus Disease, Malaysia. Immediate Notification. [Cited 10 Jan 2018.] Available from https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEve ntReport&reportid=24809.

OIE 2018. Tilapia Lake Virus, Peru. Immediate Notification. [Cited 10 Apr 2018.] Available from http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=26027.

Okaemae AN and Olufemi BE 1997. Fungi associated with tilapia culture ponds in Nigeria. Journal of Aquaculture in the Tropics. 12: 267-274.

Okaeme AN and Okojie PU 1989. Parasites and diseases of feral Oreochromis niloticus, Sarotherodon galilaeus and Clarias sp. of Lake Kainja. In: Annual Report.   National Institute of Freshwater Fisheries Research, New Bussa, Nigeria. 41-43.

Omoregie E, Ufodike EBC and Onwuliri COE 1995. A comparative survey of helminth parasites of the Nile tilapia, Oreochromis niloticus from a fish farm and petroleum polluted freshwater. Journal of Aquatic Sciences. 10: 7-14.

Pantoja MF W, Fishing Engineer LNR, Fishing Engineer MDR, Zoo Technician DM and Tavares-Dias M 2012. Protozoan and metazoan parasites of Nile tilapia Oreochromis niloticus cultured in Brazil. Revista MVZ Córdoba. 17(1): 2812-2819.

Paperna I and Di Cave D 2001. Branchiomycosis in an amazonian fish, Baryancistrus sp. (Loricariidae). Journal of Fish Diseases. 24(7): 417-420.

Piamsomboon P and Wongtavatchai J 2021. Detection of Tilapia Lake Virus (TiLV) in healthy fish from the pre-existing disease environment using different RT-PCR methods. . Turkish Journal of Fisheries and Aquatic Sciences. 21: 205-209.

Pierezan F, Yun S, Surachetpong W and Soto E 2019. Intragastric and intracoelomic injection challenge models of tilapia lake virus infection in Nile tilapia (Oreochromis niloticus L.) fingerlings. . Journal of Fish Diseases. 42(9): 1301–1307.

Pillay TVR 1990. Aquaculture: Principles and Practices.

Ponzoni RW, Nguyen NH, Khaw HL, Hamzah A, A.; BKR and Yee HY 2011. Genetic improvement of Nile tilapia (Oreochromis niloticus) with special reference to the work conducted by the WorldFish Center with the GIFT strain. Reviews in Aquaculture 3: 27–41.

Pulido LLH, Chìo MM, Chaparro ALH, Dong HT and Senapin S 2019. Tilapia lake virus (TiLV) from Peru is genetically close to the Israeli isolates, . aquaculture. 510: 61-65.

Rakus K, Mojzesz M, Widziolek M, Pooranachandran N, Teitge F, Surachetpong W, Chadzinska M, Steinhagen D and Adamek M 2020. Antiviral response of adult zebrafish (Danio rerio) during tilapia lake virus (TiLV) infection. Fish Shellfish Immunol. 101: 1-8.

Ramadan HH 1991. Effect of host species, sex, length, diet and different seasons on the parasitic infection of Tilapia fish in Lake Manzala. J. King Abdulaziz Univ. Marine Sci. 2: 81-91.

Roos N, Wahab MA, Chamnan C and Thilsted SH 2007. The role of fish in food-based strategies to combat vitamin A and mineral deficiencies in developing countries. Journal of Nutrition. 137(4): 1106–1109.

Scott PW 1977. Preliminary studies on disease in intensively farmed tilapia in Kenya.

Senapin S, Dong HT, Meemetta W, Siriphongphaew A, Charoensapsri W, Santimanawong W, Turner WA, Rodkhum C, Withyachumnarnkul B and Vanichviriyakit R 2016. Hahella chejuensis is the etiological agent of a novel red egg disease in tilapia (Oreochromis spp.) hatcheries in Thailand. Aquaculture. 454: 1-7.

Shelton WL 2002. Tilapia culture in the 21st century. In:. Proceedings of the International Forum on Tilapia Farming in the 21st Century (Tilapia Forum 2002). (ed. by R.D.I. Guerrero & M.R. Guerrero-Del Castillo), pp:1– 20, Philippines Fisheries Association Inc., Los Banos, Laguna, Philippines.

Shlapobersky M, Sinyakov MS, Katzenellenbogen M, Sarid R, Don J and Avtalion R 2010. Viral encephalitis of tilapia larvae: primary characterization of a novel herpes-like virus. Virology. 399: 239–247.

Shoemaker CA, Evans JJ and Klesius PH 2000. Density and dose: factors affecting mortality to Streptococcus iniae-infected tilapia (*Oreochromis niloticus*). Aquaculture. 188: 229-235.

Situmorang ML, Dierckens K, Mlingi FT, Van Delsen B and Bossier P 2014. Development of a bacterial challenge test for gnotobiotic Nile tilapia Oreochromis niloticus larvae. Diseases of Aquatic Organisms. 109(1): 23-34.

Smith SA 1997. Mycobacterial infections in pet fish. Seminars in Avian and Exotic Pet Medicine. 6(1): 40-45.

Soto-Rodriguez SA, Cabanillas-Ramos J, Alcaraz U, Gomez-Gil B and Romalde JL 2013. Identification and virulence of Aeromonas dhakensis, Pseudomonas mosselii and Microbacterium paraoxydans isolated from Nile tilapia, Oreochromis niloticus, cultivated in Mexico. Journal of Applied Microbiology. 115(3): 654-662.

Soto E, Abrams SB and Revan F 2012a. Effects of temperature and salt concentration on Francisella noatunensis subsp. orientalis infections in Nile tilapia Oreochromis niloticus. Diseases of Aquatic Organisms. 101(3): 217-223.

Soto E, Griffin M, Arauz M, Riofrio A, Martinez A and Cabrejos ME 2012b. Edwardsiella ictaluri as the causative agent of mortality in cultured nile tilapia. Journal of Aquatic Animal Health. 24(2): 81-90.

Soto E, Griffin M, Arauz M, Riofrio A, Martinez A and Cabrejos ME 2012c. Edwardsiella ictaluri as the causative agent of mortality in cultured Nile tilapia. Journal of Aquatic Animal Health. 24: 81-90.

Soto E, Hawke JP, Fernandez D and A Morales J 2009a. Francisella sp., an emerging pathogen of tilapia, Oreochromis niloticus (L.), in Costa Rica. Journal of Fish Diseases. 32(8): 713-722.

Soto E, Hawke JP, Fernandez D and Morales JA 2009b. Francisella sp., an emerging pathogen of tilapia, Oreochromis niloticus (L.), in Costa Rica. Journal of Fish Diseases. 32: 713-722.

Soto E, Illanes O, Hilchie D, Morales JA, Sunyakumthorn P, Hawke JP, Goodwin AE, Riggs A, Yanong RP, Pouder DB, Francis-Floyd R, Arauz M, Bogdanovic L and Castillo-Alcala F 2012d. Molecular and immunohistochemical diagnosis of Francisella noatunensis subsp. orientalis

from formalin-fixed, paraffin-embedded tissues. Journal of Veterinary Diagnostic Investigation. 24(5): 840-845.

Stratev D and Odeyemi OA 2016. Antimicrobial resistance of Aeromonas hydrophila isolated from different food sources: A mini-review. Journal of Infection and Public Health. 9(5): 535-544.

Suanyuk N, Kong F, Ko D, Gilbert GL and Supamattaya K 2008. Occurrence of rare genotypes of Streptococcus agalactiae in cultured red tilapia Oreochromis sp. and Nile tilapia O. niloticus in Thailand-Relationship to human isolates? Aquaculture. 284(1-4): 35-40.

Subasinghe R, Soto D and Jia J 2009. Global aquaculture and its role in sustainable development. Reviews in Aquaculture. 1: 2-9.

Subramaniam K, Gotesman M, Smith CE, Steckler NK, Kelley KL, Groff JM and Waltzek TB 2016. Megalocytivirus infection in cultured Nile tilapia Oreochromis niloticus. . Diseases of Aquatic Organisms. 119: 253–258.

Surachetpong W, Janetanakit T, Nonthabenjawan N, Tattiyapong P, Sirikanchana K and Amonsin A 2017. Outbreaks of Tilapia Lake Virus Infection, Thailand, 2015-2016. Emerg Infect Dis. 23(6): 1031-1033.

Surachetpong W, Roy S. R. K. and P. N 2020. Tilapia lake virus: The story so far. Journal of Fish Diseases. 43: 1115–1132.

Suwannasang A, Dangwetngam M, Issaro A, Phromkunthong E and Suanyuk N 2014. Pathological manifestations and immune responses of serotypes Ia and III Streptococcus agalactiae infections in Nile tilapia (Oreochromis niloticus). Songklanakarin Journal of Science and Technology 36: 499–500.

Taengphu S, Sangsuriya P, Phiwsaiya K, Debnath PP, Delamare-Deboutteville J, Mohan CV, Dong HT and Senapin S 2020. Genetic diversity of tilapia lake virus genome segment 1 from 2011 to 2019 and a newly validated semi-nested RT-PCR method. Aquaculture. 526 735423.

Taghreed Ibrahim 2020. Diseases of Nile tilapia with special emphasis on water pollution. J. Environ. Sci. Technol. 13: 29-56.

Tattiyapong P, Dachavichitlead W and Surachetpong W 2017. Experimental infection of Tilapia Lake Virus (TiLV) in Nile tilapia (Oreochromis niloticus) and red tilapia (Oreochromis spp.). Vet Microbiol. 207: 170-177.

Thawornwattana Y, Dong HT, Phiwsaiya K, Sangsuriya P, Senapin S and Aiewsakun P 2021. Tilapia lake virus (TiLV): Genomic epidemiology and its early origin. Transbound Emerg Dis. 68(2): 435-444.

Thilsted SH, Thorne-Lyman A, Webb P, Bogard JR, Subasinghe R, Phillips MJ and Allison EH 2016. Sustaining healthy diets: The role of capture fisheries and aquaculture for improving nutrition in the post-2015 era. . Food Policy. 61: 126–131.

Tonguthai K and Chinabut S 1997. Disease of tilapia. In: Dynamics of Pond Aquaculture. 1st ed. H.S. Egna and C.E. Boyd (eds). CRC Press, New York, New York.

Ukoli FMA 1966. On the Life History, Growth and Development from the Metacercarial Stage to Adulthood, of Clinostomum tilapiae Ukoli, 1966. Journal of Helminthology. 40(1-2): 215-226.

Valladão GMR, Alves LO and Pilarski F 2016. Trichodiniasis in Nile tilapia hatcheries: diagnosis, parasite:host-stage relationship and treatment. Aquaculture. 451: 444-450.

Valladão GMR, Pádua SB, Gallani SU, Menezes-Filho RN, Dias-Neto J, Martins ML, Ishikawa MM and Pilarski F 2013. Paratrichodina africana (Ciliophora): a pathogenic gill parasite in farmed Nile tilapia. Veterinary Parasitology Today. 197: 705-710.

Widziolek M, Janik K, Mojzesz M, Pooranachandran N, Adamek M, Pecio A, Surachetpong W, Levraud J-P, Boudinot P, Chadzinska M and Rakus K 2021. Type I interferon-dependent response of zebrafish larvae during tilapia lake virus (TiLV) infection. Developmental & Comparative Immunology. 116: 103936.

Yamkasem J, Tattiyapong P, Gorgoglione B and Surachetpong W 2021. Uncovering the first occurrence of Tilapia parvovirus in Thailand in tilapia during co-infection with Tilapia tilapinevirus. Transboundary and Emerging Diseases.

Yamkasem J TP, Kamlangdee A, Surachetpong W 2019. Evidence of potential vertical transmission of tilapia lake virus. Journal of Fish Diseases. 42: 1293–1300.

Yokota M, Watanabe S, Hatai K, Kurata O, Furihata M and Usui T 2008. Transmission of the parasite Ichthyophonus hoferi in cultured rainbow trout and comparison of epidemic models. Journal of Aquatic Animal Health. 20(4): 207-214.

Zimmermann S and New MB 2000. Freshwater Prawn Culture (ed. by M.B. New & W.C. Valenti). 187-202, Blackwell Science, Oxford, UK.

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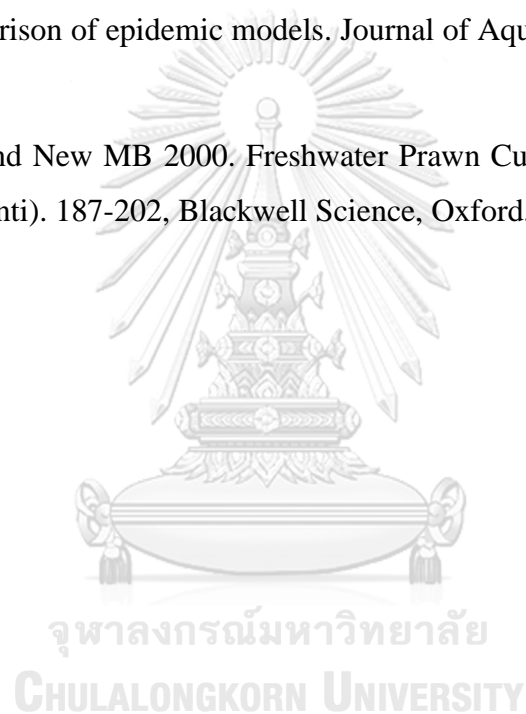
Chapter 3

Is tilapia mortality a latent concern for the aquaculture sector of Bangladesh? An epidemiology and health economic impact study

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Abstract

Tilapia are the most widely farmed finfish in global aquaculture, produced in over 170 countries. Bangladesh is the world's fourth-largest tilapia producer and yet only few studies have been conducted to understand factors associated with tilapia mortality and economic losses. Using an online tilapia epidemiology and health economics survey tool, we surveyed 565 tilapia farms in 15 of Bangladesh's most important tilapia-producing districts. The study examined a range of factors, including geographic locations, farm characteristics, water source, stocking, biosecurity measures, baseline and unusual mortality levels and characteristics. For the period January 2017 to February 2019 a total of 18.2 % of farms (103 out of 565) reported having experienced unusual mortality, with an average mortality level of 23.2 percent (range 3 to 90). A number of factors were found to be significantly associated with reporting of unusual mortality occurrences, including farmer education level, farm size, farm biosecurity measures, baseline mortality level, farmer concern about baseline mortality, dead fish removal frequency and disposal method and antibiotic treatment. Farming region, water source, dead fish removal frequency, and antibiotic treatment were all found to be significantly associated with the level of unusual mortality, with water source and dead fish removal frequency remained significant in the multivariable model. Major clinical signs linked with these mortalities included skin erosions, hemorrhagic lesions, open wounds, skin discoloration, exophthalmos, abdominal distension, swelling, scale protrusion and eye opacification. Based on baseline and unusual mortality in tilapia, a total hidden loss of 875.7 million USD annually was estimated. To ensure the future sustainability of tilapia production, the authors recommend more investigations of unusual mortalities events with collection of metadata and clinical samples for disease diagnostics, coupled with nationwide farmer awareness campaigns.

Key Words:

1. Introduction

The proportion of global aquaculture to world fish production has continuously increased, reaching 46.0 % in 2016–18, up from 25.7 % in 2000 (FAO, 2020). Globally, about 59.51 million people were associated with fishing or aquaculture in 2018 (FAO, 2020). Fish is an important and significant source of animal protein for 4.5 billion people who rely on them (FAO, 2020; Beveridge et al., 2013; Thilsted et al., 2016). Demand for fish will continue to increase as the population inevitably grows. In 2018, the world population reached 7.6 billion and is projected to reach 8.5 billion by 2030, 9.7 billion by 2050, and more than 11 billion in 2100 (DESA, 2019), which means that global food production must increase by 50% to feed the entire world. Aquaculture will play a major role in meeting this demand and is seen as the next, and perhaps last, major opportunity for animal protein production to limit livestock expansion and end overfishing of the oceans (FAO, 2020). Globally, tilapia are the most widely farmed finfish, now produced in over 170 countries (FAO, 2020b) and considered a key fish group species to meet the increasing global demand for protein, vitamin and mineral sources (Ahern et al., 2021; Amal et al., 2018; Ng & Romano, 2013). In 2018, global tilapia production was estimated at 6.5 million metric tons (MMT) valued at US$ 7.9 Billion with the top four producers being China (1.78 MMT), Indonesia (1.11 MMT), Egypt (0.88 MMT) and Bangladesh (0.32 MMT) (FAO, 2020). Bangladesh ranks fifth largest aquaculture producer (FAO, 2020), with a total annual fisheries production of 4.38 MMT, of which aquaculture accounts for 56.4% and tilapia 10.6% (DoF, 2019). In 2017-2018, Bangladesh earned USD 513 million by exporting nearly 68,940 MT of fish and other aquatic animal products from the aquaculture and fishery sectors (DoF, 2019). Over the last 10 years, the annual growth of Bangladeshi aquaculture sector has averaged 5.4% (DoF, 2019). The success of tilapia farming comes from its adaptability to a variety of environmental culture conditions, fast growth and from genetic breeding programs that select animals that are more resilient against both infectious disease and poor water quality (Ponzoni et al., 2011). Nevertheless, intensification of tilapia farming, lack of biosecurity with cross-border movements of live fish and their products, and climate change have led to the emergence of infectious diseases (Kibenge, 2019; Surachetpong & Sirikanchana, 2020). There are no population-based data on the

major risk factors associated with mortalities and economic impact of diseases in Bangladeshi tilapia aquaculture. To address these knowledge gaps, we collected extensive data on tilapia epidemiology and health economics from 565 farms in 15 major tilapia producing districts of the country.

2. Material and Methods

2.1. Case definition of tilapia farmers

Farmers who culture tilapia with or without combination of other fish species where the production volume of tilapia is 20% or more of the total fish production were considered as tilapia farmers in this study

2.2. Study area and farms selection

Using district-wise tilapia production data from the Department of Fisheries (DoF, 2018), out of the 64 districts of Bangladesh, only the top 15 tilapia producing districts from five regions namely Barisal, Chittagong, Dhaka, Khulna and Mymenshingh were selected for this study (Fig.1). A primary census list was complied to create a database of 1536 known farms holding at least 20% tilapia. To facilitate identification of each farm, the database also contained information on farmer's name, mobile phone number and farm location (GPS coordinates). From each district 37% of the farms were randomly selected from the census using an online tool (https://www.randomizer.org/). Based on past farmer surveys, up to 30% of farmers are usually untraceable or likely to decline participatation in survey studies. To adress this potential issue, a separate backup list of replacement farms was generated (randomly) from the database and used as needed. Figure 1 shows the geographic distribution of 565 tilapia polyculture farms surveyed in this study. Data were collected for the last completed production cycle (all-in all-out production) or calendar year (continous production).

2.3. Questionnaire and Online survey tool

WorldFish, in close collaboration with the Norwegian Veterinary Institute (NVI), developed a detailed questionnaire for tilapia epidemiology and health economics (TEHE) surveys (Khor et al., 2021), also referred as the "online survey tool" (Fig. 2). Field implementation of the TEHE surveys was performed by enumerators using the

Open Data Kit (ODK) Collect mobile application (ODKCMA) designed to work with the KoBoToolbox. This is a free and open-source suite of tools developed by the Harvard Humanitarian Initiative for field data collection and analysis (https://www.kobotoolbox.org/) (https://github.com/kobotoolbox) that hosts an online server used for data storage, data management and data analysis. The TEHE survey questionnaire was designed to capture a range of variables (Table 1) and follow a conversation style between enumerators and farmers. Data on baseline mortality was collected when farmers reported mortality (with or without clinical signs) of only few fish dying each day that did not seem unusual to them. Data on unusual mortality was collected for farmers reporting any rapid or steady increase of fish mortality with the typical clinical signs of diseases.

Fifty eight of the 103 farmers (add relative % = 88/103 x 100) that reported unusual mortality were able to estimate the level of unusual mortality. The highest, lowest, and average daily mortality, as well as the number of days with active mortality, were used to compute overall farm mortality levels (%). The absolute total mortalities for a given farm was then calculated by multiplying all categories by the number of days with active mortalities and used to derive the relative percentage losses from initial stock. To standardize and facilitate data collection on clinical signs, leaflets — in English and Bangla — were created with colored pictures of the major clinical signs of different tilapia diseases (English version: https://hdl.handle.net/20.500.12348/3423 and Bengali version: https://hdl.handle.net/20.500.12348/3425). These were shown to farmers for them to easily identify any clinical signs they would have observed on moribund and/or freshly dead fish during baseline or unusual mortality events.

2.4 Field team preparation for survey implementation

In October 2018, four data enumerators were hired after developing the TEHE survey tool. Prior to the start of the survey, enumerators were trained on the use of the ODKCMA, overall understanding of the TEHE survey questionnaire (including different aquaculture and fish disease terminology), and how to conduct the surveys with farmers.

From November 2018 to May 2019, a total of 565 farmers from 15 districts were interviewed (Figure 1). Upon completion of field surveys, all forms were saved on tablets, allowing offline review of entered data by enumerators and project leads before being submitted to the online database of the KoboToolbox platform.

2.5. Data analysis

Prior to analysis, data were reviewed for accuracy and variables combined where possible to form biologically relevant groups and to avoid small categorical groups of data (fewer than ten entries). From the original data set, the farmer's education level was regrouped as up to primary by combining two groups: none or some primary and primary, up to higher secondary by combining junior secondary, senior secondary, and technical college, and finally up to graduation by combining all who completed graduation and masters from university. Again, for the data of source of water, the farmer who used ground, river, canal or rain water in a different combination was grouped as ground, rain and river/canal water. Regrouping also has been done for co-cultivated species as where all species reported such as Indian Major carp, Chinese major carp, puntius, pangasius are grouped as freshwater species while along with fresh water species farm who stocked shrimp, seabass etc. were grouped as freshwater and brackish water species.

Data processing was performed in Excel MS Office 2013 and analysis in SPSS (IBM SPSS Statistics 22). Pearson Chi-square and Fishers exact tests were used to assess statistical differences in characteristics between farms that reported unusual mortality in the previous year and those that did not (yes/no, binomial variable). Kruskal Wallis and Mann Whitney U tests were used to assess factors significantly associated with reported mortality (percent, continuous variable). A statistically significant p-value of 0.05 was used.

Based on the initial data assessment, number of observations in each category (categorical variables) and biological plausibility, a number of characteristics were considered as potential confounders for occurrence and level of unusual mortality. In order to facilitate results interpretations, farm water areas were converted from decimal to the larger unit hectare (ha). For analysis and interpretation of occurrence of unusual mortalities, assessed characteristics on farmer education, farm water area

(ha), farm baseline mortality level, farmer concern about baseline mortality and antibiotics treatment were used. For unusual mortality level, assessed characteristics were farming region, farmer experience, farm water area (ha), farm water source, stocking density (first cycle), farm baseline mortality level, frequency of dead fish removal and antibiotics treatment. Regression analysis with logistic regression or generalised linear models was used to assess the effect of confounding variables on observed differences in the occurrence of unusual mortality and the reported unusual mortality levels. Percentage of unusual mortality were log-transformed prior to analyses. Confounding factors with a p-value of 0.2 in the univariable regression analyses were included in the multivariable regression using backward selection followed by forward selection with a p-value of 0.05 considered statistically significant. The Akaike information criterion (AIC) was used for final model selection. Analyses were performed in R, version 4.0.3.

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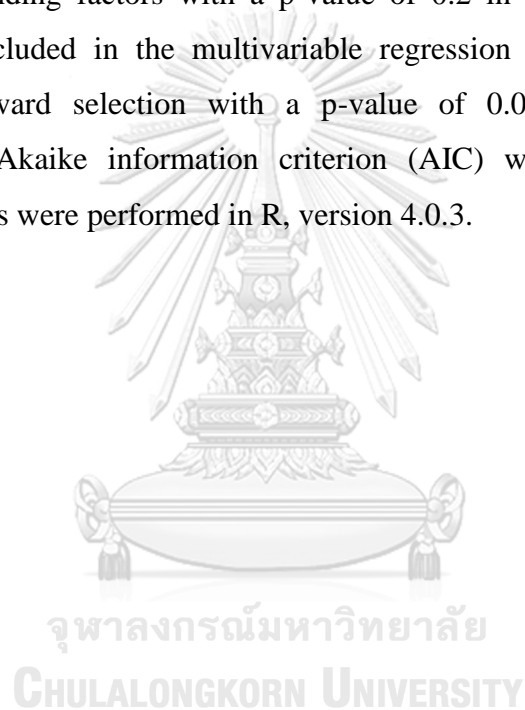
3. Results

3.1. Regional information and farmer profile

For this study, 565 tilapia farms from 15 districts in five different regions of Bangladesh were surveyed (Fig. 1). Among the five regions, the highest number of farms studied were in the Khulna region (228 farms from seven districts) while the least number of farms were in the Dhaka region (31 farms from one district) (Fig. 3). While all of the farmers interviewed were farm owners except one farmer (partner), 50.4% of them were not engaged in full-time farming. Only 1.2% (n = 7) of the farmers were female, all found in the Barisal and Khulna regions and all stated that they were the primary decision makers on their farms. Nearly all male farmers (98.8%) were primary decision makers. The mean age of farmers did not differ significantly between regions (data not shown). The average age of farmers was 42 years (range 28–65) for women and 44 years (range 18–85) for men. Majority of farmers had education up to upper secondary level (48.4%), followed by primary level (34.6%) and graduate level (17%) (Data on education level was missing for one farmer). The educational level of farmers differed significantly between farming regions (p = 0.002, Table 2). The mean farming experience was nine years, with minimum and maximum experience of 1 and 28 years found in Khulna. The only significant difference in farming experience of farmers was found to be higher in the Chittagong region when compared to farmers from the other four regions of Barisal, Dhaka, Khulna and Mymensingh (p < 0.001) (Table 2).

Khulna region had the highest mean baseline mortality level of 2.3% (range 0–20%), while Dhaka region had the lowest mean baseline mortality level of 1.1% (range 0.2–4.3%, Table 2). A total of 103 farms (18.23%) reported having experienced unusual mortality in the past production cycle or year. No association was found between the reporting of unusual mortality events and farming region (p = 0.785, Table 3a), however a significant relationship was identified between farmers' education level and the occurrence of unusual mortality on the farm (p = 0.041, Table 3a). Farmers with education level up to primary level (28.4%) recorded the highest level of unusual mortality followed by those with education level up to higher secondary (22.9%) and those with education level up to graduation (15.6%), however the differences were not

statistically significant (Table 3a). In addition, farming experience was not significantly associated with experiencing unusual mortality (p = 0.741, Table 3a).

3.2. Farming systems

Only 16.6% of the farms operated under the all-in, all-out system, while most (83.4%) operated under the continuous culture system (Table 3a). All farms used earthen ponds. The main farming system was commercial farming (97%), while 1.4% and 1.6% of the farms were classified as homestead farms and combination of homestead and commercial farms, respectively (data not shown). Mean farm size was 441.7 decimal (range 8 to 15 600 decimal), with mean farm size in Khulna region significantly larger (580.0 decimal) compared to Barisal (150.0 decimal) (p = 0.015); no significant size differences between the other regions. The average number of ponds in a farm was 4 (range 1 to 86), with 60% having two to five ponds, followed by 28% with a single pond, 8% with six to ten ponds and 4% more than 10 ponds (data not shown). The survey data revealed that 86% of the ponds were perennial while 14% were seasonal. Khulna region had the highest number of seasonal ponds (10%), while the number of seasonal ponds in all other regions was less than 1% (data not shown). Amongst farms using an all-in, all-out system 20.2% reported unusual mortality on their farm, while 17.8% of farms using a continuous culture system reported unusual mortality (p = 0.586, Table 3a). The mean farm size of farms reporting unusual mortality was significantly larger (668.8 decimals) than farms not reporting unusual mortality (391.0 decimals) (p = 0.027).

3.3. Water management

Groundwater, rainwater, river or canal and a combination of groundwater and river or canal were defined as the main water sources reported in this survey. Groundwater was used by the majority of farms (74.6%), followed by river or canal water (13.8%), rainwater (7.3%), and a combination of groundwater and river or canal (4.3%) (Table 3a). All farms in the Mymensingh region used groundwater, while the other four regions used all four water sources in varying proportions for their farms (Table 2). Farm water supply varied significantly by region (p < 0.001). The study found no association between water source and the occurrence of unusual mortality (p = 0.738, Table 3a). However, for farms with unusual mortality, the mortality level differed

significantly depending on the water source (p = 0.044). Only 9% of farms reported using a shared waterbody, of which only three reported having experienced unusual mortality (data not shown).

3.4. Stocking information

Typically, 97.5% of the farms practiced tilapia polyculture, while 1.6% and 0.9% of the farms practiced tilapia monoculture and a mixture of monoculture and polyculture, respectively (Table 3a). The highest unusual mortality cases were found in farms with a mixture of monoculture and polyculture at 60% (3 out of 5 farms), followed by farms with polyculture at 18% (99 out of 551 farms) and farms with monoculture at 11.11% (1 out of 9 farms) (Table 3a). The mean percentage of tilapia stocked was 64% (range 20 to 100) and the majority of farms (98%) raised monosex tilapia (Fig. 4). Only 5 farms (0.9%) used tilapia seed from their own stock, all other farms obtained their seed from hatcheries (Fig. 4). Most farmers collected their seed through middlemen (39%), while 33% of farmers collected their seed directly from hatchery and 28% farms were supplied by hatcheries (Fig. 4). The majority of farmers (83%, n = 460) stocked their farms once a year, followed by 16% (n = 97) twice a year and only 1% (n = 8) three times a year (Fig. 4). In the first production cycle, the mean stocking density was 137 tilapia/decimal, while the minimum and maximum stocking densities were 7 and 800 tilapia/decimal, respectively; 101, 8 and 300 tilapia/decimal in the second production cycle, respectively and 95, 45 and 250 tilapia/decimal in the third production cycle, respectively (Table 3b). For the first, second, and third production cycles, the average stocking size of tilapia was 3.9 ± 2.2 cm, 4.1 ± 2.1 cm, and 3.8 ± 2.1 cm respectively and weight of tilapia 6.6 ± 12.4 g, 9.0 ± 16.5 g and 9.2 ± 17 g respectively (data not shown). The minimum and maximum stocking size and weight of tilapia were 1 to 15 cm, 1 to 10 cm and 2 to 8 cm respectively and 0.03 to 125 g, 0.12 to 90 g and 0.16 to 50 g for the first, second and third production cycle (data not shown). Most farmers (95.4%) cultured tilapia with freshwater species as a co-cultivated species for polyculture; only 4.6% farm reported to use mix of freshwater and brackish water species. In the Khulna region, brackish water species coexisted with freshwater species (25 out of 26 farms); only one farm in Mymensingh

stocked brackish water species, and the other regions stocked only freshwater species (data not shown).

However, there was no association between the percentage of tilapia stocked and experiencing unusual mortality (p = 0.517, Table 3a). No association between stocking density and unusual mortality were found in neither of the cycles (Table 3b). There was a significant difference in tilapia stocking density by region in the first production cycle (p < 0.001), but no differences in the second (p = 0.369) and third cycles (p = 0.761) (Table 2). In the farms stocked with freshwater polyculture species, 18.4% (99 farms out of 538) reported unusual mortality, while in the farms stocked with freshwater and brackish water species, 15.4% (4 out of 26 farms) reported unusual mortality, but no association was found between species and unusual mortality in the farm (p = 0.697, Table 3b).

3.5. Biosecurity practices

Statistical analysis shows that there is a significant relationship between unusual mortality and the various biosecurity measures adopted by the farm (p = 0.002, Table 3b). Pond drying and liming were the most common biosecurity practices (67.8%) among farmers, followed by pond liming alone (14.3%) and pond drying and liming in combination with net cleaning (12.4%) (Table 3b). Other biosecurity practices including drying only, or different combination of drying, liming and cleaning of nets, were used by 4.8% (n = 27) of the farms. Only 4 farms (0.7%) did not follow any biosecurity practices (Table 3b). Farms practicing pond drying and liming between production cycles reported the highest occurrence of unusual mortality (22.5%, 86 farms out of 383), followed by farms practicing liming only (13.6%, 11 farms out of 81), drying and liming of ponds together with cleaning nets (7.1%, 5 farms out of 70) and other measures (3.7%, 1 farm out of 27). Surprisingly, no unusual mortality was recorded on any of the four farms that did not follow biosecurity measures between production cycles. The implementation of biosecurity measures between production cycles also varies greatly from region to region (P < 0.001) Table 2. The fallowing periods followed on farms were either none (10.3%), 1–7 days (7.8%), 1–2 weeks (16.3%), 3–4 weeks (34.8%), 5–10 weeks (17%) and more than 10 weeks (14%).

No significant association was found between unusual mortality and fallowing period (p = 0.301, Table 3b). Regionally, 3.5% of farmers in Barisal region did not observe any fallowing period, while the longest fallow period (>10 weeks) was observed by 8.5% of farmers in Khulna region (Table 2). Farm compliance with various fallowing periods differed significantly across regions (p < 0.001) (Table 2). Most farms (96.8%, n = 547) shared equipment with two or more farms (Table 3b). Equipment sharing or no sharing between farms had no significant association with occurrence of unusual mortality (p = 0.081, Table 3b). Most farmers 97% (n = 548) appointed harvesters on their farm but no significant associations found with experiencing unusual mortality (p = 0.529) or level of unusual mortality (p = 0.223) (Table 3b).

3.6. Baseline and unusual mortality

On-farm baseline mortality was not a concern for 90% of the farmers. Overall, baseline mortality ranged from 0 to 20% while mean baseline mortality was 2% (n = 565). Mean percentage baseline mortality was significantly higher (4.4%) among farmers who were concerned about baseline mortality level compared to farmers who were not concerned (1.8%) (p > 0.001). Farmers who were concerned about baseline mortality were also found to have significantly higher occurrence of unusual mortality (56.4%, 31 out of 55 farms, compared to those who were not concerned (15.1%, 77 out of 510) (p < 0.001) (Table 3c). In addition, the mean baseline mortality was significantly higher among farmers who faced unusual mortality (3.5%, compared to farmers who did not face unusual mortality 1.7%, p < 0.001) (Table 3c). The mean baseline mortality level differed significantly (p < 0.001) by region (Table 2). Most farmers (36.9%, n = 412) removed dead fish daily or several times a day and only 1.8% of them (n = 10) reported never removing dead fish (Fig. 5a). The mean baseline mortality level was highest on the farms (2.2 %) that practiced removal of dead fish several times a day and was the lowest (1.2%) for the farms that removed dead animals occasionally.

The methods of dead fish removal varied amongst farms (Fig. 5b and Table 3c). Frequency of dead fish removal was significantly associated with baseline mortality level (p < 0.001) (data not shown) and unusual mortality occurrence (p < 0.001, Table 3c). Farmers practicing removal of dead fish on their farms more than once a day, had

the highest unusual mortality occurrence (90.3%, n = 93). Surprisingly, no unusual mortality events reported by farmers that never discarded dead fish (Table 3c).

In this study, 18.2% (n = 103) of farms experienced unusual mortality events. No difference found (p = 0.785) in the occurrence of unusual mortality between regions (Table 3a). The mean unusual mortality level across regions was 23.2 % [range, 3% to 90 %] with significant differences found between regions (p = 0.016) (Table 3a). The highest (33.2%) and lowest (15.0%) levels of unusual mortality were reported in the Mymensingh and Dhaka regions, respectively. A weak positive correlation (r = 0.334, p = 0.000) was found between the levels of baseline and unusual mortality.

Fifty percent of farmers reported unusual mortality events to happen suddenly (21.7% mean mortality level), 48.3% gradually (mean mortality level 25.6%), and 1.7% occasionally (mean mortality level 3%) (Fig. 6a).

One point seven percent of farms experienced mortality events within a day (mean mortality 25%), 60.3% within a week (mean mortality 20.87%), 29.3% within a month (mean mortality 24.32%) and 8.6% for a period longer than a month (mean mortality 35.6%) (Fig. 6b). Data analysis shows no significant variation in mean mortality level with nature of mortality (p = 0.388) and duration of mortality (p = 0.399) (data not shown). As important disease stressors, weather stress was reported by 48% of the farmers, followed by a combination of weather and water quality stress (21%), water quality stress (19%), and other stressors (12%) (Fig. 6c). In terms of seasonal variation of unusual mortality, highest number of cases reported by 27.5% of farmers in December and lowest in January and March (Fig. 6d). Thirty eight percent of the farmers reported unusual mortality to also happen — in addition to tilapia — in co-cultured species such as Indian Major Carps, Chinese Carps, Pangasius, and Stinging catfish. Majority of farmers (82%) indicated mortalities to begin in tilapia before affecting other species (data not shown). Results of the regression analyses for the occurrence and level of unusual mortality are shown in Tables 4a and 4b, respectively. The final multivariable model found that farm water area (ha), baseline mortality level, farmer concern about baseline mortality and antibiotic treatment played a significant role in the occurrence of unusual mortality being reported (Table

4a); both water sourced from river/canal and occasional dead fish removal had an impact on the intensity of unusual mortality events (Table 4b).

3.7 Major tilapia clinical signs

Skin erosions with hemorrhagic lesions and open wounds were the two major clinical signs reported on 52% of farms experiencing unusual mortality events; followed by 47% of farmers reporting skin discoloration, 41% exophthalmos, 33% abdominal distension, swelling, and scale protrusion, and 26% eye opacification (Fig. 7).

Abnormal behaviors including loss of appetite, swimming at the surface, fish gasping for air at the surface, and additional clinical signs such as anemia with irregular gill pallor, fin rot and others (including red spot in the anus, ulcer near the anus and reddish operculum) were also seen on fish during unusual morality events (Fig. 7 and 8).

3.8 Use of Antibiotics

Overall, 31 respondents out of 565 interviewed (5.5%) reported using antibiotics on their farms (Table 3c), with majority (88%) using oxytetracycline (data not shown), followed by tetracycline, amoxicillin and a combination of erythromycin thiocyanate, sulfadiazine and trimethoprim (data not shown). Nineteen out of 31 respondents (61.3%) reported using antibiotics as prevention rather than treatment, and this was significantly higher (p = 0.006) when compared to the number of respondents (12 out of 31 farm, 38.7%) indicating the use of antibiotics for treatment of fish following the report of unusual mortality events (Table 3c).

All but one farmer mixed the antibiotic with the feed. The dose used for oxytetracycline varied from 10.5 to 0.0002 g/decimal/day (mean 1.14 g/decimal/day) (data not shown). Decision on the use of antibiotics for the treatment of sick fish was primarily made by farmers (20 out 31, 64%), while the remaining 11 farmers (36%) used antibiotics following suggestions made by feed suppliers, pharmacy owners, veterinarians and company representatives. According to respondents, in 72% of the unusual mortality cases, fish recovered with mortality reduced or stopped after using antibiotics while 28% reported that antibiotics were not effective in treating bacterial diseases and reducing fish mortality (data not shown).

3.9 Economic impact of diseases

Data on economics were collected during interviews of farmers and should be considered as the best estimates they provided at the time. Eighty percent of the farmers confirmed making a profit even with the level of baseline mortality they reported. Fifty seven percent of the farmers who experienced unusual mortality events did not make any profit. The impact of unusual mortality on staff job security was considered negligible by 86 % of farmers, low by 5%, moderate by 7% and high by 2%. Four farmers out of 58 (7%) mentioned a negative influence of unusual mortality on job security. The total tilapia production of farms reporting baseline mortality varied from 0.22 to 163.64 kg/decimal with a mean total production of 23.36 ± 22.88 kg/decimal (Table 5).

The mean loss of tilapia production for the baseline farm was 0.42 ± 0.52 kg/decimal (range 0 to 6.25 kg/decimal), with a mean loss value estimated to be 0.93 ± 0.16 USD/kg tilapia (range 0.42 to 1.45 USD/kg) (Table 5). When production loss was solely attributed to mortality, the approximate mean economic loss due to baseline mortality was estimated at 0.39 USD/decimal (data not shown). Besides cost of production loss, farmers also reported additional costs associated with biosecurity measures (range 0 to 8.0 USD/decimal), with a mean of 0.40 USD/decimal and chemotherapy costs (mean, minimum and maximum) of 0.54, 0 to 9.0 USD/decimal, respectively (Table 5). Estimated profits for farms reporting baseline mortality ranged from 0.28 to 44 USD/decimal, with a mean profit per farm of 7.24 ± 6.96 USD/decimal (Table 5).

In comparison, the mean total tilapia production for farms reporting unusual mortality events was 24.27 ± 20.52 kg/decimal (range 1 to 90 kg/decimal) (Table 5). The production loss of tilapia due to unusual mortality ranged from 1 to 933 kg/decimal with a mean of 22.40 ± 123.04 kg/decimal (Table 5). The mean expected price of tilapia lost during unusual mortality events was estimated by farmers to be 0.97 ± 0.17 USD/Kg (varied from 0.48 to 1.57 USD/kg, Table 5). The economic losses due to unusual mortality were estimated at 21.73 USD/decimal, excluding other relevant costs (data not shown).

The mean biosecurity cost on farms reporting unusual mortality was estimated at 0.16 USD/farm (range 0 to 1.0 USD/decimal) and the mean chemotherapeutics cost was estimated at 0.55 USD/decimal (range 0 to 3.0 USD/decimal) (Table 5). Estimated profit for farms facing unusual mortality ranged from 0 to 18.07 USD/decimal, with a mean profit of 5.22 ± 4.58 USD/decimal (Table 5).

4. Discussion

The current study was carried out to assess the tilapia health status, identify key risk factors contributing to health concerns (baseline and unusual mortality), and estimate the economic impact of diseases. According to Apu (2014), 11 % of Bangladesh's population is directly or indirectly dependent on the fisheries sector for livelihood, and all farmers in the current research are either completely or partially involved in aquaculture. Women's engagement in aquaculture farms or fish hatcheries in Bangladesh is virtually non-existent (Apu, 2014), as was revealed in this study where 99 % of the farmers were male. The mean farmer age observed in this study (44+. 12 years) was similar to that previously published (Ali et al., 2018). In the current study, all farmers had completed at least primary education, with 17 percent of farmers having completed up to university level. The educational level of farmers varied significantly by region and by unusual mortality occurrence. This suggest that despite having a higher level of education, few of the farmers have any subject-specific education, such as aquaculture or other related subjects as indicated by Ali et al., 2018. Again, it's probable that a farmer with a higher educational level may be more cautious and capable of recognizing baseline and unusual mortality than a farmer with a lower education level. As a result, farmers with higher levels of knowledge may report more unusual mortality cases as seen in this study. Furthermore, the farmer's average farming experience was found to be 8 ± 4.5 years in this study, which is similar to that reported by Jahan et al., 2015 for carp and tilapia farmers. Aquaculture experience were found to differ significantly by region, with no significant association with unusual mortality occurrence and level, indicating that farmers have farming experience but lack expertise in disease management and farm-level biosecurity.

In Bangladesh, majority of the farmers operate continuously (continuous stocking and harvesting), which means that farmers do not complete harvesting for each production cycle, though only 16.6% of farms use the all-in and all-out production system. However, there was no difference in unusual mortality levels reported between the two farming systems, which may indicate that the two systems do not undertake significantly different pond preparation and biosecurity management between the two production cycles. The Bangladesh Bureau of Statistics divides farms into four sizes (marginal: less than 0.20 hectares; small: 0.21–1.00 hectares; medium: 1.00–3.00 hectares; large: >3.00 hectares) (Jahan et al., 2015) and in this study mean farm size was found to be 441.7 ± 1150.4 decimal (One hectare = 247.1 decimal) which falls into the medium category. Farm size was found to be significantly larger in Khulna than in the other four regions, which may be due to gher farming, which involves converting shallow (give the mean depth??) rice field into an aquaculture farm, similar to the observations of Hinchliffe et al., 2021 and Jahan et al., 2015. Farms with unusual mortality were found to be relatively larger than farms without unusual mortality, implying that disease and biosecurity management is more challenging for larger farms. Khulna area has more seasonal ponds due to gher farming system, while all other regions in this study have mostly perennial ponds. Source of water for the farm plays the vital role in terms of biosecurity and diseases management. In this analysis, the most common source of water used by farms was groundwater (74.6%), followed by river/canal, rain and other sources. This is in contrast to Jahan et al. (2015) who found rainwater to be the most common source of water, followed by river and groundwater. This might be owing to the current technology, which mostly relies on pumping for ground water. Water sources differ by region; for example, in Khulna, the majority of farms rely on river water, while in Mymensingh, all farms rely on groundwater and rainwater (Table 3a). In addition, technology has made it easier for farmers to use groundwater instead of relying on rainwater. In this study, no association was found between the occurrence of unusual mortality and water source. However, there was an association between water source and the level unusual mortality. In the regression model, using river/canal water was found to be associated with a lower level of unusual mortality relative to the other water sources, also when dead fish removal frequency was included in the model. From an epidemiological

aspect, river/canal water is likely to carry an inherent risk of spreading disease-causing agents between farms. However, it may be that this water source allows more frequent water exchange thereby improving water quality, having a dilution effect on disease agents, contribute to flushing away dead fish or increase the likelihood of the introduction of predatory species. This aspect should be further investigated in future studies.

The most common type of aquaculture in Bangladesh is polyculture and in this study 97.5% of the farms were found to use polyculture systems. Similar results have been reported by several research groups (Faruk et al., 2017) with tilapia mainly being cultured with different species combinations in polyculture in different regions of Bangladesh (Tran et al., 2019). Statistical analysis shows that there was no association of culture type with either unusual mortality occurrences or mortality level. Majority of the farmers (98%) use mono-sex tilapia and this has also been observed by Tran et al., 2019. Almost all farms source tilapia fry from hatcheries, while less than 1% of farms use their own stock. The supply chain for tilapia fry was found to be mainly from middlemen, followed by farmers collecting themselves and hatcheries supplying directly to the farm through their agents or employees. Most of the farms (83%) used to stock once per year, followed by twice and thrice per production year. The stocking density of tilapia reported by Tran et al (2019) ranged from 125 to 270 fry/decimal, while this study found a mean stocking density of tilapia for the first cycle of 72 tilapia/decimal, 59 tilapia/decimal in the second cycle and 55 tilapia/decimal in the third cycle. Despite the fact that stocking density differed significantly by region for the first cycle t there were no variations during the second and third cycles. This could be attributable to the fact that fewer farmers followed stocking in the second (n = 97) and third (n = 8) cycles.

Pond drying, liming and cleaning nets were found to be the main biosecurity measures used by the farmers, either individually or in different combination. The majority of the farms were found using a combination of pond drying and liming as standard biosecurity interventions and this group also had the occurrence highest unusual mortality. According to (Faruk et al., 2017), farmers were found not wanting to remove soil sludge after harvesting because they considered it to be rich in nutrients.

This could be a contributing factor to unusual mortality events due to improper pond drying and subsequent infectious agent survival. Statistical analysis indicates that there is significant association in unusual mortality of tilapia with different biosecurity measures followed by the farms. Somewhat surprisingly, no unusual mortality events recorded for the farms reporting no biosecurity measures. Either these farms aren't paying attention to unusual mortality occurrences, or don't have any big disease issues, and thus get away with it. For this study, only 4 farms (0.7% of the total farm) reported under this group and difficult to make any interpretation with this negligible number of farms. Farms used fallow period practices for varying lengths of time as a biosecurity precaution; however, no association was observed between the length of the fallow period practices and the occurrences of unusual mortality. Furthermore, the majority of farms (96.8%) shared their equipment with other farms which is quite similar with the earlier publication by Faruk et al., (2017). Another biosecurity problem highlighted by this study was the use of contract harvesters, with 97% of farms hiring harvesters for the final or partial harvest of their farms. People involved in this service activity harvest fish in multiple farms each day posing a biosecurity risk as they can spread pathogens from one farm to another. Another biosecurity risk is the harvester's net, which are used in many ponds in wet conditions, allowing pathogens to spread from farm to farm, unless adequately disinfected between farms.

In this study, baseline mortality was described as mortality that appeared to be normal for the farm. Only two of the 565 farms reported a baseline mortality of zero, while the mean baseline mortality was $2 \pm 2\%$. It is likely that the farmers who reported zero baseline mortality did not properly observe their farm or that the farm had predatory fish as a true baseline mortality of zero is unrealistic. However, 90% of farmers said they were not concerned about baseline mortality. Despite the fact that about 10 percent of farmers were concerned about baseline mortality, no one kept a written or published record of it, and they all recalled it from memory. Interestingly, farmers who expressed concern about baseline mortality reported a significantly higher baseline mortality level than those who were not concerned, suggesting that their concern was legitimate. Baseline mortality level was also significantly associated with the reporting of unusual mortality, also when adjusted for farm water

area, farmer baseline mortality concern and the usage of antibiotics. For baseline dead fish disposal frequency, it was found that the farms who disposed daily or several times a day, had a significantly higher baseline mortality level compared to farms using other disposal frequencies. This is evidence of good farm management practices in order to handle the elevated levels of dead fish. A significantly higher proportion of farms reporting unusual mortality used a removal frequency of daily or several times a day compared to those not reporting unusual mortality events, which reflects the likelihood of these farms also having elevated baseline mortality levels. However, the reported unusual mortality level was significantly higher for farms reporting using occasional removal of dead fish compared to other removal frequencies, also when corrected for water source. Similarly, in farms reporting unusual mortality, dead fish disposal methods due to baseline mortality were found to be significantly higher for the group collected for discarding, followed by discarding in waterbody, and buried off farm. However, no significant relationship between different disposal methods and unusual mortality level was identified. In the absence of written mortality and disease event records it's possible that farms reporting a higher baseline mortality was really in an early stage of a disease event. They may be able to reduce or prevent unusual mortality events if baseline mortality was taken as an early warning signal and adequate diagnostic and disease investigation were followed. The absence of reported expenditure on screening, disease investigation, veterinary services suggest inadequate disease management, however it may also reflect the absence of availability of such services which needs to be addressed. Recently, with WorldFish financing and technical assistance, one private laboratory and several public laboratories have been established to provide commercial disease detection services to farmers.

Unusual mortality was reported by 18.2% of farms with a mean mortality level of 23.2% (range 3–90%). This is similar to that previously reported by Mosharraf et al., 2020, who reported 15 to 82 percent mortality in farm tilapia due to a novel RNA virus called Tilapia Lake Virus (TiLV), while Chaput et al., 2020 reported massive mortality in tilapia (15 MT tilapia lost from 28 hectare within 20 days) in Mymenshing and TiLV was identified as the causative agent of the diseases. Debnath et al., 2020, estimated a 25 to 90 percent mortality level in tilapia owing to TiLV

between 2017 and 2019. At the same time, various research groups have found unusual mortality in tilapia farms ranging from 13 to 80% farm (Faruk et al., 2017; Tran et al., 2019). This evidence suggests that unusul mortality events and disease outbreaks should be of significant concern to the Bangladeshi tilapia industry. The nature of unusual mortality was mainly sudden and gradual, while most of the mortality duration was reported within a week and data analysis revealed no significant association with unusual mortality level. There were diverse clinical signs described by the farmer for the reported mortality event where some of them resembling to gross sign of bacterial diseases such as haemorrhagic skin, scale protrusion and exophthalmia described for aeromonad septicemia (Salam et al., 2021), exophthalmia, lesion, haemorrhagic skin, lethargy described for *Streptococcus* sp. infection (Oviedo-Bolaños et al., 2021) while fin rot, pale skin and necrotic skin has been reported for columnaris (Dong et. al., 2015). Similarly, clinical signs such as lethargy, scale protrusion, skin erosion and discoloration, exophthalmia, detached scale, open wounds/lesion, and abnormal behavior were described for new emerging viral infection named TiLV which was confirmed by several research groups (Debnath et al., 2020; Chaput et al., 2020; Mosharraf et al., 2020). Considering the clinical signs identified in different studies for TiLV cases in Bangladesh and the clinical signs reported in this study, there is a high probability that the majority of unusual mortality reported by farms is related to TiLV, however some clinical sign partially matched with different bacterial diseases. Farmers identified multiple stress factors which they perceived to be related to the occurrence of unusual mortality, including weather stress, water quality stress, and a combination of both, although in this study farmer mentioned more frequently that weather stress included temperature and heavy rainfall as the most prominent stressor factor for unusual mortality. A seasonal variation, with two mortality peaks were observed, with one peak in August and another in December. In Bangladesh, July to August has weather condition that normally includes heavy rainfall with high temperatures, while November to December on the other hand is winter season with very low temperatures. Similar report found as fish disease was most frequent in the winter and late winter seasons, as well as after heavy rains and in the summer (Faruk et al., 2017). These findings indicates that this weather factor might contribute weather stress to boost up the

disease incidence as well as pathogen loads. Unusual mortality reported in both tilapia and co-cultivated species where disease and mortality started in tilapia and later spread to co-cultivated species. This data suggests that tilapia play a significant role in disease transmission to co-cultivated species, which may be a major concern for the polyculture farm if it continues to stock tilapia with other species. However, in the instance of TiLV, co-cultivated polyculture species were shown to be TiLV-resistant in both field and Challenge experiments (Debnath et. Al., 2021). On the other hand, there may be factors such as tilapia feeding behavior favoring the detection of clinical signs in tilapia as opposed to the co-cultured species.

Another key finding of this study is that the usage of antibiotics was more frequent in farms with unusual mortality than in farms without unusual mortality. This remained significant also when correcting for farm water area, baseline mortality level, and farmer baseline mortality concern. This re-emphasizes the fact that the primary driver for farmers to use/misuse antibiotics is directly linked to observing unusual mortality events rather than an informed decision following confirmation of the disease etiology. This research also show that only a small proportion of farms report the use of antibiotics, which is encouraging. On the other hand, antibiotics usage is often determined by farmers, who do not seek advice from veterinarians, implying that there is a high risk of misuse in terms of dosing, frequency, and duration. In addition, farmers have very limited access to veterinarians or aquatic animal health staff in the event of emergencies or disease events as the number of skilled veterinarian and aquatic animal health staff are very limited in this sector. This suggests that misapplication of antibiotics may encourage antimicrobial resistance in microbes, potentially jeopardizing aquaculture's sustainability.

An economic analysis was conducted to investigate the unseen economic impact of both baseline and unusual mortality on the tilapia farms as well as the tilapia sector in Bangladesh. Here, we use information provided by farmers during data collection to explain farm economics. According to our analysis, unusual mortality had a significant impact on profit making, with 23% fewer farms unable to make a profit. When losses owing to baseline (0.4 USD/decimal) and unusual mortality (21.73

USD/decimal) were compared, a considerable economic loss (21.33 USD/decimal) was discovered at the farm level due to unusual mortalities.

Bangladesh aquaculture, which includes ponds, seasonal waters, baor, shrimp/prawn farm, pen culture, and cage culture, covers an estimated area of 821 923 hectares (203 120 248.54 decimals) with tilapia being cultured in most farming systems (DoF, 2019). Based on the estimated economic impact of baseline mortality from this study is 0.4 USD/decimal, estimated losses equivalent to 81.25 million[1] USD nationwide. Similarly, based on the 18.2% of the farming area that experienced unusual mortality of tilapia in our study, could be extrapolated to 794.5 million[2] USD nationwide. Those crude estimates of economic losses of production due to both baseline and unusual mortality of tilapia, represent a total hidden loss of 875.7 million USD that should not be overlooked.

Although many still believe that tilapia is a highly resilient fish that can be farmed in many types of environments, the findings of this study contradict this notion. We suggest the following recommendations for the sustainable tilapia farming sector in Bangladesh. First of all, all farmers should have access to basic training, and provided guidance and consultation on biosecurity and fish disease management. Tilapia baseline and unusual mortality levels should be considered as indicators for proper disease investigation which include two-way reporting system (farmer to representatives of competent authority and vice versa) followed by the development of disease management strategies and surveillance programs based on diagnostic reports. This could help minimize the incidence and further spread of disease, reduce the misuse of drugs including antibiotics, and increase production and profitability. Ultimately, the sector should be able to save at least a significant proportion of this estimated hidden loss through improved fish health management with competent authorities support (action plan, development of training materials and biosecurity guidelines).

In 2017, the FAO issued a special warning to all tilapia producing countries about the emerging tilapia disease TiLV (FAO, 2017a). To date three separate research groups

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[1] Total aquaculture area in decimal × baseline loss in USD/decimal
[2] [(total aquaculture area in decimal × 18.2%) × loss in USD/decimal for unusual mortality]

confirmed TiLV infection in tilapia farms from several districts of Bangladesh (Chaput et al., 2020; Debnath et al., 2020; Mosharraf et al., 2020). This example of TiLV amongst other infectious diseases shows the importance to implement targeted surveillance programs to prevent and minimize disease transmission.

Based on farmers' reports of tilapia diseases spreading to other co-cultured species, Government Organizations, NGOs and other sector stakeholders need to develop farmer awareness programs to ensure sustainable tilapia production to feed the future world.

References:

Ahern, M. B., Thilsted, S. H., Kjellevold, M., Overå, R., Toppe, J., Doura, M., Kalaluka, E., Wismen, B., Vargas, M., & Franz, N. (2021). Locally-procured fish is essential in school feeding programmes in sub-saharan Africa [Review]. *Foods, 10*(9), Article 2080. https://doi.org/10.3390/foods10092080

Ali, H., Rahman, M. M., Rico, A., Jaman, A., Basak, S. K., Islam, M. M., . . . Mohan, C. V. (2018). An assessment of health management practices and occupational health hazards in tiger shrimp (Penaeus monodon) and freshwater prawn (Macrobrachium rosenbergii) aquaculture in Bangladesh. *Veterinary and Animal Science, 5*, 10-19. doi:https://doi.org/10.1016/j.vas.2018.01.002.

Amal, M. N. A., Koh, C. B., Nurliyana, M., Suhaiba, M., Nor-Amalina, Z., Santha, S., . . . Zamri-Saad, M. (2018). A case of natural co-infection of Tilapia Lake Virus and Aeromonas veronii in a Malaysian red hybrid tilapia (Oreochromis niloticus×O. mossambicus) farm experiencing high mortality. *Aquaculture, 485*, 12-16. doi:https://doi.org/10.1016/j.aquaculture.2017.11.019

Apu, N. A. (2014). Farmed fish value chain development in Bangladesh: Situation analysis and trends. WorldFish/ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Beveridge, M. C. M., Thilsted, S. H., Phillips, M. J., Metian, M., Troell, M., & Hall, S. J. (2013). Meeting the food and nutrition needs of the poor: The role of fish and the opportunities and challenges emerging from the rise of aquaculture. *Journal of Fish Biology, 83(4)*, 1067–1084. doi:https://doi.org/10.1111/jfb.12187

Chaput, D. L., Bass, D., Alam, M. M., Al Hasan, N., Stentiford, G. D., van Aerle, R., . . . Tyler, C. R. (2020). The Segment Matters: Probable Reassortment of Tilapia Lake Virus (TiLV) Complicates Phylogenetic Analysis and Inference of Geographical Origin of New Isolate from Bangladesh. *Viruses 12,* (doi:10.3390/v12030258), 258.

Debnath, P. P., Dinh-Hung, N., Taengphu, S., Nguyen, V. V., Delamare-Deboutteville, J., Senapin, S., Vishnumurthy Mohan, C., Dong, H. T., & Rodkhum, C. (2021). Tilapia Lake Virus was not detected in non-tilapine species within tilapia polyculture systems of Bangladesh [Article]. Journal of Fish Diseases. https://doi.org/10.1111/jfd.13537

Debnath, P. P., Delamare-Deboutteville, J., Jansen, M. D., Phiwsaiya, K., Dalia, A., Hasan, M. A., . . . Rodkhum, C. (2020). Two-year surveillance of tilapia lake virus (TiLV) reveals its wide circulation in tilapia farms and hatcheries from multiple districts of Bangladesh. . *J Fish Dis., 43*, 1381–1389. doi:https://doi.org/10.1111/jfd.13235

DESA, U. (2019). World Population Prospects 2019: Highlights. . *New York (US): United Nations Department for Economic and Social Affairs., 2019: 1-46.*

DoF. (2018). *Yearbook of Fisheries Statistics of Bangladesh 2017-18. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh : Director General, DoF, 2018. Volume 34 : p. 129.*

DoF. (2019). Yearbook of Fisheries Statistics of Bangladesh, 2018-19. *Fisheries Resources Survey System (FRSS), Department of Fisheries, Bangladesh : Ministry of Fisheries and Livestock, 36*, 135.

Dong, H. T., LaFrentz, B., Pirarat, N., & Rodkhum, C. (2015). Phenotypic characterization and genetic diversity of Flavobacterium columnare isolated from red tilapia, Oreochromis sp., in Thailand. *Journal of Fish Diseases, 38*, 901-913. doi:DOI: 10.1111/jfd.12304

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en.

Faruk, M. A. R., Rahman, N., & Patwary, Z. P. (2017). Risk factors associated with tilapia and pangasius diseases. *J Bangladesh Agril Univ 15(2)* 325–331. doi:10.3329/jbau.v15i2.35083

Hinchliffe, S., Butcher, A., Rahman, M. M., Guilder, J., Tyler, C., & Verner-Jeffreys, D. (2021). Production without medicalisation: Risk practices and disease in Bangladesh aquaculture. *Geogr J, 187*, 39-50. doi:https://doi.org/10.1111/geoj.12371

Jahan, K. M., Belton, B., Ali, H., Dhar, G. C., & Ara, I. (2015). Aquaculture technologies in Bangladesh: An assessment of technical and economic performance and producer behavior. Penang, Malaysia: WorldFish. Program Report: 2015-52.

Kibenge, F. S. (2019). Emerging viruses in aquaculture. Current Opinion in Virology, 34, 97-103. https://doi.org/10.1016/j.coviro.2018.12.008

Khor L, Delamare-Deboutteville J, Ali S and Mohan CV. 2021. Aquatic Animal Health Package of Practices: Fish epidemiology and health economics. Penang, Malaysia: WorldFish. Guidance Note. https://hdl.handle.net/20.500.12348/4900

Mosharraf, H. M. M., Imtiaz, U. M., Monjur, H. M., Islam, H., Al, A., Farjana, N., & Rukaiya, A. (2020). Molecular Detection of Tilapia Lake Virus (TiLV) in Farmed Mono-sex Nile Tilapia (Tilapia niloticus) in Bangladesh. *Asian Journal of Scientific Research, 13 (1)*, 67-78. doi:DOI: 10.3923/ajsr.2020.67.78

Ng, W. K., & Romano, N. (2013). A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Rev. Aquac., 5* pp. 220-254.

Oviedo-Bolaños, K., Rodríguez-Rodríguez, J. A., Sancho-Blanco, C., Barquero-Chanto, J. E., Peña-Navarro, N., Escobedo-Bonilla, C. M., & Umaña-Castro, R. (2021). Molecular identification of Streptococcus sp. and antibiotic resistance genes present in Tilapia farms (Oreochromis niloticus) from the Northern Pacific region, Costa Rica [Article]. Aquaculture International, 29(5), 2337-2355. https://doi.org/10.1007/s10499-021-00751-0

Ponzoni, R. W., Nguyen, N. H., Khaw, H. L., Hamzah, A., A.;, B. K. R., & Yee, H. Y. (2011). Genetic improvement of Nile tilapia (Oreochromis niloticus) with special reference to the work conducted by the WorldFish Center with the GIFT strain. *Reviews in Aquaculture 3*, 27–41.

Salam, H. S. H., Mohamed, W. M. S., Aziz, S. A. A. A., Mohammed, A. N., & Korni, F. M. M. (2021). Prevention of motile Aeromonas septicemia in Nile tilapia, Oreochromis niloticus, using thyme essential oil and its nano-emulsion [Article]. Aquaculture International, 29(5), 2065-2084. https://doi.org/10.1007/s10499-021-00735-0

Surachetpong, W., & Sirikanchana, K. (2020). Orthomyxovirosis (Tilapia Lake Virus). Climate change and infectious fish diseases, 142-156.

Thilsted, S. H., Thorne-Lyman, A., Webb, P., Bogard, J. R., Subasinghe, R., Phillips, M. J., & Allison, E. H. (2016). Sustaining healthy diets: The role of capture fisheries and aquaculture for improving nutrition in the post-2015 era. . *Food Policy, 61*, 126–131. doi:https://doi.org/10.1016/j.foodpol.2016.02.005

Tran, K., Verdegem, M., Wolkenfelt, B., Mohan, C., Verreth, J. (2019). The relation between farming practices and tilapia production in small-scale fish farms in Bangladesh The relation between farming practices and tilapia production in small-scale fish farms in Bangladesh Citation Creative Commons License Photo credits.

List of tables

Table 1: Topics and variables covered in the TEHE survey questionnaire

Topics/variables	Description
Enumerator and interviewee information	Name, email and mobile number of the enumerator and interviewee conducting the survey.
Consent	Farmers' consent to participate in the study and provide information
Geographical	Information on the region, district, sub-district, upazilla as well as farm GPS coordinates
Farmer factors	Gender, age, education, role on farm, part-time versus full-time farming, year(s) of aquaculture experience, and primary decision maker (yes/no)
Farm factors	Farming system (commercial, homestead, both), farm size, farm type (seasonal, perennial), water source, all-in all-out or continuous, culture type (monoculture or polyculture) and polyculture species
Stock factors	Tilapia sex, tilapia source, stocking density of tilapia and seed cost
Biosecurity measures	Measures taken between production cycles, fallowing duration, shared equipment between farmers and harvester use
Mortality	Baseline mortality level (%), baseline mortality concern (yes/no), baseline mortality removal frequency, mortality disposal method, unusual mortality (yes/no), unusual mortality nature, unusual mortality duration, unusual mortality level (%), unusual mortality associated stress factors and antibiotic use

Table 2: Farmers and farms characteristics by region

	Regions (Number of farms studied)					
	Barisal (91)	Chittagong (138)	Dhaka (31)	Khulna (228)	Mymensingh (77)	
	n (%) or mean (min, max)					P
Farming experiences (Years)	8.8 (3 -18)	10.7 (3 -27)	7.6 (3 -17)	8.3 (1 -28)	8.7 (2 -27)	<0.001
Farmer education level						
Missing	0	1	0	0	0	
Up to primary	40 (44.0)	51 (37.0)	11 (35.5)	55 (24.1)	38 (49.4)	
Up to higher secondary	40 (44.0)	66 (47.8)	16 (51.6)	125 (54.8)	26 (33.8)	0.002
Up to graduate	11 (12.1)	20 (14.5)	4 (12.9)	48 (21.1)	13 (16.9)	
Farm size (decimal)	150.02 ± 294.5 (10-2000)	524.42 ± 963.9 (15-7800)	413.87 ± 767.5 (50-4300)	580.02 ± 1589.7 (8-15600)	239.77 ± 222.6 (30-1300)	0.015
Water source						
Ground water	50 (54.9)	128 (93.4)	14 (45.2)	152 (67.0)	77 (100)	
River/canal	20 (22.0)	1 (0.7)	9 (29.0)	48 (21.1)	0	
Rain	11 (12.1)	7 (5.1)	1 (3.2)	22 (9.7)	0	<0.001
Ground water and river/canal	10 (11.0)	1 (0.7)	7 (22.6)	5 (2.2)	0	
Stocking density of tilapia (tilapia/decimal)						
1st cycle	181 (21-667)	110 (7-417)	167 (30-500)	133 (8-800)	133 (7-400)	<0.001
Biosecurity measures between production cycle						
No measures	2 (2.2)	0	1 (3.2)	1 (0.4)	0	
Drying and liming pond	50 (54.9)	92 (66.7)	22 (71.0)	169 (74.1)	50 (64.9)	
Liming pond	23 (25.3)	11 (8.0)	5 (16.1)	37 (16.2)	5 (6.5)	<0.001
Drying and liming pond and cleaning nets	8 (8.8)	32 (23.2)	2 (6.5)	21 (3.1)	70 (27.3)	
Others	8 (8.8)	3 (2.2)	1 (3.2)	14 (6.1)	1 (1.3)	
Fallow period						
None	20 (22.0)	15 (10.2)	3 (9.7)	16 (7.0)	4 (5.2)	
1-7 days	6 (6.6)	17 (12.4)	2 (6.5)	11 (4.8)	8 (10.4)	
1-2 weeks	5 (5.5)	33 (24.1)	8 (25.8)	32 (14.0)	14 (18.2)	
3-4 weeks	23 (25.3)	48 (35.0)	10 (32.3)	84 (36.8)	31 (40.3)	<0.001
5-10 weeks	19 (20.9)	21 (15.3)	5 (16.1)	37 (16.2)	14 (18.2)	
> 10 weeks	18 (19.8)	4 (2.9)	3 (9.7)	48 (21.1)	6 (7.8)	
Baseline mortality level*	1.8 (0-5)	1.8 (0-5)	1.1 (0.2-4.3)	2.3 (0-20)	1.9 (0.2-5)	0.01

*One missing value for mortality level for Chittagong

Table 3a: Factors examined for association with occurrence of reported unusual mortality and level of mortality

Variables	All farms	Reported unusual mortality			Reported unusual mortality level (%)	
	N = 565	No (N = 462)	Yes (N = 103)		N = 58	
	n (%) or mean (min, max)	n (%) or mean (95% CI)		P	mean (95% CI)	P
Regions						
Barisal	91 (16.1)	71 (15.4)	20 (19.4)		26.0 (7.0-75)	
Chittagong	138 (24.4)	117 (25.3)	21 (20.4)		26.1 (11.0-90.0)	
Dhaka	31 (5.5)	25 (5.4)	6 (5.8)	0.785	15.0 (10.0-20.0)	0.016
Khulna	228 (40.4)	186 (40.3)	42 (40.8)		17.1 (3.0-60.0)	
Mymensingh	77 (13.6)	63 (13.6)	14 (13.6)		33.2 (13.0-75.0)	
Farming experiences (years)	9.0 (1-28)	9.1 (1-28)	8.8 (2-20)	0.741		
Farmer education level (one missing)						
Up to primary	195 (34.6)	169 (36.7)	26 (25.2)		28.4 (11-90)	
Up to higher secondary	273 (48.4)	212 (46.0)	61 (59.2)	0.041	22.9 (6-75)	0.241
Up to graduate	96 (17)	80 (17.4)	16 (15.5)		15.6 (3-28)	
All-in All-out						
Yes	94 (16.6)	75 (79.8)	19 (20.2)	0.586	30.86 (15-90)	0.123
No	471 (83.4)	387 (82.2)	84 (17.8)		22.18 (3-75)	
Farm size (decimal)	441.7 (8-15600)	391.07 (10-8600)	668.79 (8-15600)	0.027		
Water source						
Ground water	421 (74.6)	343 (74.4)	78 (75.7)		26.1 (7-90)	
River/canal	78 (13,8)	64 (13.9)	14 (13.6)	0.936	12.2 (3-25)	0.044
Rain	41 (7.3)	33 (7.2)	8 (7.8)		16.0 (12-20)	
Ground water and river/canal	24 (4.3)	21 (4.6)	3 (2.9)		6.0 (6-6)	
Culture type						
Monoculture	9 (1.6)	8 (1.7)	1 (1.0)		N/A	
Polyculture	551 (97.5)	452 (97.8)	99 (96.1)	0.065	23.09 (3-90)	0.178
Mixculture	5 (0.9)	2 (0.4)	3 (2.9)		27.0 (25-29)	
Percentage of tilapia stocked	64 (20-100)	64 (20-100)	63 (20-100)	0.517		

Table 3b: Factors examined for association with occurrence of reported unusual mortality and level of mortality

Variables	All farms N = 565 — n (%) or mean (min, max)	Reported Unusual mortality — No (N = 462)	Yes (N = 103)	P		Reported unusual mortality level (%) N = 58 — mean (95% CI)	P
		n (%) or mean (95% CI)					
Stocking density of tilapia (tilapia/decimal)							
1st cycle	137 (7-800)	139 (7-800)	129 (23-375)	0.883			
2nd cycle	101 (8-300)	99 (8-300)	110 (14-250)	0.405			
3rd Cycle	95 (45-250)	102 (48-250)	45 (45-45)	0.250			
Species type							
Fresh water species	538 (95.4)	439 (95.2)	99 (96.1)	0.697		23.38 (3-90)	0.983
Mix of fresh and brackish water species	26 (4.6)	22 (4.8)	4 (3.9)			19.0 (13-25)	
Biosecurity measures between production cycle							
No measures	4 (0.7)	4 (0.9)	0			0	
Drying and liming pond	383 (67.8)	297 (64.3)	86 (83.5)			23.15 (3-90)	
Liming pond	81 (14.3)	70 (15.2)	11 (10.7)	0.002		22.31 (7-50)	0.363
Drying and liming pond and cleaning nets	70 (12.4)	65 (14.1)	5 (4.9)			27.60 (15-50)	
Others	27 (4.8)	26 (5.6)	1 (1.0)			12.00 (12-12)	
Fallow period							
None	57 (10.1)	4 (0.9)	0			12 (12-12)	
1-7 days	44 (7.8)	297 (64.3)	86 (83.5)			25.6 (12-50)	
1-2 weeks	92 (16.3)	70 (15.2)	11 (10.7)	0.301		23 (3-35)	0.618
3-4 weeks	196 (34.8)	65 (14.1)	5 (4.9)			24.83 (96-90)	
5-10 weeks	96 (17)	26 (5.6)	1 (1.0)			21.29 (10-50)	
> 10 weeks	79 (14)	65 (11.5)	14 (2.5)			19.50 (8-50)	
Shared equipment among farms							
Not shared	15 (2.7)	9 (1.9)	6 (5.8)			13.80(10-24)	
Shared with one farm	3 (0.5)	3 (0.6)	0	0.081		0	0.090
Shared with two or more farms	547 (96.8)	450 (97.4)	97 (94.2)			24.12 (3-90)	
Hired harvester							
Yes	548(97)	449 (97.2)	99 (96.1)	0.529		23.61 (3-90)	0.223
No	17 (3)	13 (2.8)	4 (3.9)			12.50 (12-13)	

Table 3c: Factors examined for association with occurrence of reported unusual mortality and level of mortality

Variable	All farms N= 565 n (%) or mean (min, max)	Reported unusual mortality No (N= 462) n (%) or mean (95% CI)	Reported unusual mortality Yes (N= 103)	P	Reported unusual mortality level (%) N= 58 mean (95% CI)	P
Concern about baseline mortality						
Yes	55 (9.7)	24 (5.2)	31 (30.1)	<0.001	24.34 (7-75)	0.548
No	510 (90.3)	438 (94.8)	72 (69.9)		22.18 (3-90)	
Baseline mortality level	2.07 (0-20)	1.67 (0-20)	3.47 (0.4-20)	<0.001		
Dead fish removal frequency						
Never	10 (1.8)	10 (2.2)	0		0	
Daily or several times per day	412 (73)	319 (69.2)	93 (90.3)	<0.001	21.52 (3-75)	0.030
Every 2 to 3 days interval	31 (5.5)	29 (6.3)	2 (1.9)		12 (12-12)	
Occasionally	111 (19.7)	103 (22.3)	8 (7.8)		57.67 (33-90)	
Dead fish disposal method						
Collected for discarding	213 (37.7)	165 (35.7)	48 (46.6)		22.70 (6-90)	
Discarded in waterbody	86 (15.2)	61 (13.2)	25 (24.3)		20.16 (3-60)	
Buried off farm	160 (28.3)	139 (30.1)	21 (20.4)		28.87 (10-75)	
Collected for discarding and buried off farm	26 (4.6)	22 (4.8)	4 (3.9)		20.75 (10-28)	
Fed to other animals on farm	6 (1.1)	5 (1.1)	1 (1.0)	0.003	18 (18-18)	0.583
Collected for discarding and fed to other animals on farm	18 (3.2)	18 (3.9)	0 (0)		0	
Others	41 (7.3)	37 (8)	4 (3.9)		14.50 (12-17)	
Fish not removed from pond(s)	15 (2.7)	15 (3.2)	0 (0)		0	
Use of antibiotic						
Yes	31 (5.5)	19 (4.1)	12 (11.7)	0.005	17.05 (7-60)	0.029
No	534 (94.5)	443 (95.9)	91 (88.3)		24.51 (3-90)	

Table 4a: Results from the univariable and multivariable regression analyses for the occurrence of unusual mortality (no/yes)

Univariable models	OR	95% CI	p	AIC
Farmer education				536.0
Graduate	1			
Upper secondary	1.4	0.8 - 2.7	0.241	
Primary	0.8	0.4 - 1.5	0.437	
Farm water area (ha)	1.05	0.001 - 1.1	0.051	536.8
Baseline mortality level	1.6	1.4 - 1.9	<0.001	482.4
Farmer baseline mortality concern	7.9	4.4 - 14.3	<0.001	494.6
Antibiotic treatment				
No	1			
Yes	3.1	1.4 - 6.5	0.004	532
Multivariable, final model				
	OR	95% CI	p	AIC
Intercept	0.06	0.04 – 0.10	<0.001	459.6
Farm water area (ha)	1.05	1.002 – 1.1	0.04	
Baseline mortality level	1.5	1.3 – 1.8	<0.001	
Farmer baseline mortality concern	4.4	2.3 – 8.4	<0.001	
Antibiotic treatment	2.5	1.04 – 5.9	0.03	

Table 4b: Results from the univariable and multivariable regression analyses for the level (%) of unusual mortality

Univariable models	OR	95% CI	p	AIC
Region				117.0
Barisal	1			
Chittagong	1.2	0.7 – 2.1	0.600	
Dhaka	0.7	0.3 – 2.0	0.565	
Khulna	0.7	0.4 – 1.3	0.264	
Mymensingh	1.5	0.8 – 2.8	0.208	
Years of farming experience	1.0	0.9 – 1.1	0.859	122.8
Farm water area (ha)	1.0	0.9 -1.0	0.240	121.4
Water source				114.6
Ground water	1			
Ground water and river/canal	0.3	0.1 – 0.9	0.048	
Rainwater	0.7	0.4 – 1.3	0.301	
River/canal	0.5	0.3 – 0.8	0.005	
Stocking density (1st cycle)	1.0	0.9 – 1.1	0.389	122.1
Farm baseline mortality level	1.0	0.9 – 1.1	0.770	122.8
Dead fish removal frequency				115.9
Daily or several times per day	1			
Every 2 to 3 days	0.7	0.2 - 2.4	0.552	
Occasionally	3.0	1.5 – 6.3	0.005	
Antibiotic treatment				120.1
No	1			
Yes	0.7	0.4 – 1.1	0.103	

	OR	95% CI	p	AIC
Multivariable, final model				
Intercept	20.1	16.8 – 24.0	<0.001	109.7
Water source				
Ground water	1			
Ground water and river/canal	0.3	0.1 – 0.9	0.046	
Rainwater	0.8	0.5 – 1.3	0.367	
River/canal	0.5	0.3 – 0.8	0.006	
Dead fish removal frequency				
Daily or several times per day	1			
Every 2 to 3 days	0.6	0.2 – 1.9	0.386	
Occasionally	2.6	1.3 – 5.2	0.008	

Table 5: Economics of the farm dealing with baseline and unusual mortality

Variable	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Baseline production (kg/decimal)	505	0.22	163.64	23.36	22.88	523.26
Baseline loss (Kg/decimal)	507	0.00	6.25	0.44	0.52	.27
baseline expected price of lost tilapia (USD/Kg)	507	0.42	1.45	0.93	0.16	.03
baseline biosecurity costs (USD/Decimal)	509	0	8	0.40	0.70	0.49
baseline chemotherapeutics costs (USD/Decimal)	509	0	9	0.54	0.86	0.74
Profit from baseline farm (USD/Decimal)	408	0.28	44	7.24	6.96	48.45
Tilapia production from unusual mortality farm (kg/Decimal)	58	1	90	24.27	20.52	420.95
Loss for unusual mortality (Kg/Decimal)	57	1	933	22.40	123.04	15139.18
Expected price of lost tilapia for unusual mortality (USD/Kg)	57	0.48	1.57	0.97	0.17	0.03
Biosecurity cost for unusual mortality (USD/Decimal)	58	0	1	0.16	0.25	0.06
Unusual chemotherapeutics costs (USD/Decimal)	58	0	3	0.55	0.56	0.31
Profit from Unusual mortality experienced farm (USD/Decimal)	26	0.00	18.07	5.22	4.58	20.94

List of Figures
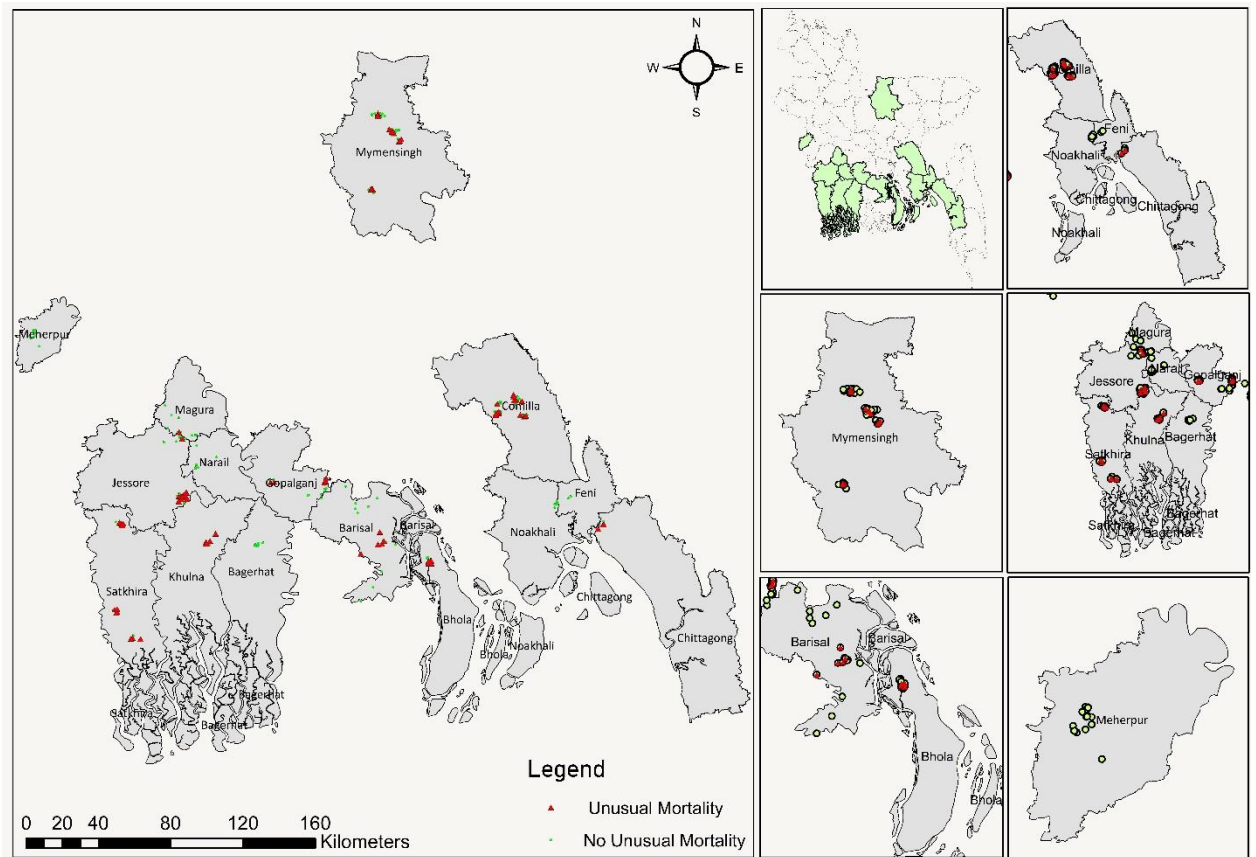
Fig. 1: Location of the five study regions of Bangladesh, namely Barisal, Chittagong, Dhaka, Khulna and Mymensingh with all the farms studied. Farms reporting unusual mortality are shown in red while farms not reporting unusual mortality evens are shown in green.
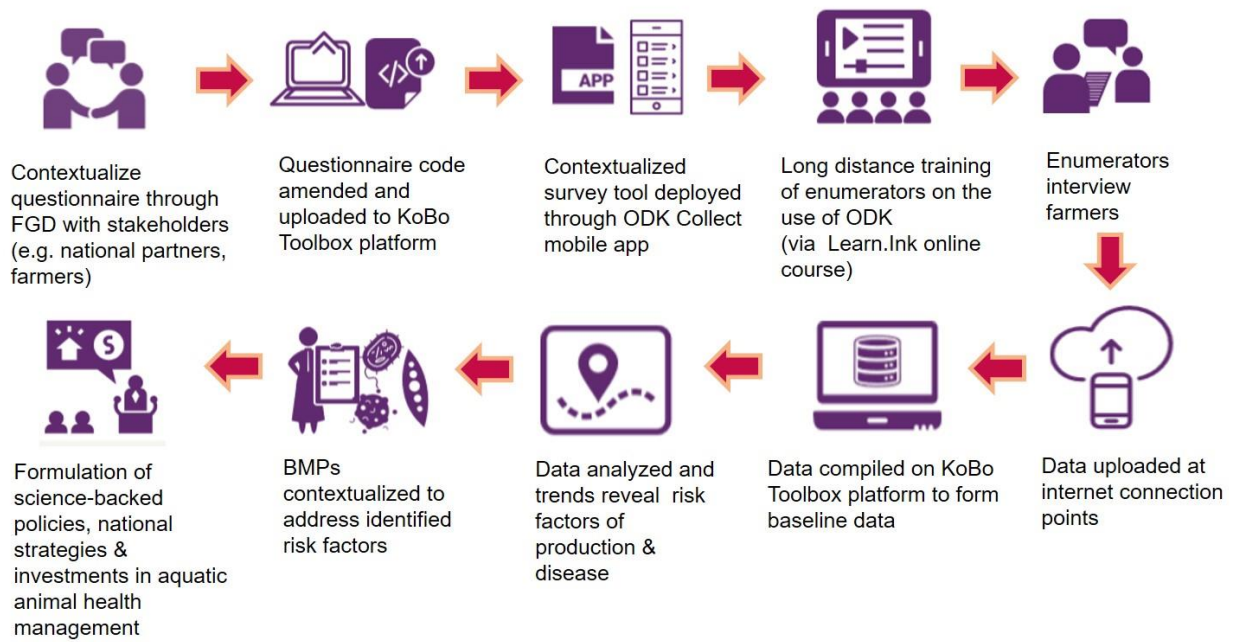
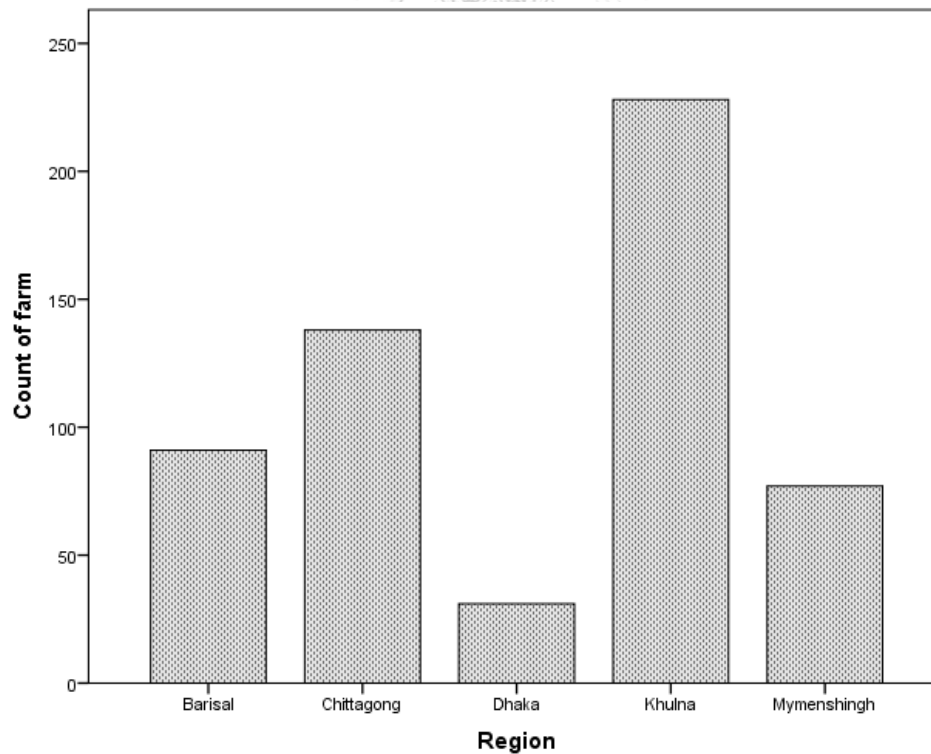Fig. 2: Conceptual framework of the survey on tilapia health epidemiology and economic impact

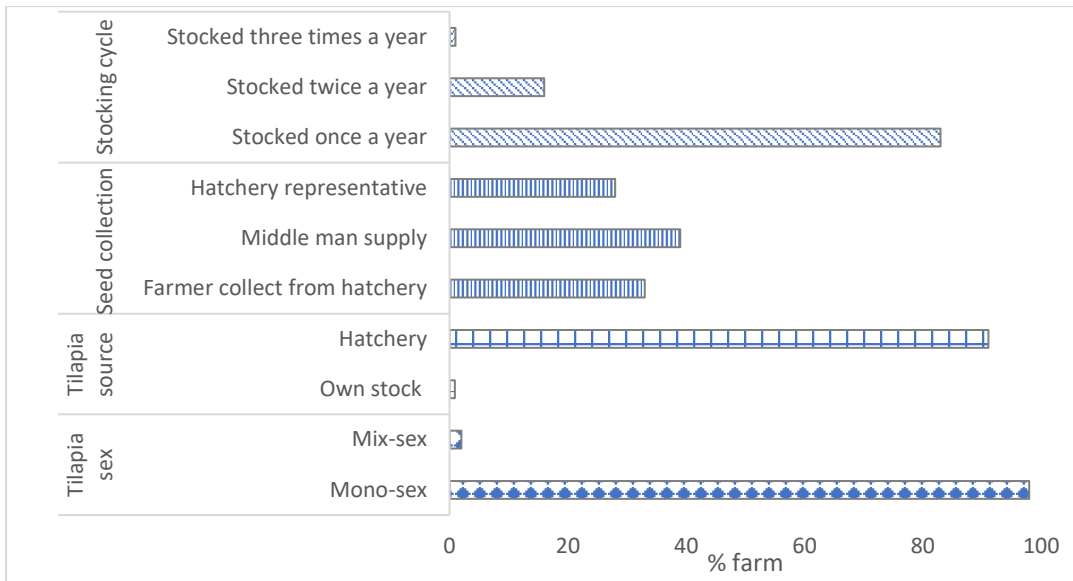Fig. 3: The number of farms studied by region

Fig. 4: Percentage of sex of tilapia, origin of tilapia, method of obtaining tilapia seed and tilapia stocking cycle in farms
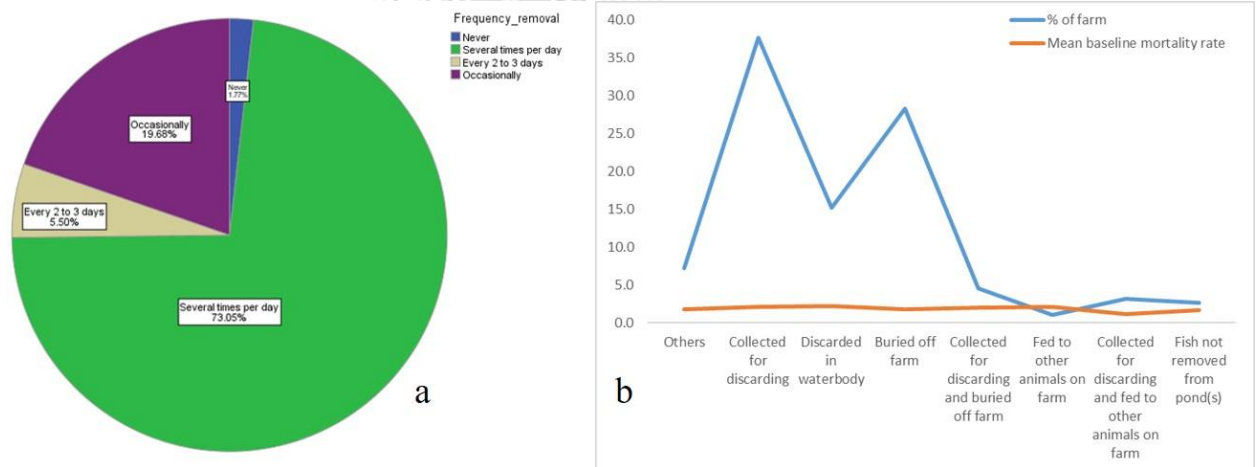
Fig. 5: (a) Different frequency duration for the removal of dead fish from the pond and (b) Percent of farms followed the dead fish disposal method and associated the mean baseline mortality rate
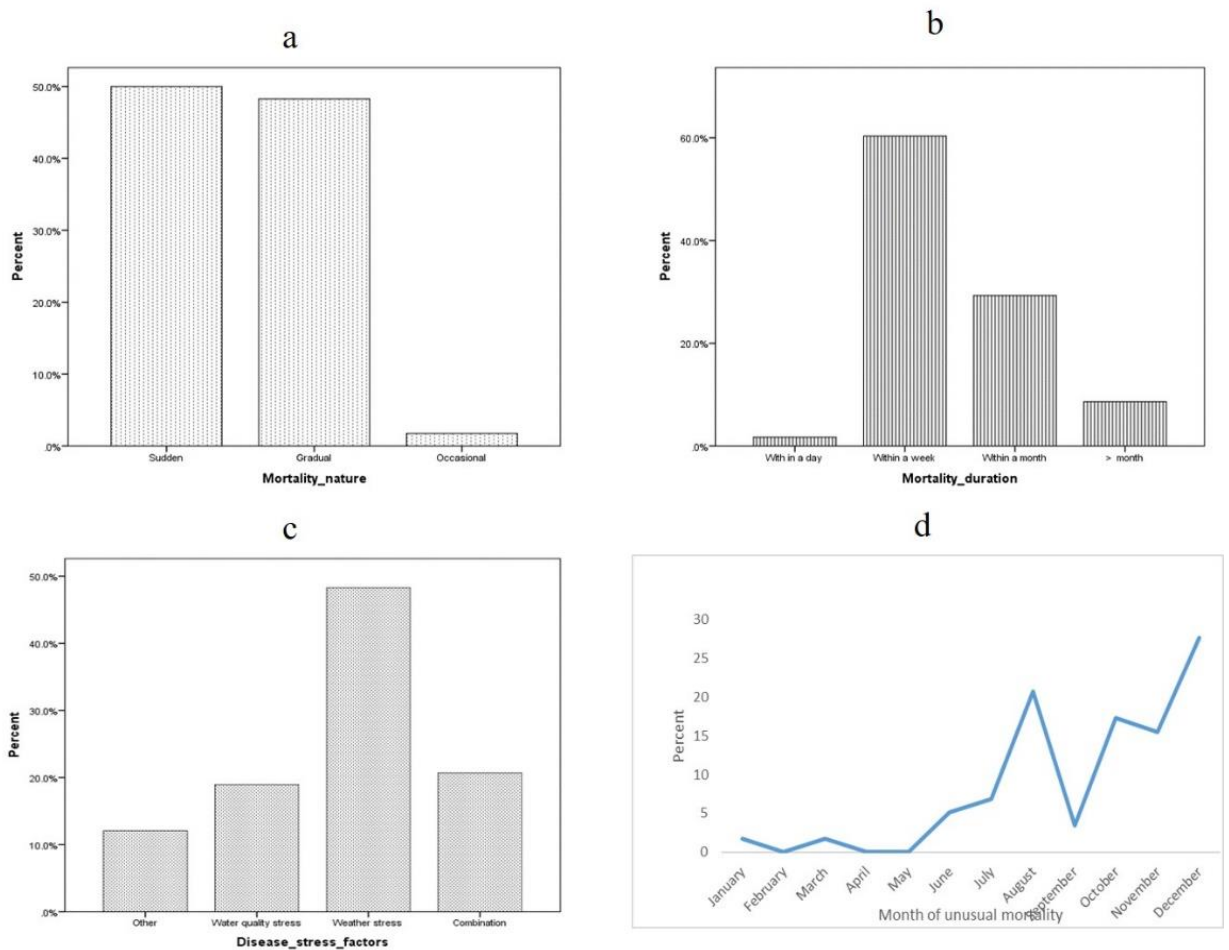
Fig. 6: (a) Percentage of farms reporting different types of mortality nature during unusual mortality; (b) Percentage of farms that reported different duration of mortality during unusual mortality; (c) Percentage of farms reporting various stressors as a cause of unusual mortality and (d) Seasonal variation of unusual mortality (data missing for the month of February, April and May)
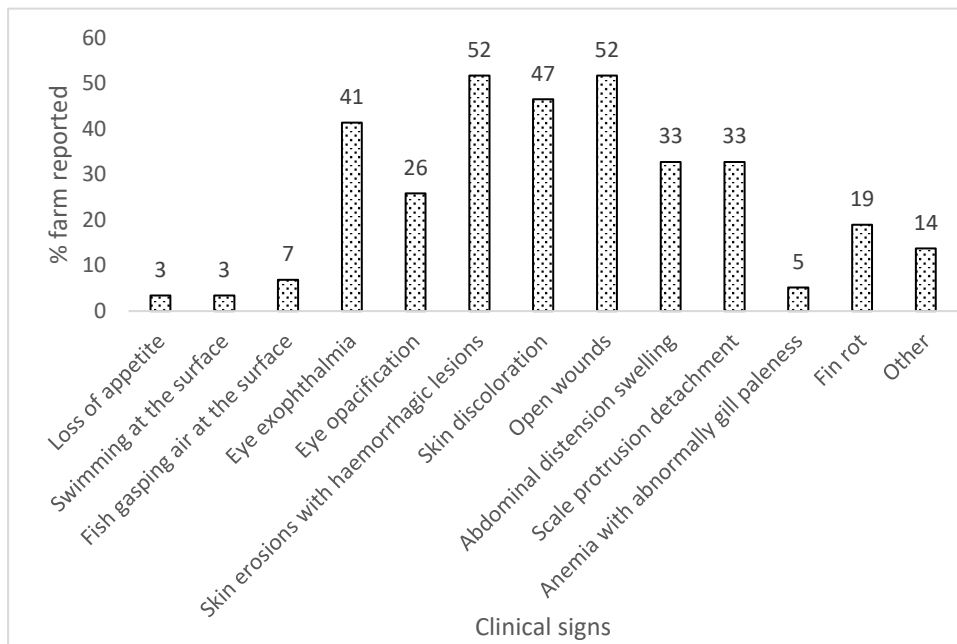
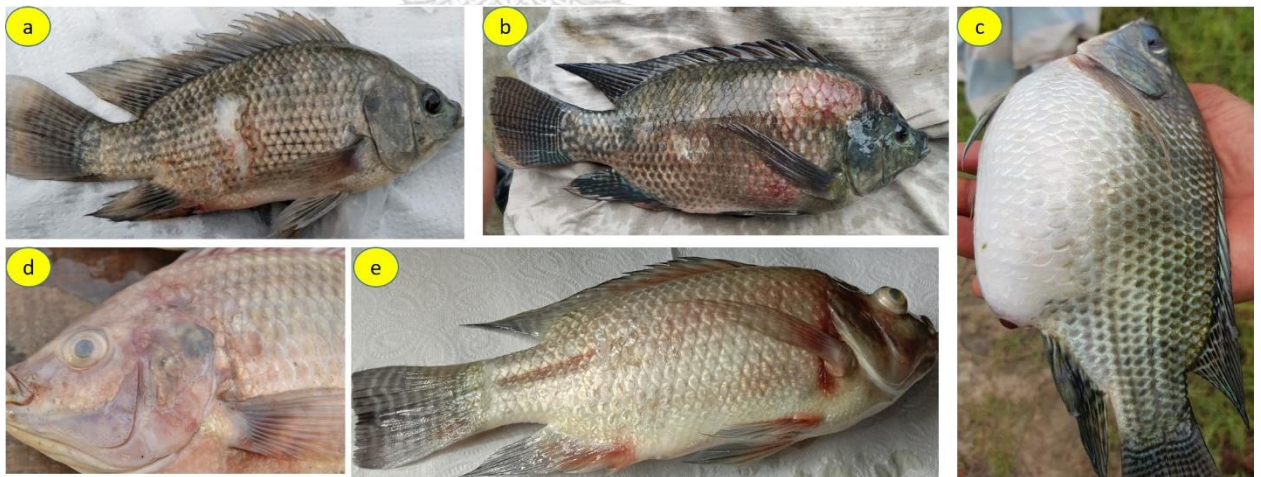Fig. 7: Percentage of farms reporting different clinical sign in case of unusual mortality

Fig.8: Photographs of the major tilapia clinical signs observed from this study (a) open wounds (b) scales protrusion and hemorrhagic lesions (c) abdominal distension/swelling (d) eye opacification and skin discoloration (e) eye exophthalmia, hemorrhagic skin and fins

Chapter 4

Two-year surveillance of tilapia lake virus (TiLV) reveals its wide circulation in tilapia farms and hatcheries from multiple districts of Bangladesh

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4.1 Abstract

Tilapia lake virus (TiLV) is an emerging pathogen in aquaculture, reportedly affecting farmed tilapia in 16 countries across multiple continents. Following an early warning in 2017 that TiLV might be widespread, we executed a surveillance programme on tilapia grow-out farms and hatcheries from 10 districts of Bangladesh in 2017 and 2019. Among farms experiencing unusual mortality, eight out of 11 farms tested positive for TiLV in 2017, and two out of seven tested positive in 2019. Investigation of asymptomatic broodstock collected from 16 tilapia hatcheries revealed that six hatcheries tested positive for TiLV. Representative samples subjected to histopathology confirmed pathognomonic lesions of syncytial hepatitis. We recovered three complete genomes of TiLV from infected fish, one from 2017 and two from 2019. Phylogenetic analyses based on both the concatenated coding sequences of 10 segments and only segment 1 consistently revealed that Bangladeshi TiLV isolates formed a unique cluster within Thai clade, suggesting a close genetic relation. In summary, this study revealed the circulation of TiLV in 10 farms and six hatcheries located in eight districts of Bangladesh. We recommend continuing TiLV-targeted surveillance efforts to identify contaminated sources to minimize the countrywide spread and severity of TiLV infection.

Keywords

Bangladesh, disease surveillance, genome, Nile tilapia, TiLV

4.2 Introduction

Tilapia, which comprises more than 100 species, is the second-most important group of farmed fish worldwide, after carp, and is considered to be among the most significant fish species to meet the rising global demand for protein, vitamin and mineral sources (Ng and Romano, 2013; Amal et al., 2018). Tilapia is farmed in over 135 countries, with global production estimated at 6.5 million metric tons (MMT) (FAO, 2017a). In 2015, the top four producers were China (1.78 MMT), Indonesia (1.11 MMT), Egypt (0.88 MMT) and Bangladesh (0.32 MMT) (FAO, 2017a). Overall, Bangladesh ranks fifth in the world in aquaculture production (FAO, 2017a) and has a total annual fisheries production of 41.34 MMT, of which aquaculture

contributes 56.44% (DOF, 2018). The country earns a significant amount of foreign exchange through exporting fish, shrimps and other aquatic animal products from the fisheries sector. In 2017–2018, Bangladesh earned USD 513 million by exporting nearly 68,940 t of fish and fishery products (DOF, 2018). For the last 10 years, the annual growth of the aquaculture sector averaged 5.43% (DOF, 2018). Moreover, tilapia makes up 10.62% of total production and ranks second in the country, after channel catfish (DoF, 2018).

Shortly after the first report of a novel disease among tilapia in Ecuador (Ferguson et al., 2014), tilapia lake virus (TiLV) was discovered as a newly emerging virus that caused mass die-offs in tilapia in Israel (Eyngor et al., 2014). Molecular analyses indicated that the same virus, TiLV, was the causative agent of these unusual mortality events in both Ecuador and Israel (Bacharach et al., 2016; Del-Pozo et al., 2016).

TiLV is an enveloped, negative sense, single-stranded RNA virus that contains 10 genome segments, with a total genome size of 10.323 kb, ranging from 465 to 1,641 bp for each of the 10 segments (Eyngor et al., 2014; Bacharach et al., 2016). The virus was initially classified as a novel *Orthomyxo*-like virus and is now officially classified as *Tilapia tilapinevirus*, the only species in the genus *Tilapinevirus*, under the new family *Amnoonviridae* (Bacharach et al., 2019). TiLV is recognized as a significant infectious agent that may threaten the development of the global tilapia industry (Bacharach et al., 2016; Jansen et al., 2018). TiLV outbreaks purportedly caused mortality in the range of 20% to 90% (Dong et al., 2017a; Surachetpong et al., 2017; Jansen et al., 2018). To date, TiLV has been detected across Asia, Africa, and North and South America in 16 tilapia producing countries: Ecuador, Israel, Colombia, Thailand, Uganda, the United Republic of Tanzania, Egypt, India, Indonesia, Chinese Taipei, the Philippines, Malaysia, Peru, Mexico, United States and Bangladesh (Jansen et al., 2018; FAO, 2019).

In early 2017, in response to the rapid spread of TiLV, several international organizations issued a disease advisory (NACA, 2017), a global special alert (FAO, 2017b), a factsheet (CGIAR, 2017) and a pathogen information sheet (OIE, 2017). At that time, over 40 countries, including Bangladesh, were forecasted to have

introduced TiLV through the translocation of live tilapia for aquaculture (Dong et al., 2017b). The scientific community urged tilapia-producing countries to quickly investigate unusual mortality events and initiate TiLV-targeted surveillance to prevent its spread and the resulting negative consequences. For this study in Bangladesh, we carried out a two-year TiLV-targeted surveillance of unusual mortalities in grow-out tilapia farms and asymptomatic broodstock from breeding nuclei (hatcheries). To better understand the origins of TiLV, we sequenced the complete genomes of three Bangladeshi TiLV isolates from 2017 and 2019 and conducted some molecular phylogenetic analyses with isolates from other countries.
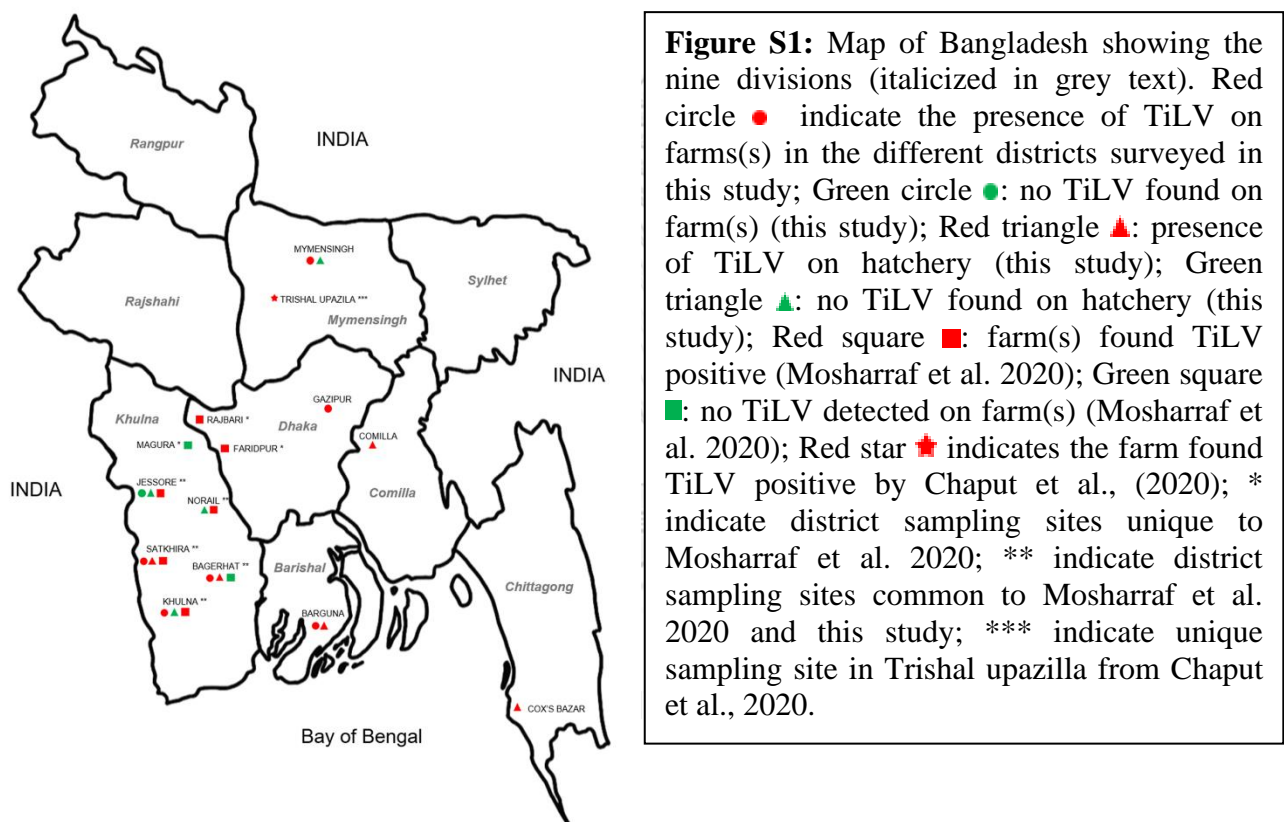
4.3 Materials and methods

4.3.1 Biological sample collection and preservation for reverse transcriptase polymerase chain reaction (RT-PCR) and histology

The use of fish in the study was approved by the National University of Malaysia (Approval no. UKM.PPI.AEC.800-4/3/1). Biological samples were collected from diseased Nile tilapia (*Oreochromis niloticus*) during unusual mortality events reported by farmers from May to December 2017 and July to November 2019. We collected a total of 85 moribund fish from 11 farms in 6 districts in 2017 and 69 moribund fish from 7 farms in 5 districts in 2019 (Table 1). Each fish was humanely euthanized using an overdose of clove oil (200 ppm). For PCR analysis, pieces of the liver, kidney, spleen and brain were collected as specimens from each fish immediately after euthanization and then pooled in RNA (Qiagen, Hilden, Germany) for preservation. In addition to the moribund fish farm samples, we also collected the same tissues from 114 clinically healthy tilapia brood fish from six tilapia hatcheries (H) in 2017 and 307 from 10 tilapia hatcheries in 2019 (Table 1). For histopathology, liver and brain specimens from individual fish were collected and preserved for 24 to 48 hours in neutral buffered formalin (10%), which was then replaced with 70% ethanol.

4.3.2 Total RNA isolation and RT-PCR diagnosis for TiLV

Total RNA from the pooled samples of liver, kidney and spleen was extracted using TRIzol (Invitrogen, Waltham, USA) based on the manufacturer's protocols. RNA

samples were quantified using the Qubit RNA HS Assay Kit with Qubit 4 (Invitrogen). In this study, to detect TiLV we used two different semi-nested RT-PCR protocols. For samples collected in 2017, we used the semi-nested RT-PCR targeting TiLV genome segment 3 as described by Dong et al. (2017a). The primers used were Nested ext.-1 (5′-TAT GCA GTA CTT TCC CTG CC-3′), ME1 (5′-GTT GGG CAC AAG GCA TCC TA-3′) and 7450/150R/ME2 (5′-TAT CAC GTG CGT ACT CGT TCA GT-3′) (Eyngor et al., 2014; Tsofack et al., 2017).

Figure S1: Map of Bangladesh showing the nine divisions (italicized in grey text). Red circle ● indicate the presence of TiLV on farms(s) in the different districts surveyed in this study; Green circle ●: no TiLV found on farm(s) (this study); Red triangle ▲: presence of TiLV on hatchery (this study); Green triangle ▲: no TiLV found on hatchery (this study); Red square ■: farm(s) found TiLV positive (Mosharraf et al. 2020); Green square ■: no TiLV detected on farm(s) (Mosharraf et al. 2020); Red star ✹ indicates the farm found TiLV positive by Chaput et al., (2020); * indicate district sampling sites unique to Mosharraf et al. 2020; ** indicate district sampling sites common to Mosharraf et al. 2020 and this study; *** indicate unique sampling site in Trishal upazilla from Chaput et al., 2020.

PCR mixtures and thermos cycling conditions were performed according to Dong et al. (2017b). As a positive control, we used a recombinant plasmid containing a 415 bp fragment of the TiLV genome segment 3 (pGEM-415_bp) (Dong et al., 2017a) and nuclease-free water as the negative control. Expected amplicon sizes from the first and nested amplification were 415 bp and 250 bp respectively.

Regarding samples collected in 2019, we used a newly published semi-nested RT-PCR targeting genome segment 1 of TiLV (Taengphu et al., 2020). This method is

highly specific for TiLV and is reported to be 100 times more sensitive than previous protocols. The primers used were TiLV/nSeg1F; 5′- TCT GAT CTA TAG TGT CTG GGC C-3′, TiLV/nSeg1R; 5′- AGT CAT GCT CGC TTA CAT GGT-3′, and TiLV/nSeg1RN; 5′- CCA CTT GTG ACT CTG AAA CAG -3′. PCR mixtures and thermocycling conditions were carried out according to Taengphu et al. (2020). This time, for the positive control, we used a plasmid containing a 620 bp fragment of the partial TiLV genome segment 1 (Taengphu et al., 2020) and a nuclease-free water template as the negative control. Expected amplicon sizes from the first and nested amplification reactions were 620 and 274 bp respectively. PCR products were electrophoresed in 1.5% agarose gel and stained with a SYBR Safe DNA gel stain before visualization under a gel documentation system (Maestrogen Inc, Model: SML-01, Hsinchu, Taiwan). To confirm the specificity of our RT-PCR detection results, we sequenced representative amplicons from 11 samples that tested positive for TiLV by Sanger sequencing (Table 1). Sequence identity with the prototype strain of TiLV (KU751816) was determined by nucleotide blast (https://blast.ncbi.nlm.nih.gov).

4.3.3 Sample selection for histopathology

Representative samples of moribund fish (n = 6) that were collected from two affected farms in 2019 tested positive for TiLV by RT-PCR (Table 1). These were investigated histopathologically to confirm pathognomonic lesions of TiLV infection. The specimens were dehydrated, embedded in paraffin, sectioned at a thickness of 5 μm, stained with hematoxylin and eosin, and scrutinized underneath a light microscope.

4.3.4 Amplification of 10 genomic segments of TiLV

Three heavily infected samples (one collected in 2017 and two collected in 2019) were used for the recovery of the 10-genome segments of TiLV. Ten primer sets targeting putative open reading frames of 10 segments were used for RT-PCR amplification as previously described by Pulido et al. (2019). Amplified DNA products were gel-purified using the FavorPrep GEL/PCR purification kit (Favorgen, Pingtung, Taiwan). The purified DNA fragments were then ligated into the pGEM-T-easy vector (Promega, Madison, USA). The recombinant plasmids containing the target DNA fragment (verified by colony PCR using vector primers) were sequenced

Table 1. Details of Nile tilapia samples collected and diagnostic results in this study

Month	Farm/ Hatchery	District	% Mortality level§	Number of TiLV positive/ tested samples	Number of sample sequenced	% Identity to the prototype strain
					Samples collected in 2017	
May	F1	Khulna	~90	2/12 (16.7%)	2 (250 bp)	97.6-98.0%
June	F2	Mymenshing	~50	2/12 (16.7%)	ND	
June	F3	Mymenshing	~10	0/3		
July	F4	Jessore	~10	0/6		
July	H-1	Bagerhat	0	3/33* (9.1%)	2 (250 bp)	97.2-98.0%
July	H-2	Jessore	0	0/10*	ND	
July	H-3	Norail	0	0/18*		
July	H-4	Mymenshing	0	0/19*		
July	H-5	Mymenshing	0	0/16*		
August	F5	Satkhira	~50	5/10 (50.0%)		
September	F6#	Bagerhat	~50	13/15 (86.7%)	5 (415 bp) 2 (250 bp)	95.0-98.0% 98.0%
September	F7	Bagerhat	~10	0/3	ND	
September	F8	Barguna	~50	2/2 (100%)		
September	F9	Satkhira	~30	2/8 (25.0%)		
September	F10	Satkhira	~50	1/5 (25.0%)		
October	F11	Khulna	~10	6/9 (66.7%)		
October	H-6	Barguna	0	12/18* (66.7%)		
					Sample collected in 2019	
January	H-7	Khulna	0	0/25		
January	H-8	Satkhira	0	10/27* (37.0%)	ND	
January	H-9	Jessore	0	0/30*		
January	H-10	Jessore	0	0/30*		
January	H-11	Mymenshing	0	0/29*		
January	H-12	Mymenshing	0	0/30*		
January	H-13	Cox's Bazar	0	4/30* (13.3%)		
February	H-14	Comilla	0	3/30* (10.0%)		
April	H-15	Bagerhat	0	0/59*		
July	F12	Satkhira	~10	0/9		
August	F13	Khulna	~5	0/10		
September	F14	Jessore	~10	0/10		
October	H-16	Bagerhat	0	2/17* (11.8%)		
November	F15	Mymenshing	~10	0/10		
November	F16#	Gazipur	~40	3/10 (30.0%)		
November	F17#	Gazipur	~25	3/10 (30.0%)		
November	F18	Satkhira	~5	0/10		

F, farm; H-, hatchery; *clinically healthy fish; §estimate by farm owner; #farms having samples subjected for 10 genome segments sequencing; ND, not done.

by Macrogen, South Korea, using T7 and SP6 primers. The obtained sequences were assembled and the vector sequence removed using Geneious software (Biomatters, Inc, Auckland, New Zealand). The identity of nucleotide and amino acid sequences were determined by Blastn and Blastp, respectively, to the GenBank database.

Table S1: Sources of TiLV sequences used for genetic analysis for this study

No	Code	Accession	Fish	Country, year	Reference
Sequences of ten genome segments (S1-S10)					
1	IL-2011-Til-4	KU751814-823	NT	Israel, 2011	Bacharach et al., 2016
2	IL-2012-AD-16	KU552131-142	HT	Israel, 2012	NCBI
3	EC-2012	MK392372-381	NT	Ecuador, 2012	Subramaniam et al., 2019
4	PE-2018-F3-4	MK425010-019	NT	Peru, 2018	Pulido et al., 2019
5	TH-2016-TV1	KX631921-930	RT	Thailand, 2016	Surachetpong et al. 2017
6	USA-2019- WVL19054	MN193523-532	NT	USA, 2019	Ahasan et al., 2020
7	USA-2019-WVL19031-	MN193513-522	NT	USA, 2019	Ahasan et al., 2020
8	TH-2018- WVL18053-	MH319378-387	NT	Thailand, 2018	Ahasan et al., 2020
9	BD-2017	MN939372-381	NT	Bangladesh,	Chaput et al., 2020
10	BD-2017-181	MT466437-446	NT	Bangladesh,	This study
11	BD-2019-E1	MT466447-456	NT	Bangladesh,	This study
12	BD-2019-E3	MT466457-466	NT	Bangladesh,	This study
Sequences of genome segment 1 (S1)					
13	TH-2013	MN687685	NT	Thailand, 2013	Taengphu et al., 2020
14	TH-2014	MN687695	NT	Thailand, 2014	Taengphu et al., 2020
15	TH-2015	MN687705	NT	Thailand, 2015	Taengphu et al., 2020
16	TH-2016-CN	MN687725	RT	Thailand, 2016	Taengphu et al., 2020
17	TH-2016-CU	MN687715	NT	Thailand, 2016	Taengphu et al., 2020
18	TH-2017	MN687735	NT	Thailand, 2017	Taengphu et al., 2020
19	TH-2018-K	MN687755	NT	Thailand, 2018	Taengphu et al., 2020
20	TH-2018-N	MN687745	RT	Thailand, 2019	Taengphu et al., 2020
21	TH-2019	MN687765	NT	Thailand, 2019	Taengphu et al., 2020
22	TH-2015-TV2	KX631931	RT	Thailand, 2015	Surachetpong et al., 2017
23	TH-2016-TV3	KX631932	RT	Thailand, 2016	Surachetpong et al., 2017
24	TH-2016-TV4	KX631933	RT	Thailand, 2016	Surachetpong et al., 2017
25	TH-2016-TV5	KX631934	RT	Thailand, 2016	Surachetpong et al., 2017
26	TH-2016-TV6	KX631935	RT	Thailand, 2016	Surachetpong et al., 2017
27	TH-2016-TV7	KX631936	NT	Thailand, 2016	Surachetpong et al., 2017
28	TH-2016-NBC02	MN602587	NT	Thailand, 2016	Ahasan et al., 2020
29	TH-2016-NBC03	MN602588	NT	Thailand, 2016	Ahasan et al., 2020
30	TH-2016-NBC06	MN602589	NT	Thailand, 2016	Ahasan et al., 2020
31	TH-2016-NBC04	MN602590	NT	Thailand, 2016	Ahasan et al., 2020

4.3.5 Phylogenetic analysis

Previous studies suggested using genome segment 1 and concatenated 10 genome segments for phylogenetic analysis of TiLV (Pulido et al., 2019; Taengphu et al.,

2020; Chaput et al., 2020). For this analysis, we used nine publicly available complete genomes of TiLV (GenBank) that originated from farmed tilapia in Israel (two), Ecuador (one), Peru (one), Thailand (two), United States (two) and Bangladesh (one) as well as three newly sequenced genomes from Bangladesh (this study, Table S1). We created 12 concatenated genomes, each with 10 coding fragments and 9,052 bp long. Following multiple sequence alignments, using the MEGAX 10.1.7 program, we constructed a maximum likelihood phylogenetic tree with the best DNA model TN93+G and bootstrap of 1,000 replicates. Similarly, we also conducted a phylogenetic analysis on genome segment 1 sequences of 31 TiLV isolates (three from this study and 28 from GenBank) (Table S1) using the best DNA model K2+G for this dataset.

4.4 Results

4.4.1 TiLV-targeted surveillance revealed its involvement in several unusual mortality events in multiple districts of Bangladesh

Molecular testing

Among the 18 sampled grow-out farms from 6 districts, 8 out of 11 farms from 5 districts tested positive for TiLV by semi-nested RT-PCR in 2017 and 2 out of 7 farms from 1 district in 2019. The percentage of positive tests for TiLV ranged from 16.7% to 100% (Table 1). To confirm RT-PCR detection results, nine representative amplicons (250 and 415 bp) from two farms that tested positive for TiLV in 2017 were chosen for sequencing (F1 and F6, Table 1). Results revealed 95% to 98% nucleotide identity to genome segment 3 of the TiLV prototype strain (KU751816). For the two farms that tested positive for TiLV in 2019 (F16 and F17, Table 1), we also have the complete TiLV genomic sequences of the 10 segments together with one sample from farm 6 (F6, Table 1) in 2017 (more information below).

Clinical signs and histopathology

From the mortality cases in 2017, we observed several major clinical signs: loss of appetite; hemorrhagic skin; lesions on the surface of the body, such as the skin, eyes, operculum and anus; rotten gills, fins and tails; swollen abdomen; red spot-on fins and tail; and fish floating at the surface of the water and swimming erratically (e.g.,

swirling). Similarly, the most prominent clinical signs observed from diseased fish collected from farms in 2019 were scale protrusion, hemorrhagic skin, pop eye, and lesions, including big open wounds in the muscle (Supplemental Figure S2). Mortality on farms with TiLV PCR-positive fish ranged from 50% to 90% in 2017 and 25% to 40% in 2019.
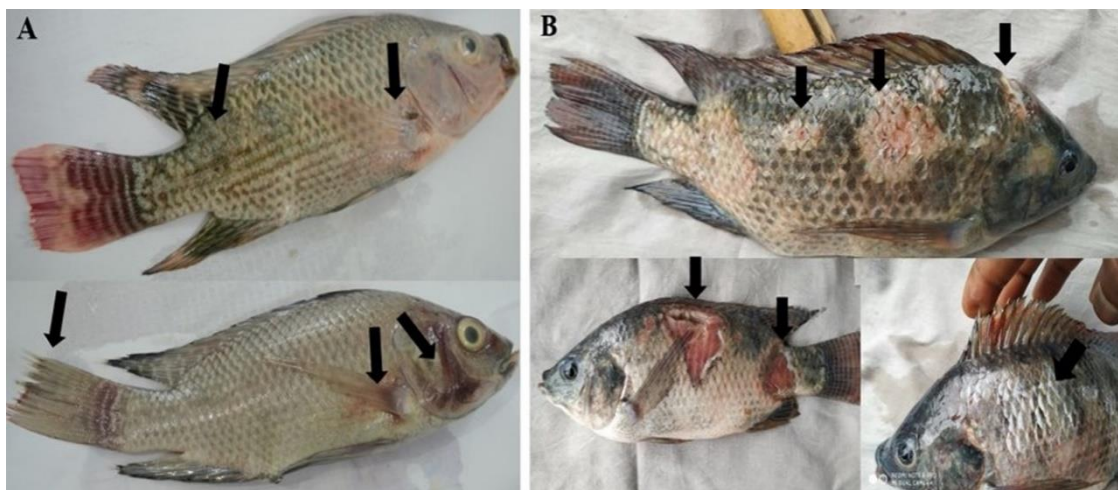

Figure S2: Major clinical sign observed from diseases affected farmed Nile tilapia in 2017 and 2019; (A) lesions on body surface, pectoral fin bottom and hemorrhagic opercula and tail rot from TiLV infected fish of 2017, (B) Scale protrusion and severe lesion on body surface in TiLV positive Nile tilapia of 2019

Histopathological examination of fish tissues collected from farms F16 and F17 in 2019 confirmed the presence of syncytial hepatitis, a typical pathognomonic lesion of TiLV (Figure 1). Two out of six tested samples from Gazipur in November 2019 showed typical syncytial giant cells with multiple nuclei and intracytoplasmic inclusion bodies in the liver, while severe infiltration of lymphocytic inflammatory cells and syncytium formation cells were observed in the brain. The remaining four samples from Gazipur showed severe hepatocyte degeneration and inflammation in both the liver and brain (figures not shown).
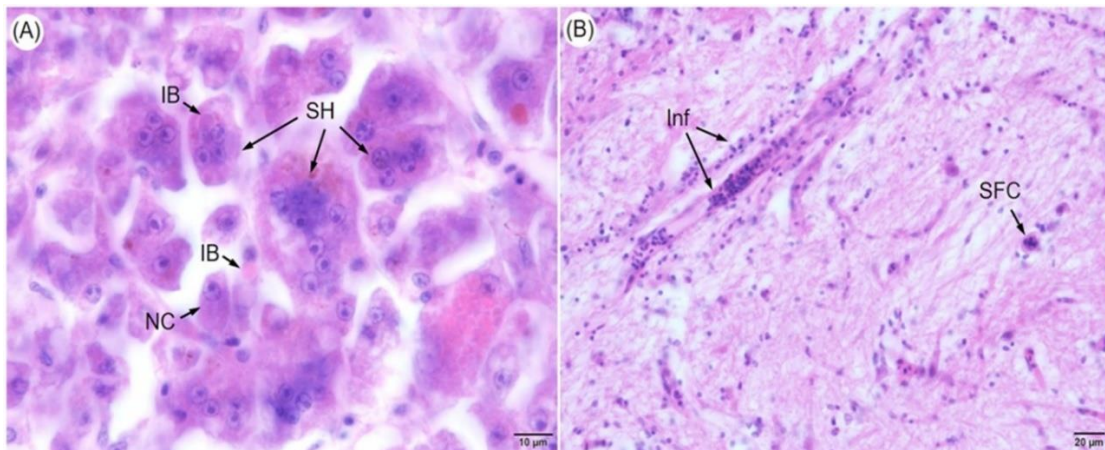
Figure 1 Photomicrographs of H&E stained sections of the liver (A) and brain (B) of the fish tested positive for TiLV by PCR. SH, syncytial hepatocytes; IB, intracytoplasmic inclusion body; Inf, infiltration of lymphocytic inflammatory cells; SFC, syncytium formation cell; NC, normal cell.

4.4.2 Asymptomatic infection detected in tilapia broodstock from breeding nuclei

To assess the potential sources of TiLV, we also surveyed 16 tilapia hatcheries. In 2017, surprisingly 18 of 114 asymptomatic broodstock samples from two out of six hatcheries (H-1 and H-6, Table 1) tested PCR positive for TiLV, with a prevalence of 9.1% and 66.7%, respectively. Similarly, 19 of 307 asymptomatic broodstock samples from four of 10 hatcheries tested positive in 2019. Sequencing of two representative amplicons (250 bp) from two PCR-positive samples of 2017 revealed 97.2% to 98% nucleotide identity to the prototype strain from Israel (KU751816) (Table 1).

4.4.3 TiLV from Bangladesh are genetically close to the Thai isolates

We successfully amplified and sequenced the complete coding region of the 10 TiLV genome segments of three TiLV isolates retrieved from three infected fish (one from 2017 and two from 2019). Sequences can be accessed on GenBank under accession numbers MT466437–MT466466. Table 2 shows the percentage comparison identity of nucleotide and amino acid sequences between the Bangladeshi isolates with the reference strains from Israel (Til-4-201) and Thailand (TV1). Based on the concatenated 10 genomes segments of three isolates from this study and nine publicly available isolates, the maximum-likelihood phylogenetic analysis revealed that the 12 TiLV isolates clustered in two distinct clades, namely Israeli and Thai (Figure 2). While the Israeli clade is comprised of four isolates from Israel, one from Ecuador

and one from Peru, the Thai clade is formed by a cluster of two isolates from Thailand, two from United States and four from Bangladesh (Chaput et al., 2020). All four isolates from Bangladesh (three from this study and one from a previous study) form a small, unique cluster that shares the same ancestral node with another cluster formed by two American isolates. On the other hand, the phylogenetic tree we constructed based on the sequencing of TiLV genome segment 1 revealed that all 31 sequences clustered in three diversified clades, namely Israeli 2011, Israeli 2012 and Thai (Figure 3). The Bangladesh TiLV sequences still nested together to form a similar cluster within the Thai clade.
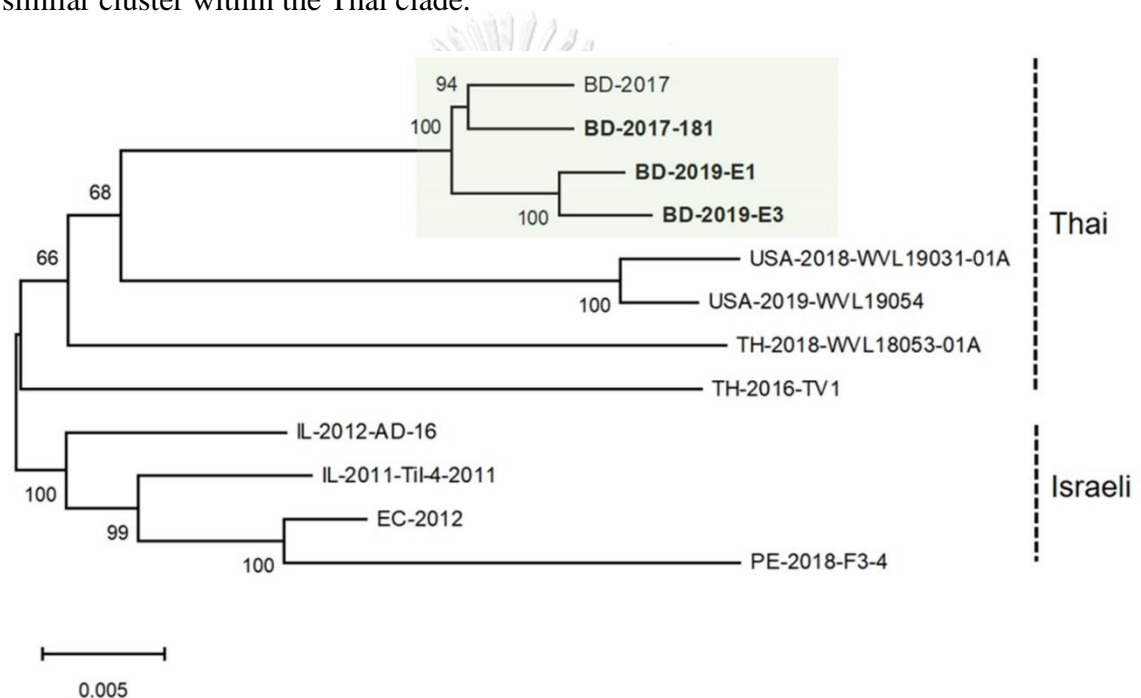
Figure 2 Maximum likelihood phylogenetic tree of the concatenated coding fragments of 10 TiLV genome segments using the best DNA model TN93+G. Bold codes represent three isolates obtained from this study in Bangladesh. Bootstrap values were performed using 1000 replicates. Scale bar shows number of substitutions per site.
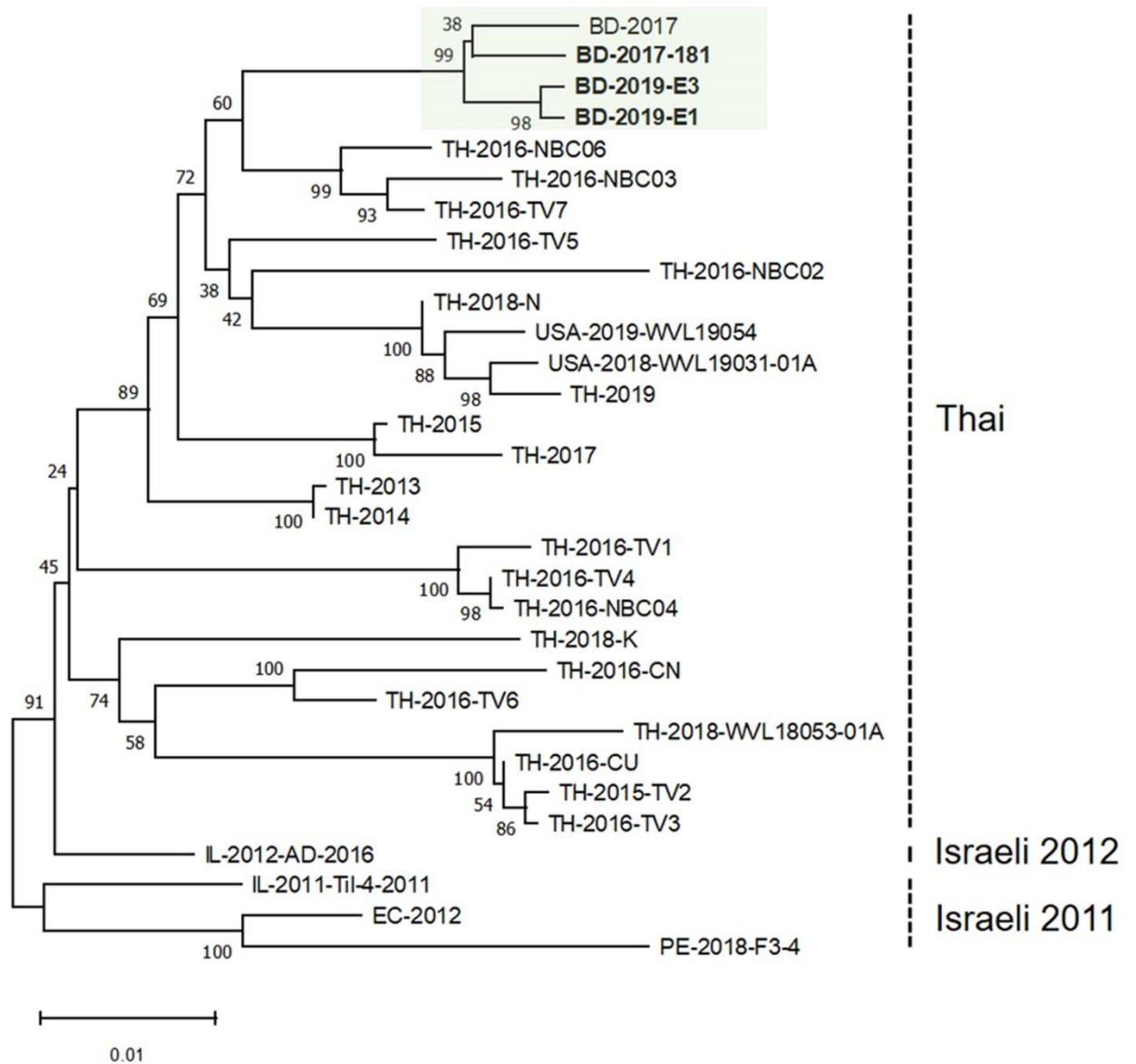
Figure 3 Maximum likelihood phylogenetic tree of the coding region of TiLV genome segment 1 using the best DNA model K2+G. Bold codes represent three isolates obtained from this study in Bangladesh. Bootstrap values were performed using 1000 replicates. Scale bar shows number of substitutions per site.

Table 2 Comparison of nucleotide and amino acid sequences of Bangladeshi TiLV
isolates to Israeli and Thai TiLV strains

Segment (S)/isolates	% Identity with the prototype Israeli strain (Til-4-2011)		% Identity with the Thai strain (TV1)	
	nt	aa	nt	aa
S1 BD-2017-181	96.03	98.27	95.32	98.65
BD-2019-E1	95.96	98.65	95.38	99.04
BD-2019-E3	95.96	98.65	95.38	99.04
S2 BD-2017-181	97.31	98.91	96.05	98.68
BD-2019-E1	97.09	99.12	95.69	98.90
BD-2019-E3	96.29	98.91	94.88	98.68
S3 BD-2017-181	96.98	99.05	95.16	98.57
BD-2019-E1	96.90	98.81	94.92	98.33
BD-2019-E3	97.06	99.52	95.08	99.05
S4 BD-2017-181	96.62	97.74	96.71	98.59
BD-2019-E1	97.09	98.31	97.37	99.15
BD-2019-E3	97.00	95.77	97.28	98.93
S5 BD-2017-181	97.48	99.13	95.41	98.24
BD-2019-E1	97.19	98.54	95.50	98.24
BD-2019-E3	97.19	98.83	95.31	97.94
S6 BD-2017-181	95.81	96.21	94.34	95.90
BD-2019-E1	95.39	96.85	94.13	96.53
BD-2019-E3	94.97	96.53	93.71	96.21
S7 BD-2017-181	96.43	98.97	95.41	98.46
BD-2019-E1	96.09	98.46	95.75	97.95
BD-2019-E3	96.26	98.46	95.92	97.95
S8 BD-2017-181	98.29	98.28	98.48	98.85
BD-2019-E1	98.29	99.43	98.48	100
BD-2019-E3	98.67	99.43	98.86	100
S9 BD-2017-181	97.72	96.55	98.29	97.41
BD-2019-E1	97.72	96.55	98.29	97.41
BD-2019-E3	97.44	95.69	98.01	96.55
S10 BD-2017-181	98.83	99.12	98.54	98.23
BD-2019-E1	99.12	100	98.83	99.12
BD-2019-E3	99.12	100	98.83	99.12

4.5 Discussion

Since TiLV was first isolated and characterized in 2014, there have been multiple warnings and reports describing this new *Tilapinevirus* as an emerging threat to the tilapia industry globally (FAO, 2017b; OIE, 2017; CGIAR, 2017; Dong et al., 2017b). Farming of tilapia has been reported in almost all 64 districts of Bangladesh (DOF, 2018). In this study, we conducted a TiLV-targeted surveillance of the virus in Bangladesh targeting unusual tilapia mortality events reported by farmers on their farms and the potential circulation of the virus among tilapia broodstocks in hatcheries.

Our findings from 2017 and 2019 revealed the presence of TiLV associated with unusual mortality events from 10 grow-out farms in six districts (Barguna, Bagerhat, Gazipur, Khulna, Mymensingh and Satkhira) and in asymptomatic infected broodstock from five hatcheries in five districts (Bagerhat, Barguna, Comilla, Cox's Bazar and Satkhira) (Supplemental Figure S1). Unbeknownst to us, TiLV investigations were also carried out by other research groups, resulting in two recent peer-reviewed publications. The first one, by Mosharraf et al. (2020), detected TiLV in mortality events from seven districts (Faridpur, Magura, Rajbari, Norail, Jessore, Khulna and Satkhira) while the second publication, by Chaput et al. (2020), identified TiLV from 2017 outbreaks in Mymensingh district (Supplementary Figure S1). Norail, Khulna, Satkhira and Mymensingh are areas that the present study had in common with these two studies. The results from these three studies in Bangladesh (from 2017 to 2019) revealed the presence of TiLV in 13 of 64 districts. In the absence of a sufficient adequate response to contain the infected areas and source of origins, this problem may persist and continue to affect new areas nationwide. There is urgency for effective strategies to control the movement of live fish from infected areas to non-infected districts to minimize further spread.

Interestingly, we found that farms experiencing unusual mortality had relatively low prevalence of fish testing positive for TiLV (less than 50% in most cases). This suggests the possible contribution of other causes to the mortality events (e.g. co-infection with other pathogens, poor water quality/feed/management practices, lack of aeration, overstocking). Awareness among farmers on the potential seriousness of

TiLV was minimal or non-existent in 2017, and this allowed mortalities to progress in farms over a longer period, leading to 50%–90% overall mortality. In 2019, farmers started using partial harvests as a strategy to minimize the impact of TiLV, and this resulted in lower reported mortalities. Recent findings from affected farms in Bangladesh identified some co-infections between TiLV, ectoparasites and bacteria (*Streptococcus* and *Aeromonas* sp.) (Mosharraf et al., 2020). Similarly, natural co-infections of TiLV and other pathogens were reported in previous disease outbreaks in Malaysia and Thailand (Surachetpong et al., 2017; Amal et al., 2018). In an experimental coinfection challenge model among tilapia, Nicholson et al. (2020) described the synergistic effects of TiLV and *Aeromonas hydrophila* resulting in higher mortality.

Most TiLV-associated outbreaks from this study occurred between July and November in both 2017 and 2019. These months are known for experiencing high temperature fluctuations caused by sudden heavy rainfall (Bangladesh Meteorological Department, 2020). These can cause stressful conditions for the fish, making them more susceptible to ubiquitous bacteria, such as the freshwater motile aeromonads. While we did not look for co-infections, it may explain the higher levels of mortality observed in those affected farms that tested positive for TiLV.

None of the earlier investigations of TiLV in Bangladesh (Mosharraf et al., 2020; Chaput et al., 2020) looked at TiLV in tilapia broodstock from hatcheries. Warnings about the risk of the transboundary spread of the virus across borders as a result of vertical transmission was reported in early 2017 following the detection of the virus in fertilized eggs, fry and fingerlings in tilapia hatcheries (Dong et al., 2017b). Further evidence supported this potential vertical route of transmission (Yamkasem et al., 2019). Our results showed the presence of TiLV in asymptomatic broodstock from several Bangladeshi tilapia hatcheries. This raises a significant concern of the virus being transmitted from broodstock to progeny at the hatcheries level and to small-scale farmers after dissemination. If not controlled, disseminating infected seeds will spread the virus to thousands of small-scale grow-out farms nationwide.

A follow-up study, led by our team, suggested that the virus caused systemic infection in experimentally infected broodstock. As further support for this hypothesis, it was

found that the virus infected the reproductive organs of these fish and was then passed on to the fertilized eggs (Dong et al., 2020). All these findings point toward strict recommendation to avoid, at all costs, the use of TiLV infected broodstock for production of tilapia fry (Yamkasem et al., 2019; Dong et al., 2020). Therefore, we strongly urge that immediate actions be taken by the relevant authorities and various aquaculture stakeholders to develop systematic testing protocols and a certification system for tilapia hatcheries as well as the implementation of proper biosecurity to ensure that tilapia fingerlings are produced from reliable sources of TiLV-negative broodstock.

Chaput et al. (2020) sequenced the first complete genome of TiLV isolated from Bangladesh in 2017 from an infected farm in Mymensingh District (Supplemental Figure S1). The different phylogenetic analyses revealed that their isolate grouped in the same clade with the Thai isolates. Here, we report three new genomes of TiLV from Bangladesh collected during farm outbreaks in September 2017 (Bagerhat District) and November 2019 (Gazipur District). Our results are consistent with Chaput et al. (2020). Our phylogenetic analysis based on the concatenated 10-genome segments also grouped the three new Bangladeshi isolates with the Thai isolates separated from the Israeli clade (Pulido et al., 2019; Chaput et al., 2020). Within the Thai clade, the Bangladeshi TiLV isolates are placed in a small, unique cluster, separated from the American isolates. These results make sense given that the two American isolates reportedly originated from a farm with a history of importing live fish from Thailand (Ahasan et al., 2020). This supports the interpretation that all isolates in this clade share the same origin. This was further supported with a segment 1 phylogenetic analysis, where the two American isolates still nested with other Thai isolates while the four Bangladesh isolates remained in a small, unique cluster within the Thai clade. Taken together, these findings suggest that Bangladesh TiLV appears to be a daughter clone that evolved from the Thai clone.

In summary, our two-year TiLV-targeted surveillance in Bangladesh revealed the circulation of the virus in eight districts in the country. The virus was detected in both grow-out farms experiencing unusual mortality but most importantly in asymptomatic broodstock hatcheries. This suggests the origin of the infection from hatcheries to

farms and warns of the potential for further spread if no control measures are implemented. Phylogenetic analyses suggest that the Bangladeshi TiLV is a daughter clone of the Thai clone. Nevertheless, continued collection of biological samples and collation of basic data on mortality events are needed. These will serve as a baseline for conducting risk-based TiLV surveillance and assessing future socioeconomic impact. Scientifically sound information on TiLV should be distributed to relevant academic institutions and be made available to all stakeholders, including information on the planned surveillance activities, ongoing and future research, and mitigation and control measures. Currently, there is a lack of any systematic TiLV-targeted surveillance systems in place for the Bangladeshi tilapia industry, as well as limited diagnostic investigations or collection of baseline information regarding adverse events. The presence of TiLV highlights the need for developing such systems. This will also aid preparations for future emerging disease problems in Bangladeshi aquaculture in general.

Our study has provided clear evidence for widespread TiLV in Bangladesh. The results of our work have been brought to the attention of national competent authority resulting in the inclusion of TiLV in the national list of diseases for consideration under the national surveillance program. We have produced knowledge products based on better management practices and disseminated them to the industry and authorities to create awareness and build capacity about this emerging pathogen in Bangladesh. These include a TiLV fact sheet in both English and Bangla (CGIAR Research Program on Fish Agri-food Systems, 2017), a tilapia biosecurity training manual (Mohamed and Subasinghe, 2017), a tilapia clinical sign poster (WorldFish, 2019) and TiLV infographics (Mohan and Delamare-Deboutteville, 2019). We are working closely with the national CA in developing and implementing a comprehensive national aquatic animal health strategy.

4.6. References

Ahasan M.S., Keleher W., Giray C., Perry B., Surachetpong W., Nicholson P., Al-Hussinee L., Subramaniam K., Waltzek T.B. (2020). Genomic Characterization of Tilapia Lake Virus Isolates Recovered from Moribund Nile Tilapia (*Oreochromis niloticus*) on a Farm in the United States. *Microbiol Resour Announc, 9*. doi:https://doi.org/10.1128/MRA.01368-19.

Amal, M. N. A., Koh, C. B., Nurliyana, M., Suhaiba, M., Nor-Amalina, Z., Santha, S., . . . Zamri-Saad, M. (2018). A case of natural co-infection of Tilapia Lake Virus and *Aeromonas veronii* in a Malaysian red hybrid tilapia (*Oreochromis niloticus×O. mossambicus*) farm experiencing high mortality. *Aquaculture, 485*, 12-16. doi:https://doi.org/10.1016/j.aquaculture.2017.11.019

Bangladesh Meteorological Department, (2020). Climate report. http://live3.bmd.gov.bd

Bacharach, E., Mishra, N., Briese, T., Zody, M. C., Kembou, J. E. T., Zamostiano, R., . . . Lipkin, W. I. (2016). Characterization of a novel Orthomyxo-like virus causing mass die-offs of tilapia. *MBio, 7*, pp. e00431-00416. doi:10.1128/mBio.00431-16.

Bacharach, E. M., Nischay; Briese, Thomas; Eldar, Avi; Lipkin, W. Ian; Kuhn, Jens H. . (2019). Create one (1) new genusincluding one (1) new species. "Virus Taxonomy: 2018b Release".*International Committee on Taxonomy of Viruses (ICTV).* Retrieved 14 March 2019. https://talk.ictvonline.org/taxonomy/

Behera, B. K., Pradhan, P. K., Swaminathan, T. R., Sood, N., Paria, P., Das, A., . . . Jena, J. K. (2018). Emergence of Tilapia Lake Virus associated with mortalities of farmed Nile Tilapia *Oreochromis niloticus* (Linnaeus 1758) in India. *Aquaculture, 484*, 168-174. doi:10.1016/j.aquaculture.2017.11.025

CGIAR. (2017). Tilapia lake virus (TiLV): What to know and do? Penang, Malaysia: CGIAR Research Program on Fish Agri-food Systems. Factsheet: FISH-2017-03 https://hdl.handle.net/20.500.12348/115.

Chaput, D. L., Bass, D., Alam, M. M., Al Hasan, N., Stentiford, G. D., van Aerle, R., . . . Tyler, C. R. (2020). The Segment Matters: Probable Reassortment of Tilapia Lake Virus (TiLV) Complicates Phylogenetic Analysis and Inference of Geographical Origin of New Isolate from Bangladesh. *Viruses 12,* 258. doi:10.3390/v12030258.

DoF. (2017). Yearbook of Fisheries Statistics of Bangladesh 2016-17. *Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh : Director General, DoF, 2017. Volume 34 : p. 129*

DoF. (2018). Yearbook of Fisheries Statistics of Bangladesh 2017-18. *Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh : Director General, DoF, 2018. Volume 34 : p. 129.*

Dong, H. T., Siriroob, S., Meemetta, W., Santimanawong, W., Gangnonngiw, W., Pirarat, N., . . . Senapin, S. (2017a). Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. *Aquaculture, 476*, 111-118. doi:10.1016/j.aquaculture.2017.04.019

Dong, H. T., Ataguba, G. A., Khunrae, P., Rattanarojpong, T., & Senapin, S. (2017b). Evidence of TiLV infection in tilapia hatcheries from 2012 to 2017 reveals probable global spread of the disease. *Aquaculture, 479*, 579-583. doi:https://doi.org/10.1016/j.aquaculture.2017.06.035

Dong, H.T., Senapin, S., Gangnonngiw, W., Nguyen, V. V., Rodkhum, C., Debnath, P. P., . . . Mohan, C. V. (2020). Experimental infection reveals transmission of tilapia lake virus (TiLV) from tilapia broodstock to their reproductive organs and fertilized eggs. *Aquaculture 515* doi:https://doi.org/10.1016/j.aquaculture.2019.734541

Eyngor, M., Zamostiano, R., Kembou Tsofack, J. E., Berkowitz, A.,Bercovier, H., Tinman, S., … Eldar, A. (2014). Identification of a novel RNA virus lethal to tilapia. *Journal of Clinical Microbiology*, *52*(12), 4137–4146. https://doi.org/10.1128/JCM.00827-14

FAO. (2017a). Food and Agriculture Organization of the United Nations. 2017. Global aquaculture production. http://www.fao.org/fshery/statistics/global-production/en.

FAO. (2017b). Outbreaks of Tilapia lake virus (TiLV) threaten the livelihoods and food security of millions of people dependent on tilapia farming GIEWS Special Alert No: 338 - Global, Food and Agriculture Organization of the United Nations (FAO) http://www.fao.org/giews/.

FAO. (2019). Food and Agriculture Organization of the United Nations. Aquaculture Newsletter. *FAO, 60th*. Retrieved from www.fao.org/fi shery/publications/fan

Fathi, M., Dickson, C., Dickson, M., Leschen, W., Baily, J., Muir, F., . . . Weidmann, M. (2017). Identification of Tilapia Lake Virus in Egypt in Nile tilapia affected by 'summer mortality' syndrome. *Aquaculture, 473*, 430-432. doi:https://doi.org/10.1016/j.aquaculture.2017.03.014

Ferguson, H. W., Kabuusu, R., Beltran, S., Reyes, E., Lince, J. A., & Del Pozo, J. (2014). Syncytial hepatitis of farmed tilapia, *Oreochromis niloticus* (L.): A case report. *Journal of Fish Diseases, 37(6)*, 583–589. doi:https://doi.org/10.1111/jfd.12142

Jansen, M. D., Dong, H. T., & Mohan, C. V. (2018). Tilapia lake virus: a threat to the global tilapia industry? *Reviews in Aquaculture* 1-15. doi:10.1111/raq.12254

Mohamed Din MS and Subasinghe R., (2017). Basic biosecurity manual for tilapia hatchery technicians in Bangladesh. Penang, Malaysia: CGIAR Research Program on Fish Agri-Food Systems. Manual: FISH-2017-10. https://hdl.handle.net/20.500.12348/664

Mohan, C.V. and Delamare-Deboutteville, J., (2019). Tilapia lake virus (TiLV): Putting a global resource at risk. Penang, Malaysia: CGIAR Research Program on Fish Agri-food Systems. Infographic. https://hdl.handle.net/20.500.12348/3816

Mosharraf, H. M. M., Imtiaz, U. M., Monjur, H. M., Islam, H., Al, A., Farjana, N., & Rukaiya, A. (2020). Molecular Detection of Tilapia Lake Virus (TiLV) in

Farmed Mono-sex Nile Tilapia (*Tilapia niloticus*) in Bangladesh. *Asian Journal of Scientific Research, 13 (1),* 67-78. doi:DOI: 10.3923/ajsr.2020.67.78

NACA. (2017). Tilapia Lake Virus (TiLV) – an Emerging Threat to Farmed Tilapia in the Asia-Pacific Region. *Network of Aquaculture Centres in Asia-Pacific.* Retrieved from https://enaca.org/?id=864

Ng, W. K., & Romano, N. (2013). A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Reviews in Aquaculture, 5* pp. 220-254. doi:https://doi.org/10.1111/raq.12014

Nicholson P, M.-o. N., Jaemwimol P, Tattiyapong P, Surachetpong W. (2020). Coinfection of tilapia lake virus and *Aeromonas hydrophila* synergistically increased mortality and worsened the disease severity in tilapia (*Oreochromis* spp.). *Aquaculture, 520* (2020) 734746. Doi: https://doi.org/10.1016/j.aquaculture.2019.734746.

OIE. (2017). Disease notification report 25278, 23/11/2017. [https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&repo rtid=25278].

Pulido L.L.H., C. M. M., Chaparro A.L.H., Dong H.T., Senapin S. (2019). Tilapia lake virus (TiLV) from Peru is genetically close to the Israeli isolates. *Aquaculture, 510,* 61-65. doi: https://doi.org/10.1016/j.aquaculture.2019.04.058

Surachetpong, W., Janetanakit, T., Nonthabenjawan, N., Tattiyapong, P., Sirikanchana, K., & Amonsin, A. (2017). Outbreaks of tilapia lake virus infection, Thailand, 2015–2016. *Emerging Infectious Diseases*, *23*(6), 1031–1033. Doi: https ://doi.org/10.3201/eid23 06.161278.

Taengphu, S., Sangsuriya, P., Phiwsaiya, K., Debnath, P. P., Delamare-Deboutteville, J., Mohan, C. V., . . . Senapin, S. (2020). Genetic diversity of tilapia lake virus genome segment 1 from 2011 to 2019 and a newly validated semi-nested RT-PCR method. *Aquaculture 526 735423* doi: https://doi.org/10.1016/j.aquaculture.2020.735423

Tsofack, J. E. K., Zamostiano, R., Watted, S., Berkowitz, E., Mishra, N., Briese, T., ... Bacharach, E. (2016). Detection of Tilapia Lake Virus (TiLV) in Clinical Samples by Culturing and Nested RT-PCR. *Journal of Clinical Microbiology, 01808-16*. doi:10.1128/JCM.01808-16

WorldFish (2019). Tilapia major clinical signs. Penang, Malaysia: WorldFish. https://hdl.handle.net/20.500.12348/3522

Yamkasem J, Tattiyapong P, Kamlangdee A, Surachetpong W. (2019). Evidence of potential vertical transmission of tilapia lake virus. *Journal of Fish Diseases, 42*, 1293–1300. doi:DOI: 10.1111/jfd.13050

Chapter 5

Tilapia Lake Virus was not detected in non-tilapine species within tilapia polyculture systems of Bangladesh.

Running Title: Insusceptibility of carps and catfish to TiLV

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5.1 Abstract

Sixteen countries, including Bangladesh, have reported the presence of tilapia lake virus (TiLV), an emerging tilapia pathogen. Fish polyculture is a common farming practice in Bangladesh. Some unusual mortalities reported in species co-cultivated with TiLV-infected tilapia led us to investigate whether any of the co-cultivated species would also test positive for TiLV and whether they were susceptible to TiLV infection under controlled laboratory experiments. Using 183 samples obtained from 15 farms in six districts across Bangladesh, we determined that 20% of the farms tested positive for TiLV in tilapia, while 15 co-cultivated fish species and seven other invertebrates (e.g., insects and crustaceans) considered potential carriers all tested negative. Of the six representative fish species experimentally infected with TiLV, only Nile tilapia showed the typical clinical signs of the disease, with 70% mortality within 12 days. By contrast, four carp species and one catfish species challenged with TiLV showed no signs of TiLV infection. Challenged tilapia were confirmed as TiLV-positive by RT-qPCR, while challenged carp and walking catfish all tested negative. Overall, our field and laboratory findings indicate that species used in polycultures are not susceptible to TiLV. Although current evidence suggests that TiLV is likely host-specific to tilapia, targeted surveillance for TiLV in other fish species in polyculture systems should continue, in order to prepare for a possible future scenario where TiLV mutates and/or adapts to new host(s).

Key words

5.2 Introduction

Tilapia lake virus (TiLV) is an enveloped, negative-sense, single-stranded RNA virus containing 10 genome segments ranging from 465 to 1641 bp, with a total genome size of 10,323 kb (Eyngor et al., 2014; Bacharach et al., 2016). The virus was first classified as a novel orthomyxo-like virus, but has now been classified as *Tilapia tilapinevirus*, the only species in the *Tilapinevirus* genus, and placed in the new *Amnoonviridae* family (Bacharach et al., 2019). TiLV is a highly contagious pathogen

that could jeopardize the growth of the tilapia industry worldwide (Bacharach et al., 2016; Jansen et al., 2019). TiLV outbreaks purportedly cause mortality in the range of 20% to 90% (Dong et al., 2017b; Surachetpong et al., 2017; Jansen et al., 2019). To date, TiLV has been detected and reported across Asia, Africa, and North and South America in 16 tilapia-producing countries: Ecuador, Israel, Colombia, Thailand, Uganda, the United Republic of Tanzania, Egypt, India, Indonesia, Chinese Taipei, the Philippines, Malaysia, Peru, Mexico, the United States, and Bangladesh (FAO, 2019; Jansen et al., 2019; Surachetpong et al., 2020).

In early 2017, in response to the rapid spread of TiLV, several international organizations issued and disseminated disease advisory alerts and information about the virus (CGIAR, 2017; FAO, 2017; NACA, 2017; OIE, 2017). At the time, it was expected that TiLV would have spread through the translocation of live tilapia for aquaculture in over 40 countries, including Bangladesh (Dong et al., 2017c). As fifth largest tilapia producer, since 1954 Bangladesh has been importing seeds and tilapia broodstock from various sources including Malaysia, Thailand, and the Philippines (Rahman, 1985). In Bangladesh, TiLV was first detected from sick Nile tilapia in 2017, but the findings were only published recently (Chaput et al., 2020,; Debnath et al., 2020; Hossain et al., 2020). The results from these three studies in Bangladesh (from 2017 to 2019) revealed the presence of TiLV in 13 of 64 districts. In the absence of adequate hatcheries and farms biosecurity and regular screening of live animals during production and before movement between production sites, TiLV may persist and continue to affect tilapia and, perhaps, new species in new locations across the country (Debnath et al., 2020).

The foremost aquaculture production systems in Bangladesh are extensive, semi-intensive, and small-scale pond-based polyculture systems (Belton & Azad, 2012). Pond polyculture systems in Bangladesh are typically optimized to produce multiple fish species together, generally tilapia, carps, and catfish (Castine et al., 2017). While tilapia production is high in Bangladesh, carp species are the primary culture crop, with tilapia serving as a surplus crop. Carp species accounted for 33.5% of entire aquaculture production (fiscal year 2018-19), with a total production volume of 1.47 million metric tons (DoF, 2019). The total value of carp produced is estimated to be

USD 2.94 billion using an average market price of USD 2/kg for 1 to 1.5 kg/fish. Prominent carp species farmed in Bangladesh include rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhinus cirrhosis*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), bighead carp (*Hypophthalmichthys nobilis*), mohashol (*Tor putitora*), and black carp (*Mylopharyngodon piceus*). Prominent catfish species include striped catfish, locally called pangas. Without further evidences to prove the contrary, TiLV must be considered as a potential threat to Bangladesh polyculture systems, with the virus's possible ability to adapt and spread from tilapia to other non-tilapine species. There are several examples of fish viruses that have jumped from one fish species to another. Infectious pancreatic necrosis virus (IPNV) and nervous necrosis virus (NNV) are notable examples of RNA viruses in fish, whereas the infectious spleen and kidney necrosis virus (ISKNV) is a good example of a DNA virus. IPNV was first isolated from a diseased rainbow trout (*Oncorhynchus mykiss*) fingerling and later discovered worldwide in a wide host range of diseased and non-diseased salmonid/non-salmonid fish species and invertebrates (Hill & Way, 1995; Reno, 1999). Viral nervous necrosis (VNN) caused by *Betanodavirus* was first reported in Australian farmed barramundi (*Lates calcarifer*), and a year later, in turbot (*Scophthalmus maximus*), European sea bass (*Dicentrarchus labrax*), red-spotted grouper (*Epinephelus akaara*), and striped jack (*Pseudocaranx dentex*) (Breuil et al., 1990; Yoshikoshi & Inoue, 1990; Bloch et al., 1991; Mori et al., 1992; Munday et al., 2002;). ISKNV has been detected from both freshwater and euryhaline fish species, including tilapia (*O. niloticus*) and farmed barramundi (*L. calcarifer*) (Suebsing et al,. 2016; Dong et al., 2017d). To date, there are a number of tilapia species known to be susceptible to TiLV, including hybrid tilapia (*O. niloticus* × *O. aureus* hybrids), Nile tilapia (*O. niloticus*), grey tilapia (*O. niloticus* × *O. aureus*), red tilapia (*Oreochromis* sp.), Mozambique tilapia (*O. mossambicus*), mango tilapia (*Sarotherodon galilaeus*), redbelly tilapia (*Tilapia zillii*), blue tilapia(*O. aureus*), and wild tilapia (*Tristamellasimonis intermedia*) (Eyngor et al., 2014; Ferguson et al., 2014; Fathi et al., 2017; Surachetpong et al., 2017; Mugimba et al., 2018; Waiyamitra, et al., 2021). In addition to tilapia, giant gourami (*Osphronemus goramy*) naturally infected with TiLV have been found (Chiamkunakorn et al., 2019) and also shown to be susceptible

to TiLV in an experimental challenge study (Jaemwimol et al., 2018). TiLV has also been identified in wild tinfoil barb (*Barbonymus schwanenfeldii)* in Malaysia (Abdullah et al., 2018) as well as in farmed barramundi (*L. calcarifer*) in Thailand (Piamsomboon & Wongtavatchai, 2021). In Israel, Egypt, and India, there have been no reports of TiLV detected in co-cultivated species during TiLV outbreaks in tilapia (Eyngor et al., 2014; Fathi et al., 2017; Behera et al., 2018). There is still a scarcity of information about the host range of TiLV.

Bangladesh is one of the very few countries where tilapia, carp, and catfish species are produced together by small scale-farmers in semi-intensive, homestead, and backyard ponds. During unusual and unexplained disease outbreaks in species produced in polyculture systems, there is often suspicions among farmers that TiLV might be the cause of the mortalities in species other than tilapia. Here, we investigated if co-cultivated species were TiLV positive in tilapia polyculture farms experiencing abnormal mortalities and also conducted controlled laboratory TiLV experiments with various carp species and walking catfish to assess their susceptibility to the virus. This research was set to investigate the TiLV status and TiLV susceptibility of non-tilapine species co-cultured with tilapia. Monoculture of tilapia is very rare in Bangladesh. Majority of freshwater farming systems undertake polyculture and it is very common to see tilapia raised with carps, catfish, amongst other species. Our preliminary results show that — in a limited number of tilapia polyculture farms experiencing abnormal mortalities and under experimental conditions—non-tilapine species were negative and not susceptible to TiLV. If future evidences point towards susceptibility of carps or catfish species to TiLV, this will have major implications to small-holder Bangladeshi farmers, management and biosecurity risk mitigation for the industry including legislating against polyculture of tilapia. Therefore, pursuing this line of research is very important for the country and also for the national Competent Authorities.

5.3 Materials and Methods

5.3.1 Field Sample Collection and Preservation

The utilization of fish in this investigation was approved by the Animal Care and Use Committee of the National University of Malaysia (approval No. UKM.PPI.AEC.800-

4/3/1). Field samples were collected from 15 polyculture farms from 2017 to 2020, where mortalities for tilapia and other co-cultivated species were documented. A total of 183 samples belonging to 23 species of fish, crustaceans, and insects were collected from 15 polyculture farms (Table S1). Samples of the affected stock included moribund fish, along with crustaceans and insects from the same ponds, while samples of the non-affected stock were clinically healthy fish. The 15 affected farms were located in six districts of Bangladesh, including Cumilla, Chandpur, Chittagong, Jashore, Satkhira, and Gazipur (Table 1). For each fish, we collected and pooled a small piece (approximately, $5 \times 5 \times 5$ mm) of liver, kidney, spleen, and brain. For crabs, snails, and bivalves, a small piece of muscle was collected. For small shrimp, copepod, and insects, whole specimens were taken. All of these tissues were preserved in RNAlater (Qiagen, Hilden, Germany) for reverse transcription-polymerase chain reaction (RT-PCR) analyses.

Table 1. Samples collected from fish farms experiencing abnormal mortalities in six districts of Bangladesh

Date-Month-Year[b]	Farm	Districts	Fish Species (Common Name) [c]	(%) Mortality	# Sample(s) Collected *	# TiLV Positive/# Sample Tested (%)
3 September 2017	Farm 1	Satkhira	Corsula mullet	~5	3	0/3 (0)
			Tilapia	~30	8	2/8 (25)
3 September 2017	Farm 2	Satkhira	Corsula mullet	~10	4	0/4 (0)
			Tilapia	~50	5	1/5 (20)
10 January 2019	Farm 3	Jashore	Gonia	~10	4	0/4 (0)
			Rohu	~10	1	0/1 (0)
			Silver carp	~10	1	0/1 (0)
			Tilapia	~10	4	0/4 (0)
28 January 2019	Farm 4	Satkhira	Rohu	~5	3	0/7 (0)
			Tilapia	5–10	7	0/7 (0)
9 November 2019	Farm 5	Gazipur	Stinging catfish	~25	5	0/5 (0)
			Gulsha	~25	4	0/4 (0)
			Tilapia	~40	10	3/10 (30)
29 September 2020	Farm 6	Cumilla	Common carp	~5	1	0/1 (0)
			Snail [a]	No mortality	2	0/2 (0)
			Tilapia	~50	5	0/5 (0)
30 September 2020	Farm 7	Cumilla	Common carp	~5	2	0/2 (0)
			Rohu	~10	2	0/2 (0)
			Bighead carp [a]	No mortality	2	0/2 (0)
			Silver hatchet chela [a]	No mortality	1	0/1 (0)
			Climbing perch [a]	No mortality	1	0/1 (0)
			Small shrimp [a]	No mortality	1	0/1 (0)
			Crab [a]	No mortality	2	0/2 (0)
			Copepod [a]	No mortality	1	0/1 (0)
			Tilapia	~80	5	0/5 (0)

5.3.2 Experimental Challenge

For the challenge experiment, we used four carp species and one catfish species commonly stocked with tilapia by polyculture farmers in Bangladesh. These were rohu (*L. rohita*), silver carp (*H. molitrix*), mrigal (*Cirrhinus cirrhosus*), mohashol or Putitor mahseer (*Tor khudree*), and walking catfish (*Clarias batrachus*). Nile tilapia (*O. niloticus*) was used as our positive control (Table 2). The number of fish used for each species was 20 for challenge and 20 for control, groups, with the exception of mrigal and walking catfish, which had a lesser number of fish utilized due to a shortage of the required number of fish at the time of the experiment (Table 2). All of the fish utilized in this experiment were of approximately similar size (5±1 cm). All non-tilapia species were sourced from a commercial Thai hatchery that was not linked with any past tilapia seed production. Tilapia were sourced from a known TiLV-negative population. All fish species were shipped to the laboratory in temperature-controlled boxes supplied with oxygen. Upon arrival to the laboratory, all fish were disinfected using 5 parts per thousand (ppt) salt water for 30 min, then left to acclimatize for 2 h in 500 L freshwater holding tanks within a quarantine room. Following the period of disinfection and acclimatization, individual species were stocked in separate 200 L fiberglass tanks with air stone and biological cotton filter units. Cotton filters were exchanged once every three days, while water was replaced with new tap water disinfected with 60 parts per million (ppm) chlorination at the rate of 50%. Prior to the infection trial, the fish were conditioned within their respective tanks for an additional seven days and fed twice daily with a commercial feed containing 28% protein at a rate of 5% body weight. Water quality parameters for the period of the experiment were recorded as were kept as follow: temperature (28128±1) °C, pH 7.6-8.4, dissolve oOxygen 8 mg/L, NH3 < 3 mg/L, and NO2 < 1 mg/L. The original TiLV stock, NV18R, was prepared as previously described (Dong et al., 2020). All fishes were divided into two groups—control and experimental groups—with one replicate tank per species (Table 2). All fish were anesthetized using 100 ppm clove oil before being injected with 0.1 mL TiLV inoculum intraperitoneally at a dose of 10^{-6} $TCID_{50}$ per fish. Control fish were injected with 0.1 mL 1× phosphate-buffered saline (PBS), pH 7.4, and stocked separately. All fish were returned to their original tank and monitored four times per day for the typical clinical

signs of TiLV disease. Any moribund fish was immediately euthanized by overdose with 250 ppm clove oil and small pieces (approximately, $5 \times 5 \times 5$ mm) of liver, kidney, spleen, and brain were collected and pooled for RT-qPCR, as previously described (Debnath et al., 2020). After 21 days post-infection, all remaining surviving fish from both groups were humanely euthanized and subjected to sampling, as described above, for RT-qPCR test.

Table 2. Experimental challenge test results of Nile tilapia, rohu, Tor khudree, silver carp, mrigal, and catfish injected with TiLV isolate (NV18R) at a dose of 10–6 TCID50 per fish in the peritoneal cavity.

Fish Species	Photograph and Body Length (cm)	Group	Number of Fish Used	Mortality	RT-qPCR Test Result (+ve/Tested Samples)	Viral Loads (Copies per Reaction)
Nile tilapia (*Oreochromis niloticus*)		PBS	20	1	0/5	0
		TiLV	20	14	9/10	6.12×10^5 to 2.35×10^8
Rohu (*Labeo rohita*)		PBS	20	0	0/5	0
		TiLV	20	0	0/10	0
mohashol or Putitor mahseer (*Tor khudree*)		PBS	20	0	0/5	0
		TiLV	20	0	0/10	0
Silver carp (*Hypophthalmichthys molitrix*)		PBS	20	0	0/5	0
		TiLV	30	0	0/10	0
Mrigal (*Cirrhinus cirrhosus*)		PBS	15	0	0/5	0
		TiLV	20	0	0/10	0
Walking catfish (*Clarias batrachus*)		PBS	7	0	0/5	0
		TiLV	8	3	0/8	0

5.3.3 Total RNA Isolation and PCR Amplification for Detection of TiLV

Field Samples Tested by Semi-Nested RT-PCR

Following manufacturer's protocol, TRIzol reagent (Invitrogen, Waltham, USA) was used for total RNA extraction of pooled samples of liver, kidney, spleen, and brain for each individual fish, crustacean, copepod, and insect species. All field samples were subjected to semi-nested RT-PCR using TiLV genome segment 1 primers (Taengphu et al., 2020). The primers used were TiLV/nSeg1F: 5′- TCT GAT CTA TAG TGT CTG GGC C-3′; TiLV/nSeg1R: 5′- AGT CAT GCT CGC TTA CAT GGT-3′; and TiLV/nSeg1RN: 5′- CCA CTT GTG ACT CTG AAA CAG -3′. PCR master mix composition and thermocycling conditions were the same as described by Taengphu et al. (2020). A plasmid with a 620 bp fragment of the partial TiLV genome segment 1 (pGEM-620 bp) (Taengphu et al., 2020) was used as the positive control, and nuclease-free water served as the negative control. Expected amplicon sizes from the first and nested reactions were 620 and 274 bp, respectively. The amplified products were electrophoresed in 1.5% agarose gel and stained with ethidium bromide.

Experimentally TiLV-Challenged Animals Tested by RT-qPCR

In addition to TiLV detection from field samples by semi-nested RT-PCR, TiLV in tissue samples from experimentally TiLV-challenged animals was detected using a newly developed quantitative one-step RT-qPCR protocol targeting TiLV genome segment 9 (Taengphu et al. 2021). This method was used as it offers quantifiable results which can describe the potential multiplication of the virus inside fish cells. TaqMan primer sequences for TiLV segment 9 were as follows: forward primer, Seg9-TaqMan-F, 5′-CTA GAC AAT GTT TTC GAT CCA G-3′; reverse primer, Seg9-TaqMan-R, 5′-TTC TGT GTC AGT AAT CTT GAC AG-3′; and probe primer, Seg9-TaqMan-Probe, 5′-6-FAM-TGC CGC CGC AGC ACA AGC TCC A-BHQ-1-3′, with a product size of 137 bp. The one-step RT-qPCR reaction was carried out in a 20 µL volume, which included 10 µL of 2X qScriptTM XLT 1-Step RT-qPCR ToughMix Low ROX (Quanta Bio, Beverly, MA), 0.9 µL each of 10 µM forward primer (450 nM) and 10 µM reverse primer (450 nM), 0.3 µL of 10 µM TaqMan probe (150 nM), 2 µL of RNA template (100 ng/µL), and 5.9 µL of RNase-free water.

Amplification was performed at 50 °C for 10 min, followed by 95 °C for 1 min and 40 cycles at 95 °C for 10 s and then 58 °C for 30 s.

5.4 Results

5.4.1 Field Samples

All investigated polyculture farms (n = 15), from the six considered districts, experienced abnormal mortality in tilapia first, i.e., before other co-cultivated species were affected. Out of 15 co-cultivated fish species and seven other aquatic organisms, only seven species (i.e., corsula mullet, gonia, rohu, silver carp, Asian stinging catfish, gulsha, and common carp) were found to experience abnormal mortalities along with tilapia. Clinical signs observed in moribund fish from affected polyculture farms are shown in Figure 1. Swollen eyes, lesions on body surface, ascitic fluid, scale protrusion, hemorrhagic skin, and loss of appetite are the major clinical signs in tilapia (Figure 1). Mortality in tilapia from all 15 affected farms ranged from 10% to 80%, with mortality in farms testing positive for TiLV (n = 3 farms) ranging from 30% to 50% (Table 1). Clinical signs found in affected co-cultivated species included lesions on the opercula, jaw, and body surface, hemorrhagic skin, and fin rot and tail rot, with mortality ranging from 5% to 40%, depending on the farm and species (Table 1 and Figure 1).
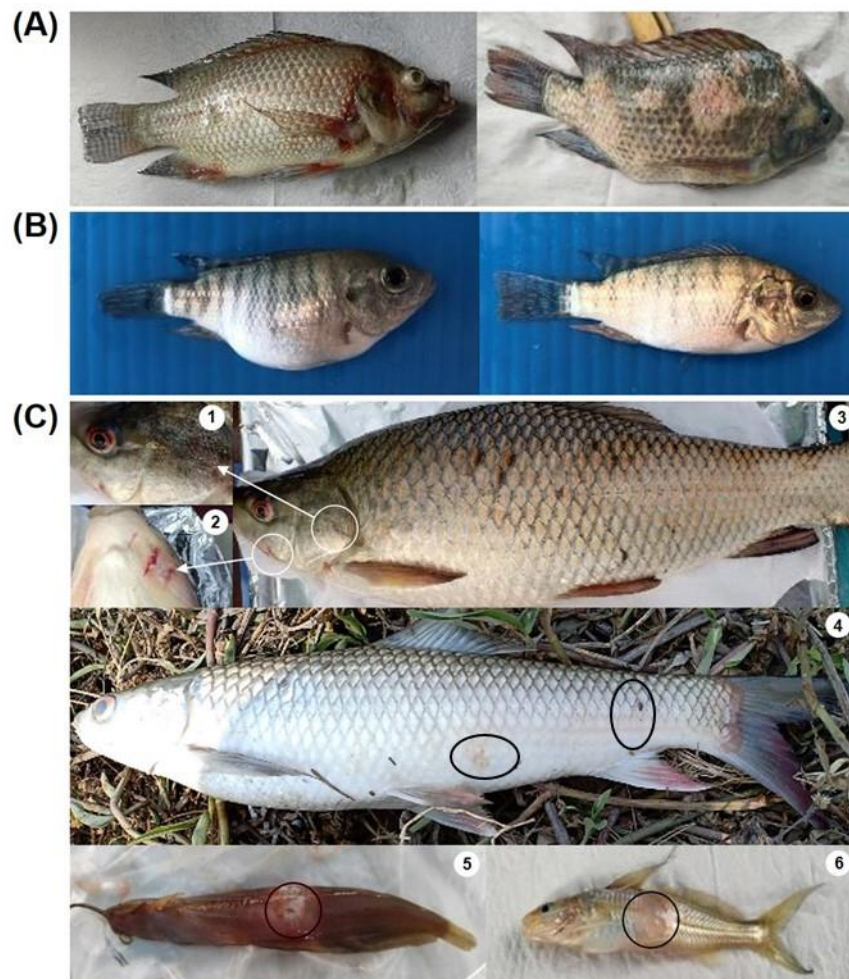
Figure 1. Pictures of the major clinical signs observed in moribund fish from affected polyculture farms and those experimentally challenged with TiLV: (A) field-collected Nile tilapia displaying swollen eyes, body lesions, and hemorrhagic skin; (B) laboratory TiLV-injected tilapia with scale protrusion, swollen eyes, and swollen abdomen; and (C) field-collected co-cultivated species (C1–3: carp, C4: mullet, C5–6: catfish), showing lesions on opercula, jaw, head region, and body surface, as well as fin rot and tails with petechial hemorrhage.

Some co-cultivated species in the affected farms were found to be clinically healthy, with no mortality or clinical signs observed (Table 1). The RT-PCR test results showed that samples from three out of 15 farms tested positive for TiLV, with 20% to 30% of tilapia samples from these farms testing positive (Table 1 and Figure 2). By contrast, 99 non-tilapia samples collected from 15 co-cultivated species and seven other aquatic organism on those same 15 farms were all negative for TiLV (Table 1). Representative test results are shown in Figure 2. TiLV-affected farms were identified in 2017 (n = 2/2) and 2019 (n = 1/3), but all farms sampled in 2020 (n= 10) were

TiLV-negative (Table 1). Within the samples obtained from 2017 to 2020, 7% of tilapia samples (6 out of 84 tilapia samples) were positive for TiLV (Table S2).
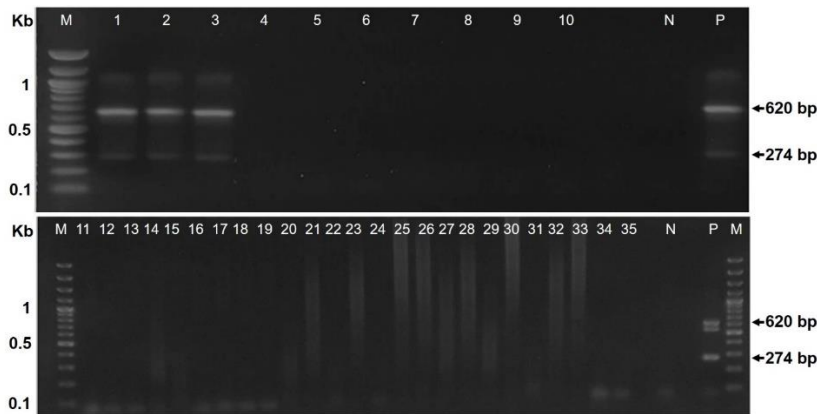

Figure 2. Analysis of 35 RT-PCR products acquired using TiLV semi-nested PCR primers electrophoresed on a 1.5% agarose gel. Lanes 1–10: field samples of tilapia; lanes 11–16: field samples of rohu; lanes 16–20: field samples of *Cyprinus carpio*; lanes 21–26: field samples of silver barb, lanes 27–32: field samples of Pangasius; lanes 32–35: field samples of damselfly larvae. M, DNA marker (New England BioLabs, Hitchin, United Kingdom); P, positive control using RNA extracted from TiLV-infected tilapia as template (note the presence of two bands at 620 and 274 bp); N, no RNA negative control, using nuclease-free water as template.

5.4.2 Carp and Catfish Species are Not Susceptible to TiLV under Experimental Challenge

None of the individual fish from the four carp species in both infected and control groups showed any clinical signs of TiLV disease manifestation, with no mortality observed until 21 days post-infection (DPI), when the experiment was terminated (Table 2). Of the walking catfish that were TiLV challenged, three out of eight individuals (37.5%) died at 13 DPI (Figure 3), with no major clinical signs but observed some injured area in head region (Table 2). None of the control (PBS injected) walking catfish died (Table 2).
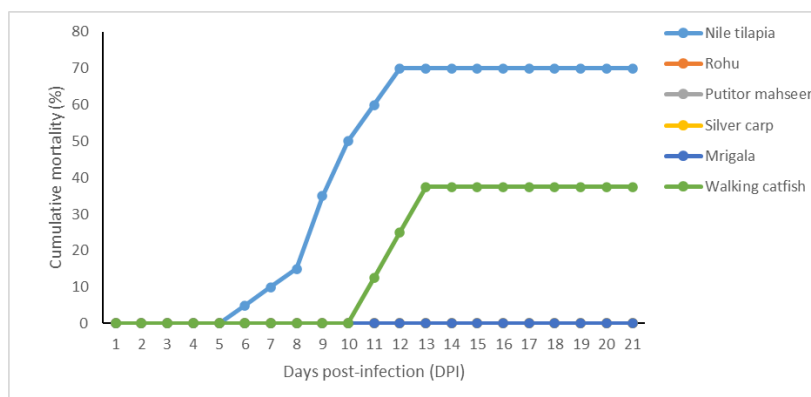
Figure 3. Cumulative mortality rate of all challenged fish species in TiLV challenge experiment. Number of fish used is summarized in Table 2.

However, after 5 DPI, positive control Nile tilapia individuals injected with TiLV started to exhibit clinical signs, including anorexia, lethargy, bilateral exophthalmia, scale protrusion, and abdominal swelling (Figure 1B). Final mortality (70%) in the infected tilapia group started at 6 DPI and continued until 12 DPI (Figure 3). No clinical signs were observed in any of the control tilapia individuals injected with PBS, with only one fish dying at 9 DPI. The RT-qPCR test results from 40 individual samples taken from 40 challenged fish of the four carp species were all TiLV-negative (40 out of 40), and similarly, all 20 individual samples collected from 20 fish of the same four carp species from the control group were also TiLV-negative (20 out of 20) (Figure 4, Table 2).Similarly, all eight walking catfish individuals (including the three dead individuals) from the challenged group and five from the control group were found to be TiLV-negative (Figure 4, Table 2). . Ninety percent (9 out of 10) of the tilapia individuals from the challenged group, where 8 samples were obtained from dead fish and one from surviving fish, were confirmed to be TiLV-positive by RT-qPCR, whereas none (0 out of 5) in the control group (Table 2, Figure 4) tested positive. For each TiLV-positive tilapia sample, the RT-qPCR result revealed a TiLV load of 6.12×10^5 to 2.35×10^8 copies per reaction containing 200 ng RNA template (Table 2).
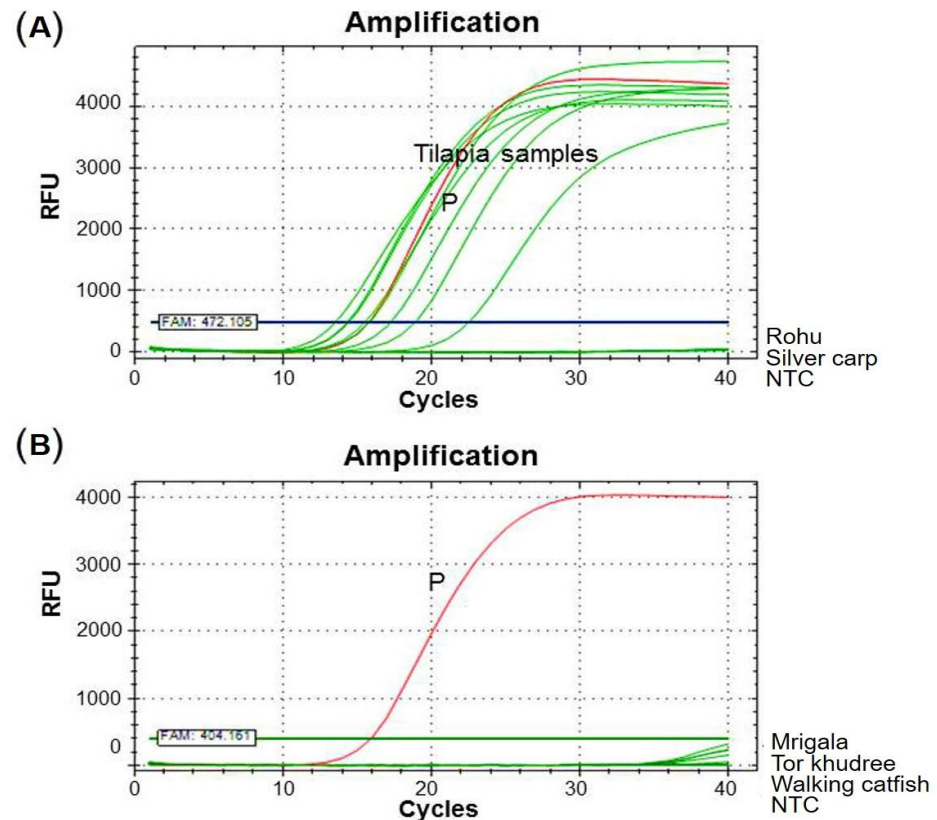
Figure 4. TiLV RT-qPCR results from TiLV experimental challenge samples. A) Detection for TiLV in Nile tilapia, rohu, and silver carp samples of both challenged and control groups. B) Detection in mrigal, Tor khudree, and walking catfish samples. Tested specimen numbers are indicated in Table 2. P, positive control using RNA template extracted from TiLV-infected tilapia; NTC, no template control.

5.5 Discussion

The use of polycultures consisting of multiple species (e.g., tilapia, carp, catfish, shrimp, prawn, and others) is the most common production strategy in Bangladesh, as it is perceived by many farmers to be a strong resilient strategy to reduce production risks (DoF, 2019). Both extensive and semi-intensive homestead to entrepreneur commercial farming practices in Bangladesh follow polyculture farming. The choice of species for polyculture farming depends on the geographical location, water type, seasonality, and market demand. Tilapia has always been considered as a hardy fish, capable of surviving and thriving in sub-optimal conditions but, with the

intensification of its production globally, there have been an increasing number of pathogens shown to infect tilapia, with TiLV being one of them. In Bangladesh, TiLV was first identified in 2017 in tilapia farmed in the district of Mymensingh (Chaput et al., 2020) then in tilapia farmed in six districts (Hossain et al., 2020). Additional cases have been recently reported in five more districts (Bagerhat, Barguna, Cumilla, Cox's Bazar, and Gazipur), in 2017 and 2019 (Debnath et al., 2020).

Among fish-farming communities and the competent authorities of Bangladesh, there is great concern that TiLV could spread to new geographies, not only affecting tilapia but potentially other major economically important co-cultivated species. Tilapia remains the major fish group susceptible to TiLV, while only rare cases have been found in non-tilapia species, such as tinfoil barb (*Puntius schwanenfeldii*) farmed in Malaysia (Abdullah et al., 2018) and giant gourami (*Osphronemus goramy*) and barramundi (*Lates calcarifer*) farmed in Thailand (Chiamkunakorn et al., 2019; Piamsomboon & Wongtavatchai, 2021). Experimental evidence has also been provided that giant gourami (*Osphronemus goramy*) is susceptible to TiLV in response to challenge in a laboratory setting (Jaemwimol et al., 2018). With increased awareness and fear among producers regarding the potential impact of TiLV, increased numbers of abnormal mortality events have been reported by farmers for tilapia, carp, and catfish, but in the absence of proper disease investigation with sample collection for diagnostic purposes, those mortalities tend to be incorrectly attributed to TiLV by farmers, often leading producers to use inadequate chemical and drug treatments.

Both our field and challenge findings revealed no evidence of TiLV infection in co-cultured fish species or other aquatic organism such as crustaceans and insects. During the challenge experiment, no mortality was recorded in carp, while 37.5% mortality was observed in walking catfish; however, the TiLV test results revealed that these samples were TiLV-negative. This unexpected mortality in walking catfish might be attributed to the fact that these species were aggressive and fought each other, leading to the death of some individuals. In Israel, during TiLV outbreaks in tilapia, other co-cultivated species, such as grey mullet (*Mugil cephalus*) and carp (*Cyprinus carpio*), did not show the clinical signs of TiLV with no mortalities

recorded (Eyngor et al., 2014). Similar observations were made in Egypt with co-cultivated grey mullet (*M. cephalus*) and thin-lipped mullet (*Liza ramada*) (Fathi et al., 2017), and in India with co-cultivated Indian major carps, including rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhinus cirrhosus*), milk fish (*Chanos chanos*), and pearl spot (*Etroplus suratensis*) (Behera et al., 2018). Supporting confirmations were found in Indian major carp (rohu), which were shown not to be susceptible to TiLV infection (Pradhan et al., 2020). Additionally, a TiLV experimental challenge in 10 warm-water fish species, including giant gourami (*Osphronemus goramy*), snakeskin gourami (*Trichogaster pectoralis*), iridescent shark (*Pangasianodon hypophthalmus*), walking catfish (*Clarias macrocephalus*), striped snakehead fish (*Channa striata*), climbing perch (*Anabas testudineus*), common carp (*Cyprinus carpio*), silver barb (*Barbodes gonionotus*), Asian sea bass (*Lates calcarifer*), and red hybrid tilapia (*Oreochromis* spp.), showed that all species, apart from giant gourami, were not susceptible to TiLV infection (Jaemwimol et al., 2018). On the other hand, adult zebrafish (*Danio rerio*) and Mozambique Tilapia (*Oreochromis mossambicus*) were found to be TiLV susceptible through experimental challenge (Rakus et. Al., 2020; Waiyamitra et. Al., 2021). Those studies are coherent and in support of our findings of our study: that all the co-cultivated species, along with other aquatic organisms (crustaceans and insects), were unlikely to benot susceptible to TiLV infection. In summary, both our field samples collected from outbreaks on farms in Bangladesh, along with our susceptibility experimental challenge findings, confirmed that those co-cultivated species, together with other aquatic organisms, presumably were not susceptible to TiLV. The lack of viral receptors or factors that allow the virus to enter and proliferate in these fish, crustacean, and insect species may be one of the reasons for them being refractory to TiLV—a hypothesis proposed by Surachetpong et al. (2020). As a result, these species may have a limited probability of becoming TiLV carriers.

While 22 species from tilapia polyculture farms were examined in this study, we acknowledge the limitation in terms of number of samples collected per species and number of TiLV-affected farms. We also targeted only TiLV, while other infectious agents and/or possible environmental factors that may have been associated with the observed mortality were not explored. Most of the farms included in this study came

from districts with previous reports of TiLV. Future disease investigations from affected polyculture farms should first confirm TiLV in tilapia, then test other species present on the farm; this with a sufficient number of samples from each species. Similarly, for future TiLV tests, relevant polyculture species can be challenged with new TiLV isolate retrieved from affected farms.

A cautionary approach should always prevail, as the nature of RNA viruses such as IPNV, avian influenza, and SARS-CoV-2 can evolve rapidly and adapt to new host(s) (Stallknecht & Shane, 1988; Hill & Way, 1995; Reno, 1999; Wu et al., 2020). While in this initial investigation, co-cultivated species and other aquatic organisms were found to be apparently not susceptible to TiLV; further research and regular disease investigations are required to validate our observations. Until now there are very little knowledge available regarding TiLV host range, evolution, transmission route, and disease pattern, so it is very important that farmers and health experts continue to report and investigate the origins of those mortalities occurring in tilapia and co-cultivated species. TiLV needs to be kept in the priority list of potential pathogens as part of the national disease surveillance program of Bangladesh and other countries where it has been reported. If implemented in the long term, this will minimize further spread of TiLV as well as limiting its potential transmission to other co-cultivated species in tilapia polyculture systems.

Table S1. List of co-cultivated fish species and potential vectors investigated for TiLV from polyculture farms in Bangladesh.

Common Name	Scientific Name	Photograph
Corsula mullet	*Rhinomugil corsula*	
Gonia	*Labeo boggut*	
Rohu	*Labeo rohita*	
Silver carp	*Hypophthalmichthys molitrix*	
Common carp	*Cyprinus carpio*	
Bighead carp	*Hypophthalmichthys nobilis*	
Mrigal	*Cirrhinus cirrhosus*	
Bata labeo	*Labeo bata*	
Pangasius	*Pangasius pangasius*	
Climbing perch	*Anabas testudineus*	
Silver barb	*Barbonymus gonionotus*	
Stinging catfish	*Heteropneustes fossilis*	

Gulsha	*Mystus cavasius*	
Silver hatchet chela	*Chela cachius*	
Flying barb	*Esomus danrica*	
Tilapia	*Oreochromis niloticus*	
Small shrimp	*Palaemon* sp.	Not available
Crab	Scylla serrata	
Snail	*Lymnaea* spp	
Bivalve	*Lamellidens* spp	Not available
Copepod	*Cyclops* spp	
Damselfly larvae	*Ischnura* spp	
Water striders	*Gerris lacustris*	

5.6 References

Abdullah, A., Ramly, R., Mohammad Ridzwan, M. S., Sudirwan, F., Abas, A., Ahmad, K., . . . Kua, B. C. (2018). First detection of tilapia lake virus (TiLV) in wild river carp (Barbonymus schwanenfeldii) at Timah Tasoh Lake, Malaysia. *J Fish Dis, 41*(9), 1459-1462. doi:10.1111/jfd.12843

Bacharach, E., Mishra, N., Briese, T., Eldar, A., Lipkin, W. I., & Kuhn, J. H. (2019). Create one (1) new genusincluding one (1) new species. In *Virus Taxonomy: 2018b Release*; International Committee on Taxonomy of Viruses (ICTV). https://ictv.global/taxonomy

Bacharach, E., Mishra, N., Briese, T., Zody, M. C., Kembou, J. E. T., Zamostiano, R., . . . Lipkin, W. I. (2016). Characterization of a novel Orthomyxo-like virus causing mass die-offs of tilapia. *MBio, 7*, pp. e00431-00416.

Behera, B. K., Pradhan, P. K., Swaminathan, T. R., Sood, N., Paria, P., Das, A., . . . Jena, J. K. (2018). Emergence of Tilapia Lake Virus associated with mortalities of farmed Nile Tilapia Oreochromis niloticus (Linnaeus 1758) in India. *Aquaculture, 484*, 168-174. doi:10.1016/j.aquaculture.2017.11.025

Belton, B., & Azad, A. (2012). The Characteristics and Status of Pond Aquaculture in Bangladesh. *Aquaculture, 358–359*, 196–204.

Bloch, B., Gravningen, K., & Larsen, J. L. (1991). Encephalomyelitis among turbot associated with a picornavirus-like agent. *Diseases Aquatic Organisms, 10*, 65-70.

Breuil, G., Bonami, J. R., Pepin, J. F., & Pichot, Y. (1991). Viral infection (picorna-like virus) associated with mass mortalities in hatchery-reared sea bass (Dicentrarchus labrax) larvae and juveniles. *Aquaculture 97*, 109–116

Castine, S. A., Bogard, J. R., Barman, B. K., Karim, M., Hossain, M. M., Kunda, M., . . . Thilsted, S. H. (2017). Homestead pond polyculture can improve access to nutritious small fish. *Food Sec., 9*, 785–801. doi:https://doi.org/10.1007/s12571-017-0699-6

CGIAR. (2017). Tilapia lake virus (TiLV): What to know and do? Penang, Malaysia: CGIAR Research Program on Fish Agri-food Systems. Factsheet: FISH-2017-03 https://hdl.handle.net/20.500.12348/115.

Chaput, D. L. B., D.; Alam, M.M.; Al Hasan, N.; Stentiford, G.D.; van Aerle, R.; Moore, K.; Bignell, J.P.; Haque, M.M.; Tyler, C.R. . (2020,). The Segment Matters: Probable Reassortment of Tilapia Lake Virus (TiLV) Complicates Phylogenetic Analysis and Inference of Geographical Origin of New Isolate from Bangladesh. *Viruses, 12*, 258.

Chiamkunakorn, C., Machimbirike, V. I., Senapin, S., Khunrae, P., Dong, H. T., & Rattanarojpong, T. (2019). Blood and liver biopsy for the non-destructive screening of tilapia lake virus. *J Fish Dis., 42*, 1629–1636. doi:https ://doi.org/10.1111/jfd.13076

Debnath, P. P., Delamare-Deboutteville, J., Jansen, M. D., Phiwsaiya, K., Dalia, A., Hasan, M. A., . . . Rodkhum, C. (2020). Two-year surveillance of tilapia lake virus (TiLV) reveals its wide circulation in tilapia farms and hatcheries from multiple districts of Bangladesh. . *J Fish Dis., 43*, 1381–1389. doi:https://doi.org/10.1111/jfd.13235

Department of Fisheries, (2019). *Yearbook of Fisheries Statistics of Bangladesh, 2018–2019*; Fisheries Resources Survey System (FRSS), Department of Fisheries, Bangladesh: Ministry of Fisheries and Livestock: Matshya Bhaban, Dhaka, Volume 36, p. 135.

Dong, H.T.; Senapin, S.; Gangnonngiw, W.; Nguyen, V.; Rodkhum, C.; Debnath, P.P.; Delamare-Deboutteville, J.; Mohan, C.V. (2020). Experimental infection reveals transmission of tilapia lake virus (TiLV) from tilapia broodstock to their reproductive organs and fertilized eggs. *Aquaculture*, *515*, 734541. doi:https://doi.org/10.1016/j.aquaculture.2019.734541

Dong, H. T., Ataguba, G. A., Khunrae, P., Rattanarojpong, T., & Senapin, S. (2017c). Evidence of TiLV infection in tilapia hatcheries from 2012 to 2017 reveals probable global spread of the disease. *Aquaculture, 479*, 579-583. doi:https://doi.org/10.1016/j.aquaculture.2017.06.035

Dong, H. T., Jitrakorn, S., Kayansamruaj, P., Pirarat, N., Rodkhum, C., Rattanarojpong, T., . . . Saksmerprome, V. (2017d). Infectious spleen and kidney necrosis disease (ISKND)outbreaks in farmed barramundi (Lates calcarifer) in Vietnam. *Fish and Shellfish Immunology, 68*, 65-73. doi:10.1016/j.fsi.2017.06.054

Dong, H. T., Siriroob, S., Meemetta, W., Santimanawong, W., Gangnonngiw, W., Pirarat, N., . . . Senapin, S. (2017b). Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. *Aquaculture, 476*, 111-118. doi:10.1016/j.aquaculture.2017.04.019

Eyngor, M., Zamostiano, R., Tsofack, J. E. K., Berkowitz, A., Bercovier, H., Tinman, S., . . . Eldar, A. (2014). Eldar Identification of novel RNA virus lethal to tilapia , ,. *J. Clin. Microbiol. , JCM. 52 (2014)*, pp. 4137-4146.

Food and Agriculture Organization of the United Nations (2019). Aquaculture Newsletter. FAO, 60th. Available online: www.fao.org/fishery/publications/fan (accessed on August 2019).

Food and Agriculture Organization of the United Nations (2017). Global information and early warning system on food and agriculture (GIEWS). In Proceedings of the 2020 Annual Meeting, AAAS, Seattle, WA, USA, pp. 1–6.

Fathi, M., Dickson, C., Dickson, M., Leschen, W., Baily, J., Muir, F., . . . Weidmann, M. (2017). Identification of Tilapia Lake Virus in Egypt in Nile tilapia affected by 'summer mortality' syndrome. *Aquaculture, 473*, 430-432. doi:https://doi.org/10.1016/j.aquaculture.2017.03.014

Glazebrook, J. S. H., M.P.; de Beer, S.W. . (1990). Picorna-like viral particles associated with mass mortalities in larval barramundi, Lates calcarifer (Bloch). *Journal of Fish Diseases, 13*, 245–249. .

Hill, B. J., & Way, K. (1995). Serological classification of infectious pancreatic necrosis (IPN) virus and other aquatic birnaviruses. *Annu. Rev. Fish Dis., 5*, 55-77.

Hossain, M. M. M., Uddin, M. I., Hossain, M. M., Islam, H., Al-Amin;, Farjana, N., & Afroz, R. (2020). Molecular Detection of Tilapia Lake Virus (TiLV) in Farmed Mono-sex Nile Tilapia (Tilapia niloticus) in Bangladesh. *Asian Journal of Scientific Research, 13 (1)*, 67-78. doi:https://10.3923/ajsr.2020.67.78

Jaemwimol, P., Rawiwan, P., Tattiyapong, P., Saengnual, P., Kamlangdee, A., & Surachetpong, W. (2018). Susceptibility of important warm water fish species to tilapia lake virus (TiLV) infection. *Aquaculture, 497*, 462-468.

Jansen, M. D., Dong, H. T., & Mohan, C. V. (2019). Tilapia lake virus: a threat to the global tilapia industry? *Reviews in Aquaculture, 11,* 725-739. doi:10.1111/raq.12254

Mori, K. I., Nakai, T., Muroga, K., Arimoto, M., Mushiake, K., & Furusawa, I. (1992). Properties of a new virus belonging to Nodaviridae found in larval striped jack (Pseudocaranx dentex) with nervous necrosis. *Virology, 187*, 368-371.

Munday, B., Kwang, J., & Moody, N. (2002). Betanodavirus infections of teleost fish: a review. *Journal of Fish Diseases, 25*, 127-142.

Network of Aquaculture Centres in Asia-Pacific. (2017). *Tilapia Lake Virus (TiLV)— An Emerging Threat to Farmed Tilapia in the Asia-Pacific Region*; Network of Aquaculture Centres in Asia-Pacific: Bangkok, Thailand. Retrieved from https://enaca.org/?id=864

OIE. (2017). Disease notification report 25278, 23/11/2017. [https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&repo rtid=25278].

Piamsomboon, P., & Wongtavatchai, J. (2021). Detection of Tilapia Lake Virus (TiLV) in healthy fish from the pre-existing disease environment using different RT-PCR methods. . *Turkish Journal of Fisheries and Aquatic Sciences, 21*, 205-209. doi:http://doi.org/10.4194/1303-2712-v21_4_05

Pradhan, P.K.; Paria, A.; Yadava, M.K.; Verma, D.K.; Gupta, S.; Swaminathan, T.R.; Rathore, G.; Sood, N.; Lal, K.K. (2020). Susceptibility of Indian major carp Labeo rohita to tilapia lake virus. *Aquaculture*, *515*, 734567. doi:https://doi.org/10.1016/j.aquaculture.2019.734567.

Rahman, A.K.A. (1985). Introduction of Exotic Fishes in Bangladesh; (booklet); Fisheries Research and Training Centre: Dhaka, Bangladesh.

Rakus, K., Mojzesz, M., Widziolek, M., Pooranachandran, N., Teitge, F., Surachetpong, W., Adamek, M. (2020). Antiviral response of adult zebrafish (Danio rerio) during tilapia lake virus (TiLV) infection *Fish & Shellfish Immunology*, *101*, pp. 1-8

Reno, P. W. (1999). Infectious pancreatic necrosis and associated aquatic birnaviruses. *Fish diseases and disorders, Volume 3: viral, bacterial and fungal infections*, 1-55.

Stallknecht D. E., & Shane S. M. (1988). Host range of avian influenza virus in free-living birds. Vet Res Commun, 12(2-3), 125-141. doi: 10.1007/BF00362792. PMID: 3055662

Suebsing R ., P. P. J., Jitrakorn S. , Sirithammajak S. ,Kampeera J. , Turner W. A. , Saksmerprome V. , Withyachumnarnkul B., Kiatpathomchai W. (2016). Detection of Natural Infection of Infectious Spleen and Kidney Necrosis Virus in Farmed Tilapia by Hydroxynapthol Blue-Loop-Mediated Isothermal Amplification Assay. *J Appl Microbiol, 121(1)*, 55-67. doi:10.1111/jam.13165. Epub 2016 Jun 9

Surachetpong W., Roy S. R. K., & Nicholson P. (2020). Tilapia lake virus: The story so far. Journal of Fish Diseases, 43, 1115–1132. doi:https://doi.org/10.1111/jfd.13237

Surachetpong, W., Janetanakit, T., Nonthabenjawan, N., Tattiyapong, P., Sirikanchana, K., & Amonsin, A. (2017). Outbreaks of Tilapia Lake Virus Infection, Thailand, 2015-2016. *Emerg Infect Dis, 23*(6), 1031-1033. doi:10.3201/eid2306.161278

Taengphu, S., Kayansamruaj, P., Kawato, Y., Deboutteville, J. D., Mohan, C. V., Dong, H. T., Senapin, S. (2021). Concentration and quantification of Tilapia tilapinevirus from water using a simple iron flocculation coupled with probe-based RT-qPCR. bioRxiv. https://doi.org/10.1101/2021.08.10.455809

Taengphu, S., Sangsuriya, P., Phiwsaiya, K., Debnath, P. P., Delamare-Deboutteville, J., Mohan, C. V., . . . Senapin, S. (2020). Genetic diversity of tilapia lake virus genome segment 1 from 2011 to 2019 and a newly validated semi-nested RT-PCR method. *Aquaculture, 526* *735423*. doi:https://doi.org/10.1016/j.aquaculture.2020.735423

Waiyamitra, P.; Piewbang, C.; Techangamsuwan, S.; Liew, W.C.; Surachetpong, W. (2021). Infection of Tilapia tilapinevirus in Mozambique Tilapia (*Oreochromis mossambicus*), a Globally Vulnerable Fish Species. *Viruses, 13*, 1104. https://doi.org/10.3390/ v13061104

Wu, L., Chen, Q., Liu, K., Wang, J., Han, P., Zhang, Y., . . . Wang, Q. (2020). Broad host range of SARS-CoV-2 and the molecular basis for SARS-CoV-2 binding to cat ACE2. Cell Discovery, 6(1), 68. doi:10.1038/s41421-020-00210-9

Yoshikoshi, K., & Inoue, K. (1990). Viral nervous necrosis in hatchery-rearded larvae and juveniles of Japanese parrotfish, Oplegnathus fasciatus (Temminck & Schlegel). *Journal of Fish Diseases, 13*, 69-77.

Chapter 6

General Conclusion

6.1 Conclusion

Bangladesh as a fourth highest tilapia producing countries in the World, received a special alert from FAO about the possible incidence of TiLV in 2017. Considering this alert and several report from scientific community, this study designed to investigate TiLV with three separate phases. In the first phase, an online tilapia epidemiology and health economics survey tool was developed and surveyed 565 tilapia farms in 15 of Bangladesh's most important tilapia-producing districts which identified 18.2% farm reported unusual mortality with an average mortality level of 23.2% along with several factors associated with this mortality. Despite the fact that the majority believe tilapia is a very resilient fish that can be farmed in any environment, the findings of this study contradict this belief and offer the following recommendations for the welfare of Bangladesh's booming tilapia industry. Tilapia baseline and unusual mortality levels should be considered as indicators for proper disease investigation and the development of disease management strategies and surveillance programs based on diagnostic reports. This could help minimize the incidence of disease, prevent the further spread of disease, reduce the misuse of drugs including antibiotics, and increase production and profitability. Ultimately, the sector should be able save at least a significant proportion of this estimated hidden loss through improved fish health management.

Considering the survey findings, we've continued a TiLV targeted surveillance activity while we've collected sample from both diseases affected grow-out farms as well as asymptomatic sample from hatcheries. Our study was successful in detecting TiLV in tilapia from disease-affected farms as well as asymptomatic broodstock from hatcheries. Simultaneously, we were able to recover full TiLV genome sequences from 2017 and 2019 samples, and identified that Bangladeshi TiLV isolates formed a distinct cluster within the Thai clade, suggesting a close genetic relation. In summary, our TiLV-targeted surveillance in Bangladesh revealed the virus's prevalence in eight

districts. The virus was found in both grow-out farms exhibiting unusual mortality and, more significantly, in asymptomatic broodstock hatcheries. This indicates that the infestation originated in hatcheries and spread to farms, and warns of the risk of future spread if no control measures are taken. Nonetheless, continuous biological sample collection and basic data gathering on mortality occurrences are required. These will serve as a baseline for performing risk-based TiLV surveillance and estimating future socioeconomic impact.

Furthermore, there is great concern among fish-farming communities and Bangladeshi competent authorities that TiLV could spread to new geographies, affecting not only tilapia but potentially other economically important co-cultivated species, prompting us to investigate whether any of the co-cultivated species would test positive for TiLV and whether they were susceptible to TiLV infection. Despite the fact that we investigated 22 co-cultivated species along with other aquatic organisms and conducted a TiLV challenge experiment with six mostly cultured polyculture species, both our field samples collected from outbreaks on Bangladesh farms and our susceptibility experimental challenge findings confirmed that those co-cultivated species, along with other aquatic organisms, presumably were not susceptible to TiLV. Because we know so little about the TiLV host range, evolution, transmission route, and disease pattern, it is essential that farmers and health professionals continue to report and research abnormal mortalities in tilapia and co-cultivated species, and that TiLV screening remains as part of national disease surveillance in Bangladesh and other countries.

6.2 Recommendations

6.2.1 Recommendation for tilapia industry in Bangladesh

➢ Farmers need to ensure proper biosecurity to keep TiLV free farm environment during culture period

➢ Ensure TiLV free fry and fingerling for stocking

➢ Report to CA immidiatley if any unusual mortality occured and submit sample to diagnostic labs

➢ All farmers need training, guidance and consultation on biosecurity and fish disease management.

➢ The competent authority must plan and implement a short, medium and long-term targeted TiLV surveillance program to confirm TiLV infection, infected area, source, disease severity and disease status, and develop action plans, training materials, biosecurity guidelines and disease management strategies to prevent and minimize disease transmission.

➢ TiLV needs to be kept in the priority list of potential pathogens as part of the national disease surveillance program of Bangladesh and other countries where it has been reported.

➢ Need to develop farmer awareness programs to ensure sustainable tilapia production to achieve the increased aquaculture production required to feed the future world.

➢ Scientifically sound information on TiLV should be distributed to relevant academic institutions and be made available to all stakeholders, including information on the planned surveillance activities, ongoing and future research, and mitigation and control measures.

➢ Future disease investigations from affected polyculture farms should first confirm TiLV in tilapia, then test other species present on the farm; this with a sufficient number of samples from each species.

Similarly, for future TiLV tests, relevant polyculture species can be challenged with new TiLV isolate retrieved from affected farms.

6.2.2 Further studies recommended:

➢ Investigate transmission of TiLV through co-habitation with polyculture species (tilapia to other species and vice versa)

➢ Determine how long TiLV can persist without a host in the environment (mostly in water and soil).

➢ Examine and identify probable TiLV reservoir hosts and vectors.

REFERENCES

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PUBLICATION   1.   Debnath PP, Dinh-Hung N, Taengphu S, Nguyen
                    VV, Delamare-Deboutteville J, Senapin S, Vishnumurthy
                    Mohan C, Dong HT and Rodkhum C 2021. Tilapia Lake
                    Virus was not detected in non-tilapine species within
                    tilapia polyculture systems of Bangladesh. J Fish Dis.
                    2.   Jerome Delamare-Deboutteville, Suwimon
                    Taengphu, Han Ming Gan, Pattanapon Kayansamruaj,
                    Partho Pratim Debnath, Andrew Barnes, Shaun Wilkinson,
                    Minami Kawasaki, Chadag Vishnumurthy Mohan,
                    Saengchan Senapin, Ha Thanh Dong (2021). Rapid
                    genotyping of tilapia lake virus (TiLV) using Nanopore
                    sequencing. J Fish Dis. 2021;00:1–12.
                    https://doi.org/10.1111/jfd.13467
                    3.   Partho Pratim Debnath, Jerome Delamare-
                    Deboutteville, Mona Dverdal Jansen, Kornsunee
                    Phiwsaiya, Afsana Dalia, Md. Abir Hasan, , Saengchan
                    Senapin, Chadag Vishnumurthy Mohan, Ha Thanh Dong ,
                    Channarong Rodkhum (2020). Two-year surveillance of
                    tilapia lake virus (TiLV) reveals its wide circulation in
                    tilapia farms and hatcheries from multiple districts of
                    Bangladesh. J Fish Dis.2020; 00:1–9.
                    4.   Mahmoud Mabrok, Sivaramasamy Elayaraja, Putita
                    Chokmangmeepisarn, Wansadaj Jaroenram, Narong
                    Arunrut, Wansika Kiatpathomchai, Partho Pratim
                    Debnath, Jerome Delamare-Deboutteville, Chadag
                    Vishnumurthy Mohan, Aml Fawzy, Channarong Rodkhum
                    (2020). Rapid visualization in the specific detection of
                    Flavobacterium columnare, a causative agent of freshwater
                    columnaris using a novel recombinase polymerase
                    amplification (RPA) combined with lateral flow dipstick
                    (LFD) assay. Aquaculture. In press.
                    5.   Hooper, Chantelle, Debnath, Partho P., Biswas,
                    Sukumar, van Aerle, Ronny, Bateman, Kelly S., Basak,
                    Siddhawartha K., Rahman, Muhammad M., Mohan,

Chadag V., Rakibul Islam, H.M., Ross, Stuart, Stentiford, Grant D., Currie, David and Bass, David (2020). A novel RNA virus, Macrobrachium rosenbergii Golda virus (MrGV), linked to mass mortalities of the larval giant freshwater prawn in Bangladesh.

6.   Taengphu, S., Sangsuriya, P., Phiwsaiya, K., Debnath, P. P., Delamare-Deboutteville, J., Mohan, C. V., Senapin, S. (2020). Genetic diversity of tilapia lake virus genome segment 1 from 2011 to 2019 and a newly validated semi-nested RT-PCR method. Aquaculture 526 735423

7.   Dong, H.T., Senapin, S., Gangnonngiw, W., Nguyen, V. V., Rodkhum, C., Debnath, P. P., Mohan, C. V. (2020). Experimental infection reveals transmission of tilapia lake virus (TiLV) from tilapia broodstock to their reproductive organs and fertilized eggs. Aquaculture 515

8.   Muhammad Meezanur Rahman, Hendrik Jan Keus, Partho Debnath, M.B. Shahrier, Rayhan Hayat Sarwer, Quazi A.Z.M. Kudrat-E-Kabir, C.V. Mohan (2018). Benefits of stocking white spot syndrome virus infection free shrimp (Penaeus monodon) post larvae in extensive ghers of Bangladesh. Aquaculture 486 (2018) 210–216

9.   Partho Pratim Debnath, Manjurul Karim, Hendrik Jan Keus, Chadag Vishnumurthy Mohan and Ben Belton (2016) Effects of White Spot Disease and Bio-Security on Shrimp Farming in Bangladesh. Fish Pathology, 51 (special-issue)

10.   Partho Debnath, Shamsul H. Khan, Manjurul Karim, Ben Belton, Chadag Vishnumurthy Mohan and Michael Phillips (2015). Review of the history, status and prospects of the black tiger shrimp (Penaeus monodon) hatchery sector in Bangladesh. Reviews in Aquaculture (2015) 0, 1–13.

11.   Debnath, P., Karim, M., Belton, B. in press. Comparative study of the reproductive performance and White Spot Syndrome Virus (WSSV) status of black tiger shrimp (Penaeus monodon) collected from the Bay of Bengal. Aquaculture

12.   Ehsanul Karim, Mohammad Assraful Haque and Partho Pratim Debnath (2012). Constraints & Prospects of Existing Shrimp Farming In Bangladesh. Lambert Academic Publishing.

13.   Partho Pratim debnath, Manjurul Karim, Quazi A. Z. M. Kudrat-E-Kabir (2013). Comparative Study on Growth Performance of Bagda (P. monodon, Fabricius, 1798) in Traditional and Semi-intensive Culture Systems. Science and Technology. 3(1): 1-16

14.   Partho Pratim Debnath, Manjurul Karim, Quazi A.Z.M. Kudrat-E-Kabir, Mohammed Ashraful Haque, Md. Shahzad Kuli Khan3 (2012). Production Performance of White Fish in Two Different Culture Systems in Patuakhali, Bangladesh. Journal of Advanced Scientific Research. J Adv Sci Res, 2012, 3(4): 55-67

15.   Partho Pratim Debnath, Ehsanul Karim, Mohammed Ashraful Haque, Md. Shahab Uddin, Manjurul Karim (2012). Prevalence of White Spot Syndrome Virus in Brood stock, Nauplii and Postlarvae of Tiger shrimp (Penaeus monodon Fabricius, 1798) in Bangladesh. Journal of Advanced Scientific Research. J Adv Sci Res, 2012, 3(3): 58-63

16.   Mahmudul Hasan, Shahajat Kuli Khan, Mohammad Assraful Haque, Ehsanul Karim and Partho Pratim Debnath (2012). Livlihood Strategies of Tiger Shrimp Post Larvae Collectors in Backkhali River Estuary, Cox's Bazar of Bangladesh. Int. J. Sustain. Agril. Tech. 8(9): 0l-07

17.   M. Mostafa, P.P. Debnath, B. Biswas and M.M. Iqbal (2007). Socio-economic Status of Shrimp (penaeus monodon) Post-Larvae (PL) Collectors in Cox's Bazar with Emphasis on Violation of Restriction on Wild PL Collection. South Asian Journal of Agriculture, Volume 2, Number 1 & 2, December 2007, Agrotechnology Discipline, Khulna University, Khulna-9208, Bangladesh.

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