Text S1 Questionnaire and Consent form.

<u>QUESTIONNAIRE FOR "TILAPIA AQUACULTURE PRODUCTION AND DISEASE STATUS IN GHANA" SURVEY</u>

Tilapia aquaculture has been identified as a major player for securing future food systems in Ghana. However, infectious diseases have become a major drawback for attaining high level of sustainable production. To come up with an appropriate solution to the disease problem facing Ghana's Tilapia aquaculture, the University of Ghana in collaboration with the Fisheries Commission (FC) and the support of the Norwegian Veterinary Institute are developing a project that seek to provide rapid diagnostics solutions and sustainable disease control strategies. This activity is part of the Fish for Development' bilateral program that aims to empower the FC to be in a better position to manage and regulate the aquaculture industry in a sustainable manner. We would like to invite you to participate in this survey, which will help us assess the recent disease status of the industry. The identity of the interviewee and the farm will be kept confidential, but you may stay anonymous if you wish. This will take only 5-10 minutes of your time. Thank you in advance for your contribution.

Please take note, filling and submission of this form indicate consent to participate in the survey.

Signature:

………………………………………….

Date:

………………………………………….

2.0 Farm Details:

2.1 Name of Farm?

……………………………………………………………………………………………..

2.2 Respondent's post (i.e., owner, farm manager, general staff...)

……………………………………………………………………………………………..

Email:

………………………………………………………………………………………….

2.3 Location of farm (Region and District)?

…………………………………………………………………………………………..

2.4 Type of holding facility?

☐ Ponds

☐ Dam/Dugout/Reservoirs

☐ Floating Cages

☐ Tanks

☐ Other…

2.5 What is the source of brood stock/ fingerlings?

………………………………………………………………………………………

2.6 What is the source of water used for culture?

………………………………………………………………………………………..

2.7 How many Cages, Ponds, Tanks and/or Reservoirs does the farm have?

………………………………………………………………………………………..

2.8 What is the estimated number of fish per Cage, Pond and/or Reservoir?

…………………………………………………………………………………………..

2.9 What is the stocking rate per cycle (MT)?

…………………………………………………………………………………………..

2.10 List the average production per annum for the last 3 years (MT)?

…………………………………………………………………………………………..

2.11 How long has the farm been operating?

○ <1 year

○ 1-5 years

○ 6-10 years

○ >10 years

2.12 What is the facility category as per production permit provided by the Fisheries Commission?

○ Small scale

○ Medium scale

○ Large scale

3.0 History of disease and production losses

3.1 Have you had any significant/major fish disease on the farm in the past five years?

◯ Yes   ✕   Continue to next section

◯ No   ✕   Go to section 9 (Partnership/Collab...on on the project)

If Yes:

3.2 How many major fish disease episodes have been recorded in the last five years?

……………………………………………………………………………………………

3.3 How long did each individual episode last? (eg. Jun 2016- Feb 2021, May 2018-date)

…………………………………………………………………………………………….

3.4 What was the normal average daily mortality rate before any major fish disease episode?

…………………………………………………………………………………………..

3.5 What was the normal average daily mortality rate during the major fish disease episodes?

…………………………………………………………………………………………..

3.6 What were the major disease symptoms observed in the tilapia?

☐ Unusual swimming

☐ skin nodules

☐ frayed fins

☐ 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

Other: ………………………………………………………………………..

3.7 Were there any significant observable changes in the water environment in the cages?

○ Yes   ✕   Continue to next section

○ No   ✕   Continue to next section

3.8 State some of these changes if Yes

………………………………………………………………………………………………..

Environmental Parameters

DO: salinity:

pH: T°:

4.0 Control strategies/farmers opinion

4.1 Did the farm use any control measures during the major fish disease episodes? (such as vaccine, antivirals, chemicals, herbs etc.)

○ Yes   ✕   Continue to next section

○ No   ✕   Continue to next section

4.2 What specific intervention was adopted for each disease episode?

………………………………………………………………………………………………..

4.3 Were the control measures used, effective in control or eradication of the disease symptoms?

○ Yes

○ No

4.4 How can the specific intervention stated in 4.2 be improved?

…………………………………………………………………………………………..

4.5 State any routine disease prevention practices used on this farm
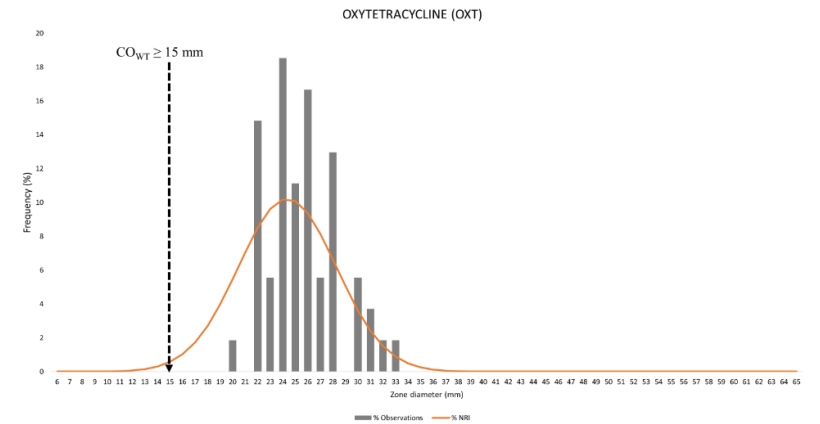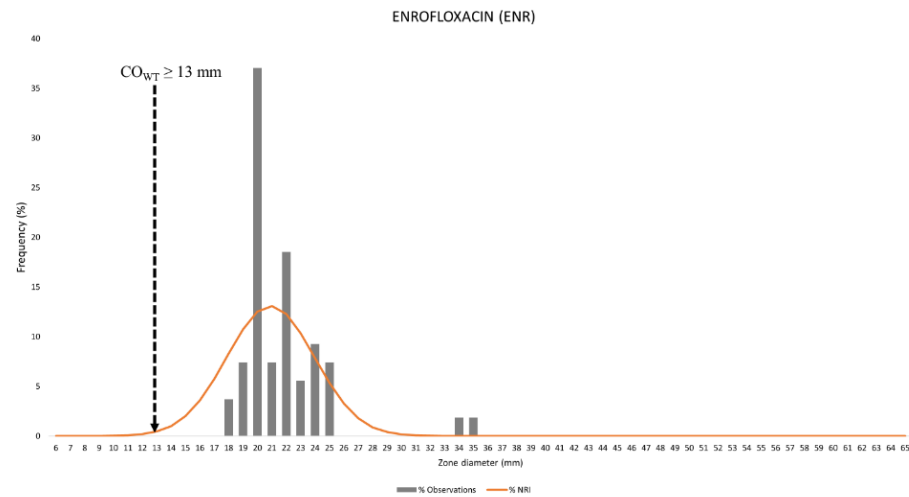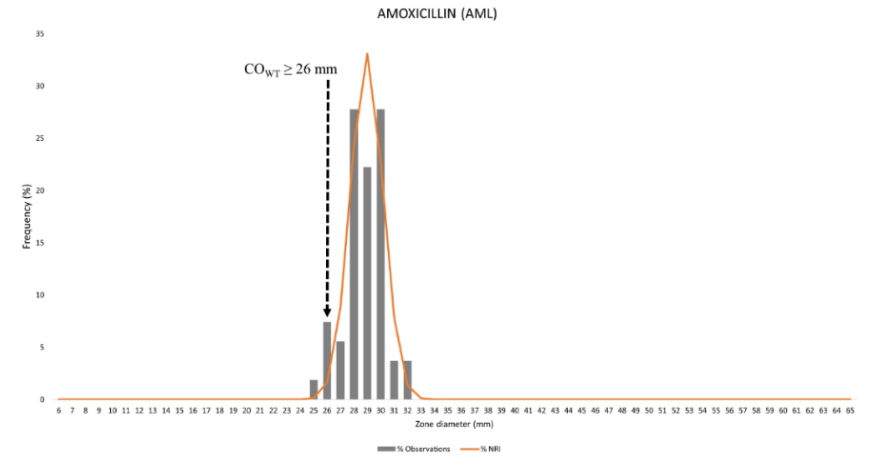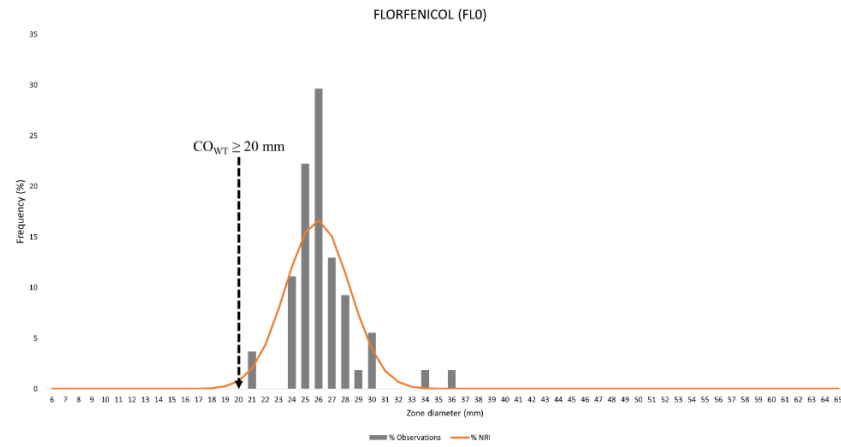
…………………………………………………………………………………………..

5.0 Partnership/Collaboration on the project

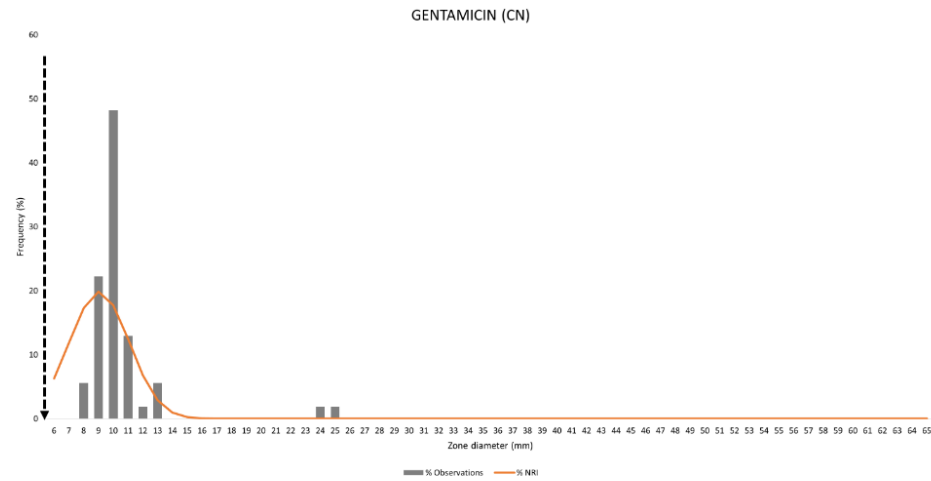Thank you for taking time to complete this questionnaire.

6.1 Are you interested in becoming a partner on this project?

◯ Yes

◯ No

Figure S1. Histograms displaying the distribution of zone sizes of different antimicrobials (florfenicol, amoxicillin, enrofloxacin, oxytetracycline, gentamicin) from disk diffusion assays using the NRI method.

Table S1. Oligonucleotides and PCR cyclic conditions for PCR amplification of genes used in this study.

Gene target	Primer	Nucleotide sequence 5´- 3´	Expected amplicon size (bp)	PCR cyclic conditions	Reference
16S rRNA	S-20 A-18	F: AGAGTTTGATCCTGGCTCAG R: GWATTACCGCGGCKGCTG	500	95°C 3 min, 30 cycles [95°C for 60 sec, 48°C for 60 sec, 72°C for 60 sec], and 72°C for 10 min	Suau et. al. (1999)
cpsL	GBS capsular gene	F: CAATCCTAAGTATTTTCGGTTCATT R: TAGGAACATGTTCATAACATAGC	688 (serotype Ia, Ib)	94°C 2 min, 30 cycles [94°C for 30 sec, 50°C for 60 sec, 72°C for 60 sec], and 72°C for 10 min	Imperi et al. (2010)
cpsG	GBS capsular gene	F: ACATGAACAGCAGTTCAACCGT R: ATGCTCTCCAAACTGTTCTTGT cpsG 2-3-6 R: TCCATCTACATCTTCAATCCAAGC	272/352 (Serotype Ia/III)	94°C 2 min, 30 cycles [94°C for 30 sec, 50°C for 60 sec, 72°C for 60 sec], and 72°C for 10 min	
cpsJ-Ib	GBS capsular gene	F: GCAATTCTTAACAGAATATTCAGTTG R: GCGTTTCTTTATCACATACTCTTG	621 (serotype Ib)	94°C 2 min, 30 cycles [94°C for 30 sec, 50°C for 60 sec, 72°C for 60 sec], and 72°C for 10 min	

cspA	Serine Protease	F: GGTCGCGATAGAGTTTCTTCCGC	104	95°C 5 min, 35 cycles [95°C for 60 sec, 55°C for 60 sec, 72°C for 60 sec], and 72°C for 10 min	Kayansamruaj et al. (2014)
		R: AACGCCTGGGGCTGATTTGGC			
fbsA	Fibrinogen-binding protein A	F: AACCGCAGCGACTTGTTA	278	95°C 5 min, 35 cycles [95°C for 60 sec, 55°C for 60 sec, 72°C for 60 sec], and 72°C for 10 min	Kannika et al. (2017), Legario et al. (2020)
		R: AAACAAGAGCCAAGTAGGTC			
cfb	CAMP factor	F: GGATTCAACTGAACTCCAAC	600	95°C 5 min, 35 cycles [95°C for 60 sec, 55°C for 60 sec, 72°C for 60 sec], and 72°C for 10 min	Kannika et al. (2017), Legario et al. (2020)
		R: GACAACTCCACAAGTGGTAA			

Table S2. Descriptive characteristics of fish sampled from *S. agalactiae* positive farms and co-infections with other pathogens. NOS 'no observable symptoms'

Farm	Fish no.	Weight	Clinical signs	Detection of *S. agalactiae*/tissue	Co-infection with other bacterial pathogens
1	Fish 5	300 g	Frayed fin, excess mucus, haemorrhages	Positive (kidney)	*Aeromonas veronii,* (kidney)
	Fish 6	350g	Opaque eyes, exophthalmia, eroded fin, swollen gall bladder, haemorrhaging (abdomen and caudal)	Positive (Kidney)	NiL
	Fish 7	150 g	Loss of scales, (whitish discolouration of affected area), haemorrhaging	Positive (kidney)	NiL
	Fish 8	300 g	Pale colouration, nodules on operculum and caudal fin	Positive (kidney)	*Chrysobacterium gambrini* (pectoral fin)
	Fish 9	200g	Loss of scales, eroded caudal fin, exophthalmia, opaque eyes, skin lesions	Positive (brain, kidney)	NiL
	Fish 10	200g	Nodules on mouth and head, caudal fin erosion, haemorrhaging near caudal fin	Positive (kidney)	Flavobacteria-like organism (skin)
2	Fish 3	285g	NOS	Positive (kidney)	NiL
3	Fish 1	51g	NOS	Positive (brain, kidney)	NiL
	Fish 3	28g	NOS	Positive (brain)	NiL
	Fish 6	1064g	Haemorrhage, excess mucus, excess fat deposition in abdomen, fish lice	Positive (brain)	NiL
	Fish 7	832g	Opaque eyes, excess mucus, eroded fin, ascites, friable liver, necrosis of viscera	Positive (brain)	NiL
	Fish 8	444g	ascites, friable liver, excess fat deposition in abdomen	Positive (kidney)	NiL

	Fish 9	552g	Excess mucus, discolouration, eroded fin, pale liver	Positive (brain, caudal fin)	NiL
	Fish 10	800g	Exophthalmia, darkened skin, excess mucus, haemorrhage on operculum, lesion on ventral skin, fish lice, eroded gills	Positive (kidney, brain, skin, pectoral, operculum)	*Pseudarthrobacter polychromogenes* (operculum)
4	Fish 1	375g	Skin lesions and nodule, opaque eyes, loss of scales, exophthalmia, discolouration of skin, eroded fin, congestion at the base of pectoral fin, congested gills, mottled friable liver	Positive (brain, kidney)	NiL
5	Fish 2	560g	Exophthalmia, eroded fin, haemorrhagic ova and intestine, darkened spleen	Positive (brain)	NiL
	Fish 4	345g	Nodule on mouth, opaque eyes, discolouration, pale gills, enlarged gall bladder	Positive (brain)	NiL
6	Fish 9	10g	NOS	Positive (brain, kidney)	
	Fish 10	7g	NOS	Positive (brain, kidney)	
7	Fish 2	340g	Skin lesions, discolouration, ascites	Positive (brain)	Flavobacteria-like organism (skin)
	Fish 3	82g	Discolouration of skin	Positive (kidney)	*Edwardsiella tarda* (kidney)
	Fish 4	274g	Haemorrhage of Heart and congested kidney	Positive (brain, kidney)	NiL
	Fish 5	112g	Opaque eyes, darkened skin, discoloration patches, excess mucus, frayed/eroded fin; Dark liver, heart haemorrhage, engorged spleen	Positive (kidney)	NiL

Table S3. Blast search results for the 500 bp 16S rRNA product sequences identified *S. agalactiae* as the most likely species, based on information obtained from GenBank. See Supplement 2 at www.int-res.com/articles/suppl/d158p027_supp2.xlsx

Table S4. Antibiotic zone of inhibition measurement of *S. pneumoniae* ATCC 49619 at 28°C (48 hours) on Mueller-Hinton agar supplemented with 5% Sheep blood.

Antibiotic	Disk contents	Zone diameter (mm)*
Enrofloxacin (ENR)	5 µg	25.0 ± 0.0
Trimethoprim- sulfamethoxazole (SXT)	25 µg	18.6 ± 0.89
Oxytetracycline (OXT)	30 µg	37.0 ± 0.71
Gentamicin (CN)	30 µg	11.7 ± 0.58
Ampicillin (AMP)	10 µg	33.3 ± 0.58
Erythromycin (ERY)	15 µg	29.0 ± 0.0
Florfenicol (FLO)	30 µg	30.0 ± 0.0
Amoxicillin (AML)	10 µg	34.0 ± 0.82

*Tests were performed in 3 biological replicates.

LITERATURE CITED

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