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A study of the occurrence of Tilapia Lake Virus in farmed tilapia in Zambia

En studie av forekomst av Tilapia Lake Virus i oppdretts-tilapia i Zambia

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Preface

Throughout the years of studying veterinary medicine, our interest in aquaculture and research has been exponential. We started our educational course knowing very little about this field of study, but thanks to inspirational and knowledgeable professors at the School of Veterinary Medicine in Norway, we now feel eager and curious to continue working within this field.

Aquaculture and salmon farming are important for the Norwegian industry, just like tilapia is important for the Zambian industry. When working with such large scale productions, different diseases and disorders are not uncommon, and it is our job as future veterinarians to help prevent and treat such diseases in cooperation with the farmers.

Over the last years while studying veterinary medicine, we have learned about diseases affecting mainly salmon, and when we were offered to participate in a study about Tilapia Lake Virus in Zambia, we were eager to expand our knowledge and engagement regarding aquaculture in other parts of the world. During this study and the field trip we conducted in Zambia, we visited various fish farms that willingly opened their doors and taught us about tilapia, aquaculture and about Zambia. We have also gotten hands-on-experience from sampling and working in the laboratory. This study has without a doubt sparked our interest in aquaculture in other parts of the world and encouraged us to continue working and doing research within this field.

Summary

Title: A study of the occurrence of Tilapia Lake Virus in farmed tilapia in Zambia

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Tilapia Lake Virus (TiLV) disease induces high mortalities in tilapia aquaculture and may result in substantial economic losses for the tilapia industry. Even with growing research about TiLV and the disease, there is still more to be learnt about the pathogenesis and the geographic distribution of the virus. Tilapia production is one of the largest aquaculture food industries worldwide, stretching from China to Colombia and Ghana. Zambia is the fourth largest tilapia producing country in Africa, preceded by Ghana, Uganda and Egypt in ascending order. Tilapia lake virus has been reported in 16 countries so far, with detections in Uganda and Egypt in 2017. Even with the presence of TiLV in surrounding countries, Zambia has not conducted a comprehensive testing of the virus nationally. In this study, we examined fish farms along the line of rail including Kabwe, Ndola, Kitwe, Kalulushi as well as Siavonga.

We sampled a total of 197 fish from eight different locations. Materials collected were from the head kidney and the spleen from apparently healthy fish. The samples were transported in RNAlater, RNA was extracted in the laboratory and measurements of concentrations were done by Nanodrop. We performed real-time PCR on all samples for the detection of TiLV genome. We did not confirm the presence of TiLV in any of our samples.

Abbreviations, acronyms and definitions

Aquaculture biosecurity	Measures put in place by an aquaculture production facility to protect fishstocks from diseases.
FAO	Food and Agriculture Organization of the United Nations
Fingerlings	Juvenile fish, when the fry is about 10-15 cm. In this thesis we are referring to juvenile tilapias.
Fry	Spawn egg with the shape of a fish, about 1-2 cm in length.
GIFT	Genetically improved farmed tilapia
Hapas	Nylon mesh net cages.
ISH	*In situ* hybridization
MMC	Melanomacrophage centers
NARDC	National aquaculture research and development center
OIE	World Organization of animal health
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase PCR
RT-qPCR	Quantitative reverse transcriptase polymerase chain reaction
SBC	Schweitzer Biotech Company
SMS	Summer mortality syndrome
Tilapia, tilapias (pl)	Species of the family *Cichlidae*.
TiLV	Tilapia Lake Virus
TOMMS	Tilapia one-month mortality syndrome
UN	United Nations
Virions	Infectious virus particle

Introduction

General background

According to the United Nations (UN), the world population reached 8 billion on November 15th 2022, and it is projected that by 2030 it will increase to 8.5 billion (Nations, 2015). The UN states in their Sustainable Development Goal 14, that to meet this growth and ensure all people enjoy peace and prosperity, we have to conserve and sustainably use the oceans, seas and marine resources for sustainable development (Publications, 2022). Fisheries and aquaculture sectors are essential for global food security and nutrition, both now and in the future. They are an important source of animal protein, and supply 17 percent of the protein in people's diets globally (FAO, 2016).

Aquaculture is one of the fastest growing sectors for animal food production. Tilapia, and other freshwater species, will be the main species to increase the aquaculture production and is reckoned to represent about 60 percent of total production in aquaculture in 2025 (FAO, 2016).

Tilapia production in the world

In the 1950s the wild and farmed production of tilapia globally was about 69 710 tonnes, and increased nearly 100 times to reach 6 882 202 tonnes by 2018. More than 4 million tonnes of the total were accounted for by Nile tilapia (*Oreochromis niloticus*), while the remaining include various species of tilapia and also mixed breeds (*Oreochromis nei*) (FAO, 2020). This increase mainly comes from tilapia aquaculture. Tilapia was farmed in more than 125 countries in 2018, Asia accounted for 63,1 percent and Africa 26,29 percent (FAO, 2020).

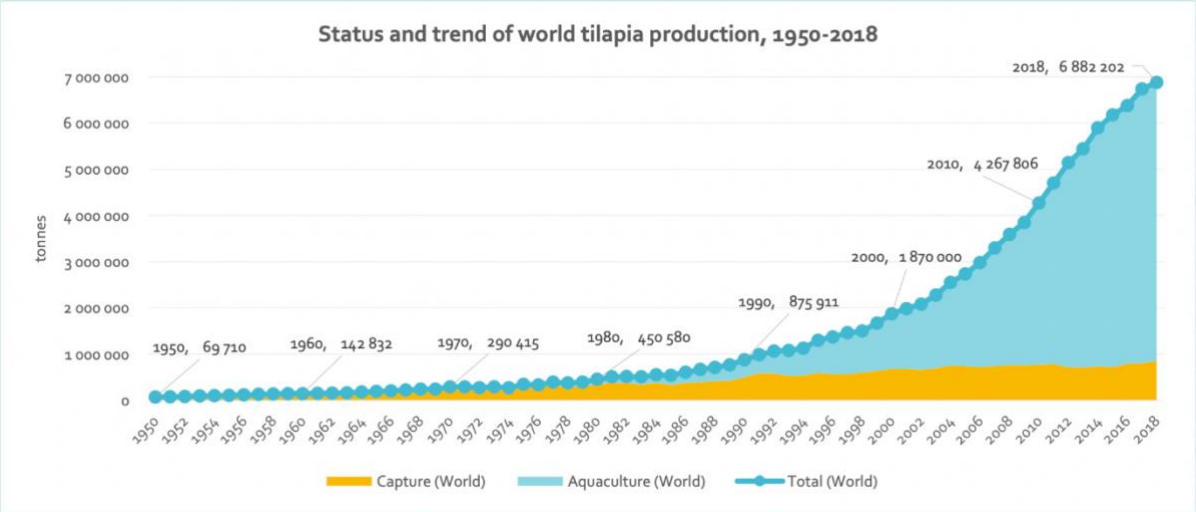

Figure 1. Status and trend of world tilapia production, 1950-2018 (FAO, 2020).

China is the top producing country of tilapia, with 23,61 percent of the total global production. Indonesia and Egypt come second and third respectively, not too far behind. Out of the top 10 tilapia producing countries, several are also among the top 15 countries with the highest population count (2018): China, Indonesia, Egypt, Bangladesh, Brazil, Philippines and Viet Nam.

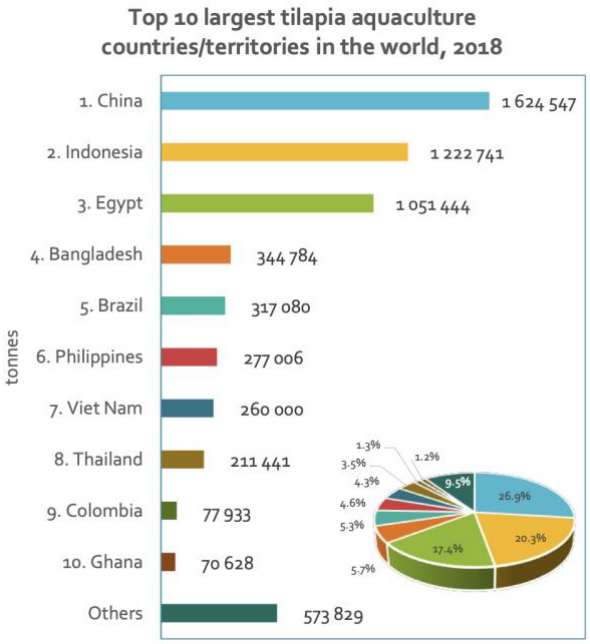

Figure 2. Top 10 largest tilapia aquaculture countries/territories in the world, 2018. (FAO, 2020)

Tilapia production in Zambia

Zambia is blessed with several large rivers and dams, giving the country a large resource of freshwater. Fish farming dates all the way back to the 1950s, when it was first attempted to raise tilapias in dams and earthen fish ponds. Back then, and today, aquaculture was and still is important for employment, earnings and as a source of food. Currently, there is about

6000 small-scale fish farmers with more than 13 000 fish ponds throughout the country (Maguswi, 2023).

The most common species include the three spotted tilapia (*Oreochromis andersonii*), the longfin tilapia (*Oreochromis macrochi*) and the redbreasted tilapia (*Coptodon rendalli*). Others include the Nile tilapia (*Oreochromis niloticus*), the common carp (*Cyprinus carpio*) and red swamp crayfish (*Procambarus clarkii*) (Maguswi, 2023).

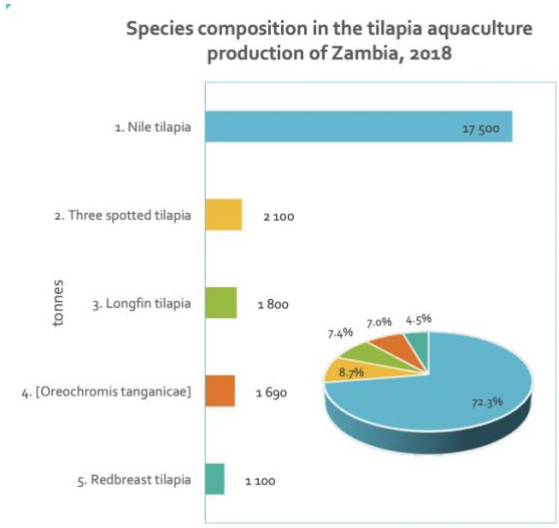

Figure 3. Species composition in the tilapia aquaculture production of Zambia, 2018 (FAO, 2020).

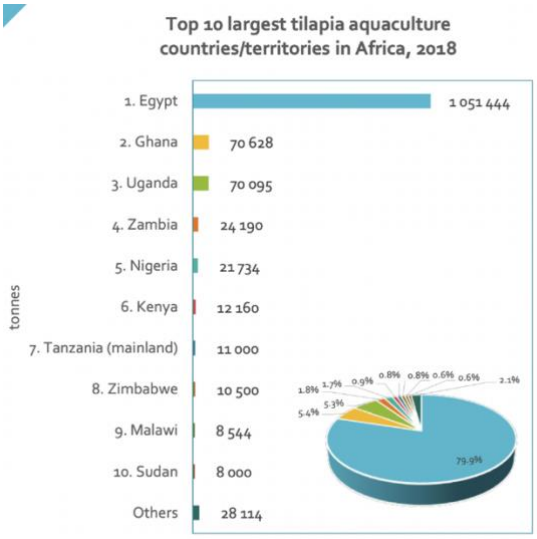

Figure 4. Top 10 largest tilapia aquaculture countries in Africa, 2018 (FAO, 2020).

Zambia was the 5th largest producer of tilapia among the developing countries in 2018. It was also ranked as the third largest tilapia producer among landlocked developing countries, and it is the fourth largest producer of tilapia in Africa (as illustrated above).

Tilapia farming

As we have already stated**,** tilapia is among the most important species produced in aquaculture worldwide. It has become popular because it is fast growing, becomes sexually mature at a young age, has a high fertility rate and has a desirable taste (Sáenz, 2021).

Tilapia is the common name used for certain freshwater species belonging to the family *Cichlidae*. They are divided into groups of mouth breeders, where the parents will incubate and protect eggs and young ones in their mouth, and those that incubate eggs in nests on the bottom of lakes or ponds (Encyclopedia, 2008). Species of the genus *Sarotherodon* and *Oreochromis* constitute the mouthbreeders, while species of the genus Tilapia constitute the latter (Lopes et al., 2015).

Spawning season begins when water temperatures rise above 22°C and continues as long as the water stays warm. In Zambia the spawning season is from August until April. In the wild, male tilapia will establish and defend territories in turbid rivers and lakes, and build nests used for courting and spawning. This is imitated in commercial production, with up to 3 females per male in a pond. Eggs are fertilized in the nest and after that mouth breeders will pick them up for incubation in their mouth, while others will leave them in the nest and incubate them by fanning water through with their fins (Chapman, 2018; Lopes et al., 2015).

In farmed production, male monosex populations are preferred because males grow approximately twice as fast as females. A mixed-sex population will create problems such as size disparity among harvested fish and uncontrolled reproduction, thus for productivity, welfare, and control of the reproduction it is common practice to raise an all-male population. After harvesting of eggs and hatching, fingerlings are administered the male sex hormone (17a-methyltestosterone) in their feed, and female tilapia will have their sexes reversed to become males (Sáenz, 2021).

Growth rates are affected by water temperature, sex, supplemental feeding and stocking density. So the estimated time to reach harvest-size fish varies, but ranges between 6 and 12 months (Chapman, 2018).

Challenges associated with tilapia production

Tilapias are cultured in several developing countries where issues of economy, education, technical competence and access to medical diagnostics and treatment hinder the desired progress. There is an ancient belief that tilapias are resistant to disease, but we know today that there is a range of diseases that affect tilapia production worldwide and cause significant losses. Examples of common bacterial pathogens are *Aeromonas hydrophila, A. veronii, Flavobacterium columnare, Streptococcus agalactiae* and. *S. iniae* (Machimbirike et al., 2019). Viruses of high and growing importance include Tilapia lake virus and infectious spleen and kidney necrosis virus (ISKNV), but others such as Aquabirnaviruses, Betanodaviruses, Herpesviruses and other Iridoviruses can also cause losses in aquaculture operations (Howell, 2019). Tilapia Lake virus has generated a lot of interest globally since it has caused substantial mortalities in some countries, reaching up to 90 percent cumulative mortality, where disease outbreaks have been reported in several countries on different continents (Aich et al., 2022). It has devastating economic effects, and pose a threat to our food security (Tang, 2021).

Discovery and geographical distribution of Tilapia Lake Virus

Tilapia lake virus has as of 2021 been reported in 16 countries (Aich et al., 2022; Surachetpong et al., 2020), but it is believed that the virus might be distributed to other countries as well due to export of fry or fingerlings (Dong et al., 2017a). With better diagnostic testing methods and surveillance for presence of virus through use of sensitive methods, the numbers of affected countries might rise (Surachetpong et al., 2020).

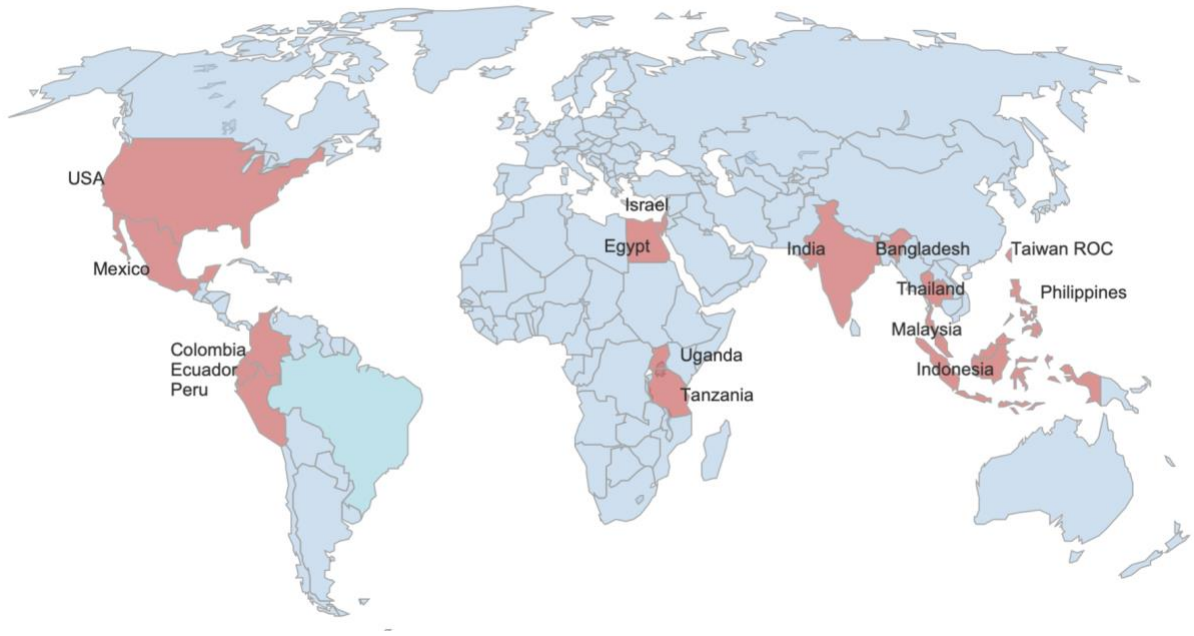

Figure 5. Geographical distribution of TiLV as of 2021 (Surachetpong et al., 2020). Map sketched with smartdraw Map Maker.

In 2009 Israel experienced production losses of wild and farmed tilapia in the Sea of Galilee (Lake Kinneret), and it was believed this was due to a viral agent (Eyngor et al., 2014; Tang, 2021). They observed that these losses occurred primarily during hot season, from May to October (Aich et al., 2022; Eyngor et al., 2014; Surachetpong et al., 2020), and they also observed that fish surviving an outbreak developed immunity against the disease (Eyngor et al., 2014). Outbreaks were reported from other parts of the world, and in Ecuador disease outbreaks were reported in tilapia farms from 2009 and onwards (Bacharach et al., 2016; Tang, 2021).

In 2011 samples were collected from clinically diseased tilapia in Israel, and in 2014 Tilapia lake virus was characterized for the first time (Eyngor et al., 2014). In 2015 Egypt began to investigate mortalities they experienced, called “Summer mortality syndrome” (SMS), because these mortalities also occurred during the hot season (Fathi et al., 2017). The same happened in Thailand, where the disease was called “Tilapia one-month mortality syndrome”

(TOMMS) (Surachetpong et al., 2020). In the wake of this it has been reported that the outbreaks in Ecuador, Egypt and Thailand also were due to TiLV (Tang, 2021). The countries where the presence of TiLV have been reported as of 2021 are Israel, Egypt, Tanzania, Uganda, Ecuador, Colombia, Peru, India, Indonesia, Malaysia, the Philippines, Thailand, Bangladesh, Chinese Taipei, Mexico and the United States (Surachetpong et al., 2020). For several of these countries, presence of the virus was based on viral genome demonstration in internal organs of tilapia by different PCR methods, and virus was not cultivated in cell culture, for example Uganda and Tanzania (Mugimba et al., 2018).

Thailand has extensive export and international trade of fry and fingerlings of tilapia, and most of the samples collected from 2012 to 2017 tested positive for TiLV, and even the earliest samples that were collected in 2012 (archival material) were positive for the virus (Dong et al., 2017a).This indicates that the virus was present in Thailand already in 2012, and some even believe that the virus came to Thailand in 2008 when the country experienced inexplicable outbreaks of disease in tilapia nationwide (Dong et al., 2017a). Over 40 countries from all over the world have imported fry and fingerlings from Thailand since 2012 (Dong et al., 2017a), and it is therefore that the virus has been spreading to many of the reported countries due to this export (Tang, 2021). While many countries have reported detection of TiLV in farmed tilapia, there might be other countries that will detect the virus in the future with better surveillance programs and methods. For example, in 2016, Zambia experienced mortalities in tilapia, but this was not investigated in depth so any possibly involvement of TiLV infection during these periods of increased losses remains unknown (Tang, 2021). Dong et al., 2017 suggested a list of 40 countries that could be at high risk of TiLV spread from positive tilapia hatcheries from Thailand including Zambia; and 11 other African countries, some of these are neighbouring countries to Zambia (Dong et al., 2017a). Two of the African countries (Tanzania and Uganda) on this list have had TiLV detected through use of PCR

methods in 2017, with Tanzania sharing borders with Zambia in the northern region. Seven other countries on this list have also had the virus detected since the article (Dong et al., 2017a) was published. In addition, Mexico, which was one of three countries on the list of countries with lower risk of TiLV spread, detected the virus in 2018 (Tang, 2021). Considering all these factors, risk evaluation of possible spread of TiLV is difficult because official tracking of trading is difficult and some movements within or between countries go unnoticed or are not accounted for.

Tilapia lake virus

Tilapia lake virus is a negative sense, segmented and single stranded RNA virus that belongs to the family *Amnoonviridae*, in genus *Tilapinevirus* and species *Tilapia tilapinevirus* (Aich et al., 2022). For a long time TiLV was classified as an «orthomyxo-like virus» because of its genomic structure, but after sequence analysis it was re-allocated as a new species in the family *Amnoonviridae* (Adams et al., 2017).

The main host of TiLV by natural infection is Nile tilapia (*O. niloticus*) and red tilapia (*Oreochromis sp*.) among other tilapia hybrids (Eyngor et al., 2014; Ferguson et al., 2014; Mugimba et al., 2018; Tattiyapong et al., 2017). Other than tilapia, natural infection of TiLV has been seen in giant gourami (*Osphronemus goramy*) (Chiamkunakorn et al., 2019). In different species of tilapia and giant gourami, a disease similar to what is seen under natural infection with TiLV has been reproduced experimentally in the laboratory (Behera et al., 2018; Eyngor et al., 2014; Mugimba et al., 2019; Tattiyapong et al., 2017). Other warm-water fish have been shown to be resistant to the virus in experimental studies, including walking catfish (*Clarias macrocephalus*) and common carp (*Cyprinus carpio*) (Jaemwimol et al., 2018). These fish are considered unlikely to be carriers of TiLV. Possible reasons for resistance in these species might be lack of viral receptors that would be necessary for the

virus to gain entrance to primary or secondary replication sites for TiLV or absence of other replication mechanisms. However, factors such as co-infections, environment and stress could increase susceptibility to the infection (Nicholson et al., 2020).

Transmission and Pathogenesis of TiLV

TiLV can be transmitted both horizontally and vertically (Eyngor et al., 2014; Liamnimitr et al., 2018). The virus has been detected in faeces following experimental challenge through the intragastric route, and also in the water of the fish tanks. Therefore, a faecal-oral route of transmission appears to be possible (Pierezan et al., 2019). It is thought that sub-clinical carriers can transmit the virus to other susceptible fish (Senapin et al., 2018). In recent studies the vertical transmission has been confirmed by identification of TiLV genome from liver and reproductive organs of infected fish (Dong et al., 2020; Yamkasem et al., 2019).

Even though the pathogenesis of TiLV disease is mostly unknown there are a few publications that have reported possible routes of entry; through direct contact of the skin and mucus, or via the oral route (Eyngor et al., 2014; Liamnimitr et al., 2018; Pierezan et al., 2019). Pierezan et al., 2019 conducted an intragastric challenge study where the epithelial barrier of the intestines was compromised by the virus and the authors also discovered high viral load in organs and histopathological changes, supporting the hypothesis that the intestinal route is a likely port of entry, followed by systemic spread of the virus (throughout the body) (Pierezan et al., 2019). Further testing of this hypothesis found detection of viral genomic RNA in multiple organs, such as liver, gills, anterior kidney, spleen and brains of infected fish (Mugimba et al., 2018; Tattiyapong et al., 2018). Eyngor and co-workers conducted a cohabitation challenge study where fingerlings were transported from hatcheries to larger ponds, and the observed mortality started 4-7 days after transfer (Eyngor et al.,

2014). They did diagnostic work–up of the fingerlings over several months observing mortality above normal range compared with a healthy control group (Eyngor et al., 2014).

In a study from 2016 it was suggested that the liver and brain could be target tissues for viral transcription and replication of TiLV due to detection of viral genomic RNA with ISH in these organs (Bacharach et al., 2016). ISH is used to examine the presence of the viral RNA in fish with disease and the results indicate that the replication and transcription of the virus take place at sites of pathology, like the liver and brain (Jansen et al., 2019). Even though the liver and brain appear to be target organs of acute infections, there is also detection of viral genome in the gills, heart, spleen and head kidney during acute stages of the disease (Jansen et al., 2019; Mugimba et al., 2018). In a study conducted only on apparently healthy farmed and wild tilapia (no signs of clinical disease), virus genome was detected most frequently in the head kidney and spleen followed by the heart while virus genome was infrequently detected in liver and brain, in contrast to what is seen under acute infection (Mugimba et al., 2020). The low prevalence of liver and brain may indicate that lymphoid organs, such as spleen and head kidney, have a higher prevalence during subclinical/chronic infections (Mugimba et al., 2020).

A recent study revealed that a lysosomotropic agent had no effect on the replication of TiLV (Chengula et al., 2019). This might suggest that TiLV can gain entrance into cells independent of classical acidification in the endosomes, unlike orthomyxoviruses and several other membrane-bound viruses (White et al., 2008). Chengula and co-workers (Chengula et al., 2019) also demonstrated that TiLV in contrast to orthomyxoviruses such as influenza PR8 or ISAV, do not agglutinate red blood cells from tilapia, Atlantic salmon or turkey. Based on hemagglutination tests, the study suggests that TiLV may use another mechanism than orthomyxoviruses for entry and replication in host cells due to the lack of a hemagglutinating

protein that would bind to RBCs and importantly, to target cells leading to endocytosis, uncoating and virus replication (Chengula et al., 2019). On this basis, receptors other than those employed by orthomyxoviruses, namely different sialic acids linked to glycoproteins (Matrosovich et al., 2015) seem to be used by TiLV and so far, these remain unknown.

Clinical signs of TiLV-infected fish

The major clinical signs of TiLV are loss of appetite, lethargy, swimming at the water surface, loss of balance, skin lesions such as discolorations or loss of scale, ocular lesions and abdominal discoloration and distention (Jansen et al., 2019; Tattiyapong et al., 2017). Other described gross lesions of infection are severe anemia, bilateral exophthalmia, loss of body condition, skin hemorrhage and erosion (Tattiyapong et al., 2017). Also unusual behavior like abnormal or erratic swimming has been described (Pierezan et al., 2019). Clinical lesions of infected fish seem to vary depending on geographic location and farmed to wild fish. Reported findings from a study in Israel showed lethargy, skin erosions and discolorations with ocular lesions in farmed tilapia (Eyngor et al., 2014). However, for wild tilapia in Israel the reported clinical signs only included skin erosion and ocular lesions (Eyngor et al., 2014). This same pattern with a wide variety of reported clinical signs has been seen around the world, from Ecuador (Eyngor et al., 2014) to India (Behera et al., 2018).

Histopathological lesions and target organs

Infection with TiLV affects many organs. Macroscopically TiLV infection often includes ocular lesions like opacity of the lens known as cataract. In the most significant clinical cases ruptured lenses have been observed with uveitis or endophthalmitis, and occasionally perforated cornea and complete loss of ocular function (Eyngor et al., 2014). Microscopically, the lenticular capsule was coiled and ruptured, surrounded by fibroplasia. Cataractous

changes within the lens were seen as infiltrates with eosinophilic granulocytic cells that extended into the anterior chamber, iris, and choroid (Eyngor et al., 2014). The disease also affects and changes the behavior of the fish, its swimming abilities and activity levels. Histopathological changes of the brain included focal hemorrhages of the leptomeninges, edema and capillary congestion throughout the brain (Eyngor et al., 2014). Also some neuronal degeneration was seen in the telencephalon, especially in the optic lobes (Eyngor et al., 2014). Another study conducted a few years later revealed massive degeneration and inflammatory cell infiltration compatible with severe meningoencephalitis, and the degenerative changes involved cellular vacuolization and cell shrinkage (Tattiyapong et al., 2017).

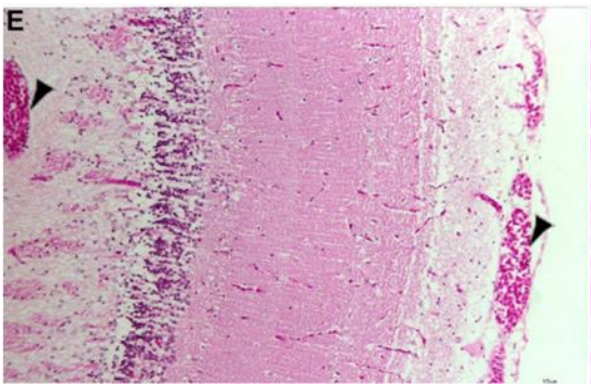

Figure 6. (E) Example of dilated blood vessels in the brain and cortex (Eyngor et al., 2014) , reprinted with permission obtained from Journal of clinical microbiology (JCM).

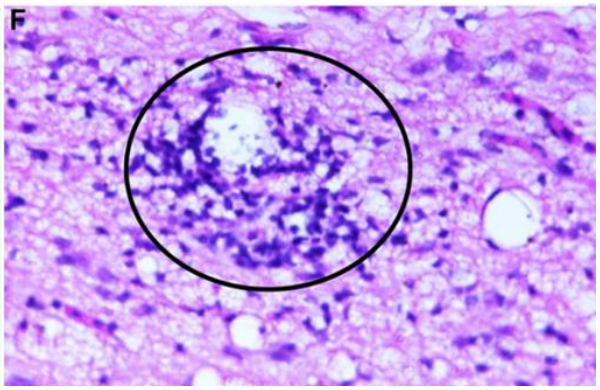

Figure 7. (F) Example of perivascular cuffs of lymphocytes in brain and cortex (Eyngor et al., 2014), reprinted with permission obtained from Journal of clinical microbiology (JCM).

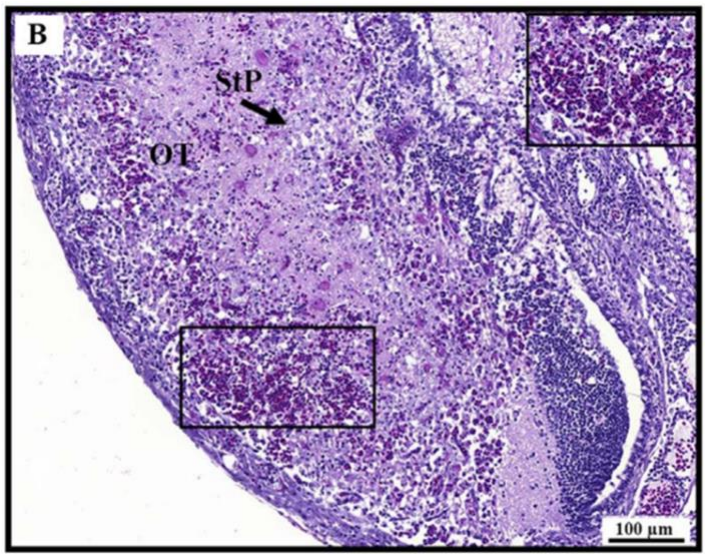

Figure 8. Multifocal hemorrhages with blood congestion in the brain, optic tectum (OT), stratum periventriculare (StP) (Tattiyapong et al., 2017), permission obtained from Veterinary Microbiology.

Regarding changes found in the hepatic parenchyma, similar lesions have been described in multiple studies. TiLV infected fish display necrosis of hepatocytes (Ferguson et al., 2014) with foci of swelling and clearing in the parenchyma (Eyngor et al., 2014), and massive degeneration and inflammatory cell infiltration with eosinophilic intracytoplasmatic inclusion bodies in hepatocytes (Tattiyapong et al., 2017). Another finding of the hepatic tissue architecture and changes, was brown or eosinophilic lipoproteinaceous material in the cytoplasm of hepatocytes and in some studies also syncytial giant cells (Eyngor et al., 2014; Ferguson et al., 2014; Mugimba et al., 2020). A distinct finding during acute stages of infection was an markedly uneven size of hepatocytes, anisocytosis, and enlarged nucleoli (see Figure 9) (Mugimba et al., 2020). Histological changes in the gastrointestinal-tract are necrosis of gastric glands that extend into the crypts (Ferguson et al., 2014) with absent or pyknotic nuclei and loss of cell-to-cell adherence (Pierezan et al., 2019). Inflammation in the submucosa sometimes accompanied the necrotic changes. Bile ducts and extrahepatic

pancreatic acini were mostly uninvolved, but pancreatic exocrine cells were sometimes affected (Ferguson et al., 2014). Multiple lesions in the spleen have been described and include hyperplastic spleen tissue and melanomacrophage centers (MMCs) in both liver and spleen were enlarged in both size and numbers (Eyngor et al., 2014). Another study revealed eosinophilic intracytoplasmic inclusion bodies in MMCs along with an increase in cells (Tattiyapong et al., 2017). Proliferation of MMCs is commonly seen in late stages of chronic infections or could be due to poor living conditions (Eyngor et al., 2014). Histopathology in TILV-challenged tilapia also showed areas of necrosis and inflammatory cell infiltrates in the anterior kidney of infected fish (Mugimba et al., 2020; Tattiyapong et al., 2017).

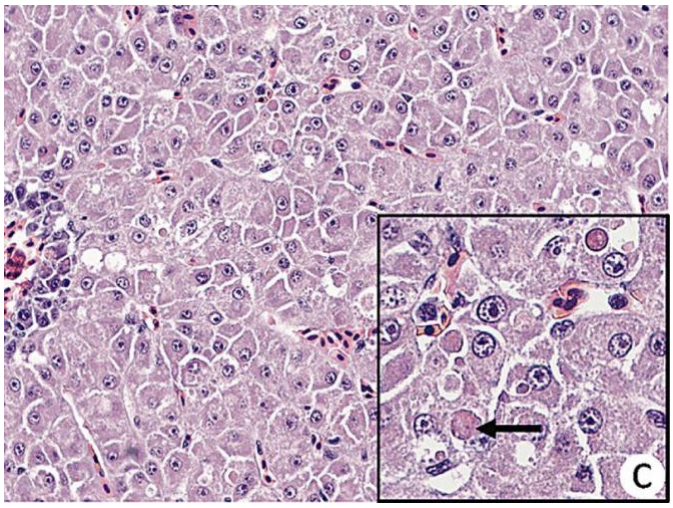

Figure 9. (C) Examples of liver changes, anisocytosis and small droplets in hepatocyte cytoplasm (Mugimba et al., 2020).

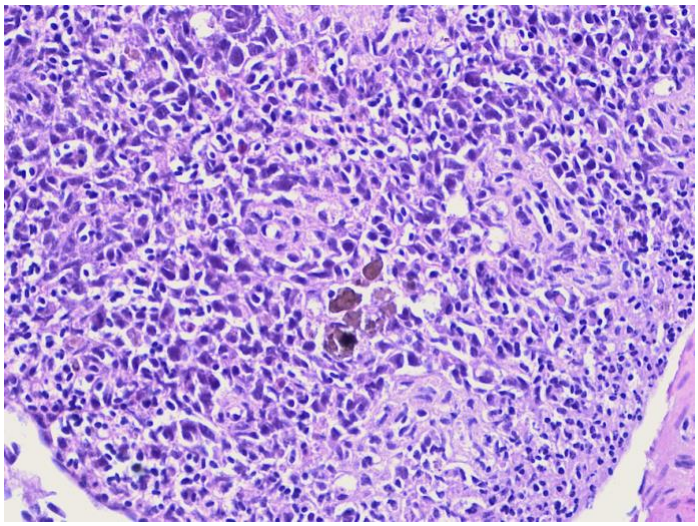

Figure 10. Example of increased melanomacrophage center (MMC) in the spleen (photo: Øystein Evensen).

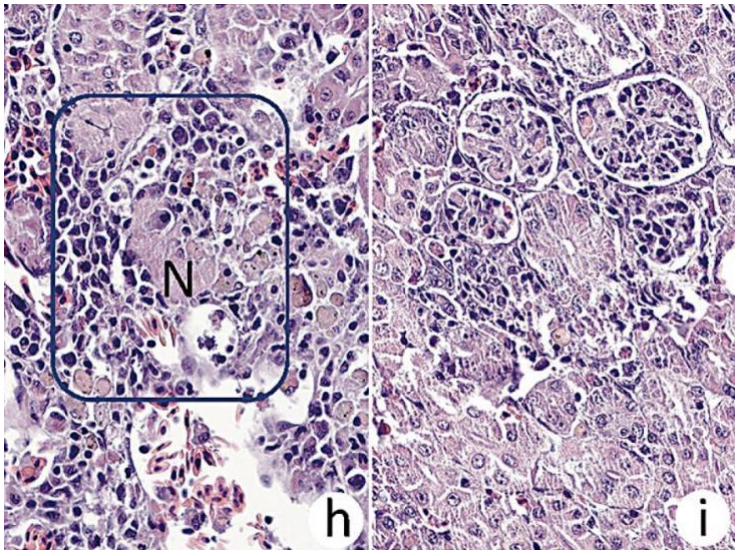

Figure 11. (H) Example of focal area of necrosis (N) and inflammatory cells infiltration of lymphocytes. (I) Examples of inflammatory changes around glomeruli (Mugimba et al., 2020).

Diagnostic methods

As already stated, infected fish exhibit clinical signs presumptive of TiLV. High levels of mortality combined with ocular alterations should particularly be considered suspicious. Further investigation of histological sections from affected organs can strengthen this suspicion albeit are not sufficient to make a definite diagnosis.

Eyngor et al. (2014) cultured the virus from diseased fish in primary tilapia brain cells and E-11 cell lines, which induced cytopathic effect in 5 to 10 days after infection. Supernatant harvested from these cultures, resulted in induction of disease when inoculated into naive tilapia. TiLV virions were demonstrated by electron microscopy, and RT-PCR analysis confirmed TiLV as a causative pathogen (Eyngor et al., 2014; Tattiyapong et al., 2017) . Nested and semi-nested RT-PCR assays have been developed to increase sensitivity when applied to clinical samples (Dong et al., 2017b; Kembou Tsofack Japhette et al., 2017). To further increase sensitivity, molecular accuracy and accessibility a few RT-qPCR methods have been established (Tattiyapong et al., 2018). Because of the benefits of sensitivity with RT-qPCR, and its rapid time to result, this is the method recommended for the detection of the virus.

It is possible to diagnose an infection with TiLV using PCR methods or virus detection through cell culture. PCR assays are used as the confirmatory test methods, and today there are four commercial PCR assays suitable for detection of TiLV. Cefas RT-qPCR and Hong RT-qPCR assays, both real time probe-based, show the highest sensitivity and are the ones recommended by OIE (2021). SYBR-based PCR assays also show acceptable results, and are considered appropriate for use in laboratories that cannot access real-time probe-based assays.(WOAH, 2021; WOAH, 2022).

Research questions

The overall aim of this study was to investigate the presence of subclinical infections of TiLV in farmed tilapia in Zambia. If present, the sub-goal was to evaluate whether the spleen or the head kidney contains the highest concentration of virus during subclinical infections.

Materials and Methods

Material

Groups

This study included species from the cichlidae family, commonly called tilapias. We included three Oreochromis species, *O. andersonii, O. niloticus*, *O. macrochir* and one *coptodonine, C. rendalli*. We collected farmed tilapia from eight different locations, and the groups we collected varied between fingerlings and older fish, at one location we sampled from both groups. The collection of fish consisted of ordinary production fish in the standards of farmed tilapia in Zambia.

Anamnestic information/questionnaire

We included eight different fish farms in two regions in Zambia, in the Copperbelt area and in the Siavonga district. None of these had previously been diagnosed with Tilapia Lake Virus infection.

On all fish farms we obtained anamnestic information about the farm based on a pre-defined questionnaire and this was done prior to the sampling being carried out. The questionnaire included relevant questions with regard to management, production and occurrence of

disease. Figure 12 illustrates locations we visited, and the Questionnaire in Table 3.

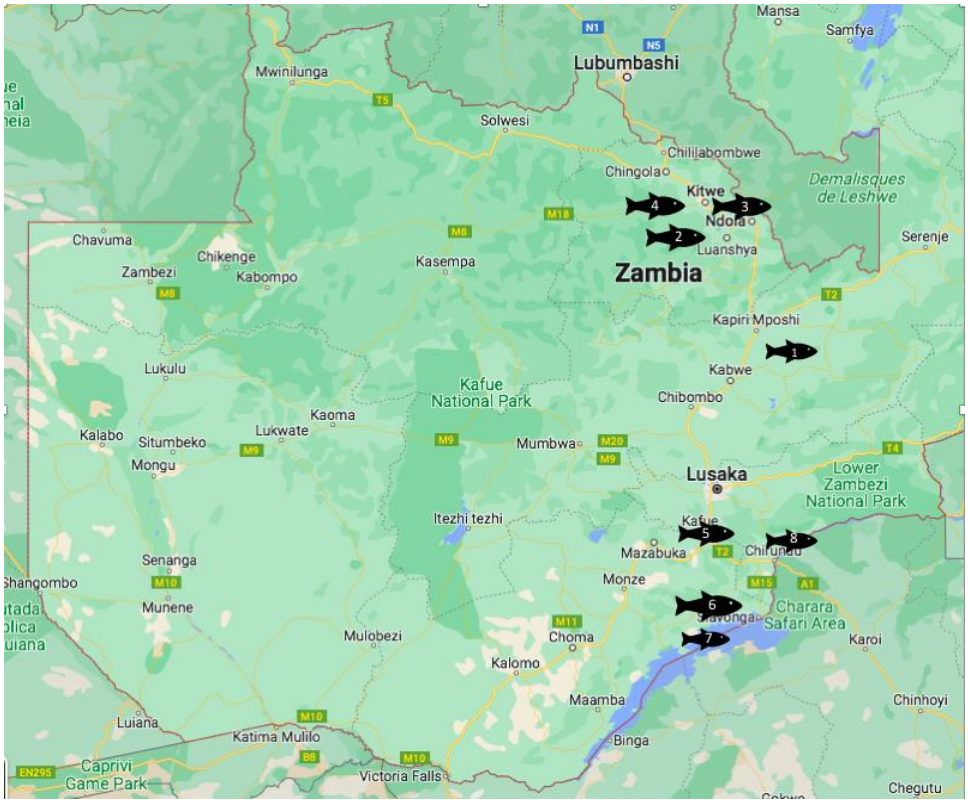

Figure 12. Map with the sites we visited, site 1-8. Map courtesy of Google.

Inclusion and exclusion criteria

The inclusion criteria for this study were farmed tilapia in Zambia, and the farmers' willingness to participate in the study. The exclusion criteria were cadaverous fish, individuals displaying macroscopic lesions or other signs of sickness as well as the farmers' reluctance to participate.

Sampling

Samples at each farm were collected with the help of farm employees. The employees collected the required quantity of fish using nets. The fish were sedated in buckets by clove powder mixed in water, and then euthanized by cutting off the gills with a scalpel. Dissection was done by laying the fish on its right side. An incision was then made laterally, from the

anus to the gills, and then along the upper abdominal cavity cranially from the anus. From there the cut was made towards the gills to expose the abdominal cavity by removing the muscle and skin from the left side of the fish. The organs needed were excised and removed with a set of forceps.

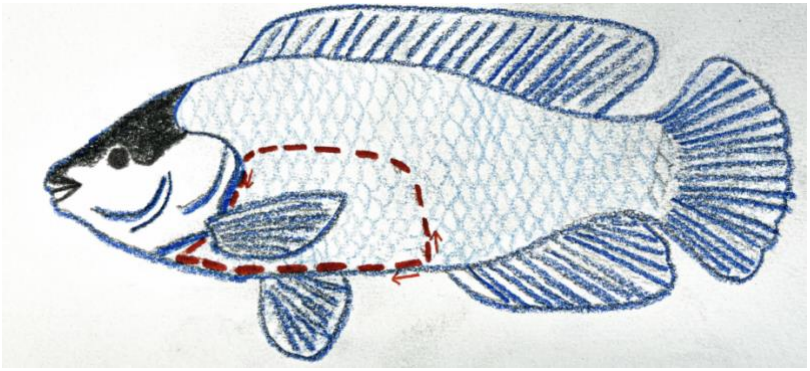

Figure 13. Illustration showing how the incisions were made for sampling of adult fish.

Fingerlings were dissected differently since the size made it difficult to extract the preferred organs, i.e. the head and tail were cut off, and then the body cut in half lengthwise. Aseptic protocols were achieved by cleaning the scalpel, forceps, scissors and cutting board with chlorine between fish.

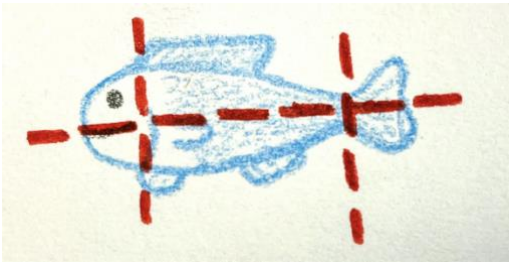

Figure 14. Sketch illustrating where the incisions were made for sampling of fingerlings.

The head kidney and spleen from larger fish were homogenized and used as template for real-time PCR, while for fingerlings, the dorsal section including the head kidney and the ventral section including the spleen were used. The tissues were kept in tubes containing RNA-later and kept cool until RNA extraction.

Labeling

The first number in the labels indicates at what site the sample was taken. The second number and letter represented the fish number and organ, respectively, that the sample contained. For example, 1-F1K equals a sample gathered from site 1, containing kidney tissue from fish 1. Another example is 3-F2S representing a sample obtained from site 3 and containing spleen tissue from fish 2.

Methods

Homogenization

Samples stored in RNA later at 4°C were used as starting materials. Two samples per fish, spleen and head kidney, in separate tubes (max 30 mg tissue per organ per fish) was used. To cut the tissue, we used sterile scalpel blades and forceps, one set per organ type and sterilized with chlorine in between each sample. 350 µl lysis buffer (RLT buffer) from the RNeasy mini kit (QIAGEN®) per < 20 mg of tissue or 600 µl for the tissue weighing 20 mg or above was used. The tissue was then homogenized by adding 1-2 beads to each tube and debris removed by centrifuging for 3 min at maximum speed. The supernatant was carefully removed using a pipette and transferred into new tubes.

RNA extraction

The RNeasy mini kit from QIAGEN® was used to perform RNA extraction of the samples in preparation to run real-time PCR.

An equal volume of 70% ethanol (equal to 350 µl or 600 µl RLT buffer used in the homogenization step above depending on tissue weight) was added to the lysate mix and mixed by pipetting 3-5 times. We transferred up to 700 µl of the sample to a RNeasy spin

column placed in a 2 ml collection tube. The lid was closed and the sample centrifuged for 1 minute at maximum speed (8000 x *g*). The flow through was discarded after centrifugation.

Next, 700 µl buffer RW1 was added to each RNeasy spin column to wash membrane-bound RNA. The lid was closed and the tube centrifuged for 1 minute at maximum speed (8000 x *g*). After centrifugation, the flow through was discarded. 500 µl buffer RPE was then added to the RNeasy spin column for additional washing of membrane-bound RNA. The lid was then closed before centrifuging for 2 minutes at 8000 x *g*. The flow through was discarded.

Then the RNeasy spin columns were placed in new 1,5 ml collection tubes before adding 50 µl RNase-free water to each RNeasy spin column. The RNase-free water was added directly onto the spin column membrane. The lid was closed before centrifuging for 1 minute at 8000 x *g*. Then we were finished with the RNA extraction. The last step with added RNase-free water could be repeated, if necessary, but we concluded not to after measuring concentrations of elutes by nanodrop.

Nanodrop

The concentration of RNA after extraction was measured by using the Nanodrop ND1000 (NanoDrop technologies, Wilmington, DE, USA). We selected a total of 25 samples, distributed among the various farms, to evaluate the concentration of RNA.

Real-time PCR

We used SBC's Tilapia Lake virus qPCR kit (Schweitzer Biotech Company, Taiwan) for qualitative detection of TiLV. This is a TaqMan probe-based real time qPCR assay where the presence of TiLV nucleic acids was detected using a fluorescent probe. The kit consists of PCR Enzyme Mix, PCR Master Mix including FAB probe and primers, Positive Control and Negative Control, all of these presented in foil and kept in the freezer before use.

For each sample we added 1,5 µl of the PCR Enzyme Mix with 18,5 µl of the PCR Master Mix into a sterile container and with a pipette we transferred 20 µl of this mix into each qPCR reaction well. We used 96-well PCR plates, however not all wells were used for every PCR run, see Table 2. We always made sure the last well (H12) was saved for positive control. Due to a system error, well F9 could not be used in the qPCR machine. Thereafter, we added 5 µl of template into each well, including unknown samples, positive control and negative control. We mixed the contents in each well with a pipette. This resulted in a 25 µl volume in each well.

We sealed the PCR plate after checking for air-bubbles and ensuring that the liquid content was at the bottom of each well. Finally, we placed the reaction plate into a qPCR device and proceeded with the amplification protocol.

Step	**Temp**	**Time**	**Cycle**
1	50 °C	15 min	1
2	95 °C	5 min	1
3	95 °C 60 °C	10 s 40 s	40

Table 1. Amplification conditions (cycling program). From instructions of use SBC Tilapia Lake Virus qPCR Kit.

Samples	**Location**	**Date for sampling**	**Date for RNA extraction**	**Date for PCR**
1-24 (24 samples)	Site 1 (24)	1.11.22	14.11.22	15.11.22 (pilot)
25-116 (92 samples)	Site 1 (6), Site 2 (60), Site 3 (26)	1.11.22 - 3.11.22	16.11.22	22.11.22 (plate 1)
117-209 (93 samples)	Site 3 (26), Site 4 (60), Site 5 (7)	3.11.22 - 7.11.22	17.11.22	22.11.22 (plate 2)

210-302 (93 samples)	Site 5 (7), Site 6 (58), Site 7 (28)	7.11.22 - 9.11.22	18.11.22	23.11.22 (plate 3)
303-393 (91 samples)	Site 7 (32), Site 8 (60)	9.11.22 - 10.11.22	21.11.22	23.11.22 (plate 4)

Table 2. Dates for laboratory procedures.

Results

Survey

Information on-site was obtained by use of a standard questionnaire where the manager or owner of the farm was asked a number of questions. In the table below, we have summarized the most important findings from the survey. Some of the columns are somehow incomplete, this is due to language barrier, generally lack of knowledge regarding production and often the manager was not on site to clarify. Important to note is that three of the farms had fish originating from other farms, and that none seemed to have experienced problems associated with TiLV disease previously. At one of the fish farms, site 3, they have had a previous outbreak with suspicious deaths of fish, resulting in them contacting a veterinarian and testing for TiLV although a negative result was obtained.

Location	Species	Origin	Farming type	Specific problems	Dead fish
Site 1	Tilapia and catfish	Broodstock on site	Cages and hapas	Gasbubble disease, fungi, ammonia build-up, predators	Counts dead fish every morning and afternoon. Burn dead fish.
Site 2	Tilapia	Broodstock on site	Ponds and hapas	Fungi, predators	*
Site 3	Tilapia	Broodstock gathered from all over Zambia	Ponds and hapas	Fungi, predators	Does not count dead fish daily. Burn dead fish.
Site 4	Tilapia. Also catfish and carp, but do not sell these.	*	Ponds	Algae, fungi, predators	*
Site 5	GIFT	Broodstock	Ponds	Predators	*

		on site			
Site 6	Tilapia	Fry from Zimbabwe	Cages	*Streptococcus agalactica* and *lactococcus*, fungi, predators, theft	Collect floating dead fish
Site 7	Tilapia	Different hatcheries	Cages	Pop eye infection, fungi, predators	Collect floating dead fish
Site 8	Tilapia	Broodstock on site	Hapas	Predators, heat, fungi	Counts dead fish every day

*Table 3. A summary of site details and key questionnaire results from each farm. * = not answered during the survey.*

Information about the fish

In the table below we have summarized information about the fish we sampled in this study. There is a complete table with every fish collected in Appendix 1. The main information to draw from this is that we sampled fingerlings at three of the farms, and adult fish at the others. *O. niloticus* was the most represented species.

Locations	**Number of sampled fish**	**Sampling date**	**Mean weight (gram)**	**Mean length (cm)**	**Fish group**	**Species**
Site 1	1-15 (15)	01.11.22	33	12,6	Older fish	*O. andersonii*
Site 2	16-45 (30)	02.11.22	Not measured	Not measured	Fingerlings	*O. niloticus* *O. andersonii*
Site 3	46-71 (26)	03.11.22	100	19,7	Older fish	*O. macrochir* *C. rendalli* *O. andersonii*
Site 4	72-101 (30)	04.11.22	Only adults: 66	Only adults: 16,2	Older fish 72-81 Fingerlings 82-101	*O. andersonii*
Site 5	102-108 (7)	07.11.22	140	19,0	Older fish	*O. niloticus*

Site 6	109-137 (29)	08.11.22	292,5	23,0	Older fish	*O. niloticus*
Site 7	138-167 (30)	09.11.22	220,6	19,9	Older fish	*O. niloticus*
Site 8	168- 197 (30)	10.11.22	Not measured	Not measured	Fingerlings	O. niloticus

Table 4. A table summarizing information about the tilapia used in this study.

RNA purity after extraction

RNA purity was determined by spectrophotometric measurement (NanoDrop). Pure RNA is measured at an absorbance of 260/280, and pure RNA ranges between 1,8 and 2,1. All of our samples fell within this range (Table 5 below). The 260/230 ratio is utilized as a secondary measure of purity, where a generally acceptable range is 2,0-2,2. Even so, there is no consensus on the acceptable lower limit of the 260/230 ratio. Only three of our samples fell within the range, while the rest were below (QIAGEN, 2010).

Sample number	Date	Tissue (mg)	ng/µl	260/280	260/230
1F1K	14.11.2022		313,2	2,04	
1F4K	14.11.2022		118,9	2,04	
1F7S	14.11.2022		240,7	1,93	
1F9S	14.11.2022		16,8	2,05	
1F12S	14.11.2022		19,8	1,96	
1F14K	22.11.2022	14	164	2,06	1,09
2F6K	22.11.2022	27	129,3	1,99	0,46
2F10K	22.11.2022	26	269,2	2,06	2,18
2F19S	22.11.2022	25	109,7	2,03	0,26
3F11S	22.11.2022	23	58,4	2,08	0,95
3F20K	22.11.2022	27	186,6	2,1	1,64

4F1S	22.11.2022	20	244	1,92	0,65
4F18S	22.11.2022	26	909	2,09	2,16
5F1K	22.11.2022	26	532,7	2,1	2,01
5F4S	22.11.2022	25	901,9	2,04	1,85
5F6K	23.11.2022	20	209,8	2	0,7
6F2S	23.11.2022	21	569,7	1,98	1,27
6F15S	23.11.2022	23	26,8	1,91	0,2
7F4K	23.11.2022	20	380,2	2,04	1,68
7F11S	23.11.2022	30	298,7	1,92	1,85
7F19S	21.11.2022	21	1510,2	1,97	1,72
7F25S	21.11.2022	27	363,8	1,95	0,87
7F29K	21.11.2022	29	367,4	2,05	1,74
8F19S	21.11.2022	28	578,5	2,09	1,23
8F29K	21.11.2022	29	705,4	2,09	1,74

Table 5. Samples that were evaluated using NanoDrop, the date they were evaluated, the tissue weight we started with and the results. The blank fields in the table are due to lack of notes during the pilot.

No detection of TiLV genome in examined samples

A total of 197 tilapia were tested and both head kidney and spleen were sampled from each fish. During extraction, eight samples were lost due to errors in the lab, making the final number of samples run in the PCR machine 386. See Appendix 2 under for an overview of all the samples.

SBC´s “Instruction for use” got requirements for data interpretation, which is summarized below:

Target (FAM)	Positive control (FAM)	Negative control (FAM)	Interpretation
+	+	-	Positive for TiLV
-	+	-	Negative for TiLV
+/-	-	+/-	Invalid result
+/-	+/-	+	Invalid result

Table 6. SBC´s requirements for data interpretation. From SBC Tilapia Lake Virus qPCR Kit Instruction for Use

Positive results were defined as Ct values ≤ 40, and negative results as Ct values > 40 or not detected at all. From the 386 samples, TiLV was not detected in any of the samples. The heat map registered two samples as positive, but these did not meet the Ct value criteria, and were therefore not classified as positive (false positive, Figure 16 and 17).

Validity of the data

The validity of the data was decided based on SBC´s "Instruction for use". The requirements were for the positive control to show a typical qPCR amplification curve with a Ct value ≤ 40, and for the negative control to display no typical qPCR amplification curve and no Ct/Ct value > 40. Both requirements were met on every plate run in the PCR machine.

	Negative control	Positive control	False positive
Pilot	not detected	21,66	
Plate 1	not detected	10,56	
Plate 2	not detected	20,45	1,97
Plate 3	not detected	21,17	2,33
Plate 4	not detected	22,28	

Table 7. Table showing the Ct values. For the negative controls, no Ct values were obtained, and the positive controls had Ct values below 40.

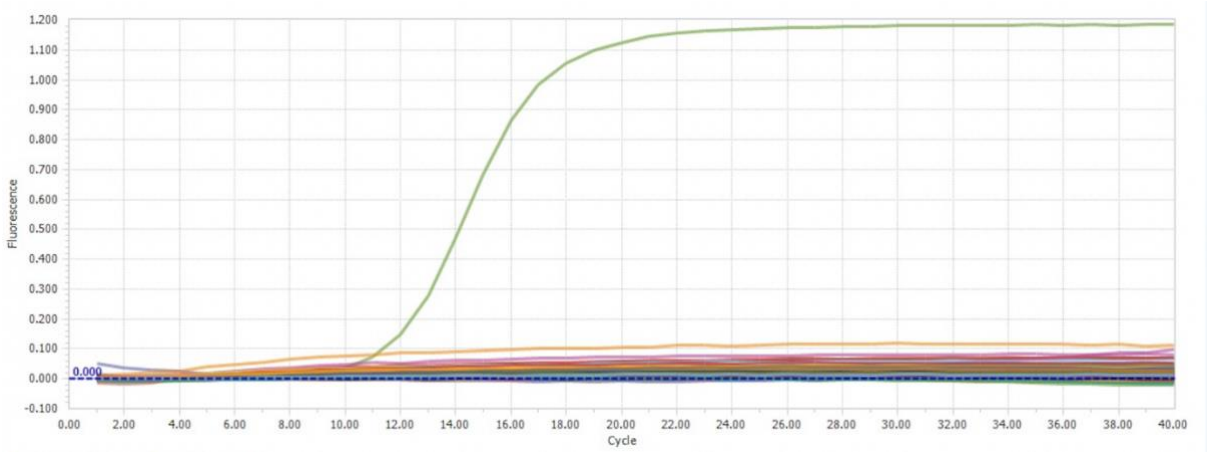

Figure 15. *Photo illustrating the amplification curve of the positive control (green), and the rest of the plate with negative results.*

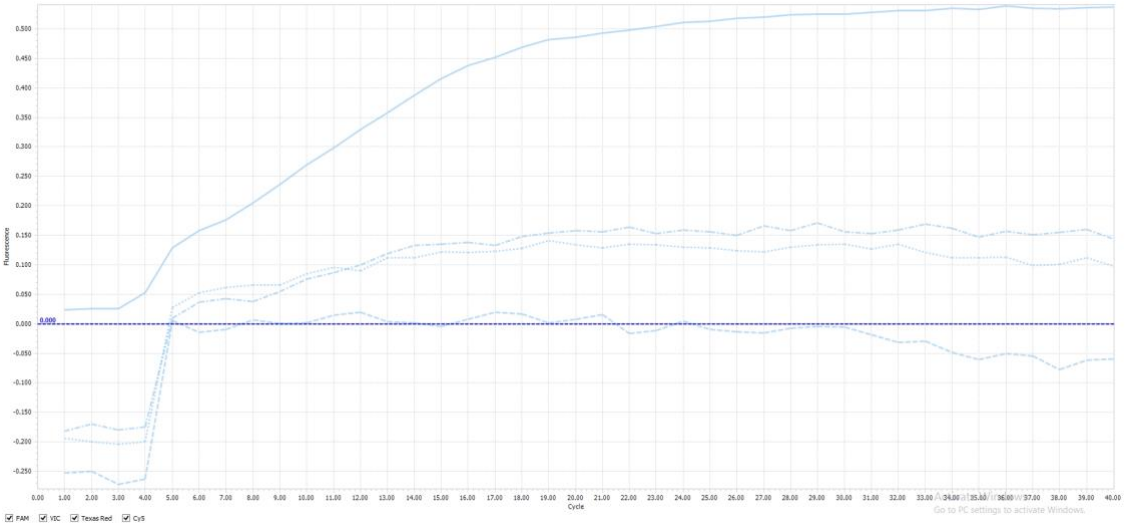

Figure 16. *Photo of the amplification curve of 3F21K, a false positive sample. There is no typical amplification curve.*

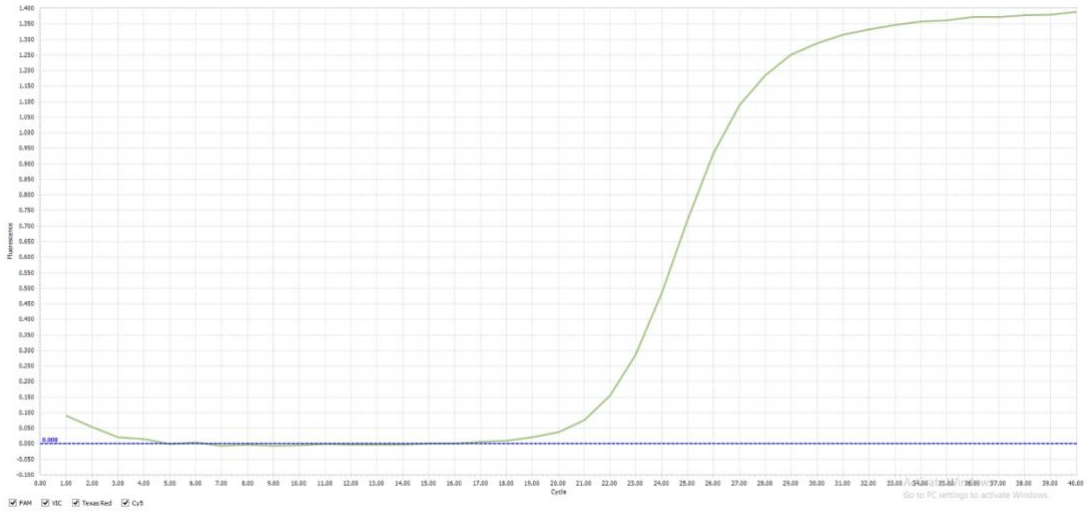

Figure 17. Photo of the amplification curve of the positive control on the same plate as 3F21K. This illustrates a correct amplification curve with a baseline, exponential curve and plateau.

Discussion

TiLV was first detected in 2014 (Eyngor et al., 2014), but archived samples suggest that it has been present for years (Tang, 2021). The countries with reported presence of TiLV as of 2021 are Israel, Egypt, Tanzania, Uganda, Ecuador, Colombia, Peru, India, Indonesia, Malaysia, the Philippines, Thailand, Bangladesh, Chinese Taipei, Mexico and the United States (Surachetpong et al., 2020). The tilapia industry is global, with main producers being large both financially and distributionally, and international trade has gone global. For years we have not had any readily available diagnostics to detect TiLV and have not been able to map the geographical distribution thoroughly. This study aimed at detecting the TiLV genome in apparently healthy fish in Zambia, where the virus has not been detected yet. Samples from the immune organs, head kidney and spleen, were examined through RT-qPCR, but the TiLV genome was not detected.

Nanodrop

All of our samples were within the ratio of pure RNA of absorbance at 260/280. However, the 260/230 ratio was in the lower than expected range, which may indicate contaminants in the sample that absorb at 230 nm. This contamination in RNA samples is often due to guanidine thiocyanate, but other possible contaminants are carbohydrates, peptides and phenol (QIAGEN, 2010). We find guanidine thiocyanate at high concentrations in the Buffer RLT, used during the homogenizing for lysing cells and tissues prior to RNA isolation (QIAGEN, 2013-23a). It is also present in the Buffer RW1 used for washing membrane-bound RNA during the RNA extraction (QIAGEN, 2013-23b). Therefore, it is possible that guanidine thiocyanate caused the low 260/230 values. Qiagen's own experiments revealed that even sub-milimolar concentrations of the salt will solidly reduce the 260/230 ratio (QIAGEN, 2010). Nevertheless, the experiments also showed that concentrations of the salt up to 100mM will not compromise the reliability of RT-PCR.

The 260/230 ratio also depends on the RNA concentration, and contaminants will have a major impact if the concentration is low. When we sampled fingerlings, our source of material was limited. Therefore, some of our samples had quite low amounts of material, the lowest being 3 mg (Appendix 2).

QIAGENs own analysis and experiments show that a low 260/230 ratio will not influence downstream applications, and that samples with a low ratio still had typical amplification and melting curves (QIAGEN, 2010).

False positive samples

Sample 3F21K and 6F4K showed positive results on the heat map after PCR. These samples lack the standard three phases of a qPCR amplification curve. There is no baseline, exponential curve or plateau. That tells us that the result is a false positive, most likely an

artifact. The Ct values do not match either, our positive controls have a Ct value ranging from 10.56 to 22.28, while the false positive samples have a Ct value at 1.97 and 2.33, see Table 7.

We used a Taqman probe-based qPCR for this study, which is a highly specific and sensitive method based on dual-labeled probes which degrade and release fluorophore for detection of accumulated DNA (Udayangani, 2017). An alternative approach would have been to use a SYBR Green-based detection of the amplicon, which also is a very sensitive method (Cao & Shockey, 2012). SYBR green is based on a fluorescent dye, and binds to the double-stranded DNA (the amplicon), but will bind to the dsDNA generated through the PCR process irrespective of the obtained sequences being specific for the target or not (Artika et al., 2022). For this reason, to ensure that the obtained result does not reflect a binding to a nonspecific product, the recommendation is to carry out a melting curve analysis to ensure the specificity of the amplified DNA sequences (Artika et al., 2022).

As mentioned, SYBR Green binding is not sequence specific, and binds non-specifically to double-stranded DNA and while a melting curve can be done using SYBR green, this is not possible when a Taqman probe setup is used, and related to our findings, this is a drawback when false positive samples are obtained, as seen in our study. An analysis of the form of the amplification curve is the approach used and will support the conclusion drawn in this instance.

Tissue tropism

Mugimba et al. (2018) found in their study that fish with subclinical infections had a high prevalence of the TiLV virus in their lymphoid organs and heart. In terms of tissue distribution, the highest prevalence was observed in head kidney and spleen, followed by the heart. And contrary to acute infections where the target organs, liver and brain, show a high prevalence (Mugimba, 2020) for subclinical infections, detection of viral genomes was low in

the liver and absent in the brain (Mugimba et al., 2018). Other studies have also found that the head kidney and spleen have the highest prevalence during subclinical infections, while it has been low/absent in the liver and brain (Chengula, 2021). And this constituted the basis for selecting spleen and head kidney in our study.

Why is TiLV not yet present/detected in Zambia?

As mentioned in the introduction, Zambia is at high risk of TiLV spread through import of fry and fingerlings from Thailand, where the virus has been detected. There are also nine other African countries at high risk, where the virus is not yet detected, many of these geographically close to Zambia. Two of the countries mentioned in the "high risk" list, Uganda and Tanzania (Dong et al., 2017a), have on the other hand had the virus nucleic acids detected since the article was published. Why is it that the virus is not yet present or detected in Zambia? Why is risk evaluation of possible TiLV spread so comprehensive?

One theory regarding the risk of TiLV spread is the size of tilapia aquaculture in Zambia versus other countries who have had the virus detected. According to an article posted by FAO in 2020, with information from 2018 (FAO, 2020), tilapia aquaculture is significantly less in Zambia than for example in Uganda and Egypt, who both have had the virus detected. Tilapia aquaculture in Uganda is almost three times as big as in Zambia in terms of tonnes, and in Egypt the production is nearly 44 times larger. One factor that might explain the absence of TiLV infection in Zambia is a lower production, and with the expected increase in production it will be important to keep a close eye on biosecurity, particularly if fish are imported. On the other hand, Ghana has around the same size of tilapia aquaculture as Uganda (around 70 000 tonnes per year), but TiLV infection has not been reported, and Tanzania has less than half of the production of Zambia and PCR positive results were reported in 2018. Thus, there is no clear relationship between size of the tilapia aquaculture

and the risk of TiLV occurrence, and several factors play a role related to introduction or occurrence of TiLV infection. It should also be added that Tanzania and Uganda share water bodies through Lake Victoria, and PCR positive fish were detected on each side of the border in Lake Victoria (Mugimba et al., 2018).

Another theory concerns import of fry and fingerlings. Finding exact numbers regarding import of fry and fingerlings and the production of tilapia in Zambia has proven difficult. Nevertheless, information from The Department of Fisheries in Chilanga was that there has been no importation of tilapia to Zambia since 2019 due to the risk of importing infected seeds (Namufaka, pers com). Therefore, Zambia has extensive self-production of fry and fingerlings. We also obtained information from one of the fish farms we visited, site 5, and they had previously imported fertilized eggs from Thailand, but due to the ban in 2019 they stopped. On the other hand, other trade agreements, for example the Common Market of East and Central Africa (COMESA) dictate for example that importation of fish to site 6 from a neighboring country was handled differently, and this import was not affected by the ban in 2019, thus treated differently from importation from other countries, for example from Thailand.

There are many aquaculture operations in Zambia and we sampled from a very limited number of fish farms, i.e. eight farms were examined in the present study. The farms included were selected randomly and with consideration of disease occurrence or previously reported disease outbreaks, *i.e.*, the sampling was not risk-based. Furthermore, the local farmers / managers decided from which group, pond, hapas or cages fish were sampled, and they also collected the fish from these entities. Therefore, it is difficult to determine to what extent our samples were selected and/or how representative they are. The fish farms from which sampling was conducted were geographically in proximity to each other, as presented in

Figure 12. Thus, the limited number of farms and also the fact that we covered only a small proportion of the fish farming regions/areas in Zambia puts some limitation to results obtained from this study. That said, we were informed that the farm on site 8, ships large amounts of fingerlings nationally within Zambia. In addition, from the surveys conducted, two of the other fish farms, site 3 and site 7, import broodstock and fingerlings from other fish farms in Zambia. Indirectly we have covered larger areas of Zambia than we initially assumed, and our selection might not have been too narrow.

Over the years there have been reports of disease outbreaks in farmed tilapia in Zambia, for example the significant mortality recorded in tilapia farms in 2016 (Tang, 2021), that was not examined in depth and no cause was determined. There could be other similar cases where either the testing methods were not adequate or non-existing, or the veterinary services were not involved to further examine the cause of mortality or there was a lack of finances to conduct a thorough examination. From the survey we conducted at the different fish farms, few farmers indicated that they contacted veterinarians in cases of disease. When we asked if the different fish farms contacted veterinarians in case of sick fish/disease, five sites answered no, one site answered sometimes, one site answered yes and one site did not answer this during the survey. This indicates that few veterinarians are involved in tilapia aquaculture in Zambia, and that it is not common to request assistance from a veterinarian to investigate disease outbreaks or conduct preventive work at the fish farms.

Another factor that would impact on risk of TiLV spread is presence or absence of surveillance programs, generally within aquaculture and specifically for TiLV. We have not been able to find any information regarding general or specific surveillance programs for diseases in aquaculture operations in Zambia. The reasons are likely many, and would depend on a willingness from the farmers to test for diseases, economy (government and private

sector), and knowledge regarding the importance of testing and monitoring diseases in such large scale productions. Another reason can be politics, considering that such decisions must come from departments. It may be discussed if Zambia should introduce a surveillance program, but the implementation would require involvement from government and farmers and would have to cover all farms to be effective. WorldFish published at fact sheet called "Biosecurity practices for tilapia hatcheries: A case of Zambia" in December 2022 (Basiita K. R, 2022). This fact sheet contains important biosecurity measures, procedures for obtaining new broodstock, management of both indoor and outdoor facilities, disinfection, feeding and how to develop a biosecurity plan. Even though this is not a surveillance program, this is a step in the right direction of developing good biosecurity and management practices, and it also shows that disease prevention is on the radar for tilapia aquaculture in Zambia.

Systematic errors and internal validity

The internal validity is the extent to which the conclusion of our research is valid for the study population. We want to acknowledge factors and other variables that might have influenced the causal relationship to our testing, like systematic errors.

In this study, we have not detected TiLV with the chosen method, and reviewing our own work, we have identified procedures used during the process that possibly could have affected our results. As of our sampling methods they varied depending on the size of the fish, since we were not always given fish of sufficient size to sample the organs specified in the protocol. Therefore, we had to divide the smallest fish in two parts, separating the head kidney and the spleen. Although we did this dissection as precisely as we could, it is possible that we have missed the essential organs since they were not identifiable. Another aspect is the stabilization of the RNA in our RNAlater cooling chain. After each sampling we kept our finalized RNAlater cooled in a box with ice that were later put in the fridge. The environmental

temperatures were very high and therefore the ice occasionally melted down before the samples reached the refrigerator. Also, the amount of time the different samples had to be stored before RNA extraction varied a lot, and so did the time before refrigerating, although storage at 4°C for 1-2 weeks would in general not represent a critical factor and the guidelines from Thermofisher state that tissue stability is one day when stored at 37 °C, 1 week when left in room temperature and 1 month at 4 °C (Inc., 2006-2023). None of our samples were left in the fridge for more than two weeks before beginning the RNA extractions, but the temperatures up until refrigerating could in theory have affected the tissues stability for some of the samples on the warmest days.

In the laboratory, we had to deviate from our protocol for one specific part of the laboratory work due to lack of optimal equipment. The available centrifuge could not centrifuge for less than one minute. When our protocol required 15 seconds of centrifugation, one minute was used. Apart from this, we only had minor technical issues with a few leaks from our test-tubes during centrifugation that led to loss of less than 10 samples. This has been described under results and since few samples were affected, we do not consider this an error that has impacted the results. Also, these events have less impact since all samples were negative. However, had samples been positive for TiLV, these samples would have had to be re-examined.

In retrospect, we recognize that we potentially would have benefited from sampling more material from each fish. If two samples from the same organs per fish were analyzed, we could have claimed with more certainty that the PCR results were valid. In addition, more material could have been used as controls if required after PCR runs. Since our selected organs of testing oftentimes were small, and the size of the fish we sampled from varied a lot, we did not always have the opportunity to sample enough material to do control testing or the

possibility to run paired PCR tests to validate our results. On the other side, cost of analysis puts a cap on how many samples can be included.

Conclusion

The main conclusion that can be drawn from this study is that TiLV was not detected in Zambia as of November 2022. This study is based on eight fish farms, and geographically they do not cover the whole of Zambia. However, due to national movements of fish and locally across borders, we have reasons to believe that a greater area than initially thought was covered. We cannot conclude that the virus is not present in Zambia, although not detected yet. To make such a conclusion, a greater study needs to be done, covering larger parts of Zambia.

Although not detected, there is a fear of the virus being spread to Zambia due to earlier import from countries where TiLV has been reported. Today there is a ban on import of tilapia, established in 2019, but there is a chance of the virus being introduced and established prior to that. Furthermore, there is the issue of different trade agreements that apply, there means that while there is a ban on importation of fish in Zambia, if there is no ban in a neighboring country and that country imports infected fish, there is a risk that the infection could eventually find itself in Zambia. This is an issue that the government of Zambia needs to take into account in combating fish disease control.

The tilapia production is of great importance for Zambia, so further development of high biosecurity standards and control measures should be prioritized. Systematic collection of data, and organizing an integrated surveillance infrastructure, would be beneficial to secure early warnings if the virus should appear. A national emergency disease response system targeting TiLV if it is detected should be prioritized, with restrictions on movement of live

tilapines, generic biosecurity measures and appropriate disinfection protocols incorporated. Communication and cooperation from the farmers is essential, especially to stress immediate notification to competent authorities if unexplained tilapia mortalities arise.

Given tilapia's importance internationally, there should be a global interest to manage the disease. To date, there are no measures available to prevent TiLV disease through vaccines and outbreaks have the potential to have serious impact on production. Further studies on development of vaccines and breeding programs for resistance should be investigated.

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Sammendrag

Tittel: En studie av forekomst av Tilapia Lake Virus i oppdretts-tilapia i Zambia

Forfattere: Erle Amalie Fuglesteg, Elisabeth Søsæter Stenseth og Margrete Krage

Veiledere: Professor Øystein Evensen og Professor Stephen Mutoloki, Institutt for parakliniske fag

Sykdom som følge av TiLV gir høy dødelighet i tilapia akvakultur og kan resultere i betydelig økonomisk tap for industrien. Det er blitt et økende fokus på forskning rundt TiLV og sykdommen, men det er fortsatt mer å lære om patogenesen og den geografiske distribusjonen av viruset. Tilapia produksjon er en av verdens største matindustrier innen akvakultur og produksjon finnes i land som Kina til Colombia og Ghana. Zambia er det fjerde største tilapia-produserende landet i Afrika, etterfulgt av Ghana, Uganda og Egypt på topp. Hittil er viruset rapportert i 16 land og Uganda og Egypt fikk viruset påvist i 2017. Det har ikke blitt gjennomført omfattende testing i Zambia selv med tilstedeværelse av TiLV i flere naboland. I denne studien undersøkte vi fiskeoppdrett i Zambia, inkludert områder som Kabwe, Ndola, Kitwe, Kalulushi og Siavonga.

Vi har prøvetatt totalt 197 fisk fra åtte ulike lokalisasjoner og samlet inn vev fra hodenyre og milt fra klinisk frisk fisk. Prøvene ble transportert i RNAlater, RNA ble ekstrahert på laboratoriet og målinger av konsentrasjoner ble vurdert med Nanodrop. Vi brukte real-time PCR på alle prøver for deteksjon av TiLV genom. Vi bekreftet ikke tilstedeværelse av TiLV i noen av våre prøver.

References

Adams, M. J., Lefkowitz, E. J., King, A. M. Q., Harrach, B., Harrison, R. L., Knowles, N. J., Kropinski, A. M., Krupovic, M., Kuhn, J. H., Mushegian, A. R., et al. (2017). Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Arch Virol*, 162 (8): 2505-2538. doi: 10.1007/s00705-017-3358-5.

Aich, N., Paul, A., Choudhury, T. G. & Saha, H. (2022). Tilapia Lake Virus (TiLV) disease: Current status of understanding. *Aquaculture and Fisheries*, 7 (1): 7-17. doi: https://doi.org/10.1016/j.aaf.2021.04.007.

Artika, I. M., Dewi, Y. P., Nainggolan, I. M., Siregar, J. E. & Antonjaya, U. (2022). Real-Time Polymerase Chain Reaction: Current Techniques, Applications, and Role in COVID-19 Diagnosis. *Genes*, 13 (12). doi: 10.3390/genes13122387.

Bacharach, E., Mishra, N., Briese, T., Zody, M. C., Kembou Tsofack, J. E., Zamostiano, R., Berkowitz, A., Ng, J., Nitido, A., Corvelo, A., et al. (2016). Characterization of a Novel Orthomyxo-like Virus Causing Mass Die-Offs of Tilapia. *mBio*, 7 (2): e00431-16. doi: 10.1128/mBio.00431-16.

Basiita K. R, M. T., Hampuwo B, Chungu P and Songe M. (2022). *Biosecurity practices for tilapia hatcheries: A case of Zambia*. Penang, Malaysia: WorldFish.

Behera, B. K., Pradhan, P. K., Swaminathan, T. R., Sood, N., Paria, P., Das, A., Verma, D. K., Kumar, R., Yadav, M. K., Dev, A. K., et al. (2018). Emergence of Tilapia Lake Virus associated with mortalities of farmed Nile Tilapia Oreochromis niloticus (Linnaeus 1758) in India. *Aquaculture*, 484: 168-174. doi: https://doi.org/10.1016/j.aquaculture.2017.11.025.

Cao, H. & Shockey, J. M. (2012). Comparison of TaqMan and SYBR Green qPCR Methods for Quantitative Gene Expression in Tung Tree Tissues. *Journal of Agricultural and Food Chemistry*, 60 (50): 12296-12303. doi: 10.1021/jf304690e.

Chapman, F. A. (2018). *CULTURE OF HYBRID TILAPIA: A REFERENCE PROFILE*. Gainesville, FL 32611: School of Forest Resources and Conservation, Fisheries and Aquatic Sciences Program, UF/IFAS Extension. Available at: https://edis.ifas.ufl.edu/publication/FA012 (accessed: 02/03/2023).

Chengula, A. A., Mutoloki, S., Evensen, Ø. & Munang'andu, H. M. (2019). Tilapia Lake Virus Does Not Hemagglutinate Avian and Piscine Erythrocytes and NH(4)Cl Does Not Inhibit Viral Replication In Vitro. *Viruses*, 11 (12). doi: 10.3390/v11121152.

Chengula, A. A. (2021). *Tilapia Lake Virus (TiLV) – development of PCR-based diagnostic assays and insights into infection mechanisms*. Adamstuen: Norwegian University of Life Sciences Faculty of Veterinary Medicine.

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 (11): 1629-1636. doi: 10.1111/jfd.13076.

Dong, H. T., Ataguba, G. A., Khunrae, P., Rattanarojpong, T. & Senapin, S. (2017a). 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., Siriroob, S., Meemetta, W., Santimanawong, W., Gangnonngiw, W., Pirarat, N., Khunrae, P., Rattanarojpong, T., Vanichviriyakit, R. & Senapin, S. (2017b). Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. *Aquaculture*, 476: 111-118. doi: https://doi.org/10.1016/j.aquaculture.2017.04.019.

Dong, H. T., Senapin, S., Gangnonngiw, W., Nguyen, V. V., Rodkhum, C., Debnath, P. P., Delamare-Debouteville, 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.

Encyclopedia, N. W. (2008). *"Tilapia," New World Encyclopedia*. Available at: https://www.newworldencyclopedia.org/p/index.php?title=Tilapia&oldid=742867 (accessed: 20/2/2023).

Eyngor, M., Zamostiano, R., Kembou Tsofack Japhette, E., Berkowitz, A., Bercovier, H., Tinman, S., Lev, M., Hurvitz, A., Galeotti, M., Bacharach, E., et al. (2014). Identification of a Novel RNA Virus Lethal to Tilapia. *Journal of Clinical Microbiology*, 52 (12): 4137-4146. doi: 10.1128/JCM.00827-14.

FAO. (2016). *The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all.* Rome.

FAO. (2020). *Tilapia production and trade with a focus on India. WAPI factsheet to facilitate evidence-based policymaking and sector management in aquaculture*: FAO Global Fishery and Aquaculture Production Statistics. Available at: https://www.fao.org/common-pages/search/en/?q=Tilapia%20production%20and%20trade%20with%20a%20focus%20on%20India&fbclid=IwAR29j2jdtwXS76ak8HKLoGEeiyWO34qb09mep15j02RqDwcHQ40aP8NHLhg (accessed: 13/02/2023).

Fathi, M., Dickson, C., Dickson, M., Leschen, W., Baily, J., Muir, F., Ulrich, K. & 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. *J Fish Dis*, 37 (6): 583-9. doi: 10.1111/jfd.12142.

Howell, M. (2019). *Seven deadly fins: study sheds light on key tilapia viruses*: The Fish Site. Available at: https://thefishsite.com/articles/seven-deadly-fins-study-sheds-light-on-key-tilapia-viruses (accessed: 11/03/2023).

Inc., T. F. S. (2006-2023). *RNAlater Solutions for RNA Stabilization and Storage*. Available at: https://www.thermofisher.com/no/en/home/brands/product-brand/rnalater.html (accessed: 06/04/2023).

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. doi: https://doi.org/10.1016/j.aquaculture.2018.08.028.

Jansen, M. D., Dong, H. T. & Mohan, C. V. (2019). Tilapia lake virus: a threat to the global tilapia industry? *Reviews in Aquaculture*, 11 (3): 725-739. doi: https://doi.org/10.1111/raq.12254.

Kembou Tsofack Japhette, E., Zamostiano, R., Watted, S., Berkowitz, A., Rosenbluth, E., Mishra, N., Briese, T., Lipkin, W. I., Kabuusu Richard, M., Ferguson, H., et al. (2017). Detection of Tilapia Lake Virus in Clinical Samples by Culturing and Nested Reverse Transcription-PCR. *Journal of Clinical Microbiology*, 55 (3): 759-767. doi: 10.1128/JCM.01808-16.

Liamnimitr, P., Thammatorn, W., U-thoomporn, S., Tattiyapong, P. & Surachetpong, W. (2018). Non-lethal sampling for Tilapia Lake Virus detection by RT-qPCR and cell culture. *Aquaculture*, 486: 75-80. doi: https://doi.org/10.1016/j.aquaculture.2017.12.015.

Lopes, T. M., Garcia de Oliveira, F., Bialetzki, A. & Agostinho, A. A. (2015). Early development in the mouth-brooding cichlid fish Satanoperca pappaterra (Perciformes: Cichlidae). *Revista de Biología Tropical*, 63: 139-153.

Machimbirike, V. I., Jansen, M. D., Senapin, S., Khunrae, P., Rattanarojpong, T. & Dong, H. T. (2019). Viral infections in tilapines: More than just tilapia lake virus. *Aquaculture*, 503: 508-518. doi: https://doi.org/10.1016/j.aquaculture.2019.01.036.

Maguswi, C. T. (2023). *National Aquaculture Sector Overview- Zambia*. Rome: FAO, Fisheries and Aquaculture Division. Available at: https://www.fao.org/fishery/en/countrysector/naso_zambia (accessed: 15/02).

Matrosovich, M., Herrler, G. & Klenk, H. D. (2015). Sialic Acid Receptors of Viruses. *Top Curr Chem*, 367: 1-28. doi: 10.1007/128_2013_466.

Mugimba, K., Chengula, A., Wamala, S., Mwega, E., Kasanga, C., denis, K., Mdegela, R., Tal, S., Bornstein, B., Dishon, A., et al. (2018). Detection of tilapia lake virus (TiLV) infection by PCR in farmed and wild Nile tilapia (Oreochromis niloticus) from Lake Victoria. *Journal of Fish Diseases*, 41. doi: 10.1111/jfd.12790.

Mugimba, K. K., Tal, S., Dubey, S., Mutoloki, S., Dishon, A., Evensen, Ø. & Munang'andu, H. M. (2019). Gray (Oreochromis niloticus x O. aureus) and Red (Oreochromis spp.) Tilapia Show Equal Susceptibility and Proinflammatory Cytokine Responses to Experimental Tilapia Lake Virus Infection. *Viruses*, 11 (10). doi: 10.3390/v11100893.

Mugimba, K. K. (2020). *The infection biology of tilapia lake virus: tissue tropism, host susceptibility and immune responses*. Oslo, Norway: Norwegian University of Life Sciences, Faculty of Veterinary Medicine.

Mugimba, K. K., Lamkhannat, M., Dubey, S., Mutoloki, S., Munang'andu, H. M. & Evensen, Ø. (2020). Tilapia lake virus downplays innate immune responses during early stage of infection in Nile tilapia (Oreochromis niloticus). *Scientific Reports*, 10 (1): 20364. doi: 10.1038/s41598-020-73781-y.

Nations, U. (2015). *Population 2030: Demographic challenges and opportunities for sustainable development planning (ST/ESA/SER.A/389)*. Department of Economic and Social Affairs, P. D. New York.

Nicholson, P., Mon-on, 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: 734746. doi: https://doi.org/10.1016/j.aquaculture.2019.734746.

Pierezan, F., Yun, S., Surachetpong, W. & 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*. doi: 10.1111/jfd.13052.

Publications, U. N. (2022). *The Sustainable Development Goals Report 2022* New York, NY, 10017, United States of America. Available at: https://unstats.un.org/sdgs/report/2022/ (accessed: 08/02/2023).

QIAGEN. (2010). *What are the effects of low A260/A230 ratios in RNA preparations on downstream applications?* Available at: https://www.qiagen.com/us/resources/faq?id=c59936fb-4f1e-4191-9c16-ff083cb24574&lang=en&fbclid=IwAR0oqd3CKb4Wi40YJO1nfoemIpl5VoYfhQe6FDHODQgnlTa-zolqSBsoqdw (accessed: 30/03/2023).

QIAGEN. (2013-23a). *Buffer RLT. For lysis of cells and tissues before RNA isolation.* Available at: https://www.qiagen.com/de-us/products/discovery-and-translational-research/lab-essentials/buffers-reagents/buffer-rlt (accessed: 30/03/2023).

QIAGEN. (2013-23b). *Buffer RW1. For washing of membrane-bound RNA in RNeasy and AllPrep procedures.* Available at: https://www.qiagen.com/de-us/products/discovery-

and-translational-research/lab-essentials/buffers-reagents/buffer-rw1 (accessed: 30/03/2023).

Sáenz, J. A. C. (2021). *Tilapia reproductive cycle*. Panama City (Panama): Veterinaria Digital S.A. Available at: https://www.veterinariadigital.com/en/articulos/tilapia-reproductive-cycle/ (accessed: 20/2/2023).

Senapin, S., Uthaman, S., Meemetta, W., Rattanarojpong, T. & Dong, H. (2018). Inapparent infection cases of tilapia lake virus (TiLV) in farmed tilapia. *Aquaculture*, 487. doi: 10.1016/j.aquaculture.2018.01.007.

Surachetpong, W., Roy, S. R. K. & Nicholson, P. (2020). Tilapia lake virus: The story so far. *J Fish Dis*, 43 (10): 1115-1132. doi: 10.1111/jfd.13237.

Tang, K. F. J., Bondad-Reantaso, M.G., Surachetpong, W., Dong, H.T., Fejzic, N., Wang, Q., Wajsbrot, N. & Hao, B. (2021). *Tilapia lake virus disease strategy manual*. FAO Fisheries and Aquaculture Circular Rome, Italy.

Tattiyapong, P., Dachavichitlead, W. & 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. doi: 10.1016/j.vetmic.2017.06.014.

Tattiyapong, P., Sirikanchana, K. & Surachetpong, W. (2018). Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish. *J Fish Dis*, 41 (2): 255-261. doi: 10.1111/jfd.12708.

Udayangani, D. S. (2017). *Difference Between SYBR Green and Taqman*: Difference Between. Available at: https://www.differencebetween.com/difference-between-sybr-green-and-vs-taqman/ (accessed: 01/05/2023).

White, J. M., Delos, S. E., Brecher, M. & Schornberg, K. (2008). Structures and mechanisms of viral membrane fusion proteins: multiple variations on a common theme. *Crit Rev Biochem Mol Biol*, 43 (3): 189-219. doi: 10.1080/10409230802058320.

WOAH, W. O. f. A. H. (2021). *REPORT OF THE VIRTUAL MEETING OF THE OIE AD HOC GROUP ON INFECTION WITH TILAPIA LAKE VIRUS*. Paris, France. Available at: https://doc.woah.org/dyn/portal/listalo.xhtml?page=listalo&pgnum=1&req=103&va_0=REPORT%20OF%20THE%20VIRTUAL%20MEETING%20OF%20THE%20OIE%20AD%20HOC%20GROUP%20ON%20INFECTION%20WITH%20TILAPIA%20LAKE%20VIRUS&fbclid=IwAR1-W8Ye5JCaJyZqZ0E9y4SnHJq9UVsV7614k2yPMO1KvtobOCMU2-nHP24 (accessed: 13/03/2023).

WOAH, W. O. f. A. H. (2022). *INFECTION WITH TILAPIA LAKE VIRUS (TiLV)- A NOVEL ORTHOMYXO-LIKE VIRUS*. Paris, France. Available at: https://www.woah.org/en/document/infection-with-tilapia-lake-virus-tilv/ (accessed: 14/03/2023).

Yamkasem, J., Tattiyapong, P., Kamlangdee, A. & Surachetpong, W. (2019). Evidence of potential vertical transmission of tilapia lake virus. *Journal of Fish Diseases*, 42 (9): 1293-1300. doi: https://doi.org/10.1111/jfd.13050.

Appendices

Appendix 1. Sample information.

Sample number	Date of sampling	Weight (gram)	Length (cm)	Species	Result of PCR
1-F1	01/11/2022	44	13.4	O. Andersonii	Negative
1-F2	01/11/2022	38	13.5	O. Andersonii	Negative
1-F3	01/11/2022	56	15.5	O. Andersonii	Negative
1-F4	01/11/2022	60	15.7	O. Andersonii	Negative
1-F5	01/11/2022	38	12.8	O. Andersonii	Negative
1-F6	01/11/2022	30	12.4	O. Andersonii	Negative
1-F7	01/11/2022	62	16.2	O. Andersonii	Negative
1-F8	01/11/2022	34	13.5	O. Andersonii	Negative
1-F9	01/11/2022	18	10.5	O. Andersonii	Negative
1-F10	01/11/2022	24	11.5	O. Andersonii	Negative
1-F11	01/11/2022	22	12	O. Andersonii	Negative
1-F12	01/11/2022	24	11.5	O. Andersonii	Negative
1-F13	01/11/2022	22	11.5	O. Andersonii	Negative
1-F14	01/11/2022	8	9	O. Andersonii	Negative
1-F15	01/11/2022	20	10	O. Andersonii	Negative
2-F1	02/11/2022	Fingerling	Fingerling		Negative
2-F2	02/11/2022	Fingerling	Fingerling		Negative
2-F3	02/11/2022	Fingerling	Fingerling		Negative
2-F4	02/11/2022	Fingerling	Fingerling		Negative
2-F5	02/11/2022	Fingerling	Fingerling		Negative
2-F6	02/11/2022	Fingerling	Fingerling		Negative
2-F7	02/11/2022	Fingerling	Fingerling		Negative
2-F8	02/11/2022	Fingerling	Fingerling		Negative
2-F9	02/11/2022	Fingerling	Fingerling		Negative
2-F10	02/11/2022	Fingerling	Fingerling		Negative
2-F11	02/11/2022	Fingerling	Fingerling		Negative
2-F12	02/11/2022	Fingerling	Fingerling		Negative
2-F13	02/11/2022	Fingerling	Fingerling		Sample K and S lost in extraction
2-F14	02/11/2022	Fingerling	Fingerling		Negative
2-F15	02/11/2022	Fingerling	Fingerling		Negative
2-F16	02/11/2022	Fingerling	Fingerling		Negative
2-F17	02/11/2022	Fingerling	Fingerling		Negative
2-F18	02/11/2022	Fingerling	Fingerling		Negative
2-F19	02/11/2022	Fingerling	Fingerling		Negative
2-F20	02/11/2022	Fingerling	Fingerling		Negative
2-F21	02/11/2022	Fingerling	Fingerling		Negative
2-F22	02/11/2022	Fingerling	Fingerling		Negative
2-F23	02/11/2022	Fingerling	Fingerling		Negative
2-F24	02/11/2022	Fingerling	Fingerling		Negative
2-F25	02/11/2022	Fingerling	Fingerling		Negative
2-F26	02/11/2022	Fingerling	Fingerling		Negative
2-F27	02/11/2022	Fingerling	Fingerling		Negative
2-F28	02/11/2022	Fingerling	Fingerling		Negative
2-F29	02/11/2022	Fingerling	Fingerling		Negative
2-F30	02/11/2022	Fingerling	Fingerling		Negative
3-F1	03/11/2022	155	20	O. macrochir	Negative
3-F2	03/11/2022	175	20.1	C. rendalli	Negative
3-F3	03/11/2022	135	20	O. macrochir	Negative
3-F4	03/11/2022	130	19	C. rendalli	Negative
3-F5	03/11/2022	130	21	O. andersonii	Negative
3-F6	03/11/2022	105	19	O. andersonii	Negative
3-F7	03/11/2022	60	15.2	O. macrochir	Negative
3-F8	03/11/2022	100	17.5	O. macrochir	Negative
3-F9	03/11/2022	85	17.5	C. rendalli	Negative
3-F10	03/11/2022	95	18.5	C. rendalli	Negative
3-F11	03/11/2022	60	14.5	C. rendalli	Negative
3-F12	03/11/2022	45	13	C. rendalli	Negative
3-F13	03/11/2022	90	17.5	O. andersonii	Negative
3-F14	03/11/2022	95	17	O. andersonii	Negative
3-F15	03/11/2022	70	16.5	O. macrochir	Negative
3-F16	03/11/2022	75	16	O. andersonii	Negative

3-F17	03/11/2022	60	15.5	O. macrochir	Negative
3-F18	03/11/2022	115	20	O. andersonii	Negative
3-F19	03/11/2022	125	18.5	C. rendalli	Negative
3-F20	03/11/2022	115	18	O. macrochir	Negative
3-F21	03/11/2022	140	19.5	C. rendalli	Sample K: False positive
3-F22	03/11/2022	155	21	O. macrochir	Negative
3-F23	03/11/2022	170	22	C. rendalli	Negative
3-F24	03/11/2022	125	20.5	O. macrochir	Sample K lost in extraction
3-F25	03/11/2022	135	19	O. macrochir	Negative
3-F26	03/11/2022	100	18	O. macrochir	Negative
4-F1	04/11/2022	85	17.4	O. andersonii	Negative
4-F2	04/11/2022	77	17.4	O. andersonii	Negative
4-F3	04/11/2022	79	17.3	O. andersonii	Negative
4-F4	04/11/2022	63	15.5	O. andersonii	Negative
4-F5	04/11/2022	60	15.3	O. andersonii	Negative
4-F6	04/11/2022	45	14.7	O. andersonii	Negative
4-F7	04/11/2022	79	18	O. andersonii	Negative
4-F8	04/11/2022	58	15	O. andersonii	Negative
4-F9	04/11/2022	77	17	O. andersonii	Negative
4-F10	04/11/2022	37	14	O. andersonii	Sample K lost in extraction
4-F11	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F12	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F13	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F14	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F15	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F16	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F17	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F18	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F19	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F20	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F21	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F22	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F23	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F24	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F25	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F26	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F27	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F28	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F29	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
4-F30	04/11/2022	Fingerling	Fingerling	O. andersonii	Negative
5-F1	07/11/2022	120	18.3	O. niloticus	Negative
5-F2	07/11/2022	182	20.7	O. niloticus	Negative
5-F3	07/11/2022	138	18	O. niloticus	Negative
5-F4	07/11/2022	158	20	O. niloticus	Negative
5-F5	07/11/2022	160	20	O. niloticus	Negative
5-F6	07/11/2022	97	17.2	O. niloticus	Negative
5-F7	07/11/2022	125	19	O. niloticus	Negative
6-F1	08/11/2022	249	21.3	O. niloticus	Negative
6-F2	08/11/2022	281	23.8	O. niloticus	Negative
6-F3	08/11/2022	395	25.3	O. niloticus	Negative
6-F4	08/11/2022	351	24.2	O. niloticus	Sample K: False positive
6-F5	08/11/2022	288	24	O. niloticus	Negative
6-F6	08/11/2022	219	21.8	O. niloticus	Negative
6-F7	08/11/2022	245	22.5	O. niloticus	Negative
6-F8	08/11/2022	432	27	O. niloticus	Negative
6-F9	08/11/2022	372	24.5	O. niloticus	Negative
6-F10	08/11/2022	407	25.5	O. niloticus	Negative
6-F11	08/11/2022	237	21.1	O. niloticus	Negative
6-F12	08/11/2022	489	26.9	O. niloticus	Negative
6-F13	08/11/2022	214	21.7	O. niloticus	Negative
6-F14	08/11/2022	138	19	O. niloticus	Negative
6-F15	08/11/2022	201	20.5	O. niloticus	Negative

6-F16	08/11/2022	498	27	O. niloticus	Negative
6-F17	08/11/2022	417	26.2	O. niloticus	Negative
6-F18	08/11/2022	424	26	O. niloticus	Negative
6-F19	08/11/2022	444	26.1	O. niloticus	Negative
6-F20	08/11/2022	230	22	O. niloticus	Negative
6-F21	08/11/2022	190	21.4	O. niloticus	Negative
6-F22	08/11/2022	277	23	O. niloticus	Negative
6-F23	08/11/2022	240	22.4	O. niloticus	Negative
6-F24	08/11/2022	116	18	O. niloticus	Negative
6-F25	08/11/2022	204	21	O. niloticus	Negative
6-F26	08/11/2022	259	23	O. niloticus	Negative
6-F27	08/11/2022	429	26	O. niloticus	Negative
6-F28	08/11/2022	50	14	O. niloticus	Negative
6-F29	08/11/2022	189	21	O. niloticus	Negative
7-F1	09/11/2022	139	18	O. niloticus	Negative
7-F2	09/11/2022	628	29	O. niloticus	Negative
7-F3	09/11/2022	342	25	O. niloticus	Negative
7-F4	09/11/2022	262	23.5	O. niloticus	Negative
7-F5	09/11/2022	482	27.2	O. niloticus	Negative
7-F6	09/11/2022	216	20.3	O. niloticus	Negative
7-F7	09/11/2022	62	14	O. niloticus	Sample S lost in extraction
7-F8	09/11/2022	397	25	O. niloticus	Negative
7-F9	09/11/2022	344	25	O. niloticus	Negative
7-F10	09/11/2022	163	19.1	O. niloticus	Negative
7-F11	09/11/2022	337	24	O. niloticus	Negative
7-F12	09/11/2022	456	26	O. niloticus	Negative
7-F13	09/11/2022	422	26	O. niloticus	Negative
7-F14	09/11/2022	186	20	O. niloticus	Negative
7-F15	09/11/2022	25	11	O. niloticus	Negative
7-F16	09/11/2022	158	19	O. niloticus	Negative
7-F17	09/11/2022	178	19.5	O. niloticus	Negative
7-F18	09/11/2022	207	22	O. niloticus	Negative
7-F19	09/11/2022	154	19.3	O. niloticus	Negative
7-F20	09/11/2022	24	10	O. niloticus	Negative
7-F21	09/11/2022	31	12	O. niloticus	Negative
7-F22	09/11/2022	118	17	O. niloticus	Negative
7-F23	09/11/2022	60	14	O. niloticus	Negative
7-F24	09/11/2022	112	17	O. niloticus	Sample K lost in extraction
7-F25	09/11/2022	87	15.5	O. niloticus	Negative
7-F26	09/11/2022	94	16.5	O. niloticus	Negative
7-F27	09/11/2022	153	18	O. niloticus	Negative
7-F28	09/11/2022	303	22.7	O. niloticus	Negative
7-F29	09/11/2022	301	22.5	O. niloticus	Negative
7-F30	09/11/2022	176	19.5	O. niloticus	Negative
8-F1	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F2	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F3	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F4	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F5	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F6	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F7	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F8	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F9	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F10	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F11	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F12	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F13	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F14	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F15	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F16	10/11/2022	Fingerling	Fingerling	O. niloticus	Sample K lost in extraction
8-F17	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F18	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative

8-F19	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F20	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F21	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F22	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F23	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F24	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F25	10/11/2022	Fingerling	Fingerling	O. niloticus	Sample K lost in extraction
8-F26	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F27	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F28	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F29	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative
8-F30	10/11/2022	Fingerling	Fingerling	O. niloticus	Negative

Appendix 2. Laboratory sample information.

Sample number	Organ	Sampling	RNA-extraction	Tissue (mg)	PCR	Results
1-F1 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F1 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F2 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F2 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F3 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F3 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F4 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F4 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F5 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F5 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F6 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F6 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F7 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F7 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F8 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F8 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F9 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F9 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F10 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F10 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F11 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F11 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F12 K	Kidney	01/11/2022	14/11/2022		15/11/2022	Negative
1-F12 S	Spleen	01/11/2022	14/11/2022		15/11/2022	Negative
1-F13 K	Kidney	01/11/2022	16/11/2022	11	22/11/2022	Negative
1-F13 S	Spleen	01/11/2022	16/11/2022	25	22/11/2022	Negative
1-F14 K	Kidney	01/11/2022	16/11/2022	14	22/11/2022	Negative
1-F14 S	Spleen	01/11/2022	16/11/2022	27	22/11/2022	Negative
1-F15 K	Kidney	01/11/2022	16/11/2022	20	22/11/2022	Negative
1-F15 S	Spleen	01/11/2022	16/11/2022	12	22/11/2022	Negative
2-F1 K	Kidney	02/11/2022	16/11/2022	13	22/11/2022	Negative
2-F1 S	Spleen	02/11/2022	16/11/2022	27	22/11/2022	Negative
2-F2 K	Kidney	02/11/2022	16/11/2022	22	22/11/2022	Negative
2-F2 S	Spleen	02/11/2022	16/11/2022	14	22/11/2022	Negative
2-F3 K	Kidney	02/11/2022	16/11/2022	27	22/11/2022	Negative
2-F3 S	Spleen	02/11/2022	16/11/2022	23	22/11/2022	Negative
2-F4 K	Kidney	02/11/2022	16/11/2022	26	22/11/2022	Negative
2-F4 S	Spleen	02/11/2022	16/11/2022	25	22/11/2022	Negative
2-F5 K	Kidney	02/11/2022	16/11/2022	22	22/11/2022	Negative
2-F5 S	Spleen	02/11/2022	16/11/2022	20	22/11/2022	Negative
2-F6 K	Kidney	02/11/2022	16/11/2022	27	22/11/2022	Negative
2-F6 S	Spleen	02/11/2022	16/11/2022	7	22/11/2022	Negative
2-F7 K	Kidney	02/11/2022	16/11/2022	20	22/11/2022	Negative
2-F7 S	Spleen	02/11/2022	16/11/2022	21	22/11/2022	Negative
2-F8 K	Kidney	02/11/2022	16/11/2022	26	22/11/2022	Negative
2-F8 S	Spleen	02/11/2022	16/11/2022	12	22/11/2022	Negative
2-F9 K	Kidney	02/11/2022	16/11/2022	18	22/11/2022	Negative
2-F9 S	Spleen	02/11/2022	16/11/2022	12	22/11/2022	Negative
2-F10 K	Kidney	02/11/2022	16/11/2022	26	22/11/2022	Negative
2-F10 S	Spleen	02/11/2022	16/11/2022	7	22/11/2022	Negative
2-F11 K	Kidney	02/11/2022	16/11/2022	28	22/11/2022	Negative
2-F11 S	Spleen	02/11/2022	16/11/2022	16	22/11/2022	Negative
2-F12 K	Kidney	02/11/2022	16/11/2022	18	22/11/2022	Negative
2-F12 S	Spleen	02/11/2022	16/11/2022	7	22/11/2022	Negative
2-F13 K	Kidney	02/11/2022	16/11/2022	28	22/11/2022	Lost in extraction
2-F13 S	Spleen	02/11/2022	16/11/2022	15	22/11/2022	Lost in extraction
2-F14 K	Kidney	02/11/2022	16/11/2022	24	22/11/2022	Negative
2-F14 S	Spleen	02/11/2022	16/11/2022	7	22/11/2022	Negative
2-F15 K	Kidney	02/11/2022	16/11/2022	26	22/11/2022	Negative
2-F15 S	Spleen	02/11/2022	16/11/2022	15	22/11/2022	Negative

2-F16 K	Kidney	02/11/2022	16/11/2022	28	22/11/2022	Negative
2-F16 S	Spleen	02/11/2022	16/11/2022	20	22/11/2022	Negative
2-F17 K	Kidney	02/11/2022	16/11/2022	24	22/11/2022	Negative
2-F17 S	Spleen	02/11/2022	16/11/2022	6	22/11/2022	Negative
2-F18 K	Kidney	02/11/2022	16/11/2022	29	22/11/2022	Negative
2-F18 S	Spleen	02/11/2022	16/11/2022	25	22/11/2022	Negative
2-F19 K	Kidney	02/11/2022	16/11/2022	28	22/11/2022	Negative
2-F19 S	Spleen	02/11/2022	16/11/2022	25	22/11/2022	Negative
2-F20 K	Kidney	02/11/2022	16/11/2022	14	22/11/2022	Negative
2-F20 S	Spleen	02/11/2022	16/11/2022	9	22/11/2022	Negative
2-F21 K	Kidney	02/11/2022	16/11/2022	19	22/11/2022	Negative
2-F21 S	Spleen	02/11/2022	16/11/2022	25	22/11/2022	Negative
2-F22 K	Kidney	02/11/2022	16/11/2022	17	22/11/2022	Negative
2-F22 S	Spleen	02/11/2022	16/11/2022	21	22/11/2022	Negative
2-F23 K	Kidney	02/11/2022	16/11/2022	28	22/11/2022	Negative
2-F23 S	Spleen	02/11/2022	16/11/2022	28	22/11/2022	Negative
2-F24 K	Kidney	02/11/2022	16/11/2022	28	22/11/2022	Negative
2-F24 S	Spleen	02/11/2022	16/11/2022	19	22/11/2022	Negative
2-F25 K	Kidney	02/11/2022	16/11/2022	27	22/11/2022	Negative
2-F25 S	Spleen	02/11/2022	16/11/2022	12	22/11/2022	Negative
2-F26 K	Kidney	02/11/2022	16/11/2022	20	22/11/2022	Negative
2-F26 S	Spleen	02/11/2022	16/11/2022	3	22/11/2022	Negative
2-F27 K	Kidney	02/11/2022	16/11/2022	23	22/11/2022	Negative
2-F27 S	Spleen	02/11/2022	16/11/2022	8	22/11/2022	Negative
2-F28 K	Kidney	02/11/2022	16/11/2022	27	22/11/2022	Negative
2-F28 S	Spleen	02/11/2022	16/11/2022	12	22/11/2022	Negative
2-F29 K	Kidney	02/11/2022	16/11/2022	29	22/11/2022	Negative
2-F29 S	Spleen	02/11/2022	16/11/2022	22	22/11/2022	Negative
2-F30 K	Kidney	02/11/2022	16/11/2022	28	22/11/2022	Negative
2-F30 S	Spleen	02/11/2022	16/11/2022	13	22/11/2022	Negative
3-F1 K	Kidney	03/11/2022	16/11/2022	28	22/11/2022	Negative
3-F1 S	Spleen	03/11/2022	16/11/2022	25	22/11/2022	Negative
3-F2 K	Kidney	03/11/2022	16/11/2022	22	22/11/2022	Negative
3-F2 S	Spleen	03/11/2022	16/11/2022	23	22/11/2022	Negative
3-F3 K	Kidney	03/11/2022	16/11/2022	13	22/11/2022	Negative
3-F3 S	Spleen	03/11/2022	16/11/2022	27	22/11/2022	Negative
3-F4 K	Kidney	03/11/2022	16/11/2022	29	22/11/2022	Negative
3-F4 S	Spleen	03/11/2022	16/11/2022	28	22/11/2022	Negative
3-F5 K	Kidney	03/11/2022	16/11/2022	29	22/11/2022	Negative
3-F5 S	Spleen	03/11/2022	16/11/2022	24	22/11/2022	Negative
3-F6 K	Kidney	03/11/2022	16/11/2022	20	22/11/2022	Negative
3-F6 S	Spleen	03/11/2022	16/11/2022	20	22/11/2022	Negative
3-F7 K	Kidney	03/11/2022	16/11/2022	20	22/11/2022	Negative
3-F7 S	Spleen	03/11/2022	16/11/2022	29	22/11/2022	Negative
3-F8 K	Kidney	03/11/2022	16/11/2022	26	22/11/2022	Negative
3-F8 S	Spleen	03/11/2022	16/11/2022	29	22/11/2022	Negative
3-F9 K	Kidney	03/11/2022	16/11/2022	21	22/11/2022	Negative
3-F9 S	Spleen	03/11/2022	16/11/2022	29	22/11/2022	Negative
3-F10 K	Kidney	03/11/2022	16/11/2022	28	22/11/2022	Negative
3-F10 S	Spleen	03/11/2022	16/11/2022	20	22/11/2022	Negative
3-F11 K	Kidney	03/11/2022	16/11/2022	24	22/11/2022	Negative
3-F11 S	Spleen	03/11/2022	16/11/2022	23	22/11/2022	Negative
3-F12 K	Kidney	03/11/2022	16/11/2022	26	22/11/2022	Negative
3-F12 S	Spleen	03/11/2022	16/11/2022	19	22/11/2022	Negative
3-F13 K	Kidney	03/11/2022	16/11/2022	30	22/11/2022	Negative
3-F13 S	Spleen	03/11/2022	16/11/2022	25	22/11/2022	Negative
3-F14 K	Kidney	03/11/2022	16/11/2022	20	22/11/2022	Negative
3-F14 S	Spleen	03/11/2022	16/11/2022	21	22/11/2022	Negative
3-F15 K	Kidney	03/11/2022	17/11/2022	15	22/11/2022	Negative
3-F15 S	Spleen	03/11/2022	17/11/2022	29	22/11/2022	Negative
3-F16 K	Kidney	03/11/2022	17/11/2022	25	22/11/2022	Negative
3-F16 S	Spleen	03/11/2022	17/11/2022	16	22/11/2022	Negative

3-F17 K	Kidney	03/11/2022	17/11/2022	27	22/11/2022	Negative
3-F17 S	Spleen	03/11/2022	17/11/2022	26	22/11/2022	Negative
3-F18 K	Kidney	03/11/2022	17/11/2022	13	22/11/2022	Negative
3-F18 S	Spleen	03/11/2022	17/11/2022	30	22/11/2022	Negative
3-F19 K	Kidney	03/11/2022	17/11/2022	20	22/11/2022	Negative
3-F19 S	Spleen	03/11/2022	17/11/2022	20	22/11/2022	Negative
3-F20 K	Kidney	03/11/2022	17/11/2022	27	22/11/2022	Negative
3-F20 S	Spleen	03/11/2022	17/11/2022	28	22/11/2022	Negative
3-F21 K	Kidney	03/11/2022	17/11/2022	21	22/11/2022	False positive
3-F21 S	Spleen	03/11/2022	17/11/2022	30	22/11/2022	Negative
3-F22 K	Kidney	03/11/2022	17/11/2022	30	22/11/2022	Negative
3-F22 S	Spleen	03/11/2022	17/11/2022	28	22/11/2022	Negative
3-F23 K	Kidney	03/11/2022	17/11/2022	30	22/11/2022	Negative
3-F23 S	Spleen	03/11/2022	17/11/2022	26	22/11/2022	Negative
3-F24 K	Kidney	03/11/2022	17/11/2022	8	22/11/2022	Lost in extraction
3-F24 S	Spleen	03/11/2022	17/11/2022	17	22/11/2022	Negative
3-F25 K	Kidney	03/11/2022	17/11/2022	8	22/11/2022	Negative
3-F25 S	Spleen	03/11/2022	17/11/2022	29	22/11/2022	Negative
3-F26 K	Kidney	03/11/2022	17/11/2022	5	22/11/2022	Negative
3-F26 S	Spleen	03/11/2022	17/11/2022	24	22/11/2022	Negative
4-F1 K	Kidney	04/11/2022	17/11/2022	24	22/11/2022	Negative
4-F1 S	Spleen	04/11/2022	17/11/2022	20	22/11/2022	Negative
4-F2 K	Kidney	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F2 S	Spleen	04/11/2022	17/11/2022	30	22/11/2022	Negative
4-F3 K	Kidney	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F3 S	Spleen	04/11/2022	17/11/2022	28	22/11/2022	Negative
4-F4 K	Kidney	04/11/2022	17/11/2022	24	22/11/2022	Negative
4-F4 S	Spleen	04/11/2022	17/11/2022	23	22/11/2022	Negative
4-F5 K	Kidney	04/11/2022	17/11/2022	23	22/11/2022	Negative
4-F5 S	Spleen	04/11/2022	17/11/2022	28	22/11/2022	Negative
4-F6 K	Kidney	04/11/2022	17/11/2022	23	22/11/2022	Negative
4-F6 S	Spleen	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F7 K	Kidney	04/11/2022	17/11/2022	23	22/11/2022	Negative
4-F7 S	Spleen	04/11/2022	17/11/2022	23	22/11/2022	Negative
4-F8 K	Kidney	04/11/2022	17/11/2022	28	22/11/2022	Negative
4-F8 S	Spleen	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F9 K	Kidney	04/11/2022	17/11/2022	22	22/11/2022	Negative
4-F9 S	Spleen	04/11/2022	17/11/2022	27	22/11/2022	Negative
4-F10 K	Kidney	04/11/2022	17/11/2022	0	22/11/2022	Lost in extraction
4-F10 S	Spleen	04/11/2022	17/11/2022	23	22/11/2022	Negative
4-F11 K	Kidney	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F11 S	Spleen	04/11/2022	17/11/2022	30	22/11/2022	Negative
4-F12 K	Kidney	04/11/2022	17/11/2022	24	22/11/2022	Negative
4-F12 S	Spleen	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F13 K	Kidney	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F13 S	Spleen	04/11/2022	17/11/2022	30	22/11/2022	Negative
4-F14 K	Kidney	04/11/2022	17/11/2022	20	22/11/2022	Negative
4-F14 S	Spleen	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F15 K	Kidney	04/11/2022	17/11/2022	21	22/11/2022	Negative
4-F15 S	Spleen	04/11/2022	17/11/2022	24	22/11/2022	Negative
4-F16 K	Kidney	04/11/2022	17/11/2022	26	22/11/2022	Negative
4-F16 S	Spleen	04/11/2022	17/11/2022	28	22/11/2022	Negative
4-F17 K	Kidney	04/11/2022	17/11/2022	23	22/11/2022	Negative
4-F17 S	Spleen	04/11/2022	17/11/2022	28	22/11/2022	Negative
4-F18 K	Kidney	04/11/2022	17/11/2022	22	22/11/2022	Negative
4-F18 S	Spleen	04/11/2022	17/11/2022	26	22/11/2022	Negative
4-F19 K	Kidney	04/11/2022	17/11/2022	30	22/11/2022	Negative
4-F19 S	Spleen	04/11/2022	17/11/2022	18	22/11/2022	Negative
4-F20 K	Kidney	04/11/2022	17/11/2022	21	22/11/2022	Negative
4-F20 S	Spleen	04/11/2022	17/11/2022	28	22/11/2022	Negative
4-F21 K	Kidney	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F21 S	Spleen	04/11/2022	17/11/2022	21	22/11/2022	Negative

4-F22 K	Kidney	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F22 S	Spleen	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F23 K	Kidney	04/11/2022	17/11/2022	30	22/11/2022	Negative
4-F23 S	Spleen	04/11/2022	17/11/2022	22	22/11/2022	Negative
4-F24 K	Kidney	04/11/2022	17/11/2022	20	22/11/2022	Negative
4-F24 S	Spleen	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F25 K	Kidney	04/11/2022	17/11/2022	28	22/11/2022	Negative
4-F25 S	Spleen	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F26 K	Kidney	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F26 S	Spleen	04/11/2022	17/11/2022	20	22/11/2022	Negative
4-F27 K	Kidney	04/11/2022	17/11/2022	15	22/11/2022	Negative
4-F27 S	Spleen	04/11/2022	17/11/2022	29	22/11/2022	Negative
4-F28 K	Kidney	04/11/2022	17/11/2022	28	22/11/2022	Negative
4-F28 S	Spleen	04/11/2022	17/11/2022	30	22/11/2022	Negative
4-F29 K	Kidney	04/11/2022	17/11/2022	23	22/11/2022	Negative
4-F29 S	Spleen	04/11/2022	17/11/2022	30	22/11/2022	Negative
4-F30 K	Kidney	04/11/2022	17/11/2022	24	22/11/2022	Negative
4-F30 S	Spleen	04/11/2022	17/11/2022	30	22/11/2022	Negative
5-F1 K	Kidney	07/11/2022	17/11/2022	26	22/11/2022	Negative
5-F1 S	Spleen	07/11/2022	17/11/2022	28	22/11/2022	Negative
5-F2 K	Kidney	07/11/2022	17/11/2022	25	22/11/2022	Negative
5-F2 S	Spleen	07/11/2022	17/11/2022	28	22/11/2022	Negative
5-F3 K	Kidney	07/11/2022	17/11/2022	20	22/11/2022	Negative
5-F3 S	Spleen	07/11/2022	17/11/2022	29	22/11/2022	Negative
5-F4 K	Kidney	07/11/2022	17/11/2022	30	22/11/2022	Negative
5-F4 S	Spleen	07/11/2022	17/11/2022	25	22/11/2022	Negative
5-F5 K	Kidney	07/11/2022	18/11/2022	28	23/11/2022	Negative
5-F5 S	Spleen	07/11/2022	18/11/2022	23	23/11/2022	Negative
5-F6 K	Kidney	07/11/2022	18/11/2022	20	23/11/2022	Negative
5-F6 S	Spleen	07/11/2022	18/11/2022	27	23/11/2022	Negative
5-F7 K	Kidney	07/11/2022	18/11/2022	26	23/11/2022	Negative
5-F7 S	Spleen	07/11/2022	18/11/2022	21	23/11/2022	Negative
6-F1 K	Kidney	08/11/2022	18/11/2022	25	23/11/2022	Negative
6-F1 S	Spleen	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F2 K	Kidney	08/11/2022	18/11/2022	26	23/11/2022	Negative
6-F2 S	Spleen	08/11/2022	18/11/2022	21	23/11/2022	Negative
6-F3 K	Kidney	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F3 S	Spleen	08/11/2022	18/11/2022	26	23/11/2022	Negative
6-F4 K	Kidney	08/11/2022	18/11/2022	29	23/11/2022	False positive
6-F4 S	Spleen	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F5 K	Kidney	08/11/2022	18/11/2022	21	23/11/2022	Negative
6-F5 S	Spleen	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F6 K	Kidney	08/11/2022	18/11/2022	28	23/11/2022	Negative
6-F6 S	Spleen	08/11/2022	18/11/2022	25	23/11/2022	Negative
6-F7 K	Kidney	08/11/2022	18/11/2022	29	23/11/2022	Negative
6-F7 S	Spleen	08/11/2022	18/11/2022	25	23/11/2022	Negative
6-F8 K	Kidney	08/11/2022	18/11/2022	26	23/11/2022	Negative
6-F8 S	Spleen	08/11/2022	18/11/2022	26	23/11/2022	Negative
6-F9 K	Kidney	08/11/2022	18/11/2022	20	23/11/2022	Negative
6-F9 S	Spleen	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F10 K	Kidney	08/11/2022	18/11/2022	23	23/11/2022	Negative
6-F10 S	Spleen	08/11/2022	18/11/2022	25	23/11/2022	Negative
6-F11 K	Kidney	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F11 S	Spleen	08/11/2022	18/11/2022	20	23/11/2022	Negative
6-F12 K	Kidney	08/11/2022	18/11/2022	23	23/11/2022	Negative
6-F12 S	Spleen	08/11/2022	18/11/2022	28	23/11/2022	Negative
6-F13 K	Kidney	08/11/2022	18/11/2022	27	23/11/2022	Negative
6-F13 S	Spleen	08/11/2022	18/11/2022	29	23/11/2022	Negative
6-F14 K	Kidney	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F14 S	Spleen	08/11/2022	18/11/2022	28	23/11/2022	Negative
6-F15 K	Kidney	08/11/2022	18/11/2022	29	23/11/2022	Negative
6-F15 S	Spleen	08/11/2022	18/11/2022	23	23/11/2022	Negative

6-F16 K	Kidney	08/11/2022	18/11/2022	27	23/11/2022	Negative
6-F16 S	Spleen	08/11/2022	18/11/2022	23	23/11/2022	Negative
6-F17 K	Kidney	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F17 S	Spleen	08/11/2022	18/11/2022	25	23/11/2022	Negative
6-F18 K	Kidney	08/11/2022	18/11/2022	28	23/11/2022	Negative
6-F18 S	Spleen	08/11/2022	18/11/2022	22	23/11/2022	Negative
6-F19 K	Kidney	08/11/2022	18/11/2022	27	23/11/2022	Negative
6-F19 S	Spleen	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F20 K	Kidney	08/11/2022	18/11/2022	26	23/11/2022	Negative
6-F20 S	Spleen	08/11/2022	18/11/2022	26	23/11/2022	Negative
6-F21 K	Kidney	08/11/2022	18/11/2022	27	23/11/2022	Negative
6-F21 S	Spleen	08/11/2022	18/11/2022	29	23/11/2022	Negative
6-F22 K	Kidney	08/11/2022	18/11/2022	27	23/11/2022	Negative
6-F22 S	Spleen	08/11/2022	18/11/2022	28	23/11/2022	Negative
6-F23 K	Kidney	08/11/2022	18/11/2022	27	23/11/2022	Negative
6-F23 S	Spleen	08/11/2022	18/11/2022	25	23/11/2022	Negative
6-F24 K	Kidney	08/11/2022	18/11/2022	26	23/11/2022	Negative
6-F24 S	Spleen	08/11/2022	18/11/2022	28	23/11/2022	Negative
6-F25 K	Kidney	08/11/2022	18/11/2022	29	23/11/2022	Negative
6-F25 S	Spleen	08/11/2022	18/11/2022	25	23/11/2022	Negative
6-F26 K	Kidney	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F26 S	Spleen	08/11/2022	18/11/2022	20	23/11/2022	Negative
6-F27 K	Kidney	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F27 S	Spleen	08/11/2022	18/11/2022	28	23/11/2022	Negative
6-F28 K	Kidney	08/11/2022	18/11/2022	22	23/11/2022	Negative
6-F28 S	Spleen	08/11/2022	18/11/2022	29	23/11/2022	Negative
6-F29 K	Kidney	08/11/2022	18/11/2022	30	23/11/2022	Negative
6-F29 S	Spleen	08/11/2022	18/11/2022	30	23/11/2022	Negative
7-F1 K	Kidney	09/11/2022	18/11/2022	23	23/11/2022	Negative
7-F1 S	Spleen	09/11/2022	18/11/2022	20	23/11/2022	Negative
7-F2 K	Kidney	09/11/2022	18/11/2022	24	23/11/2022	Negative
7-F2 S	Spleen	09/11/2022	18/11/2022	27	23/11/2022	Negative
7-F3 K	Kidney	09/11/2022	18/11/2022	15	23/11/2022	Negative
7-F3 S	Spleen	09/11/2022	18/11/2022	28	23/11/2022	Negative
7-F4 K	Kidney	09/11/2022	18/11/2022	20	23/11/2022	Negative
7-F4 S	Spleen	09/11/2022	18/11/2022	25	23/11/2022	Negative
7-F5 K	Kidney	09/11/2022	18/11/2022	26	23/11/2022	Negative
7-F5 S	Spleen	09/11/2022	18/11/2022	26	23/11/2022	Negative
7-F6 K	Kidney	09/11/2022	18/11/2022	26	23/11/2022	Negative
7-F6 S	Spleen	09/11/2022	18/11/2022	25	23/11/2022	Negative
7-F7 K	Kidney	09/11/2022	18/11/2022	28	23/11/2022	Negative
7-F7 S	Spleen	09/11/2022	18/11/2022	13	23/11/2022	Lost in extraction
7-F8 K	Kidney	09/11/2022	18/11/2022	24	23/11/2022	Negative
7-F8 S	Spleen	09/11/2022	18/11/2022	24	23/11/2022	Negative
7-F9 K	Kidney	09/11/2022	18/11/2022	29	23/11/2022	Negative
7-F9 S	Spleen	09/11/2022	18/11/2022	25	23/11/2022	Negative
7-F10 K	Kidney	09/11/2022	18/11/2022	24	23/11/2022	Negative
7-F10 S	Spleen	09/11/2022	18/11/2022	26	23/11/2022	Negative
7-F11 K	Kidney	09/11/2022	18/11/2022	30	23/11/2022	Negative
7-F11 S	Spleen	09/11/2022	18/11/2022	30	23/11/2022	Negative
7-F12 K	Kidney	09/11/2022	18/11/2022	30	23/11/2022	Negative
7-F12 S	Spleen	09/11/2022	18/11/2022	25	23/11/2022	Negative
7-F13 K	Kidney	09/11/2022	18/11/2022	25	23/11/2022	Negative
7-F13 S	Spleen	09/11/2022	18/11/2022	26	23/11/2022	Negative
7-F14 K	Kidney	09/11/2022	18/11/2022	27	23/11/2022	Negative
7-F14 S	Spleen	09/11/2022	18/11/2022	27	23/11/2022	Negative
7-F15 K	Kidney	09/11/2022	18/11/2022	27	23/11/2022	Negative
7-F15 S	Spleen	09/11/2022	21/11/2022	24	23/11/2022	Negative
7-F16 K	Kidney	09/11/2022	21/11/2022	25	23/11/2022	Negative
7-F16 S	Spleen	09/11/2022	21/11/2022	21	23/11/2022	Negative
7-F17 K	Kidney	09/11/2022	21/11/2022	25	23/11/2022	Negative
7-F17 S	Spleen	09/11/2022	21/11/2022	29	23/11/2022	Negative

7-F18 K	Kidney	09/11/2022	21/11/2022	29	23/11/2022	Negative
7-F18 S	Spleen	09/11/2022	21/11/2022	29	23/11/2022	Negative
7-F19 K	Kidney	09/11/2022	21/11/2022	29	23/11/2022	Negative
7-F19 S	Spleen	09/11/2022	21/11/2022	21	23/11/2022	Negative
7-F20 K	Kidney	09/11/2022	21/11/2022	28	23/11/2022	Negative
7-F20 S	Spleen	09/11/2022	21/11/2022	28	23/11/2022	Negative
7-F21 K	Kidney	09/11/2022	21/11/2022	26	23/11/2022	Negative
7-F21 S	Spleen	09/11/2022	21/11/2022	23	23/11/2022	Negative
7-F22 K	Kidney	09/11/2022	21/11/2022	21	23/11/2022	Negative
7-F22 S	Spleen	09/11/2022	21/11/2022	25	23/11/2022	Negative
7-F23 K	Kidney	09/11/2022	21/11/2022	30	23/11/2022	Negative
7-F23 S	Spleen	09/11/2022	21/11/2022	30	23/11/2022	Negative
7-F24 K	Kidney	09/11/2022	21/11/2022	30	23/11/2022	Lost in extraction
7-F24 S	Spleen	09/11/2022	21/11/2022	22	23/11/2022	Negative
7-F25 K	Kidney	09/11/2022	21/11/2022	26	23/11/2022	Negative
7-F25 S	Spleen	09/11/2022	21/11/2022	27	23/11/2022	Negative
7-F26 K	Kidney	09/11/2022	21/11/2022	25	23/11/2022	Negative
7-F26 S	Spleen	09/11/2022	21/11/2022	20	23/11/2022	Negative
7-F27 K	Kidney	09/11/2022	21/11/2022	30	23/11/2022	Negative
7-F27 S	Spleen	09/11/2022	21/11/2022	27	23/11/2022	Negative
7-F28 K	Kidney	09/11/2022	21/11/2022	30	23/11/2022	Negative
7-F28 S	Spleen	09/11/2022	21/11/2022	28	23/11/2022	Negative
7-F29 K	Kidney	09/11/2022	21/11/2022	29	23/11/2022	Negative
7-F29 S	Spleen	09/11/2022	21/11/2022	22	23/11/2022	Negative
7-F30 K	Kidney	09/11/2022	21/11/2022	21	23/11/2022	Negative
7-F30 S	Spleen	09/11/2022	21/11/2022	27	23/11/2022	Negative
8-F1 K	Kidney	10/11/2022	21/11/2022	23	23/11/2022	Negative
8-F1 S	Spleen	10/11/2022	21/11/2022	27	23/11/2022	Negative
8-F2 K	Kidney	10/11/2022	21/11/2022	20	23/11/2022	Negative
8-F2 S	Spleen	10/11/2022	21/11/2022	27	23/11/2022	Negative
8-F3 K	Kidney	10/11/2022	21/11/2022	24	23/11/2022	Negative
8-F3 S	Spleen	10/11/2022	21/11/2022	19	23/11/2022	Negative
8-F4 K	Kidney	10/11/2022	21/11/2022	26	23/11/2022	Negative
8-F4 S	Spleen	10/11/2022	21/11/2022	17	23/11/2022	Negative
8-F5 K	Kidney	10/11/2022	21/11/2022	27	23/11/2022	Negative
8-F5 S	Spleen	10/11/2022	21/11/2022	23	23/11/2022	Negative
8-F6 K	Kidney	10/11/2022	21/11/2022	26	23/11/2022	Negative
8-F6 S	Spleen	10/11/2022	21/11/2022	28	23/11/2022	Negative
8-F7 K	Kidney	10/11/2022	21/11/2022	23	23/11/2022	Negative
8-F7 S	Spleen	10/11/2022	21/11/2022	25	23/11/2022	Negative
8-F8 K	Kidney	10/11/2022	21/11/2022	22	23/11/2022	Negative
8-F8 S	Spleen	10/11/2022	21/11/2022	23	23/11/2022	Negative
8-F9 K	Kidney	10/11/2022	21/11/2022	22	23/11/2022	Negative
8-F9 S	Spleen	10/11/2022	21/11/2022	21	23/11/2022	Negative
8-F10 K	Kidney	10/11/2022	21/11/2022	24	23/11/2022	Negative
8-F10 S	Spleen	10/11/2022	21/11/2022	19	23/11/2022	Negative
8-F11 K	Kidney	10/11/2022	21/11/2022	19	23/11/2022	Negative
8-F11 S	Spleen	10/11/2022	21/11/2022	27	23/11/2022	Negative
8-F12 K	Kidney	10/11/2022	21/11/2022	26	23/11/2022	Negative
8-F12 S	Spleen	10/11/2022	21/11/2022	29	23/11/2022	Negative
8-F13 K	Kidney	10/11/2022	21/11/2022	16	23/11/2022	Negative
8-F13 S	Spleen	10/11/2022	21/11/2022	15	23/11/2022	Negative
8-F14 K	Kidney	10/11/2022	21/11/2022	27	23/11/2022	Negative
8-F14 S	Spleen	10/11/2022	21/11/2022	28	23/11/2022	Negative
8-F15 K	Kidney	10/11/2022	21/11/2022	25	23/11/2022	Negative
8-F15 S	Spleen	10/11/2022	21/11/2022	19	23/11/2022	Negative
8-F16 K	Kidney	10/11/2022	21/11/2022	27	23/11/2022	Lost in extraction
8-F16 S	Spleen	10/11/2022	21/11/2022	26	23/11/2022	Negative
8-F17 K	Kidney	10/11/2022	21/11/2022	30	23/11/2022	Negative
8-F17 S	Spleen	10/11/2022	21/11/2022	30	23/11/2022	Negative
8-F18 K	Kidney	10/11/2022	21/11/2022	23	23/11/2022	Negative
8-F18 S	Spleen	10/11/2022	21/11/2022	24	23/11/2022	Negative

8-F19 K	Kidney	10/11/2022	21/11/2022	26	23/11/2022	Negative
8-F19 S	Spleen	10/11/2022	21/11/2022	28	23/11/2022	Negative
8-F20 K	Kidney	10/11/2022	21/11/2022	24	23/11/2022	Negative
8-F20 S	Spleen	10/11/2022	21/11/2022	30	23/11/2022	Negative
8-F21 K	Kidney	10/11/2022	21/11/2022	28	23/11/2022	Negative
8-F21 S	Spleen	10/11/2022	21/11/2022	26	23/11/2022	Negative
8-F22 K	Kidney	10/11/2022	21/11/2022	28	23/11/2022	Negative
8-F22 S	Spleen	10/11/2022	21/11/2022	26	23/11/2022	Negative
8-F23 K	Kidney	10/11/2022	21/11/2022	21	23/11/2022	Negative
8-F23 S	Spleen	10/11/2022	21/11/2022	28	23/11/2022	Negative
8-F24 K	Kidney	10/11/2022	21/11/2022	21	23/11/2022	Negative
8-F24 S	Spleen	10/11/2022	21/11/2022	30	23/11/2022	Negative
8-F25 K	Kidney	10/11/2022	21/11/2022	25	23/11/2022	Lost in extraction
8-F25 S	Spleen	10/11/2022	21/11/2022	16	23/11/2022	Negative
8-F26 K	Kidney	10/11/2022	21/11/2022	30	23/11/2022	Negative
8-F26 S	Spleen	10/11/2022	21/11/2022	23	23/11/2022	Negative
8-F27 K	Kidney	10/11/2022	21/11/2022	29	23/11/2022	Negative
8-F27 S	Spleen	10/11/2022	21/11/2022	20	23/11/2022	Negative
8-F28 K	Kidney	10/11/2022	21/11/2022	19	23/11/2022	Negative
8-F28 S	Spleen	10/11/2022	21/11/2022	27	23/11/2022	Negative
8-F29 K	Kidney	10/11/2022	21/11/2022	29	23/11/2022	Negative
8-F29 S	Spleen	10/11/2022	21/11/2022	29	23/11/2022	Negative
8-F30 K	Kidney	10/11/2022	21/11/2022	29	23/11/2022	Negative
8-F30 S	Spleen	10/11/2022	21/11/2022	29	23/11/2022	Negative

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