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Baseline Study Phase II: Fish Diseases Affecting Pond Cultures in Ghana

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Content

Summary

A survey of disease pathogens in pond cultures of catfish and tilapia was undertaken by a research group from the University of Ghana (UG) in collaboration with Fisheries Commission (FC) and with the support of the Norwegian Veterinary Institute (NVI) from 21 December 2022 to 13 April 2023. This activity was the second phase of the baseline study under the Fish for Development project that aims to empower the FC to be in a better position to manage and regulate the aquaculture industry in a sustainable manner. In all, 30 fish farms randomly selected in nine (9) regions (Western, Central, Ashanti, Bono-East, Bono, Ahafo, Northern, Upper East and North-East) across Ghana were included in this survey. Farm interviews were conducted to gather epidemiological information using a structured questionnaire. Biological fish samples were screened for bacterial and viral agents and specific organs were preserved for histopathological analysis. While bacterial and viral analyses were performed at the University of Ghana, formalin-fixed samples were sent to NVI, Norway for further processing and histological analyses. The virology testing focused on the presence of two key pathogens; Infectious spleen and kidney necrosis virus (ISKNV) and tilapia lake virus (TiLV) using the multiplex conventional and real time qPCR protocols developed by the University of Ghana with financial and material support from NVI. The laboratory analysis revealed a range of potential bacterial pathogens. *Edwardsiella* and *Aeromonas* were the major pathogens associated with catfish, the predominant species cultured in ponds. Neither *Streptococcus* spp. nor TiLV were detected in any of the samples analysed. Nevertheless, ISKNV-like organisms were detected in seven (7) farms, mainly in Ashanti and the three (3) farms in the Northern regions from catfish. This ISKNV-like virus seems to affect largely adult grow out fish. Most farmers however had no information or suspicion of ISKNV disease on their farms. It appears a new variant of ISKNV is circulating in ponds, which might be different from the ones detected in tilapia from caged cultures in Lake Volta. The outcome from this study provides a holistic view of fish diseases affecting pond cultures in Ghana, which adds to the existing knowledge to improve on the fish health situation in the Ghanaian aquaculture sector.

1 Introduction

Ghana is rapidly expanding in cultured fish production, driven mainly by Nile tilapia and African catfish (MoFAD, 2022 report). Unfortunately, disease occurrence poses a serious threat to this promising industry. Disease outbreaks causing high mortalities of cultured tilapia being reported over the years (Verner-Jeffreys et al., 2018; Ramírez-Paredes et al., 2020; Ayiku et al., 2024; Duodu et al., 2024). Until recently, investigations to determine the main disease problems have only been restricted to few farms on the Southern part of Lake Volta, thereby limiting the sampling scope for monitoring the presence and spread of existing and emerging pathogens. To address this surveillance gap, a baseline study was carried out in 2021 to map the disease occurrence in tilapia farms on Lake Volta. This was a collaborative effort between the Fisheries Commission of Ghana (FC) and Norwegian Veterinary Institute (NVI), Norway under the 'Fish for Development' (FfD) bilateral program. Through this study, several disease-causing pathogens of tilapia were identified (Norwegian Veterinary Institute, Report 36 – 2022). Although a substantial volume of cultured fish in Ghana comes from intensive caged systems along the Volta Lake, inland production from ponds/tanks, dams, reservoirs, and dugouts also makes some significant contributions to the total aquaculture output (MoFAD, 2022). Traditionally, pond aquaculture in Ghana is extensive and has limited external inputs, making it suitable for most people interested in small-scale operations. Some of these small-scale operations have transformed into semi and intensive systems over the past decade (Ragasa et al. 2022). Some practices including lack of biosecurity measures or non-adherence to these measures and the concomitant abuse or misuse of antibiotics are likely to heighten in pond aquaculture, which may promote the evolution of virulent pathogens and antimicrobial resistance at the farm level. To uncover the pathogens affecting pond cultures, the second phase of the Fish for Development baseline study was carried out in 2022/2023 across several farms and ecological zones in nine (9) regions of Ghana with heavy pond aquaculture activity. Although, bacterial, parasitic and fungal diseases affecting pond systems occur in Ghana, this is the first comprehensive study with a broader geographic coverage to show the extent of these infections in the country. The study had three sub-objectives; (1) administer questionnaire for epidemiological information, (2) identify and map out the distribution of bacterial and viral pathogens in major pond farming areas and (3) enhancing collaboration in laboratory diagnosis of fish diseases.

2 The study

2.1 Farm interviews and gathering of epidemiological information

Field visits were carried out between 21 December 2022 and 13 April 2023 in 30 randomly selected fish farms in 9 regions (Western, Central, Ashanti, Bono-East, Bono, Ahafo, Northern, Upper East and North-East) across Ghana (Table 1, figure 1). A structured questionnaire developed jointly by the University of Ghana CEFAS sponsored ISKNV project and the Norwegian Veterinary Institute was administered to collect epidemiological information from all farms visited. The questionnaire included questions relating to farm operations, production data, disease episodes, mortalities, biosecurity, and control measures against infectious agents. For all the farms visited, the farm managers or their contact persons consented to participate in the study after the reasons behind the survey were clearly outlined in the questionnaire (Supplementary data file) was explained. The farms were small-to-medium scale with 80% of farmers operating for not less than 5 years. The fish holding facilities were mainly concrete or earthen ponds. Few farms cultured in tarpaulin tanks, cages on dams and reservoirs. The general impression was that disease was not a major issue affecting production with more than 60% of the farms not having noticed any significant fish disease episode in the past five years. Contrary to earlier findings with cage farms in Lake Volta, farmers reported no suspected cases of ISKNV infection. Skin lesions and nodules, distended abdomen (ascites), exophthalmia (bulging eyes) and unusual swimming behaviour were some major disease symptoms reported (Supplementary data file). Although disease do not seem to have a major impact on production, mortality in some cases could go up to 50%. Majority of the farmers depended on the few existing commercial hatcheries for their fingerlings. About 30% of farms had established in-house fingerling productions from locally obtained brood stocks. Some farmers adopted the use of antibiotics (tetracycline, penicillin), salts, probiotics (bactinol aqua) and herbs (bitter leaf; *Vernonia amygdalina*) to control infections in their farms. Routine disease prevention practices mentioned included washing of concrete ponds and nets, removal of dead fish, disinfection, non-sharing of equipment across different ponds and regular change of water. Maintaining good water quality was critical for increased production. Different sources of water used for culturing included boreholes, spring, well, river, stream, dam and pipe-borne water. Monitoring pH and water temperatures or testing of water quality for basic chemical constituents and microbial content was rarely performed since only few farmers had the ability to do any testing.

Table 1: List of farms visited and their GPS Coordinates

SAMPLING DATE	FARM No.	FARM CODE	GPS COORDINATES	LOCATION/DISTRICT	REGION
Dec 21-22, 2022	1	GFC	N4°56'49.8" 1°46'08.8"W	WEST ANAJI	WESTERN
	2	DKF	N4°56' 26.1" 1° 46' 52.0"W	SHAMA	WESTERN
	3	EPF	N4°56'38.1" 1°46'16.0"W	SEKONDI-TAKORADI METROPOLIS	WESTERN
	4	ABCF	N5°05'56.2" 1°28'57.0"W	KOMANDA-EDINA-EGUAFO-ABIRIM	CENTRAL
	5	CF	N5°18' 57.5" 0° 53' 25.6"W	EKUMFI	CENTRAL
	6	RBF	N5°28'05.4" 0°38'10.5"W	GOMOA CENTRAL	CENTRAL
Dec 28-29, 2022	7	RF	N6°56' 23.7" 1° 39' 57.3"W	OFFINSO MUNICIPAL	ASHANTI
	8	ANF	N6°56' 23.6" 1° 39' 57.3"W	OFFINSO MUNICIPAL ASSEMBLY	ASHANTI
	9	SNF	N6°47' 38.4" 1° 43' 14.8"W	ATWEMA NWABIAGYA NORTH	ASHANTI
	10	VF	N6°47' 38.4" 1° 43' 14.8"W	ATWEMA NWABIAGYA NORTH	ASHANTI
	11	LRF	N6°44'43.4" 1°36'13.3"W	KWABRE	ASHANTI
	12	PF	N6°39' 14.9" 1° 40' 22.7"W	ATWIMA KWAWOMA	ASHANTI
Jan 16-18, 2023	13	AF	N7°35' 50.3" 1° 51' 10.5"W	TECHIMAN MUNICIPAL	BONO-EAST
	14	NG	N7°33' 24.5" 1° 53'05.9"W	TECHIMAN MUNICIPAL	BONO-EAST
	15	JF	N7°33' 24.6" 1° 53' 05.8"W	TECHIMAN MUNICIPAL	BONO-EAST
	16	JV	N7°20' 33.4" 2° 21' 34.6"W	SUNYANI WEST	BONO
	17	EF	N7°21' 34.9" 2° 16' 09.4"W	SUNYANI MUNICIPAL	BONO
	18	DH	N7°16' 28.2" 2° 53' 00.1"W	DORMAA MUNICIPAL	BONO
	19	NP	N7°10'53.1" 2°06'00.6"W	TANO NORTH	AHAFO
	20	SF	N7°11' 06.9" 2° 06'24.5"W	TANO	AHAFO
	21	BF	N7°10' 49.1" 2° 06' 5.8"W	TANO NORTH	AHAFO
April 11-13, 2023	22	AGF	N9°27'10.0" 0°51'36.2"W	SAGARIGU	NORTHERN
	23	PAF	N9°27'10.0" 0°51'36.2"W	SAGNERIGU	NORTHERN
	24	ABF	N9°24'49.0" 0°50'12.9"W	TAMALE CENTRAL	NORTHERN

	25	KF	N10°46'37.3" 0°51'23.1"W	BOLGA MUNICIPAL	UPPER EAST
	26	CPF	N10°46'37.3" 0°51'23.1"W	NAVRONGO MUNICIPAL	UPPER EAST
	27	PFF	N10°46'37.3" 0°51'23.1"W	KASSENA NANKA	UPPER EAST
	28	TF	N10°30'52.9" 0°21'58.0"W	EAST MAMPRUSI MUNICIPAL	NORTH-EAST
	29	ZF	N10°21'18.8" 0°43'28.0"W	WEST MAMPRUSI MUNICIPAL	NORTH-EAST
	30	SBF	N10°31' 52.0" 0°22' 47.6"W	NALERIGU	NORTH-EAST

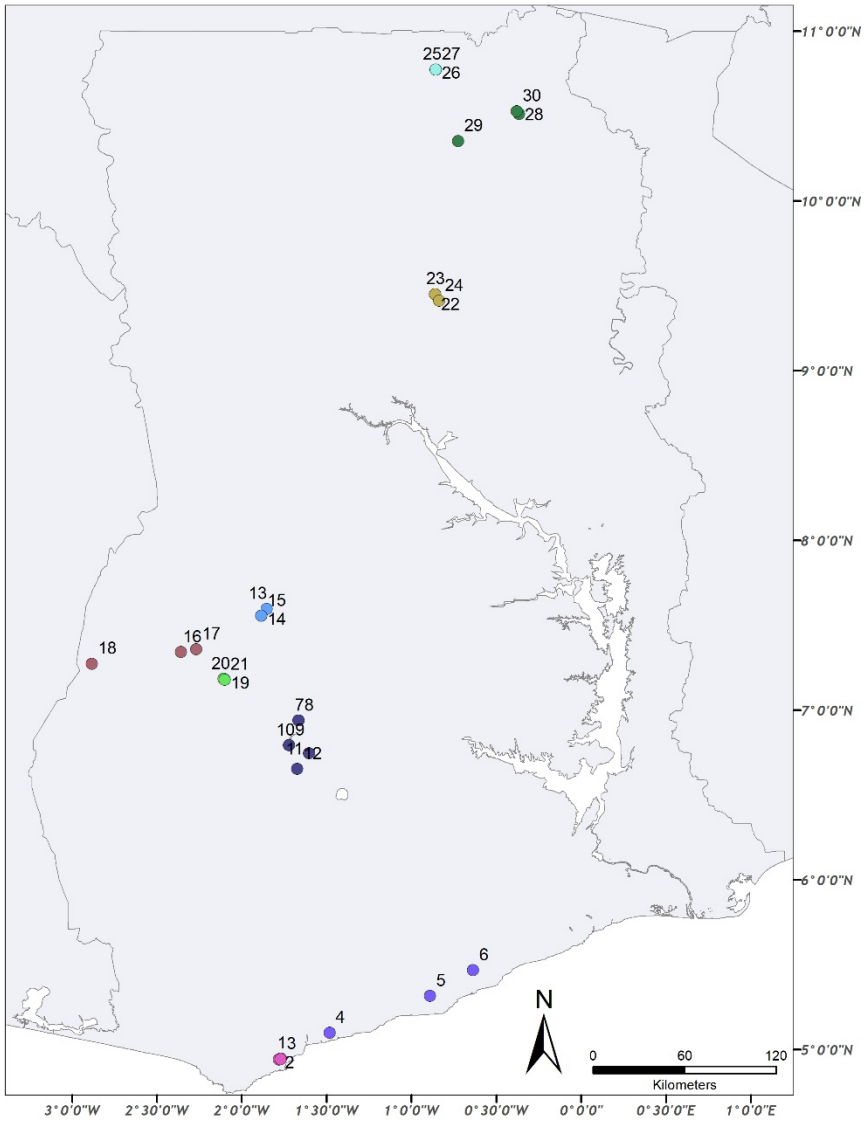

Figure 1: Map showing the distribution of pond farms included in the study. Farms are numbered 1-30, and the colours represents farms in different regions. The pink/magenta shaded circles (Western), violet (Central), navy blue (Ashanti), blue (Bono-East), maroon/reddish brown (Bono), green (Ahafo), olive/dark yellowish green (Northern), cyan/blue green (Upper East), green (North-East).

2.2 Identifying and mapping out the distribution of bacterial and viral pathogens

2.2.1 Biospecimen sample collection

The FC aided in the collection of samples from 30 random selected farms and facilitated easy access to these farms. Community entry and farmers' trust were crucial for the operation. FC regional directors and officers ensured availability of the necessary field equipment/gadgets needed for sampling. Except in Ashanti where, sampling took place on six (6) farms, the other regions had only three (3) farms. On the average, 5-10 biological samples including frys, fingerlings, moribund grow-out adult fish and broodstocks were collected at each farm depending on the type of facility (i.e., grow-out and/or hatchery) in operation. For farms with both tilapia and catfish, samples of 5 - 6 fish of each species were collected by convenience. All samples were labelled with a unique farm identification number and running fish identification numbers. Samples from the same fish carried the same identification number thus enabling assignment of laboratory results at fish-level. Fish of different sizes and culture age were considered. Samples from head kidney, brain and external lesions were cultured directly on appropriate growth media (blood agar, Trypone Soya Agar (TSA) and/or Tryptone Yeast Extract supplemented with tobramycin; TYES) for bacteriology. Fish tissues (kidney, brain and spleen) were preserved in RNA later for PCR detection of Infectious Spleen Kidney Necrosis Virus (ISKNV) and Tilapia Lake Virus (TiLV), using in-house multiplex conventional and real time qPCR protocols. Several tissues (kidney, liver, heart, brain, spleen, pancreas, gills, skin muscle, eye, intestines) and whole fish (<1 g) samples were fixed in 10% buffered formalin for histopathological analysis. Bacteriological and virological analyses were done at the University of Ghana (UG), whereas the formalin-fixed histological samples were sent to Norway, NVI for processing (slide digitalization) and further analyses.

2.2.2 Laboratory diagnostics

Bacteriology

Several bacterial pathogens causing relevant diseases in tilapia and catfish were detected across many sampling sites (Table 2). The significant isolates detected included *Edwardsiella tarda*, *Plesiomonas shigelloides* formerly known as *Aeromonas shigelloides*, *Aeromonas veronii*, *Aeromonas jandaei*, *Aeromonas hydrophila* and *Chrysobacterium* spp.

Edwardsiella tarda was the predominant pathogen identified (*n*=9) in seven (7) farms. The organism was isolated from the brain and kidney tissues of both juvenile and adult fish, predominantly in catfish than tilapia. Catfish infected with *E. tarda* presented non-specific clinical signs including enlarged kidney, orange liver, small spleen and eroded tails. Skin lesions, pale gills, frayed pectoral, and pelvic fin were the signs observed on the single tilapia sample affected. This gram-negative, cocci-bacilli and non-lactose fermenting bacteria showed varied morphology on TSA appearing mostly as whitish-creamy mucoid colonies.

P. shigelloides was the second most abundant bacterial pathogen detected (*n*=8 isolates) in seven (7) farms, mostly from catfish. Fish infected with *P. shigelloides* either showed no signs or showed non-specific clinical signs, which included loss of scales, pale gills, haemorrhaging of skin and internal organs (heart, kidney and intestines), friable liver, darkened spleen and ascites. This bacterium was also isolated from cultured tilapia in Lake Volta. The high isolation rate from the kidney of moribund fish suggested that it might play an important role in causing disease.

Aeromonas spp. (*n*=8 isolates) were also detected in five (5) farms mainly from catfish. *A. veronii*, *A. jandaei*, *A. hydrophila* were the major species isolated. This confirms the prevalence of *Aeromonas* infections in Ghanaian waters, most likely as opportunistic pathogens capable of causing a wide range of conditions including septicemia. Importantly, motile aeromonad septicemia caused by *A. hydrophila*, is an emerging global disease threatening the aquaculture industry, affecting both tilapia and catfish (Hemstreet, 2010; Pridgeon and Klesius, 2011; Xu et al., 2023)

Despite the predominance of skin lesions and other external abnormalities such as fins or gills and opercula very few flavobacteria-like organisms were isolated on TYES. It is possible the external lesions were due to cannibalism or predation, especially within the catfish culture systems. Although of less pathogenic importance, some *Chrysobacterium* spp. (*C. gambrini*) isolates (*n*=4) were identified from catfish brain in a single farm that showed the characteristic yellow pigmented colonies and filamentous gram-negative cell morphology.

It is also important to highlight the many emerging fish pathogens and human commensals that were isolated (Table 2). *Citrobacter*, *Acinetobacter*, *Cronobacter*, *Pseudomonas* have all been associated with cultured fish. Some of the organisms identified including *Brevibacterium casei*, *B. megaterium, Corynebacterium stationis and Comamonas aquatica* have their natural habitat in the aquatic environment but never associated with any known fish diseases. *C. stationi*s and *Lactococcus lactis* are probiotic dietary supplements that support digestive health. Their detection in the kidney and brain of the fish could be due to their probable use as probiotics in the feed. The coagulase negative Staphylococci isolates (*n*=13) identified may have been introduced through human contact since they are major part of the skin microbiota. It is worth mentioning that most of the ponds visited were within the residence of farmers and therefore most likely affected by normal household routines/chores. This is especially so when effective biosecurity measures were absent on the farms to control introduction and spread of disease-causing organisms from humans and other household domestic animals.

Table 2: Pathogen isolation and detection

Farm Code	No. fish samples taken	Fish species	No. purified bacterial isolates	Bacterial Pathogen Identification by MALDI-TOF	No. ISKNV positive samples/tissues	N0. TiLV positive samples/tissues
GFC	10	Catfish	7	**Fish 4** (***Plesiomonas shigelloides***, Kidney) Fish 8 (*Bacillus cereus*, Brain)	0	0
DKF	5	Catfish	13	Fish 4 (*Microbacterium testaceum*/Kidney) **Fish 4** (***Aeromonas veronii/ Aeromonas hydrophila***, Brain)	0	0
EPF	10	Catfish (5), Tilapia (5)	15	Fish 1 (*Citrobacter braakii*, Brain). Fish 2 (Citrobacter freundii, Kidney) Fish 3 (*Citrobacter freundii*, Kidney) Fish 8 (*Citrobacter braakii*, Kidney) Fish 9 (***Aeromonas veronii***, Kidney)	0	0

				Fish 10 (***Aeromonas veronii***, Brain)		
ABCF	5	Catfish	6	Fish 1 (*Staphylococcus sciuri*, Pseudomonas monteilii, brain) Fish 5 (*Staphylococcus epidermidis*; *Staphylococcus sciuri*, Brain) Fish 5 (Bacillus megaterium, kidney)	0	0
CF	10	Catfish	9	Fish 4 (*Staphylococcus haemolyticus*, kidney) Fish 4 (*Brevibacterium casei*, Brain) Fish 5 (*Citrobacter freundii*, *Citrobacter braakii*, Kidney)	0	0
RBF	10	Catfish (5), Tilapia (5)	6	**Fish 2** (***Plesiomonas shigelloides***, Kidney and Brain)	0	0
RF	10	Catfish	6	**Fish 7** (***Edwardsiella tarda***, kidney) Fish 10 (*Acinetobacter variabilis*, kidney)	6	0
ANF	6	Catfish	5	(No Organism Identification Possible)	0	0
SNF	5	Tilapia (2), Catfish (3)	3	Fish 3 (*Corynebacterium stationis*, kidney) Fish 5 (***Edwardsiella tarda***, kidney)	2	0
VF	2	Catfish	0	N/A	0	0
LRF	9	Catfish	18	Fish 1 (Acinetobacter schindleri, Brain) **Fish 4** (***Aeromonas* spp**. Kidney) Fish 5 (*Pseudomonas fragi*, kidney) Fish 7 (*Staphylococcus warneri*, kidney)	0	0
PF	5	Catfish	8	Fish 1 (*Staphylococcus epidermidis*, kidney), (*Comamonas aquatica*, brain) **Fish 3** (***Plesiomonas shigelloides***, kidney) Fish 4 (***Edwardsiella tarda***, kidney)	0	0
AF	10	catfish	11	Fish 1 (*Rhodococcus hoagii*, brain) Fish 5 (*Acinetobacter indicus,* Brain)	0	0

				Fish 6 (Staphylococcus cohnii, brain)		
NG	6	Tilapia	7	(No Organism Identification Possible, Brain)	0	0
JF	10	Tilapia (5), Catfish (5)	8	Fish 10 (*Citrobacter freundii*/*Citrobacter braaki*i, brain)	0	0
JV	10	Catfish	14	Fish 3 (*Corynebacterium stationis*, kidney) Fish 7 (*Staphylococcus arlettae*, kidney) **Fish 7** (***Edwardsiella tarda***, brain) Fish 8 (*Lactococcus lactis*, kidney)	0	0
EF	10	Tilapia (5), Catfish (5)	8	Fish 6 (*Weissella confusa*, brain) Fish 8 (Staphylococcus warneri, brain)	0	0
DH	10	Tilapia (5), Catfish (5)	2	N/A	0	0
NP	10	Tilapia (5), Catfish (5)	5	Fish 5 (*Staphylococcus kloosii*, kidney) Fish 7 *(Staphylococcus arlettae*, kidney)	0	0
SF	5	Catfish	8	Fish 2 (*Staphylococcus epidermis*, brain) Fish 4 (*Staphylococcus epidermidis*, kidney)	0	0
BF	5	tilapia	2	N/A	0	0
AGF	3	Catfish	13	**Fish 1** (***Chryseobacterium*** *spp*., brain) **Fish 2** (***Plesiomonas shigelloides***, brain 1) ***Chryseobacterium spp, Chryseobacterium gambrini/Edwardsiella tarda***, brain 2) **Fish 3** (***Edwardsiella tarda***, kidney)	0	0
PAF	5	Catfish	10	Fish 3 (*Cronobacter spp*, brain 1) Fish 3 (*Pseudomonas fragi*, brain 2) **Fish 5** (***Plesiomonas shigelloides***, kidney)	0	0
ABF	7	Catfish	25	**Fish 2** (***Edwardsiella tarda***, kidney) **Fish 4** (***Edwardsiella tarda***, brain) **Fish 5** (***Aeromonas jandaei***, kidney) Fish 6 (*Pseudomonas fragi* 2. Pseudomonas chlororaphis, kidney)	1	0

				Fish 7 (*Comamonas aquatica*, brain) (*Staphylococcus kloosii*, brain)		
KF	3	Catfish	8	Fish 1 (Staphylococcus arlettae, kidney) **Fish 2** (***Aeromonas jandaei***, kidney (***Aeromonas jandaei*/*Aeromonas veronii***, brain) **Fish 3** (***Aeromonas jandaei***, brain)	0	0
CPF	10	Catfish	4	N/A	9	0
PFF	11	Tilapia (6), Catfish (5)	9	**Fish 9** (***Plesiomonas shigelloides***, kidney) **Fish 10** (***Plesiomonas shigelloides***, kidney)	3	0
TF	3	Catfish	0	N/A	3	0
ZF	5	Tilapia	8	**Fish 2** (***Edwardsiella tarda***, brain)	0	0
SBF	9	Tilapia	8	**Fish 3** (***Plesiomonas shigelloides***, kidney)	6	0

N/A: Not available

Virology analysis

Out of the 30 farms screened, ISKNV-like virus was detected in seven (7) farms (Table 2), mainly in the Ashanti and the three Northern regions. Six **(**6) catfish and two (2) tilapia samples were positive for the virus in two farms in Ashanti region. A single farm with catfish (1 positive sample) in the Northern region was also positive for the virus. Two farms tested positive for ISKNV, both in the Upper East and North East regions. In total, twelve (12) ISKNV positive samples (catfish and tilapia) were detected in the Upper East region, whilst nine (9) samples were positive in the North East region. Generally, ISKNV was predominately detected in adult grow-out catfish (>100 g). Majority of fish that tested positive for the virus had no observable signs, whilst few showed typical ISKNV signs, including exophthalmia and distended abdomen (ascites). Other common clinical signs of disease observed includes dark liver, hepatitis, yellowish bile, friable and darkened spleen, congested heart, friable liver, splenomegaly, necrotic gills with brown reddish spots, widespread brown lesions on body surface, eroded caudal fins, gill and skin haemorrhages (figure 2).

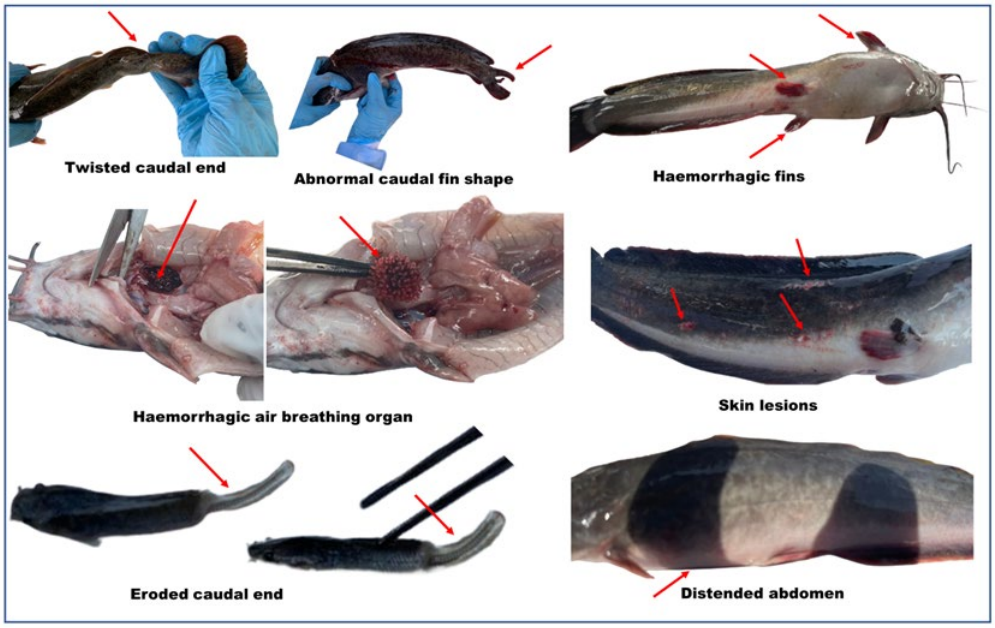

Figure 2: Common clinical signs and abnormalities observed in catfish

Notably, the detection of ISKNV-like virus by the multiplex PCR assay failed to amplify all four putative genes. Only the MCP gene with a fragment size of ~ 300 bp was detected (figure 3). To confirm the identity of the ISKNV-like isolates, amplicons from the positive samples were sent for Sanger sequencing in South Africa through Inqaba Biotech. The raw sequences received were edited using BioEdit 7.2 software and BLAST used to identify homologous sequences in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The top 10 hits from the BLAST analysis were all ISKNV isolates with 100% nucleotide sequence identity (figure 4). Compared to the other three putative genomic targets (TNFR, ATPase and VEGF), the MCP is a relatively conserved gene among iridoviruses (Fu et al., 2011). Previously, all four genes amplified in the virus were from tilapia in caged farms on Lake Volta (Ayiku et al., 2024).

The complete genome analysis of ISKNV from infected Nile tilapia and catfish were therefore carried out. Two positive samples (amplifying ~ 300 bp of the MCP gene) were whole genome sequenced using the Illumina platform (Illumina iSeq 100 system). The assembled genomes were then extracted and subjected to further genomic analysis. NCBI BLAST analysis confirmed the isolates to be indeed ISKNV with a 99.95% identity. The comparison of the MCP sequences showed no differences between the two isolates. The phylogenetic analysis showed that the assembled ISKNV genomes are different variants from the outbreak strain detected in cultured Nile tilapia on Lake Volta in 2019 (Figure 4).

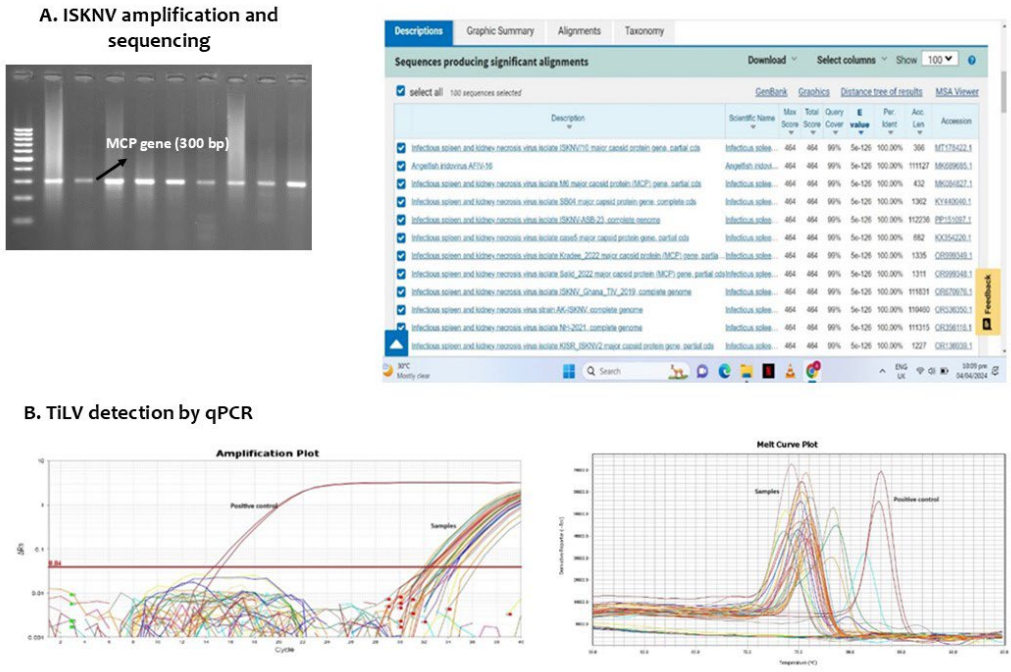

*Figure 3: Detection of viral pathogens. Multiplex PCR amplification and sequencing of the MCP gene of ISKNV (**A**), quantitative PCR amplification and melt curve analysis of TiLV (**B**).*

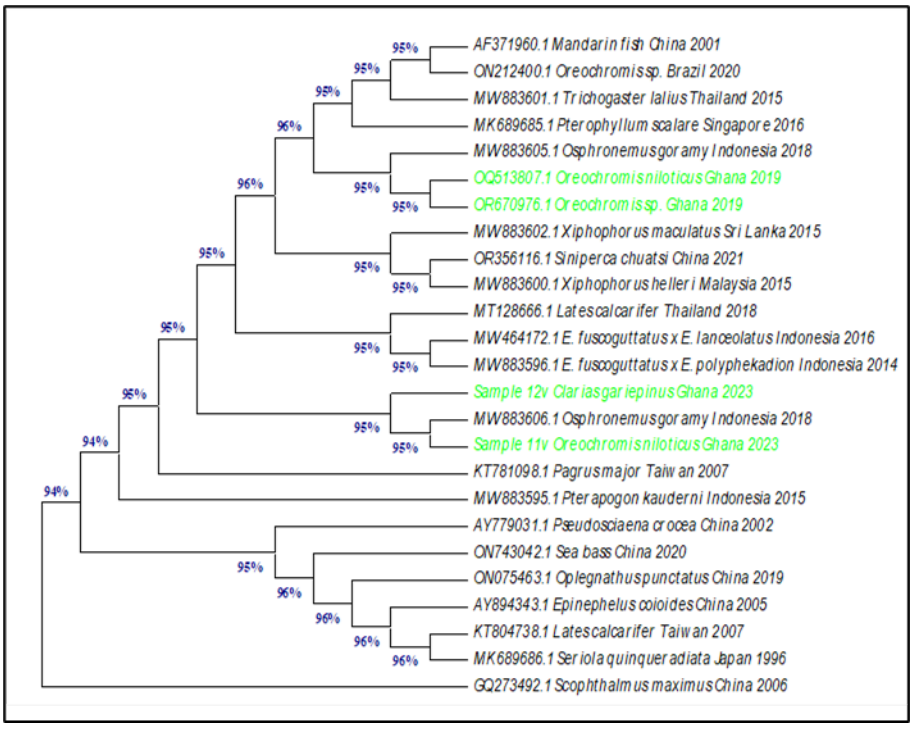

Figure 4: Neighbour-joining phylogenetic tree constructed for 25 ISKNV isolates. The Ghanaian isolates shown in green.

In the performance of epidemiologic or pathogenic investigations, it is important to know the source of infection. Besides farmers developing their own brood stocks and producing fingerlings in-house (supplementary data file), they also relied on private and public hatcheries for their supplies. Coincidentally, some of these ISKNV- positive farms outsourced fingerlings from the same supplier. For instance, SBF and CPF sourced fingerlings from supplier 1, whilst PFF and TF derived their fingerlings from Supplier 2. Since these farms are all located in the Northern part of Ghana and receive supplies from two (2) main hatcheries, it may be prudent to follow-up on these hatcheries to understand the transmission of the virus.

We also screened for the presence of TiLV in forty-three (43) tissue samples selected across the 30 farms. The tissues of choice were spleen and kidney. From the qPCR results, all samples were negative for TiLV. The positive control yielded a cycle threshold (C_t) value of 15, whiles C_t values ≥32 (cut-off value of detection) was observed for all test samples. As expected, a melting temperature (Tm) of ~83°C was recorded for the positive control (figure 3), whilst non-uniform peaks between 73°C and 81°C were observed for all the field samples, further confirming their negativity for TILV.

Histopathological analysis

The formalin fixed fish tissues were sent to the Norwegian Veterinary Institute for processing and histopathological analysis. The material consisted of 10% buffered formalin fixed samples of various internal organs, skin and skeletal muscle, eye, brain and gills from 139 catfish and 78 tilapia, as well as additional nine fish samples whose species identification proved difficult. The histopathological assessment was done mainly on digitalized haematoxylin-eosin (HE) slides, with special staining on relevant samples.

Viral disease

ISKNV was detected through virological analysis in 22 individuals, both tilapia and catfish. The histopathological findings varied in these fish, from none or mild tissue abnormalities to lesions indicating an infectious process but being too unspecific to determine the cause. There was one tilapia where the findings suggest possible clinical disease. Some individual samples with tissue changes associated with ISKNV, or other possible viral disease, but no viral agents were detected.

Parasitic infestations

Thirty-six (36) individuals had parasitic infestation/infections. These included individuals infected with coccidians (n=1), trichodina (n=4), myxozoa (n=19) and monogeneans (n=12).

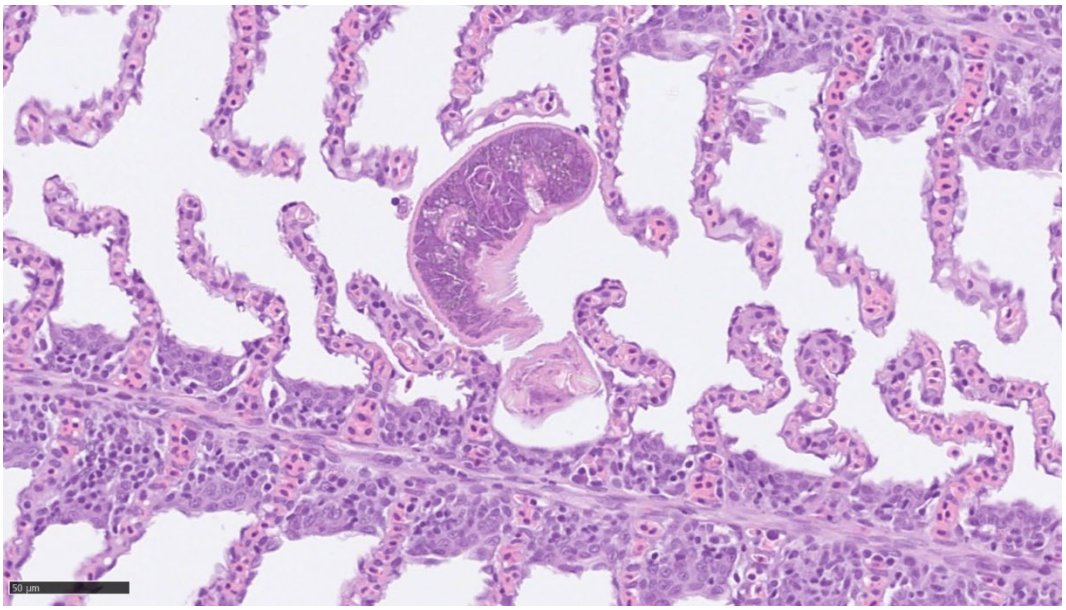

Figure 5: Histology section of gills from a catfish (farm CPF12.4.23) showing a single monogenean parasite in-between the gill lamellae. HE-stain, 40x magnification. Scalebar: 50 µm.

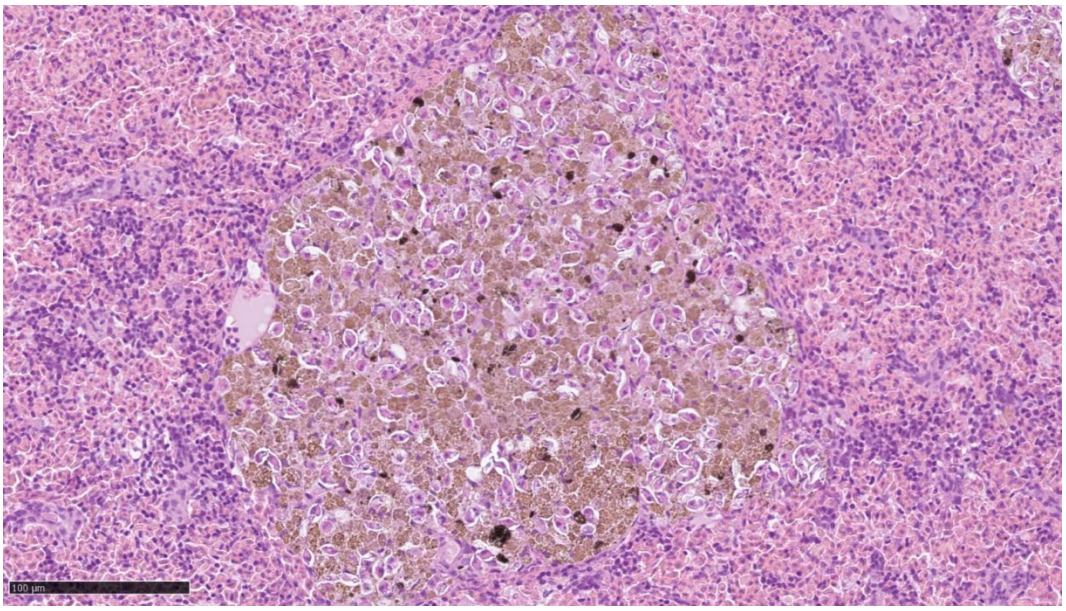

Figure 6: Histology section of spleen from a tilapia (farm RF28.12.22) showing a MMC with several myxozoan structures. HE-stain, 30x magnification. Scalebar: 100 µm.

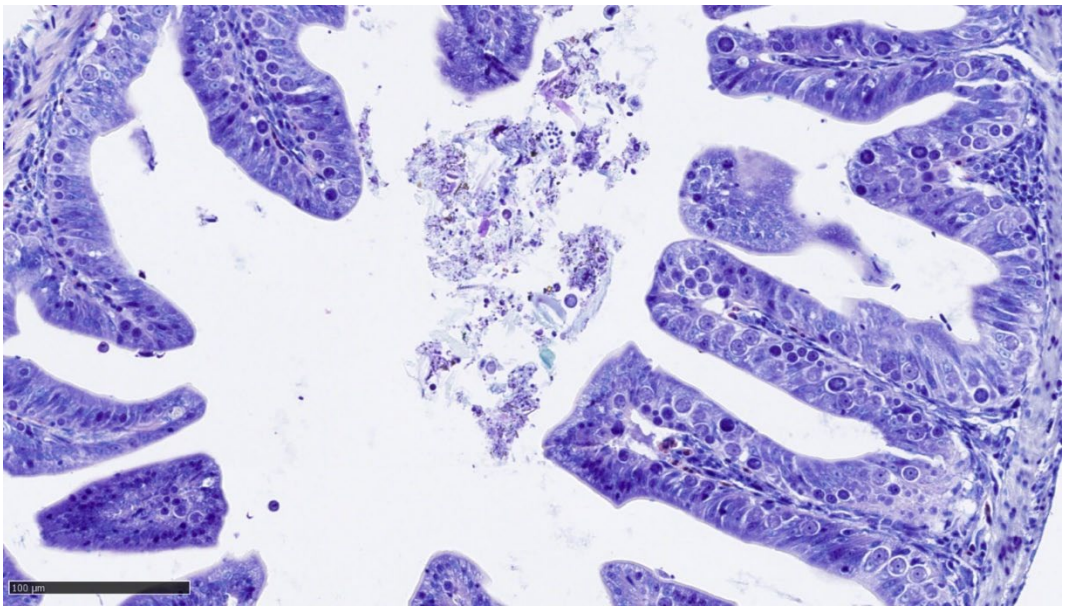

Figure 7: Histology section of intestines from a tilapia (farm DH17.1.23) showing coccidian structures in the intestinal epithelium. Giemsa stain, 30x magnification. Scalebar: 100 µm.

Bacterial infections

Catfish

Bacterial agents were detected during the bacteriological investigation in 53 catfish samples. Some of these are known fish pathogens, others regarded as emerging fish pathogens, some human commensals, and environmental bacteria not known to cause fish disease (see more information regarding the different bacteria in the section on laboratory diagnostics). The main relevant fish pathogens detected encompassed *Edwardsiella tarda*, *Plesiomonas shigelloides* and *Aeromonas* spp. Histopathological findings support possible clinical disease in some of the individuals where these agents were detected, while others showed only mild to no tissue abnormalities.

In the case of *E. tarda* positive samples, bacteria were detected in the kidney or brain tissues of nine fish in seven farms. There were no tissue abnormalities in four of these, and mild or unspecific/inconclusive tissue changes in an additional four. There was one individual with inflammation of the meninges, epicardium, liver, spleen and kidney, as well as necrosis in both liver and spleen and circulatory disturbance in the kidney. The combined bacteriological and histopathological findings suggest the presence of possible clinical disease.

Aeromonas spp. (*A. jandaei* in three cases, *A. veronii*/*A. hydrophila* in one) was detected in five fish from five farms. Histopathological findings indicated a possible infectious process in all individuals, of either suspected bacterial (2) or unspecific nature (3). In one individual, there was co-infection with ISKNV.

There were eight individuals with detected *Plesiomonas shigelloides,* originating from seven farms. In one of these, there was co-infection with ISKNV. Histopathological findings indicated a possible infectious process in seven individuals of either suspected bacterial (4) or unspecific nature (3).

Chrysobacterium sp. was detected in two individuals. There were no tissue abnormalities in one, while there were indications of an infectious process in the other.

Epitheliocystis (bacterial gill cysts) was observed in four catfish, originating from three farms. Histopathological investigations also suggested possible bacterial infection in some individuals/specimen based on observations in either gills and/or internal organs (including some individuals/specimen with inflammatory changes in the brain), but where there were no detected bacterial agents.

Finally, there was a notable difference between farms, both concerning identification of bacterial agents and also, with respects to the number of individuals with histopathological changes indicating possible infectious disease, with some farms appearing more affected than others.

Tilapia
Various bacteria were detected in six fish, originating from four farms. One individual showed signs of possible bacterial infection, the others had no significant lesions. There were no detections of *Edwardisella tarda* or *Plesiomonas shigelliodes*. *Aeromonas veronii* was detected in a few individuals where there were no samples for histology.

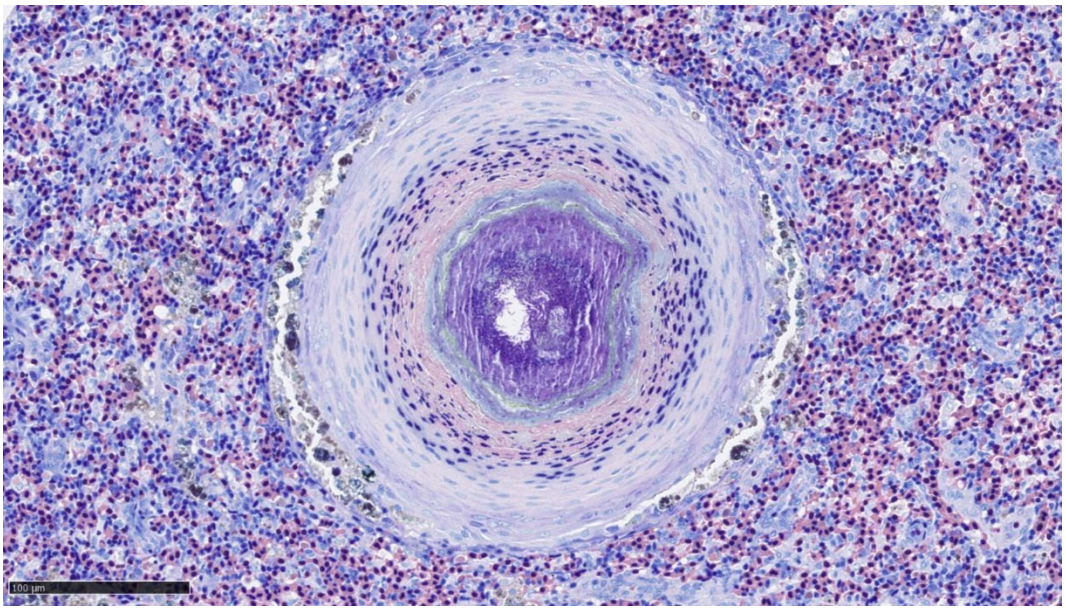

Figure 8: Histology section of spleen from a tilapia (farm DH17.1.23) showing a granuloma with several bacterial structures located in the centre. There were no bacteriology results from this individual. Giemsa stain, 30x magnification. Scalebar: 100 µm.

Epitheliocystis was observed in 16 tilapia originating from nine farms. Taking the number of sampled tilapia and catfish into account, the prevalence was notably higher in tilapia.

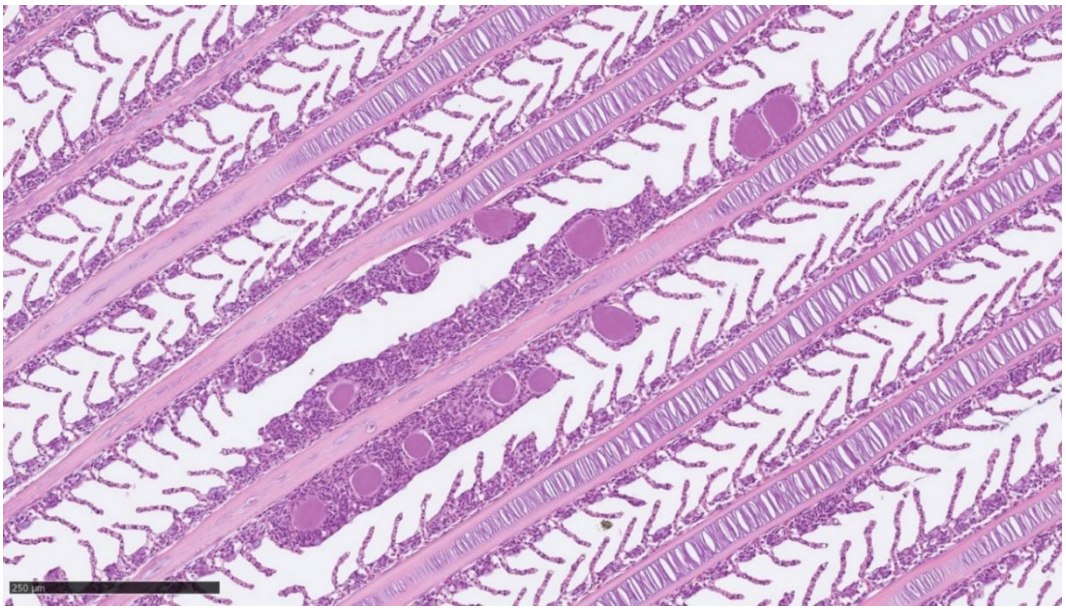

Figure 9: Histology section of gills from a tilapia (farm BF18.1.23) showing several epitheliocysts in the gill lamellae. HE-stain, 14x magnification. Scalebar: 250 µm

Fungal infections

There were no findings of fungal infections in the sampled catfish, but there was one tilapia with fungal infection in the heart (Figure 10).

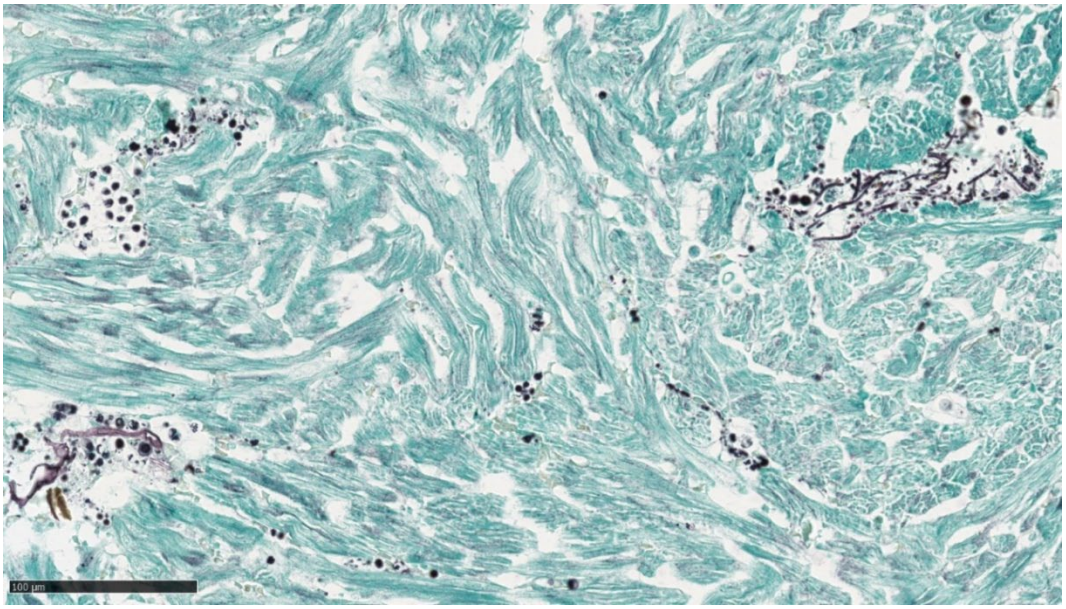

Figure 10: Histology section of heart from a tilapia (farm DH17.1.23) with several multifocal GMS+ fungal structures in the myocardium. GMS stain, 30x magnification. Scalebar: 100 µm.

Non-infectious conditions

Nephrocalcinosis

There was a modest number of individuals with nephrocalcinosis, a disease characterised with deposition of Calcium-compounds in the kidney, often related to water quality.

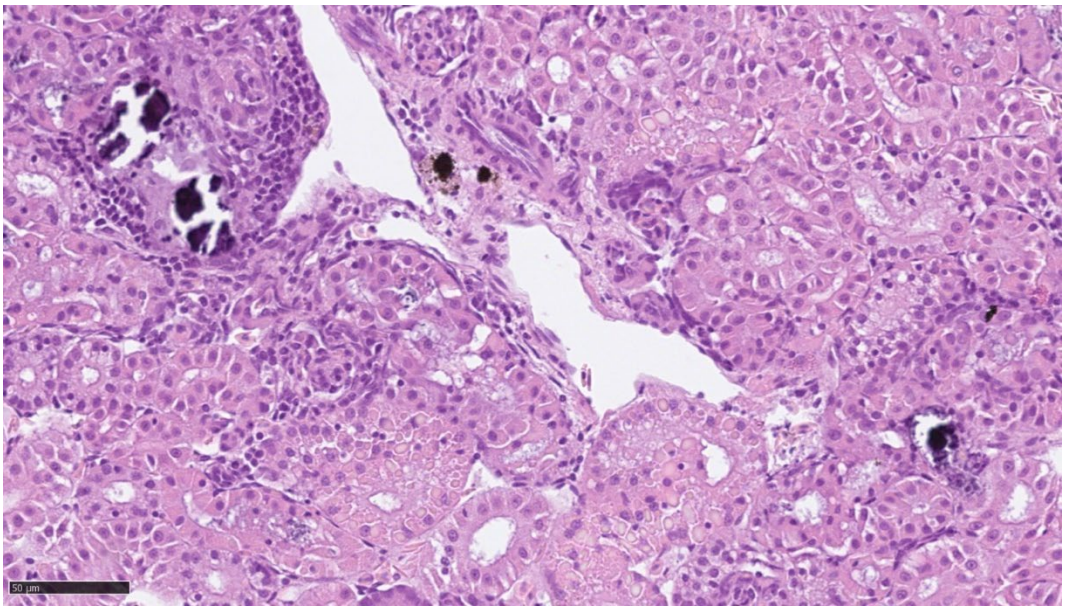

Figure 11: Histology section of kidney from a tilapia (farm AF16.1.23) showing nephrocalcinosis and tubular degeneration. HE-stain, 40x magnification. Scalebar: 50 µm.

General comments

A few individuals had ectopic thyroid tissue in the epicardium, in the bulboventricular area. In one individual, this also infiltrated into the myocardium, with accompanying inflammatory tissue response (Figure 12). On a general note, ectopic thyroid tissue may be present in the heart, as well as in the kidney, spleen, eyes and other tissues, and is a common finding in some species. Both thyroid neoplasia and hyperplastic thyroid lesions are known to occur. Follicles may extend into normal tissues in cases with extensive hyperplasia (Fournie et al., 2005; Ferguson et al., 2006).

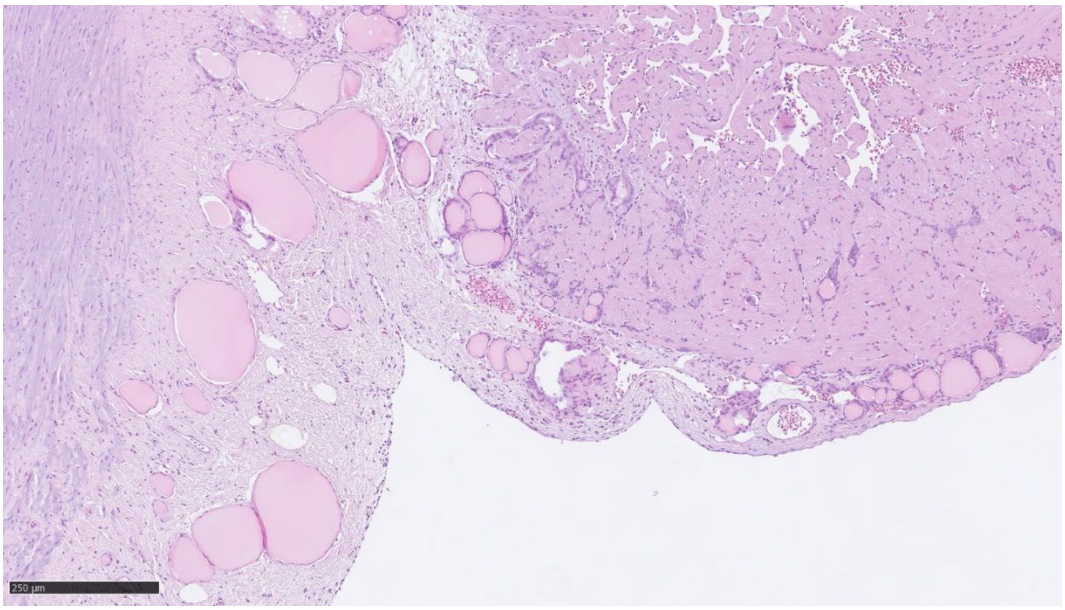

Figure 12: Histology section of heart from a catfish (farm EPF) showing multiple eosinophilic follicles (thyroid tissue) of varying sizes in the epicardium, which infiltration into the myocardium. HE-stain, 10x magnification. Scalebar: 250 µm.

Several individuals had granulomatous inflammation in one or multiple internal organs. There may be several explanations for this, including bacterial or fungal infection. In the case of a potential bacterial infection, *Mycobacterium* spp. is one possible culprit. Mycobacteria are facultative intracellular bacteria, and both these and other intracellular bacteria require special growth media for culture and bacteriological investigation. Special growth media are also recommended to investigate fungal infections.

2.3 Enhancing collaboration in fish disease laboratory diagnostics

The field experience gained by the FC veterinarians in baseline I was put to test in this survey. For each sampling trip, we had one veterinarian joining the team. These veterinarians took leading roles in performing necropsy and harvesting relevant organs/tissues for isolation and detection of bacterial and viral agents. They also assisted in tissue sampling and preservation for histopathological analysis. The UG team at the University of Ghana primarily carried out follow-up activity in the laboratory for identification of bacterial pathogens and processing samples to detect the viral agents (ISKNV and TiLV). The FC veterinarians have already been trained on basic procedures of pathogen identification under the baseline I study, which can easily be deployed in their newly established laboratories with little supervision. We intend to enhance the collaborative working relationship between the University and the FC, especially in developing SoPs and providing services for molecular diagnostics.

3 Challenges encountered

Very few challenges were encountered during field sampling. This is probably due to the experiences gained from the baseline I survey, and the better coordination established with the FC regional directors/officers. Sometimes, we had to travel long distances to the sampling sites in the various regions across the country. Consequently, additional systems were put in place to allow us to work on the samples, especially bacterial cultures while still in the field. We had some issues accessing the MALDI-TOF MS instrument at the University of Ghana. Delays in the MALDI results for the bacterial cultures affected the progress of this work. Moreover, the instrument was not able to identify correctly some of the isolated pathogens, which might be due to insufficient coverage of the required spectra for aquatic organisms in the database. Establishment of a reliable biobank of fish isolates would upgrade the existing database of the MALDI TOF MS instruments in the country.

4 Conclusion and recommendations

This was an important and relevant exercise with a broader geographic coverage of pond fish farms in Ghana. Data on the disease situation of catfish and tilapia in pond cultures pinpointed *Edwardsiella* and *Aeromonas* as major catfish pathogens. Contrary to earlier findings in Lake Volta, *Streptococcus* was not isolated from fish grown in ponds. A new variant of ISKNV seems to be associated with catfish. Since we could only amplify the MCP gene, further research may be necessary to confirm the genotype of this newly identified ISKNV-like pathogen. Due to constant exposure to human activities, pond culture environments require biosecurity measures throughout the entire production cycle.

Together, we must say that information generated from this study is a good representation of the likely pathogens affecting pond cultures in Ghana. The study has consolidated a strong working relationship between FC (the government agency), academia and researchers at the University of Ghana and fish farmers, fostering innovations to help tackle the problems associated with aquaculture in Ghana and beyond. A special forum to share the data generated from this study with farmers is necessary and strongly recommended.

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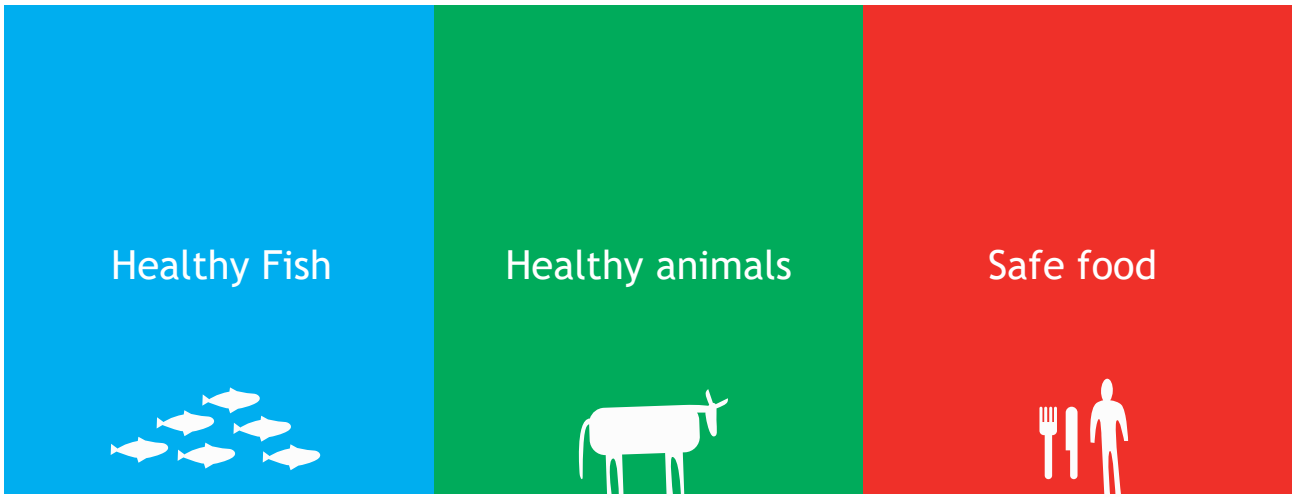

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