Veterinærinstituttet
Norwegian Veterinary Institute

Report 36 - 2022

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REPORT 36/2022

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Suggested citation

Samuel Duodu, Mona Dverdal Jansen, Mary Nkansa, Saraya Tavornpanich, Kari Nordheim, Jacob Zornu, Edgar Brun, Angela Naa Amerley Ayiku, Abigail Akosua Adelani, Toni Erkinharju, Julie Svendsen, Camilla Fritsvold, Raoul Valentin Kuiper, Kofitsyo S. Cudjoe. Fish for Development baseline study to map the disease occurrence in tilapia farms on Lake Volta. VI Report 36. Veterinærinstituttet 2022.
© Norwegian Veterinary Institute, copy permitted with citation

Quality controlled by

Edgar Brun, Director of Aquatic Animal Health, Norwegian Veterinary Institute

Published

2022 on www.vetinst.no

ISSN 1890-3290 (electronic edition)

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Commissioned by / In collaboration with

Fisheries Commission Ghana and the University of Ghana

Colophon

Cover design: Reine Linjer

Cover photo: Kofitsyo S. Cudjoe

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Content

Summary

The Norwegian Veterinary Institute and the Fisheries Commission, in collaboration with the University of Ghana, conducted a baseline survey of pathogens in tilapia farms on Lake Volta as part of the Fish for Development, Ghana (gha-17/0005) project.

Thirty farms were selected for inclusion, representing key operation types (hatchery, nursery, grow-out) and farm sizes. Farm visits and sampling was conducted between 25.08.21 and 07.12.21. Samples included tissues/eggs in RNAlater® for virological analyses, tissues in 10% buffered formalin for histopathological analyses and samples on various growth media for bacteriological analyses. In addition, farm data was collected via a questionnaire.

Protocols developed and validated for the detection of Infectious Spleen and Kidney Necrosis Virus (ISKNV) and Tilapia Lake Virus (TiLV) in Ghanaian farmed tilapia by the University of Ghana West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), were used to test the survey samples. Bacteriological assessments were conducted by a combination of bacterial colony morphology, staining and MALDI-TOF analysis (mass spectrometry), with additional characterisation through 16S sequencing for *Streptococcus agalactiae* isolates. Histopathological assessments were conducted by the NVI, with digital slides to be shared with Ghana.

ISKNV was found to be widespread and was detected in 80% of sampled farms. It was primarily detected in smaller fish, however, adult fish, broodstock and eggs were also found positive. The detection of ISKNV in eggs provides an opportunity to break the infection circle before frys are transferred to grow-out facilities. Not all ISKNV-positive fish displayed clinical signs of infection. In one group of fry, typical histological signs of ISKNV associated disease were found, while PCR detected no virus in cohabiting fry (though ISKNV was detected in some of the tested adults from the same farm). Co-infections with ISKNV and bacterial pathogens were found in a number of farms. On the other hand, TiLV was not detected in any of the analysed samples.

A wide spectrum of bacterial pathogens was detected in samples from 19 out of 28 farms (68%), with significant isolates including *S. agalactiae*, *Edwardsiella tarda*, *Plesiomonas shigelloides* (formerly known as *Aeromonas shigelloides)*, *Aeromonas veronii*, *Aeromonas jandaei*, *Aeromonas hydrophila*, *Chrysobacterium gambrini* and Flavobacteria-like organisms. The predominant bacterial pathogen, *S. agalactiae*, was mainly detected in adult fish and co-infections with other bacterial pathogens, as well as ISKNV, were found. *P. shigelloides* was the second most abundant bacterial pathogen, occurring both as single infection and as mixed co-infections. *E. tarda* was detected in four farms, mainly large-scale farms, and was found as both as single infection and as mixed co-infections. Bacterial branchial epitheliocysts were frequently observed histologically, but causative organisms were not identified to the species level. In general, not all infected fish showed gross clinical signs of bacterial infection/disease.

Histopathological examination revealed a wide range of parasites in different life stages, which may be subclinical. Branchial epitheliocysts were among the most common histologic signs of bacterial infection of the gills, which may also not be clinically apparent at low-intermediate levels of infection. There were also multiple cases of bacterial infections with accompanying septicaemia that have an immediate correlation to clinical disease. Further studies of selected

cases using special histochemical staining (i.e. Gram, PAS and Giemsa) revealed more information about the bacteria observed. Typical findings of necrosis in spleen and kidney generally correlated well with PCR results for ISKNV. Calcification of excretory kidney tissue (nephrocalcinosis) was observed in some individuals. Granulomatous inflammation of several organs was a common finding, as were circulatory disturbances of internal organs and gills. A summary of histopathology results for the most common, identified pathogens is included. Participating farms received their farm-ID together with an earlier version of this report where, anonymised, individual farm-level results were included.

Farm manager interviews revealed that the baseline mortality on studied farms ranged from 0,1 to 65%, with a median of 1% and a mean of 7%, while disease-associated mortality ranged from 1-95%, with a median of 70% and a mean of 63%. Ninety-three percent (n=28) of farms reported having experienced significant disease problems in the past five years, with 70 % (median) reporting they have experienced large financial losses (range 7,5 – 90%). A wide range of clinical signs had been observed in conjunction with disease events. Vaccination and heat shock treatment were the most reported ISKNV-specific interventions, with half (7 farms) reporting no improvement in tilapia health following vaccination while the other half (7 farms) reported improvement. When asked how interventions could be improved, key inputs were improved diagnostics, vaccine administration and heat treatments, regulation of fingerling production and sales, fingerling health certification, regulation of chemicals and reduced stocking density.

A large proportion of studied farms were found positive for ISKNV and/or pathogenic bacteria, showing that disease events are significantly affecting fish health and welfare in tilapia farms in Lake Volta. The current report includes some general recommendations in relation to disease control. These aspects will be further elaborated on in the context of overall tilapia health in Ghana once a similar study of tilapia pond farms has been completed.

List of abbreviations

EGC	Eosinophilic granular cells
FC	Fisheries Commission of Ghana
ISKNV	Infectious spleen and kidney necrosis virus
MMC	Melanomacrophage centers
NOS	No observable signs
NVI	Norwegian Veterinary Institute
TiLV	Tilapia Lake Virus
UG	University of Ghana
UG-WACCIBIP	University of Ghana West African Centre for Cell Biology of Infectious Pathogens

Introduction

Disease occurrence is a major threat to the sustainability of aquaculture worldwide. In addition to causing reduced revenue for farmers, disease events significantly affect fish welfare and may pose risks to the health of humans and other animals in relation to consumption. Evidence-based knowledge about the current disease situation is required to facilitate successful implementation of appropriate disease control measures. While there have been numerous investigations in relation to disease in farmed tilapia in Ghana, the information in many cases is not publicly available, hampering effective disease control strategies.

A baseline survey of pathogens in tilapia farms on Lake Volta was undertaken as a part of the bilateral program agreement between the Norwegian Ministry of Foreign Affairs and the Ghanaian Ministry of Finance regarding Fish for Development, Ghana (gha-17/0005).

The survey was planned to be conducted by the Norwegian Veterinary Institute (NVI) in collaboration with the Fisheries Commission (FC). Due to Covid 19 travel restrictions, NVI found it expedient to collaborate with the University of Ghana (UG) to carry out the practical aspects of this study in collaboration with the FC.

This report aims to inform participating farmers of study outcomes and summarise the laboratory results. In addition, a preliminary version of the report served as a basis for discussions during a physical meeting between international and local researchers and participating farmers in Ghana in the autumn of 2022. Input from farmers is essential to successfully develop effective disease control and biosecurity strategies.

Sampling

The on-going PhD study on ISKNV conducted by Angela Naa Amerley Ayiku at UG in collaboration with CEFAS formed an integral part of this sampling. The FC and the Ghanaian team randomly selected farms from the farm census list, stratified by location (upstream/downstream), for inclusion in the study. The inclusion criteria were tilapia farms in Lake Volta (tilapia monoculture or polyculture with a majority of tilapia).

Thirty (30) farms were selected for inclusion in the Fish for Development baseline study (Figure 1). Where selected farms had more than one facility (hatchery, nursery, grow-out) in operation, all facilities were included. The 23 farms in the Eastern region were located in 4 different districts, namely Asuogyaman (n=15), Lower Manya Krobo (n=5), Upper Manya Krobo (n=2) and Kwahu South (n=1). In the Volta region, the 7 selected farms were located in South Dayi (n=1), Afadjato South (n=4), South Tongu (n=1) and Agodomi (n=1) districts. Selected farms covered key operation types (hatcheries to grow-out and various hatchery – nursery – grow-out combinations). Farm size according to the licence permit and scale of production amongst selected farms was relatively evenly split (large scale, over 100 tons (30%, n=9), medium scale, under 100 tons (40%, n=12) and small scale, less than 50 tons (30%, n=9)).

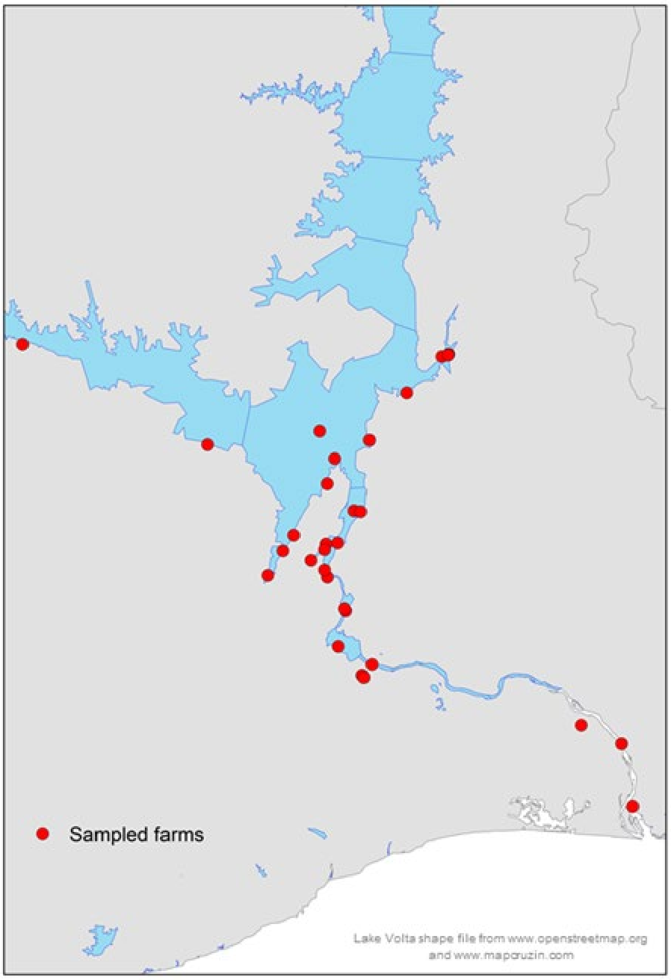

Figure 1*: Map of farms selected for inclusion in the study. Due to the proximity of some farms, some dots are overlapping.*

Farm visits and sampling was conducted on seven different trips (V1 to V7) between 25.08.21 and 07.12.21. The Fisheries Commission facilitated farm access, while the University of Ghana team, in collaboration with the Fisheries Commission, conducted the sampling.

On average, 10 biological samples including eggs, fry, fingerlings, moribund grow-out adult fish and broodstock were collected at each farm depending on the type of facility in operation. Moribund fish were selected so that they collectively represented the main fish age groups where moribund fish were observed at the time of sampling. For farms with multiple facilities, representative samples were collected across the entire production cycle.

The following samples were collected:

- Kidney, brain, spleen and eggs in RNAlater® for virological analyses
- Kidney, liver, heart, brain, spleen, pancreas, gills, skin muscle, eye and intestines, or whole fish where <1g, in 10% buffered formalin for histopathological analyses
- Head kidney, brain and external lesions on appropriate growth media (blood agar, TSA and/or tryptone yeast extract supplemented with Tobramycin) for bacteriological analyses

Each sample was labelled with a unique farm identification number and running fish identification numbers to allow laboratory results to be assigned at fish-level.

In addition to biological samples, farm data was collected through the "Tilapia Aquaculture Production and Disease Status in Ghana" questionnaire developed by the UG and NVI.

Laboratory analyses

The University of Ghana West African Centre for Cell Biology of Infectious Pathogens (UG-WACCIBIP) conducted the virological and bacteriological analyses of the collected samples. The Norwegian Veterinary Institute supplied all media and reagents used except those few available locally.

Prior to the initiation of the study, upon instigation of NVI, the UG-WACCIBIP developed and validated PCR protocols for the detection of Infectious spleen and kidney necrosis virus (ISKNV) and Tilapia lake virus (TiLV) in Ghanaian farmed tilapia (Duodu *et al.*, 2021). Based on these validations a multiplex PCR detection assay was used for ISKNV analyses and real-time qPCR for TiLV analyses. Samples for ISKNV were analysed as individual samples, while samples for TiLV were analysed as a combination of individual samples and pooled samples (up to 5 samples from one farm analysed together).

Bacteriological assessments were conducted by a combination of bacterial colony morphology, Gram staining and MALDI-TOF analysis (mass spectrometry). *Streptococcus agalactiae* isolates were further characterised through 16S sequencing.

Samples in formalin were shipped to NVI in Norway where slides have been prepared and digital images generated. In addition to assessing histopathological changes associated with infection by the identified viral and bacterial pathogens, the examination also aimed at describing additional histopathological findings in the examined fish. Digital slides for now will be shared with the UG/FC for joint histopathological analyses until the required computers/internet access have been installed in Ghana.

Laboratory results

The following sections summarise the farm-level laboratory findings.

Virology results

ISKNV was detected in 80% (24/30) of sampled farms. An approximately even proportion of large scale (89%, 8/9), medium scale (75%, 9/12) and small-scale (78%, 7/9) farms were affected, representing facilities from hatcheries to grow-out farms. The distribution of ISKNV positive and negative farms is shown in Figure 2. ISKNV was primarily detected in smaller fish, with a median weight of 21g (range 0,6 – 515g, mean 79g). However, adult fish, broodstock and eggs were also found positive. Fish testing positive were found amongst fish without gross clinical signs and amongst fish displaying signs of ISKNV infection such as production of excess skin mucus, exophthalmia and distended abdomen (ascites). Co-infections with ISKNV and bacterial pathogens were found in a number of farms.

TiLV was not detected in any of the analysed samples.

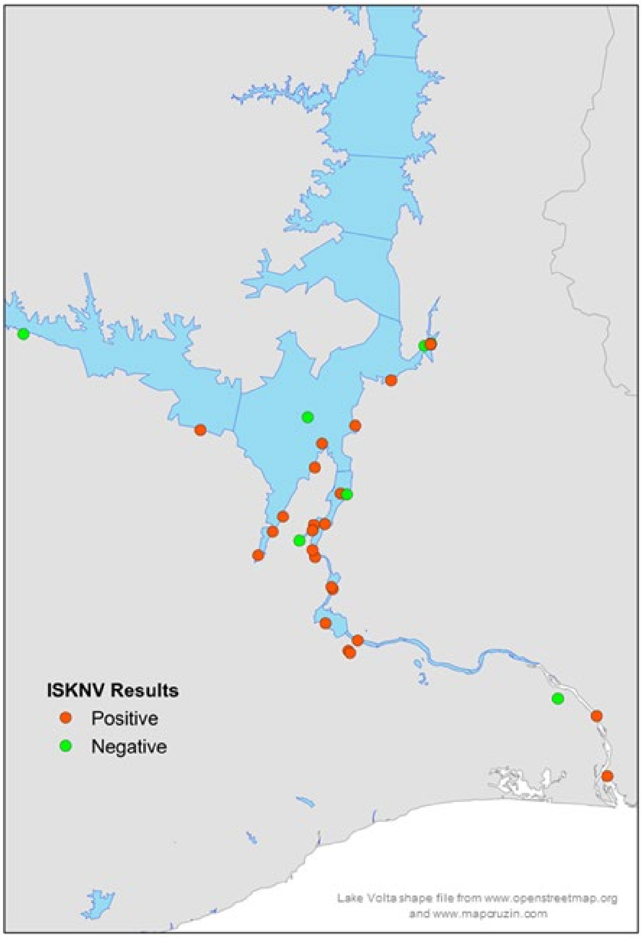

***Figure 2**: Map showing the farm status based on ISKNV laboratory results. Farms testing positive for ISKNV shown as red dots while ISKNV-negative farms are shown as green dots.*

Bacteriology results

A wide spectrum of bacterial pathogens was detected in samples from 19 out of 28 farms (68%). Three farms, including one farm within an epidemiological unit of two farms, were not sampled for bacteriology. Figure 3 shows the distribution of farms by overall bacteriology result. The significant isolates detected included *S. agalactiae*, *E. tarda*, *P. shigelloides* (formerly known as *Aeromonas shigelloides)*, *A. veronii*, *A. jandaei*, *A. hydrophila*, *Chrysobacterium gambrini* and Flavobacteria-like organisms.

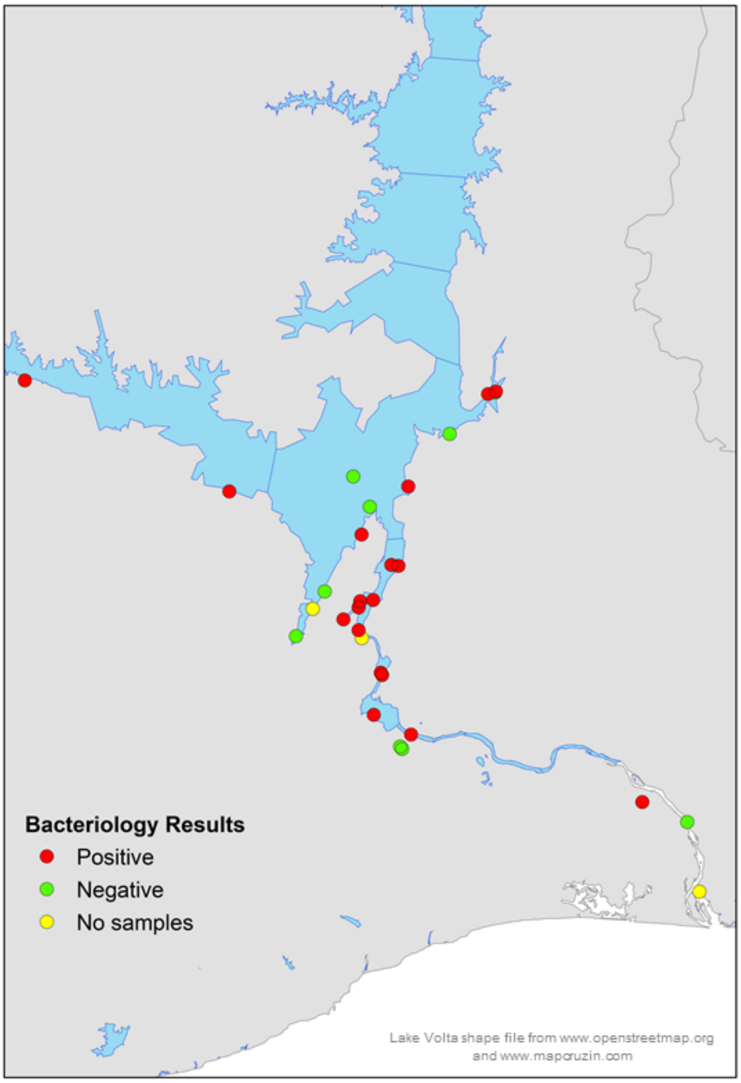

Figure 3*: The distribution of farms based on their overall bacteriology results: bacteriology positive (red dots) or bacteriology negative (green dots). For completeness, the three farms without samples for bacteriological analyses are also shown (yellow dots).*

S. agalactiae was the predominant bacterial pathogen. It was found in samples from 7 farms (Figure 4), all in the lower Volta or the south-eastern part of Lake Volta. The majority were large scale farms (71%, n=5), but medium scale farms (29%, n=2) were also represented. Positive farms included a hatchery as well as hatchery, nursery, grow-out combinations. *S. agalactiae* was isolated from samples from brain, kidney and skin, with positive samples yielding 32 isolates in total. Positive samples originated mainly from adult fish, with a median weight of 300g (range 7 – 1 064g, mean 321g). Tilapia infected with *S. agalactiae* presented with a range of gross clinical signs including haemorrhages in skin and internal organs, distended abdomen (ascites), opaque eyes, exophthalmia, necrosis of viscera, mottled friable liver, enlarged gall bladder, congested kidney and splenomegaly. Some fish found positive for *S. agalactiae* had no detectable gross signs. The bacteria displayed varied morphological features in culture and were either ß-haemolytic or non-haemolytic. *S. agalactiae* was detected both as single infections and in combination with other bacterial pathogens. Three of the 7 farms tested positive also for ISKNV, with co-infections of *S. agalactiae* and ISKNV found in fish from one farm.

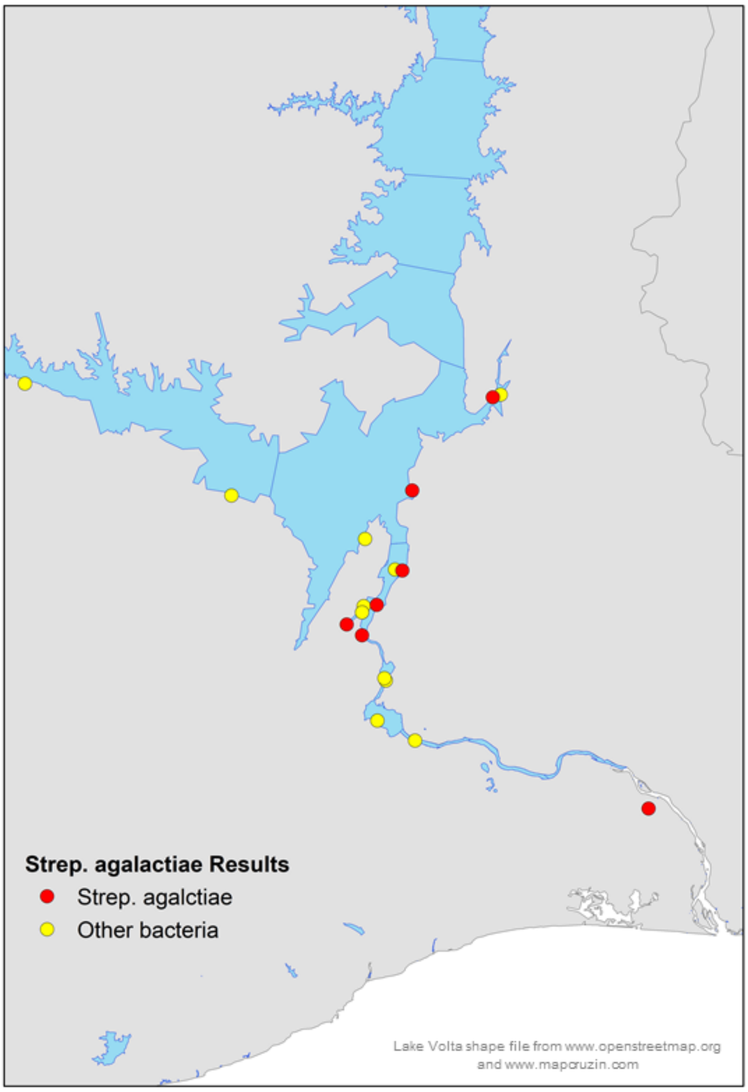

Figure 4*: Map of farms testing positive for S. agalactiae (red dots). Farms which tested positive for other bacteria are shown as yellow dots.*

P. shigelloides was the second most abundant bacterial pathogen, with 11 recovered isolates from 8 different farms (Figure 5). Affected farms were located throughout most of the study area. Half of the affected farms were medium scale farms (50%, n=4), with large scale (38%, n=3) and small scale (12%, n=1) farms making up the rest. Affected farms ranged from hatcheries to grow-out operations, including various hatchery, nursery, grow-out combinations. *P. shigelloides* was isolated from brain and kidney samples of fish with a median fish weight of 159g (range 1 - 620g, mean 218g). Some tilapia infected with *P. shigelloides* were without gross clinical signs while others showed a range of signs including loss of scales, pale gills, haemorrhaging of skin and internal organs (heart, kidney and intestines), friable liver, darkened spleen and ascites. *P. shigelloides* occurred both as single and mixed infections, including as co-infection with *E. tarda*. Two fish from 2 different farms were found to be co-infected with ISKNV.

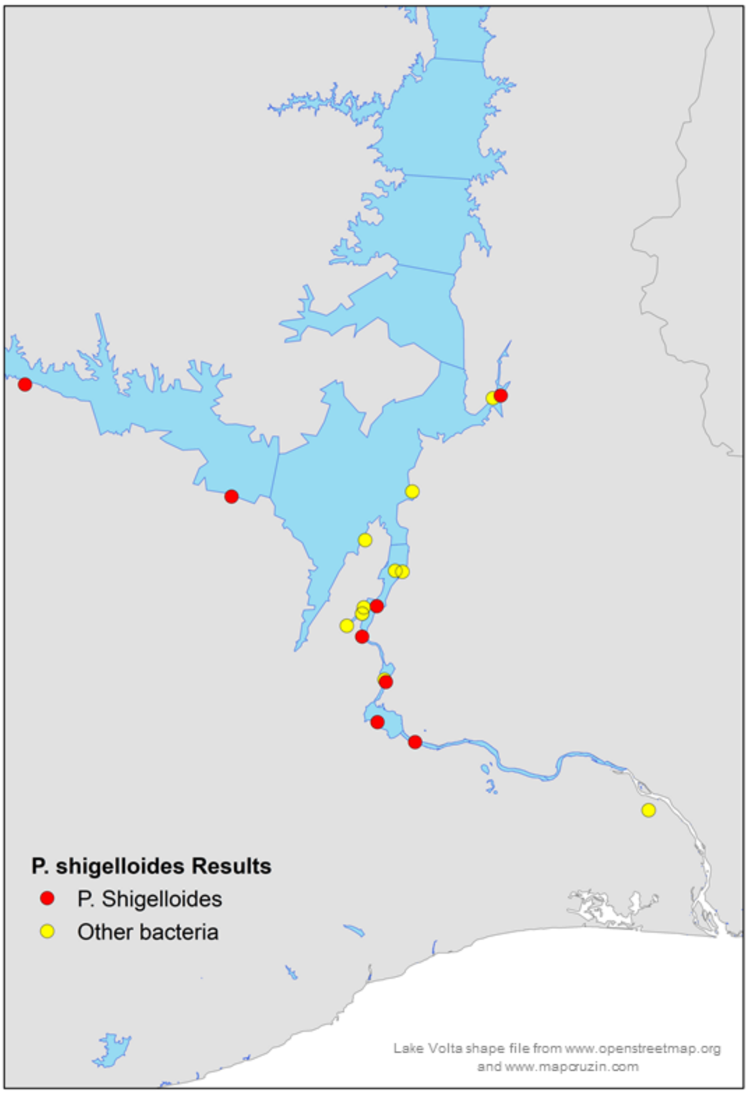

Figure 5*: Map of farms testing positive for P. shigelloides (red dots). Farms that tested positive for other bacteria are shown as yellow dots.*

E. tarda was isolated from 5 fish from 4 different farms (Figure 6). The majority were large-scale farms (75%, n=3) while one (25%) was a small-scale farm. Both hatcheries and nursery – grow-out operations were represented. Kidney samples from both juvenile and adult fish were found positive, with a median fish weight of 74g (range 1 – 240g, mean 91g). Affected fish were either without gross clinical signs or presented with signs such as skin discolouration, scale loss and haemorrhages. *E. tarda* was found both as a single infection and as co-infection with *P. shigelloides*, *S. agalactiae* and *Moraxella* sp. The majority (75%, n=4) of fish found positive for *E. tarda* tested negative for ISKNV, however at farm-level three out of four *E. tarda*–positive farms also had ISKNV detected in one of more fish.

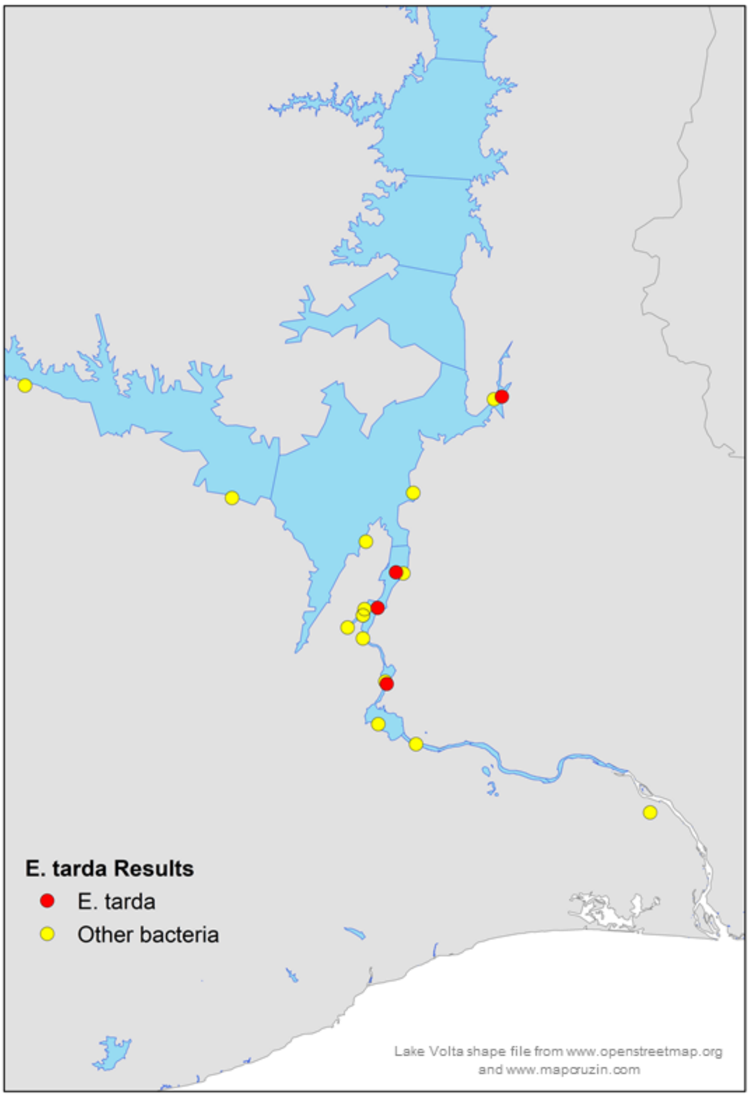

Figure 6*: Map of farms testing positive for E. tarda (red dots). Farms, which tested positive for other bacteria are shown as yellow dots.*

Bacteria belonging to *Aeromonas* spp. were detected in 6 farms, and included *A. jandaei* (5 isolates), *A. veronii* (2 isolates) and *A. hydrophila* (1 isolate). Half of the affected farms were large scale farms (50%, n=3), but medium scale (17%, n=1) and small scale (33%, n=2) farms were also represented. Affected farms ranged from hatcheries to grow-out operations, including various hatchery, nursery, grow-out combinations. *A. jandaei* were mainly isolated from smaller fish, with a median fish weight of 10g (range 1-90g, mean 25g). *A. veronii* was detected in both juvenile and adult fish (15g and 300g, respectively), while *A. hydrophila* was detected in an adult fish (170g). Affected fish presented either without gross clinical signs or with a range of clinical signs including exophthalmia, ascites, loss of scales, haemorrhages, ulcers and skin discolouration. The majority presented as single bacterial infections, however many of the *Aeromonas*-positive fish (63%, n=5) were also found to be positive for ISKNV.

C. gambrini (5 isolates from 4 farms) and Flavobacteria-like organisms (7 isolates from 6 farms) were isolated from the surface of fish with skin lesions, ulcerations, fin rot and nodules. Other skin-associated organisms were *Sphingobacterium multivorum* (caudal fin, 1 farm) and *Pseudarthrobacter polychromogenes* (operculum, 1 farm), may represent opportunistic infections rather than true pathogens.

Emerging fish pathogens such as *Moraxella* sp. (3 isolates from 1 farm, brain and kidney), *Stenotrophomonas maltophila* (1 isolate, brain), *Acinetobacter* spp. (3 isolates from 3 farms, brain and kidney), *Citrobacter* spp. (2 isolates from 2 farms, kidney) and *Kocuria rhizophila* (1 isolate, kidney) were also isolated. Human commensals like *Staphylococcus* sp. were identified in 2 fish from different farms (brain and kidney).

Lactococcus lactis, detected in one kidney sample from a juvenile fish (7 g) with no observable symptoms and no co-infections, likely reflects probiotic used in the feed.

Histopathology results

The following is a summary of the key histopathological findings observed in the collected material.

Viral disease

The major identified viral disease in the material was changes associated with infection with ISKNV, genus Megalocytivirus (one of 3 genera within the Iridovirus family). Splenic and/or renal necrosis in combination with typical megalocytic cells were found in 6 out of 28 samples identified as positive (Figure 7). Except for 3 fry, detailed below, similar changes were not observed in IKSNV negative fish. Because these organs play a central role in the physiology of the fish, and comorbidities were frequent, with general signs in other organs accompanied the more typical histopathological findings in spleen and kidney presented here. Extensive spleen and renal necrosis were found in 1 adult and 3 fry from 53 samples that tested negative for ISKNV by PCR; the adult had septicaemic signs and *S. agalactiae* (see below) was cultured from this fish, while the 3 fry were from a farm where other (adult) fish tested positive for ISKNV by PCR.

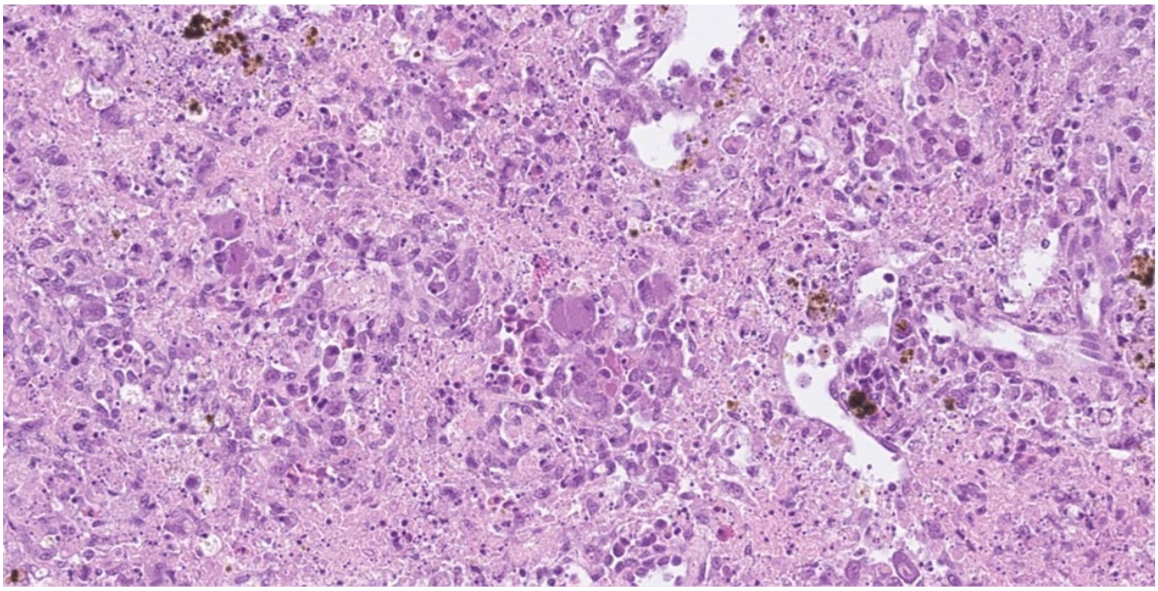

***Figure7A**: V1F3, fish 6. Necrosis in the spleen. This individual tested positive for ISKNV through a multiplex PCR detection assay*

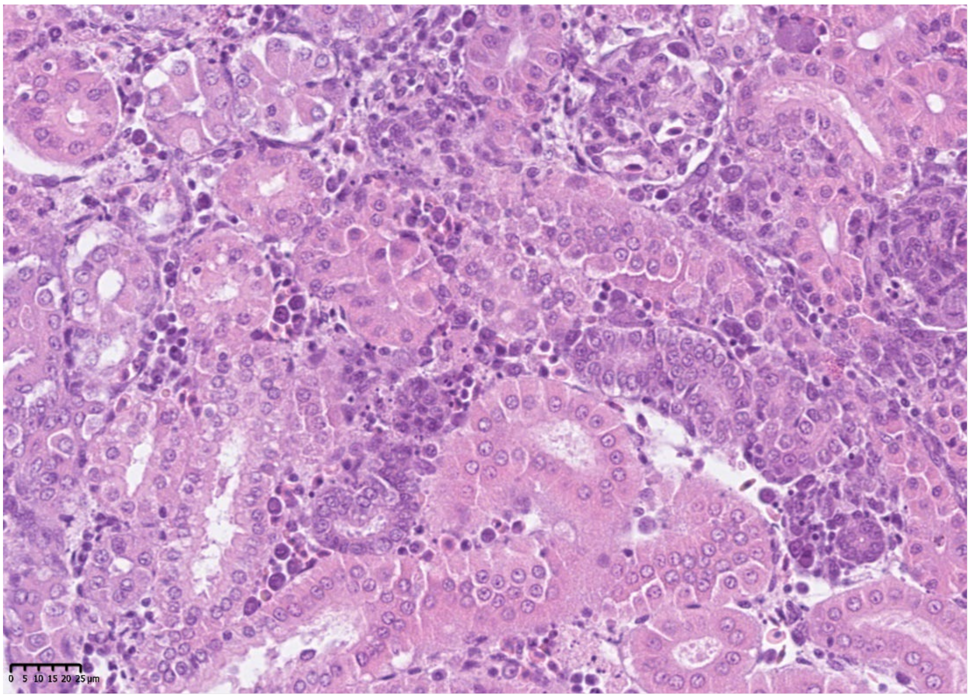

***Figure 7B**: V1F3, fish 6. Necrosis in the kidney. This individual tested positive for ISKNV through a multiplex PCR detection assay*

Parasitic infestations

A wide range of parasites in different life stages affected a high proportion of the evaluated tissue samples. Possible myxozoan spores were found in spleen/kidney, gills and muscle tissue, ciliates, flagellates, monogeneans (Figure 8) and crustaceans in gills, tentative coccidia stages and helminths in the gastrointestinal tract, encapsulated metacercarian cysts in internal organs as well as gills. The latter is an example of a myxozoan plasmodium in an intrahepatic bile duct (Figure 9), and several cases of parasitic structures of unknown character in the gastrointestinal tract and gills.

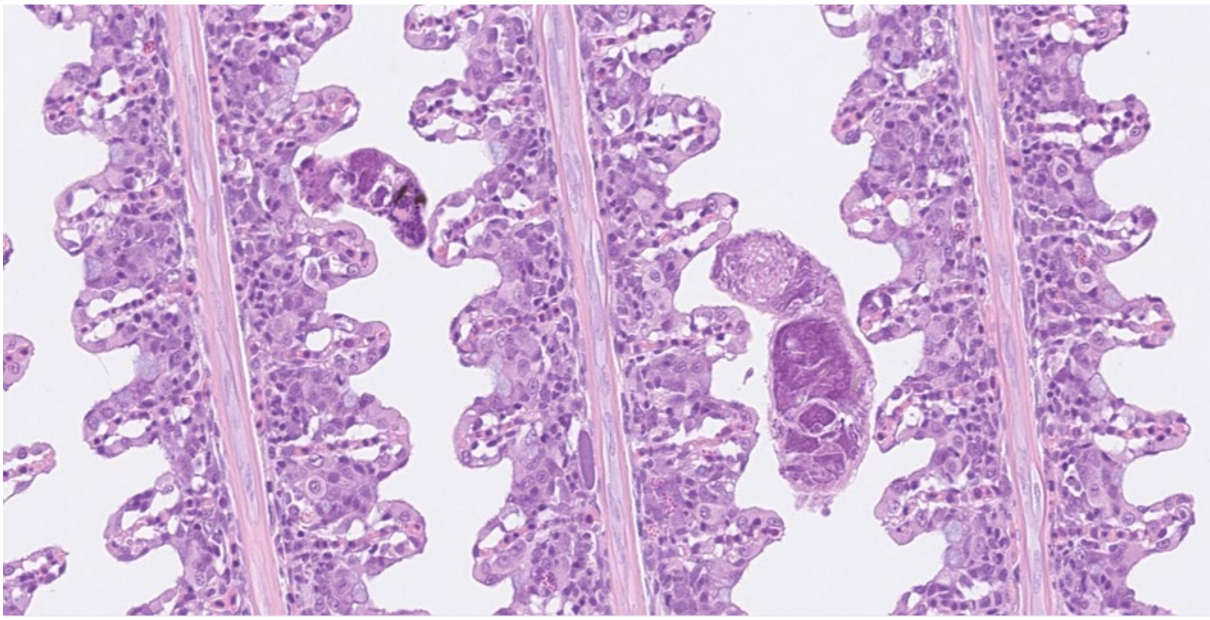

Figure 8*: V1F9, fish 5. Gills: metazoan parasites, possible monogeneans. HE-stain.*

Figure 9*: V3F1, fish 1. Liver: posssible myxozoan parasite plasmodium in bile canal. HE-stain.*

Bacterial infections

As for bacterial infections, epitheliocysts in the gills were among the most common histopathological findings. Epitheliocystis disease affects the gills and skin of many species of freshwater and marine fish, and the associated pathogens appear to cover a wide and increasing range of intracellular bacteria including various species in the phylum *Chlamidiae*, and β-, and γ-proteobacteria (reviewed in Blandford et al., 2018). The observed cyst-like lesions consist of intracellular colonies of tightly packed Gram-negative organisms within hypertrophied epithelial cells (Figure 10). Heavy gill infestations are thought to compromise gas- and metabolite exchange and may contribute to episodes of mortality in cultured fish, with juveniles representing the most sensitive stage; high stocking densities, water temperatures and soiled water presenting further risk factors (Blandford et al., 2018). In routine HE stained histological sections they appear as rounded or "shrivelled" basophilic finely granular structures along the lamella. Although causative agents have largely not been characterized for Tilapia, lesions with consistent morphology and histochemical staining properties are being reported in cultured *Oreochromis niloticus* in the New World (Pádua et al., 2015; Abad-Rosales et al., 2022). Morphology and localisation of similar cysts found in the studied material from Lake Volta is consistent with earlier reports. Epitheliocysts were histologically observed in 45 out of 73 sufficient quality samples from adult fish, and 9 out of 26 juvenile samples; 6 out of 14 samplings of juveniles and 15 out of 20 samplings of adults detected epitheliocystis at farm level.

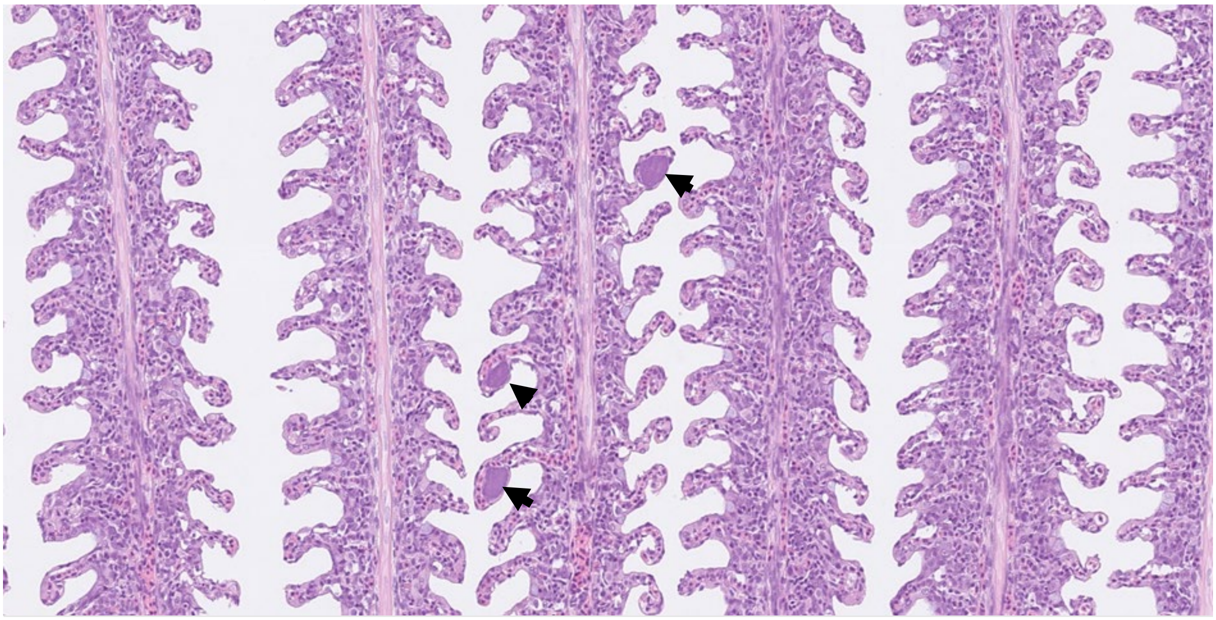

Figure 7*: V1F9, fish 5 (same as Figure 8). Gill lamellae with moderate diffuse epithelial hyperplasia and occasional epitheliocysts (arrowheads). HE-stain.*

There were also multiple cases of bacterial infections with accompanying septicaemias: some of these were suspected bacterial wound infections, some were related to infections of the eye and some were suspected intracellular bacterial infections. Coccoid, Gram-positive bacteria were histologically observed in multiple organs in a number of cases, where *S. agalactiae* was also cultured.

Histological changes vary with the number of bacteria present at the affected sites. Involved serosal membranes show oedema, fibrin deposits and occasional haemorrhage, and are further expanded by a mixed inflammatory infiltrate, with a tendency to become (pyo)granulomatous. In many of the confirmed cases, extracellular colonies of bacteria were found in abundance. In some cases, bacteria appeared mostly phagocytosed and were less easy to detect in H&E stained histological sections. In particular, within the epicard and meninges, the appearance can be more fibrinopurulent and large numbers of cocci can be present (Figure 10).

Haematogenous spread to parenchymatous organs including liver (Figure 11) and kidney may result in focal bacterial aggregates, necrosis, haemorrhage, and/or inflammation that may also be visible with the naked eye (mottled appearance; petechial haemorrhages). (Thrombo)-emboli may lodge in the meshwork of trabecular cardiac muscle; in *Oreochromis* spp., the atrial endothelium is highly responsive and often shows marked, diffuse hypertrophy in response to septicaemia, as has also been shown in systemic francisellosis (Soto et al., 2011). Ocular involvement is also consistent with haematogenous spread and frequently characterized by haemorrhages and uveitis, with necrosis and aggregates of cocci within the retrobulbar vascular network. Damage to the globe may progress to involve corneal oedema and keratitis, and panophthalmitis, matching macroscopic finds of ocular bleeding and corneal opacity.

In addition, there were some cases of possible bacterial overgrowth of the gastrointestinal tract in association with distension and excess content in fry. Further studies of selected cases using special histochemical staining (i.e. Gram, PAS and Ziehl-Neelsen) may reveal more information about the bacteria observed.

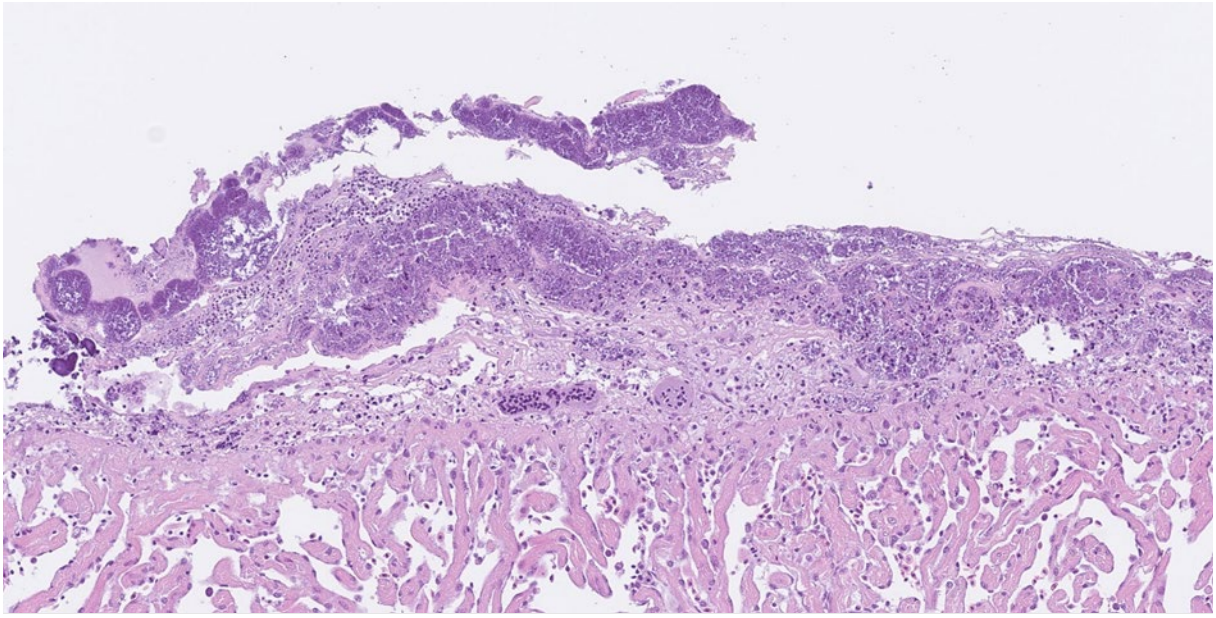

Figure 10A*: V1F2, fish 9. Heart and epicardium with bacterial infection. This individual was positive for Streptococcus agalactiae on bacteriology. HE-stain.*

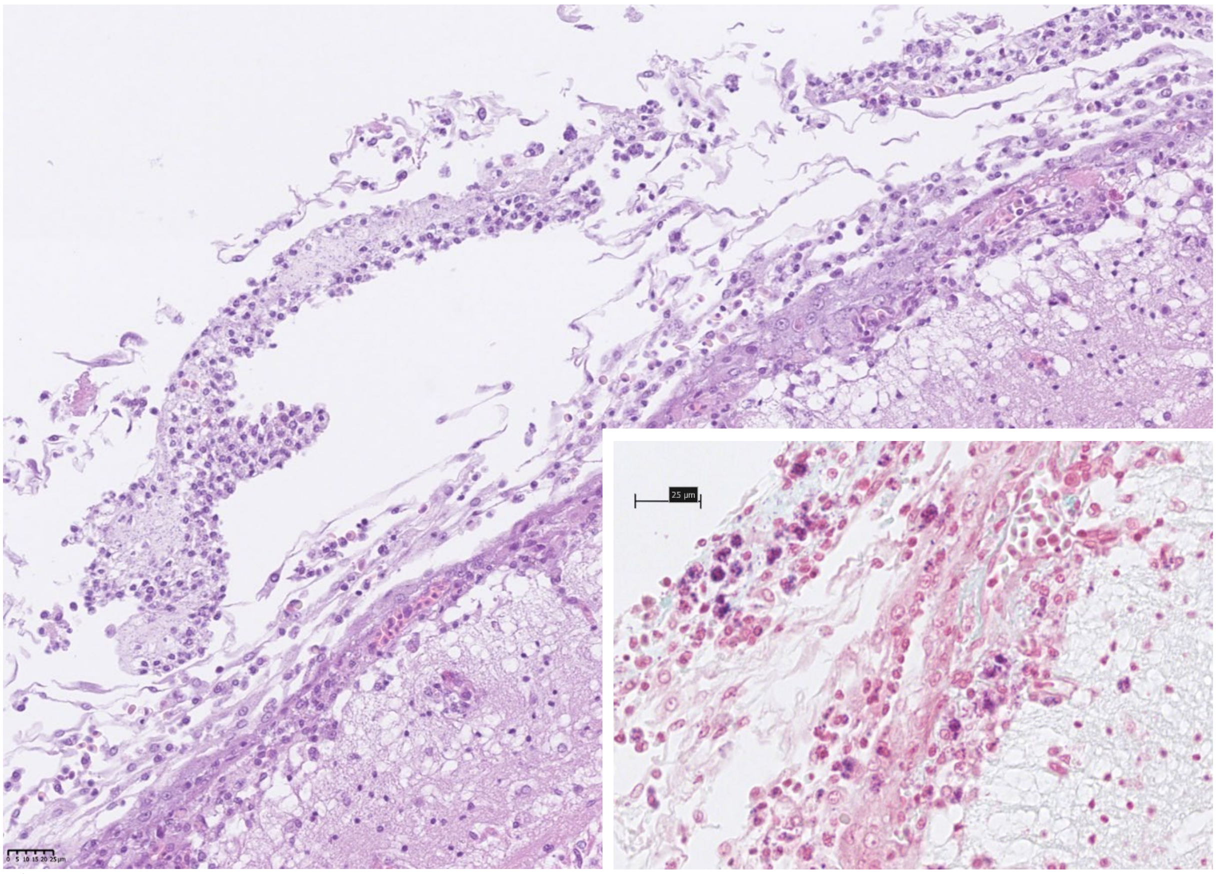

***Fig 10B**: V2F1, fish 4. Meningo-encephalitis in a fish that tested positive for Streptococcus agalactiae (PCR). Thickened, cell-rich meningi with fibrin deposition and coccoid bacteria. Detail: clusters of phagocytosed Gram+ bacteria.*

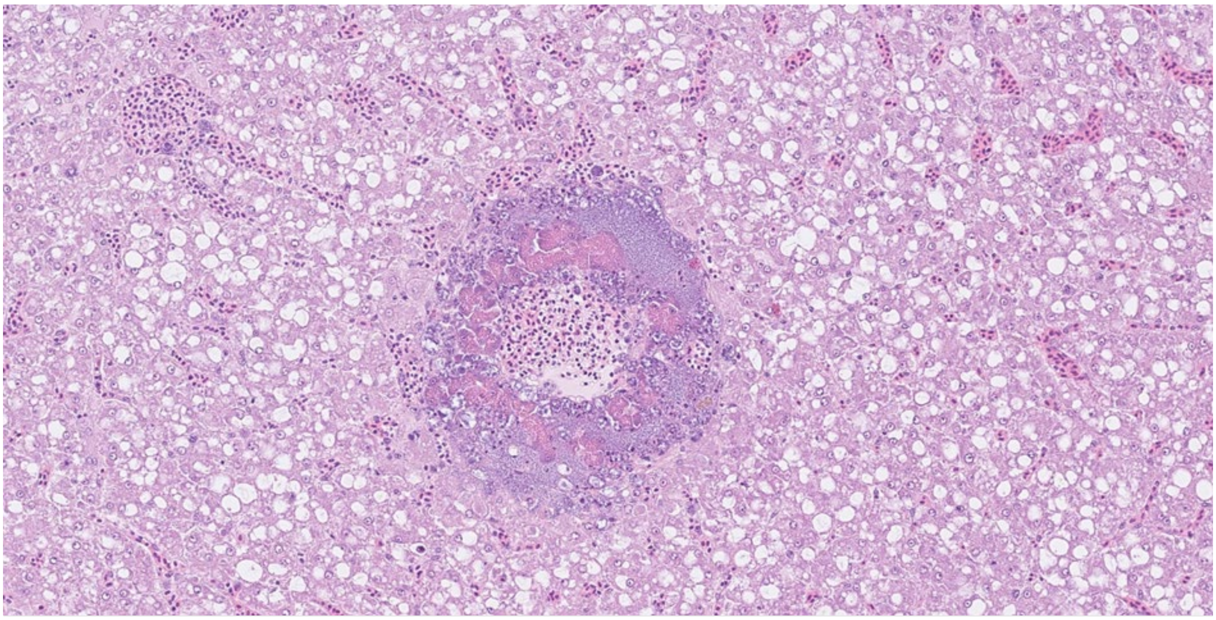

***Figure 81**: V2F1, fish 3. Liver/pancreas: bacterial infection. This individual was positive for Streptococcus agalactiae on bacteriology. HE-stain.*

Non-infectious conditions

Mineralisation of excretory kidney tissue (nephrocalcinosis; Figure 12) was observed in some individuals, but the true prevalence of this may be higher, as the majority of the kidney samples consisted of haematopoietic tissue only, hence they contained no excretory tissue for assessment. This is probably a result of how sampling was performed: kidney samples collected from head kidney will most often consist of haematopoietic tissue only, while samples of mid-kidney and more caudal parts of the kidney will consist of a mixture of both haematopoietic and excretory tissue. For future samplings, mid-kidney or more caudal parts of the kidney are recommended in addition to the head-kidney. Signs of possible calcification were also observed in the hearts of a few individuals.

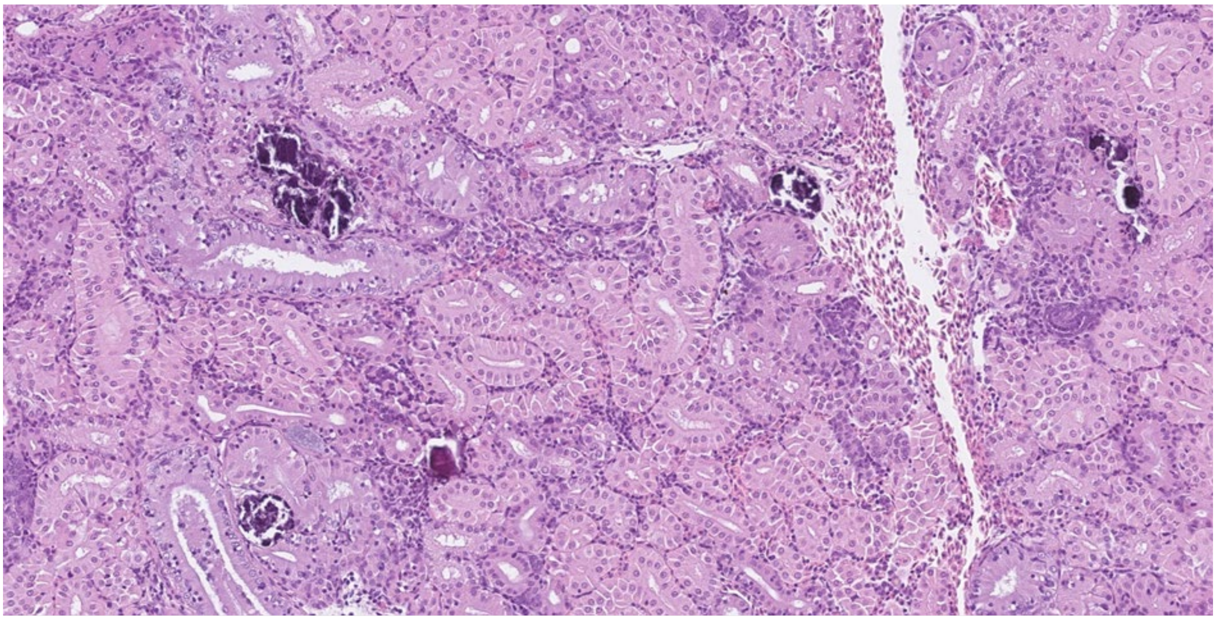

***Figure 9**: V1F1, fish 4. Nephrocalcinosis; deposits of mineral (calcium salts) in kidney. HE-stain.*

General comments

Granulomatous inflammation of several organs was among the most common findings (most frequent in spleen), as were circulatory disturbances of internal organs and gills. Possible cataracts were found in some individuals, as well as degenerative changes/necrosis (e.g. in spleen).

Several fish appear to have fed well, on a diverse diet, and suspected GI tract overfilling were observed in some fry. This poses a challenge in evaluating the gastrointestinal contents, with respect as to whether the observed contents reflect ingested material/food, or whether this represents an actual pathogen infestation. The findings must therefore be interpreted with some caution.

Spleen and kidney samples contained large numbers of melanomacrophage centers (MMC). MMC are aggregates of highly pigmented phagocytes, and they are a common finding also in some marine fish species. However, the amount of MMC does appear increased in some of the assessed individuals, which could indicate a general catabolic state and/or occur as part of an ongoing immune response (Steinel & Bolnick, 2017).

Finally, some general observations of the assessed material: the tilapia specimens in this study show prominent eosinophilic granular cells (EGC), with a raspberry-like appearance, as can also be observed in e.g. wrasse. In certain tissues and individuals, like gills and nervous tissue, the number of these cells appeared somewhat increased (example in Figure 13). It should be noted that the pancreatic tissue of tilapia is found integrated with the liver architecture, often referred to as hepatopancreas (Figure 14).

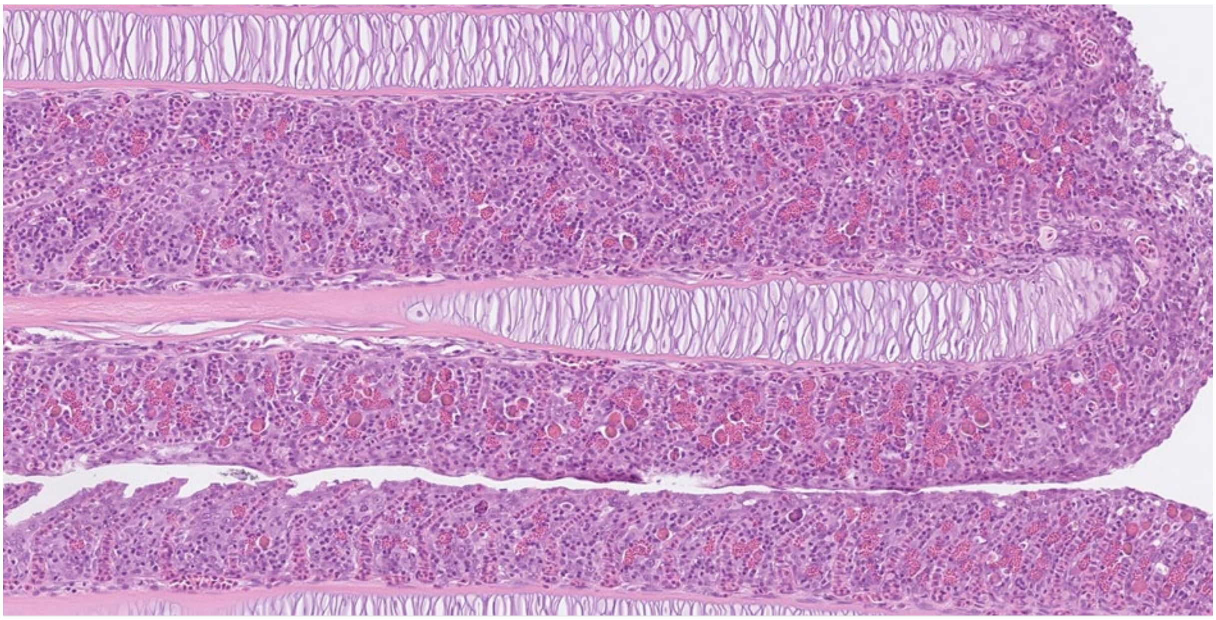

Figure 10*: V1F2, fish 7. Gills: diffuse epithelial hyperplasia and frequent eosinophilic granular cells. HE-stain.*

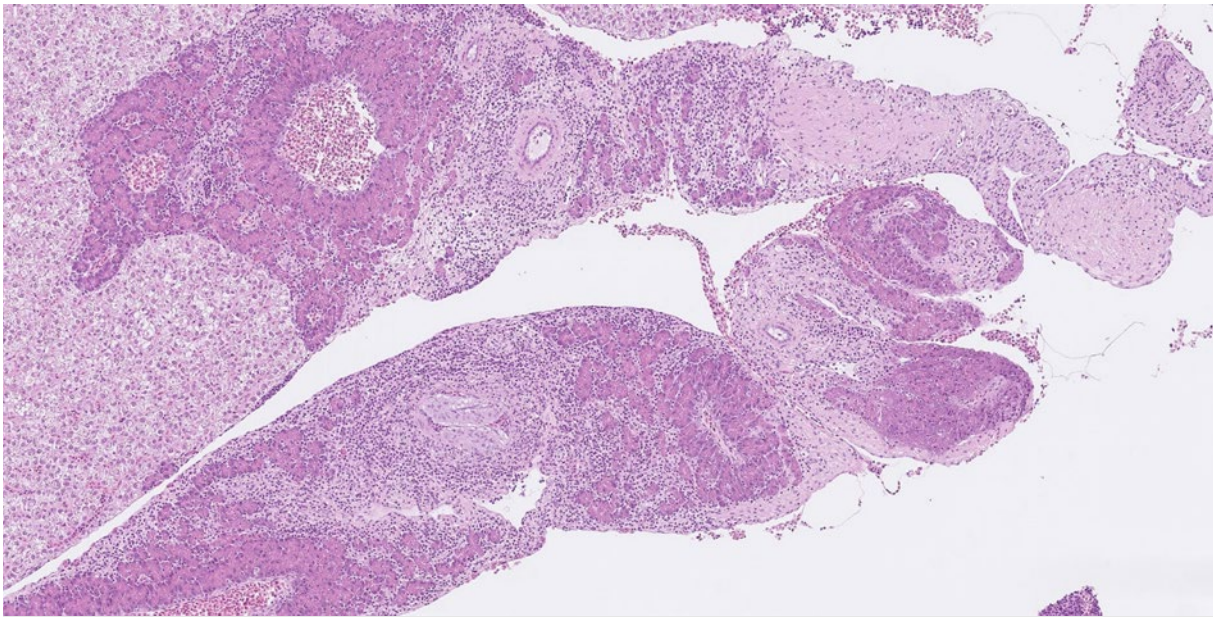

***Figure 11**: V3F5, fish 7. Liver, with integrated pancreatic tissue and possible hepatopancreatitis. HE-stain.*

Brief account of farm manager interviews

Most of the studied farms (60%, n=18) had been in operation between 1 and 10 years while the remaining 40% had been in operation for more than 10 years.

Reported baseline mortality ranged from 0,1 to 65%, with a median of 1% and a mean of 7%. Only 13% (n=4) of the farms reported a baseline mortality above 10%.

Ninety-three percent (n=28) of farms reported having experienced significant disease problems in the past five years. Impacts included high mortality and large financial losses (median 70%, range 7,5 – 90%). The number of outbreaks over the 5-year period ranged from 1 to 5, with some indicating more continuous problems.

Disease-associated mortality ranged from 1-95%, with a median of 70% and a mean of 63%. Some indicated differential mortality levels, with ISKNV infections leading to higher mortality than bacterial infections. There were also reports of mortality events with significantly higher mortality in fingerlings than juveniles and grow-outs.

Respondents typically reported having observed one or more of the following clinical signs: abnormal swimming patterns, skin nodules, loss of eyes, darkening of eyes, bulging of eyes, loss of scales, excess mucous on skin, distended abdomen, discolouration/darkened skin, weight loss, skin lesions, whitening of mouth part, frayed fins, eroded fin, fin rot and ectoparasite infestation.

Ten farms reported observable water changes prior to disease events. These were most commonly colour change, with some also reporting changes in measured parameters.

Two questions related to general disease control interventions and their effect on disease control. The eight farms reporting unsuccessful disease control most frequently mentioned the use of salt treatment, with some mentioning chemicals (potassium permanganate, hydrogen peroxide), herbal treatments, reduced feeding, antibiotics (clindamycin, oxytetracycline) and the use of autogenous vaccine. On the other hand, adopted measures deemed successful included vaccine and antibiotic (clindamycin, oxytetracycline, chloramphenicol) administration, heat shock treatment, reduced stocking density, removal of diseased fish, salt treatment, herbal treatments, reduced feeding, and the use of potassium permanganate. When asked how interventions could be improved, key inputs were improved diagnostics, vaccine administration and heat treatments, regulation of fingerling production and sales, fingerling health certification, regulation of chemicals and reduced stocking density.

Farm-level routine disease prevention practices mentioned included disinfection (salt or other disinfectant), access restriction, staff biosecurity training, heat treatment, reduced feeding, herbal remedies, removal of dead and diseased fish, net cleaning and not sharing equipment. Some mentioned "biosecurity measures" without further details.

A number of questions were specifically related to ISKNV. Twenty farms reported having suspected ISKNV on the farm while 10 had not. Vaccination and heat shock treatment were the most commonly reported ISKNV-specific interventions, with additional mentions of salt treatment, reduced feeding, use of immunostimulants, herbal treatment, reduced stocking density, in-house fingerling production, net cleaning, removal of diseased fish and antibiotic treatment.

Fourteen farms reported having used ISKNV vaccines, all administered through intra-peritoneal injection. Half (7 farms) reported no improvement in tilapia health following vaccination while the other half (7 farms) did. The majority of farms (71%, n=5) with improved tilapia health also reported a significant increase in production. When asked about improvements to ISKNV interventions, the majority of replies indicated a need for improved vaccines, vaccination strategies and heat treatment regimens.

Discussion

During the current study in 2021, infection with ISKNV was highly prevalent amongst sampled farms, with an apparent overall farm-level prevalence of 80% (detected in 24 out of 30 sampled farms). ISKNV was detected in all farm types and ISKNV-positive farms were found throughout most of the study area. In the Volta region, 4 out of 7 sampled farms were positive while in the Eastern region, 20 out of 23 sampled farms were positive. Most test-negative farms were distant from neighbouring farms, which may indicate that these farms are exposed to a lower infection pressure or may easier implement effective biosecurity measures, preventing the introduction of the virus. It should be borne in mind that the results from this study only indicate the status of the fish population in a studied farm at the time of sampling. It will therefore not say anything about the infection status of that population at a later point in time. In addition, a sample size of 10 samples/farm demands the infection to be highly contagious and present at farm or population level in order to be detected (high farm prevalence).

The first reported cases of ISKNV in Ghanaian aquaculture occurred in November 2018 (Ramirez *et al.*, 2021). The emergence of this viral infection in the Lake sheds some light on a possible year of introduction and the importance of enforcing regulations that ban the import of foreign fish species and concomitant ramifications of biosecurity breaches.

The wide geographical distribution of ISKNV within the study area suggests significant horizontal transmission between farms. Common horizontal transmission routes in aquaculture include live fish movements, fomite contact between farms and local spread through water contact. While a number of farms mention having biosecurity measures in place, a previous visit to Lake Volta found effective biosecurity measures to be largely absent (Jansen *et al.*, 2018), which may facilitate inadvertent horizontal transmission between farms. ISKNV was detected in eggs and samples from broodstock in the current study. While true vertical transmission of ISKNV has not been confirmed, its detection in broodstock and eggs highlight the importance of biosecurity measures and testing at hatcheries and disinfection of eggs to prevent spread to and from such facilities. With a limited number of operational hatcheries in Ghana, many farmers depend on a few hatcheries to source their fingerlings thereby promoting large-scale live fish movements over large geographical areas.

The high farm-level prevalence of ISKNV, and the detection of ISKNV in both smaller and larger fish, is in line with what has previously been reported from Ghana (Ramírez-Paredes *et al.*, 2021). While the high mortality levels reported in this study could not be attributed specifically to current disease events, ISKNV has previously caused high mortalities in Lake Volta (Ramírez-Paredes *et al.*, 2021). Some of the currently studied farms may also have been part of this previous study. Both the current study and the study by Ramírez-Paredes *et al* (2021) found co-infections of ISKNV and bacterial pathogens to be common, which may have exacerbated clinical disease and mortality levels.

ISKNV-positive fish presented with excess skin mucus, exophthalmia and distended abdomens (ascites). ISKNV-positive tilapia have previously been reported to display a range of clinical signs such as lethargy, abdominal distension, gill pallor, darkening, erratic swimming, opaque eyes, loss of scales, petechial haemorrhages and enlarged and haemorrhagic internal organs

(Machimbirike *et al.*, 2019; Ramírez-Paredes *et al.*, 2020). Amongst the 10 farms reporting not having had suspicion of ISKNV on farm, 9 were found ISKNV positive in the current study. Large scale, medium scale and small scale farms were all represented in this group. The lack of ISKNV suspicion may be due to the fact that none of the major pathogens detected in the current study have clear clinical signs that makes it possible to diagnose the disease based on clinical signs alone (so called pathognomonic signs). It may also indicate that farmers would benefit from regular updates on disease trends in tilapia farms in the Lake Volta area, together with relevant information about the key diseases.

Twelve of the 24 farms found ISKNV-positive in the current study reported having used a vaccine against ISKNV. It is not clear whether this referred to the sampled population or previous populations, whether the entire population had been vaccinated or when vaccination had taken place relative to the sampling point. It would be beneficial to gather further information on the vaccination status of the sampled fish populations to aid the development of ISKNV control strategies.

There is an urgent need for further knowledge about ISKNV in Ghanaian aquaculture. The on-going PhD study conducted by Angela Naa Amerley Ayiku at UG in collaboration with CEFAS will yield essential knowledge to enhance the control of ISKNV in Ghana.

TiLV was not detected in any sample, which is in line with previous publications (Jansen et al., 2018; Ramírez-Paredes et al., 2021). The diagnostic methods for ISKNV and TiLV developed and validated by UG pave the way for local surveillance for these viral pathogens.

The bacteriological investigation revealed the presence of key bacterial pathogens, with most pathogens having a wide geographical distribution. Some of the bacterial pathogens (notably streptococci, but also *Kocuria* sp and *P. shigelloides*) are potentially zoonotic, thus constituting an occupational and consumer health hazard. For the first time, bacteria from fish were extensively profiled using MALDI-TOF in Ghana. Major fish pathogens were missing from the MALDI-TOF database. This study therefore forms a solid basis to generate reliable biobanks of fish isolates that can be used to upgrade the existing database of the MALDI-TOF MS instruments in the country, enhancing future diagnostic capacity.

Streptococcal infections affect aquaculture production worldwide, can occur in most production systems and may result in high levels of mortality with a range of non-specific clinical signs (Amal & Zamri-Saad 2011; Suwannasang *et al*. 2014). *S. agalactiae* was the predominant bacterial pathogen detected in the current study. A high prevalence, and association with mortality events, has been reported from previous studies in Ghana (Verner-Jeffreys et al., 2018; VetEau, 2021). Two major serotypes of S. agalactiae (serotype 1a and 1b) have so far been confirmed to cause outbreaks in Ghana. Considering the high prevalence of *S. agalactiae* in tilapia farms across the lake and knowing that some farmers are already using a conjugate vaccine targeting three serotypes (type 1a, 1b and III) to treat or manage streptococcus infection, further genomic characterisation of these isolates is urgently needed. As a result, *S. agalactiae* study isolates were characterised through 16S sequencing. This approach enabled establishment of the genetic relationship between the *S. agalactiae* strains isolated in the present study and those previously reported. As for ISKNV, the details around the vaccination status of the sampled populations are somewhat unclear. Gathering further information on this is required to optimise control strategies.

P. shigelloides has been reported as a primary pathogen of tilapia (Liu *et al.*, 2015), and has purportedly been isolated several times previously from cultured tilapia in Lake Volta. Being isolated from brain and kidney samples in the current study, it likely contributed to clinical disease on its own or as part of identified co-infections.

E. tarda is known as a primary pathogen of fish, including tilapia, which may cause high mortality (Iregui *et al.*, 2012; Park *et al.*, 2012, El-Seedy *et al.*, 2015). In the current study, it was isolated from kidney samples of moribund fish, suggesting it to be an important contributor to clinical disease on its own or in combination with other pathogens.

This study confirms the presence of *Aeromonas* infections in cultured tilapia as previously reported by other investigators (VetEau, 2021). Infections by motile aeromonads such as *A. hydrophila, A. veronii* and *A. jandaei* may present as clinical signs of haemorrhage, exophthalmia and ascites (Li & Cai 2011, Dong *et al*. 2015) as was found for some fish in this study. However, a number of Aeromonas-positive fish presented without observable clinical signs. Aeromonads may act as important pathogens also in co-infections (Dong *et al*. 2015, Assis *et al*. 2017, Nicholson *et al*. 2017, Amal *et al*., 2018). However, there is currently a distinct lack of knowledge about the relative importance of different pathogens in bacterial and viral co-infections (Dong *et al*. 2015).

While Flavobacteria-like organisms were found in samples from this study, the MALDI-TOF analysis failed to confirm their identity. This is likely due to the inadequacy of the database and further sequencing is therefore required to identify them to the species level.

Histopathology can serve as a valuable additional diagnostic tool. For cases where infectious agents are identified by PCR, histopathology can be useful to differentiate between infections without clinical manifestations and infections causing on-going diseases of clinical importance. In this study, histopathology also detected a number of other diseases or infections not specifically targeted by PCR analysis or microbial culture, illustrated by the diversity of parasitic infections and bacterial epitheliocysts observed in this material. Branchial epitheliocysts were identified by microscopy in around half of the sampled farms. Although adults were even a bit more frequently affected, the numbers indicate high prevalence already at sensitive early life stages, sending a fair warning for outbreaks that could be especially damaging in hatcheries and subsequent juvenile stages.

Ninety-three percent of the studied farms reported having experienced significant disease problems in the past five years, with most reporting very high disease-associated mortality. A large proportion of studied farms was found positive for ISKNV and/or pathogenic bacteria. This shows that disease events are significantly affecting fish health and welfare in tilapia farms in Lake Volta. The large financial losses reported by farmers constitute a threat to individual businesses who may risk going out of business. The 30 farms included in this study alone report employing a total of 1192 full-time staff and 463 part-time staff, suggesting that large scale closures may additionally lead to significant impacts on local societies.

Recommendations

This study has revealed that ISKNV and pathogenic bacteria are prevalent amongst tilapia farms in Lake Volta.

In this report to participating farmers the listed, brief recommendations focus on aspects specifically related to farmer actions or areas of direct collaboration between farmers, FC and academic institutions. A more comprehensive discussion of recommendations, including aspects of national strategies, will be provided following the completion of a similar study in tilapia pond farms.

- All available laboratory information on detections of key pathogens in Lake Volta (e.g. ISKNV, major bacterial pathogens) should be collated as a joint effort between the FC, the industry and academic institutions to provide the best possible overview of key pathogen distributions and prevalences.

- Information on vaccinations should be included in the collected information to enhance the understanding of the effect of current vaccination strategies. If sufficient documentation of vaccine efficacy is available, vaccination should be encouraged, and possibly made mandatory, to reduce infection pressure and subsequent losses. Studies of pathogen prevalence before and after the introduction of any mandatory control measures should be conducted to monitor the effect of introduced mitigation measures.

- Fish, including fingerlings, known or suspected to be infected by ISKNV or other major pathogens should not be moved between farms. Movement restrictions are essential to prevent pathogen spread to currently unaffected areas. Any fish movements between endemic areas should be approved by the FC prior to taking place. Quarantine facilities and approved quarantine procedures may be a specific requirement for movement permits if fish have to be moved between management areas.

- Improved laboratory capacity for fish diagnostics opens for improved disease detection and fish health management. It further allows enhanced passive and active surveillance for key pathogens. This requires long-term collaboration between the competent authority, fish health personnel and the industry. Screening capacity for key pathogens can also pave the way for the introduction of health certificates (especially for fingerlings and broodstock) and enforcement of movement permits based on fish health status. The improved outbreak investigation capacity must be utilised by farmers immediately upon suspecting a disease event.

- Farmers must receive updated information on major diseases of tilapia and dynamic disease situation overviews for Lake Volta and Ghana. This requires frequent communication between the FC and the industry, including framer organisations, to allow the production of relevant information materials and efficient knowledge transfer.

- Farms should re-visit their biosecurity plans in light of received information to assess any areas for improvement to reduce the risk of pathogen entry to or exit from the farm. Regional farmer collaboration, as well as close industry – FC collaboration, will be essential to enhance the overall industry biosecurity. Specific biosecurity adaptations for Lake Volta farms, such as requirements for treatment tanks given the impracticality of treating fish in Lake Volta, should be considered.

- As stress enhances the susceptibility to pathogens, good management practices should be encouraged throughout the industry. Producer organisations must play a key role in the development of relevant guidelines. It should be remembered that most major diseases affecting tilapia may present with a range of clinical signs, requiring the use of diagnostic laboratory methods to determine the underlying cause of ill health.

- Develop procedures for mandatory disinfection of eggs in hatcheries.

Acknowledgements

This study would not have been possible without the cooperation of participating farmers. We are grateful for your essential contribution to this study.

We would like to thank all the Fisheries Commission staff who contributed to the study; namely Ewuabena Ntiamoah Bediako, Eric Boyce Sawyerr, Mimi Ama Wekem, Stanley Obresi, Janet Anchinirah¸ Patrick Appenteng, Kofi Nti Agyarko, Kwaku Appiah Duodu, Linda Eleblu, Daniel Nyanteh, Ebenezer Sarpong and David Asiama.

We are extremely grateful to the entire UG team for conducting the field trips in our absence and for analysing the samples with excellence. The core team at UG acknowledges the support of Hannah Segbefia and Dr Peter Quashie in the laboratory analyses.

The authors are grateful to the following at NVI for their support in diverse ways and excellent collaboration throughout the study: Duncan Colquhoun, Geir Bornø and Tone Mathisen Fagereng.

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