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Investigation of tilapia mortality in Ghana

Veterinærinstituttet
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Introduction

The World Bank is supporting the Government of Ghana to develop its fisheries and aquaculture sector.

Within the aquaculture sector, reports on mortality outbreaks from cage-farmed tilapia in the country are believed to be of infectious nature. Based on the current situation with global spread of Tilapia Lake Virus, which also includes the African continent, there is a need to investigate more closely the cause of this mortality. There is also a need for identification of management options that would be available to the industry and the government.

On this background, the World Bank asked the Norwegian Veterinary Institute (NVI) to do a field mission to try answer a set of questions defined in Terms of Reference (TOR) developed by the WB and approved by Ghanaian Authorities. The mission took place between May 25 and June 3, 2018. Representatives from NVI were Drs Edgar Brun, Mona Dverdal Jansen and Kofitsyo Cudjoe.

The Fishery Commission in Ghana represented by Janet Gyogluu Tuomozie and Peter Ziddah, gratefully assisted the Norwegian team by organizing the visits, giving local transport, and otherwise supported the team with all needs during the visit.

This World Bank mission paralleled both in time and in scope, a planned mission by the "Fish for Development" program funded by NORAD (The Norwegian Agency for Development Cooperation). The World Bank and NORAD therefore co-funded this mission, and the report is for mutual use.

Summary

A field trip to Lake Volta for collecting data for evaluating causes of high mortality in the Ghanaian aquaculture industry was performed in the period 28 - 31 Mai 2018. The mission was defined by Term of References given by the World Bank. During the mission seven farms in the southern Lake Volta region were visited; five grow-out farms and two hatcheries with ownership representing African, European and Chinese interests. Biological samples were taken from all the farms and analyzed in Norway by the Norwegian Veterinary Institute (histopathology and bacteriology) and the University of Life Science (virology). The virology testing focused only on potential presence of Tilapia Lake Virus.

Tilapia Lake Virus was not detected in any of the samples analyzed.

Mortality on farms may range on average from 10-20% and peak at more than 40%. This appears to be a "normal" situation that does not seem to have severe social and economic consequences on the local society. The farms also seem to sustain these losses. None of the farms visited expressed thoughts of ceasing production due to disease problems. Dead fish were collected frequently, but such high mortality is not environmentally sustainable over time and is a continuous source of maintaining a high infectious pressure on site.

Various bacteria were detected in our samples with dominance of *Streptococcus agalactiae*. This is a bacteria commonly causing infection in Tilapia and has also been identified earlier as a cause of Tilapia mortality in Lake Volta. Most farms practice vaccination against this bacterium using autogenous vaccines. The field efficacy of these vaccines is not proven. Environmental stress and low vaccine coverage (/efficacy) are factors that may be associated with this bacteria; causing high mortality in spite of vaccination. Gills were particularly examined for infections agents or environmental stress. No indications were identified to conclude that gill damages are any important cause of mortality.

Hatcheries being at the top of the production pyramid have a high potential to spread infectious diseases to all its customers. Negative TiLV findings are therefore a huge benefit for the industry as long as grow out producers are supplied from these hatcheries and they themselves manage to maintain a highly bio-secure production. Devastating spread of new infectious agents within the Volta Lake if introduced must be regarded as highly likely. It is therefore of upmost importance to prevent serious agents from being introduced into the lake. Implementation of biosecurity measures at national (like the newly introduced ban on import of ornamental fish and tilapia) and at local level, is important and need to be enforced.

The aquaculture industry lacks access to basic epidemiological competence, fish health services, properly equipped and functioning laboratory and diagnostic facilities. The Competent Authority cannot perform its national responsibility and the industry cannot sustain without access to these services. Building competence and capacity within these fields are a prerequisite for developing the industry according to governmental ambitions. (Optimal feed and genetics of brood stock are equally important but has not been accessed in this report).

We recommend;

- The development and enforcement of a national biosecurity plan
- The establishment of a field fish health service for regular monitoring of all farms
- The establishment of diagnostic and laboratory capacity to undertake primary and more advanced analyses (incl PCR-analyses) – initially under a parent institutional guided training program.
- The establishment of good baseline knowledge of the disease situation (also "production" related diseases) and the implementation of risk based surveillance programs.
- The development and implementation of an overall vaccination strategy for Lake Volta

Background

Freshwater aquaculture production in Ghana is privately driven and consists mainly of cage-culture (Lake Volta) and earthen pond-culture (Ashanti, Brong-Ahafo, Central and Eastern Regions). According to figures from the FAO, Ghana produced 43 000 tons of tilapia in 2015 (up from 37 000 tons in 2014) (FAO, 2017a), with some additional culture of catfish. The Fisheries Commission, the implementing agency of the Ministry of Fisheries and Aquaculture Development (MoFAD), consists of five divisions and four units, one of which is the Fish Health Unit (FHU). The Ghana National Aquaculture Development Plan (GNADP) has been developed to enable Ghana to bridge the gap between national fish demand and supply in the medium term. A zonation plan for the Volta Lake has been developed to guide investments in cage culture. An Aquatic Animal Health Policy was recently approved by the Government. As a result of the recent emergence of Tilapia Lake Virus (TiLV) the Ghanaian the Ministry of Fisheries and Aquaculture Development has implemented a ban on the importation of all ornamental fish species and tilapia (live, dead and reproductive material), with effect from 1.7.2018 (MoFAD, 2018).

Tilapia Lake Virus (TiLV) has recently emerged as a potentially important pathogen affecting tilapia farming worldwide. At the time of writing this report it has been shown to affect wild and farmed tilapia stocks on all of the three major tilapia-farming continents (Africa, Asia, South America) (Jansen *et al*., 2018). Affected fish present a range of non-specific clinical signs such as behavioural changes, exophthalmia or other ocular lesions, skin erosions, discolouration, skin haemorrhage, scale protrusion and/or abdominal swelling (Jansen *et al*., 2018). There is some variation in the reported mortality levels between outbreaks, however high levels of mortality (> 80%) has been reported from all affected continents (Jansen *et al*., 2018). While not yet listed, the OIE has produced a disease card (OIE, 2017) and several organizations such as the FAO (FAO, 2017b), NACA (NACA, 2017) and WorldFish (CGIAR, 2017) have produced alerts and warnings to increase the awareness regarding this potential threat. At time of writing, scientific literature has reported TiLV-presence in Sub-Saharan Africa, namely in Lake Victoria where both farmed and wild Nile Tilapia was reportedly affected in Uganda and wild Nile tilapia in Tanzania (Mugimba *et al*., 2018). Both wild and farmed fish were apparently healthy and for this reason no histopathological analyses were performed to detect evidence of clinical disease (Mugimba *et al*., 2018). Investigations of mortality events in Egypt found 50% of tested farms in 2015 (4 out of 8) and 43% of tested farms in 2016 (3 out of 7) to be positive for TiLV (Fathi *et al*., 2017; Nicholson *et al*., 2017). Sequence analyses from Egypt showed 93% nucleotide identity to Israeli reference strains (Fathi *et al*., 2017; Nicholson *et al*., 2017), while isolates from Tanzania and Uganda have been described as being "identical with" or "closely related to" the reference strains (Mugimba *et al*., 2018).

Amongst other important disease challenges in tilapia production are Streptococcal infections. These infections affect 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) which resemble those infected with TiLV. Apart from Streptococcosis, there are several common infectious diseases in farmed tilapia. Columnaris caused by *Flavobacterium columnare* often shows clinical signs of necrotic gills, fin rot, skin erosion or necrotic muscle (Figueiredo *et al*. 2005; Dong *et al*. 2015a). Francisellosis caused by *Francisella noatunensis* subsp. orientalis and Edwardsiellosis caused by *Edwardsiella ictaluri* produce clinical signs of visceral white spots in internal organs (Soto *et al*. 2009, 2012; Nguyen *et al*. 2016). Despite a wide-spread presence of Francicellosis in many aquaculture-regions around the world, there are no scientific reports on its detection in African aquaculture. Haemorrhagic septicaemia caused by motile aeromonads (*Aeromonas hydrophila*, *A. sobria*, *A. veronii* and *A. jandaei*) may present clinical signs of haemorrhage, exophthalmia and ascites (Li & Cai 2011; Dong *et al*. 2015b, 2017) and mixed clinical signs of complicated multiple infections (Dong *et al*. 2015b; Assis *et al*. 2017). Different biotypes of *Vibrio cholerae* has been isolated from diseased tilapia in Thailand together with

other bacterial species but their pathogenicity has to date not been further investigated (personal communication H. T. Dong).

In the case of TiLV, reported coinfections in TiLV-positive fish from Thailand included bacteria (Flavobacterium, Aeromonas and Streptococcus), external monogenean parasites (*Gyrodactylus* and *Dactylogyrus*) and ciliated protozoa (*Trichodina*) (Surachetpong *et al*. 2017). Several of the TiLV-positive fish from Egypt in 2015 were reported to have a coinfection of one or more Aeromonas spp. (*A. veronii, A. ichthiosmia, A. enteropelogenes and A. hydrophilia*) (Nicholson *et al*. 2017). A case of coinfection between *A. veronii* and TiLV in juvenile hybrid red tilapia (O. niloticus * O. mossambicus) has been reported in Malaysia, which resulted in a mortality rate of approximately 25% (Amal *et al*. 2018). Examination of 20 diseased fish revealed an infection rate of 20% and 50% for TiLV and *A. veronii*, respectively (Amal *et al*. 2018). In a case of TiLV infection in red tilapia juveniles, it was observed that while all clinically diseased fish tested positive for TiLV, 50% of the examined fish were also infected by an unknown microsporidian-like organism in their muscle (personal communication, H. T. Dong). The relative importance of TiLV and any coinfections in terms of clinical severity, mortality, incubation time and so on has not been determined (Jansen *et al*., 2018).

In addition to TiLV, several other viral infections have been described in tilapia, including betanodavirus and tilapia larvae encephalitis virus (TELV) which show neurological signs of erratic swimming or whirling syndrome (Shlapobersky *et al*. 2010; Keawcharoen *et al*. 2015). Betanodavirus infection resulting in clinical disease has been reported from farmed tilapia juveniles in Indonesia (Prihartini *et al*., 2015), Thailand (Keawcharoen *et al*. 2015) and Europe (Bigarré *et al*., 2009), however the prevalence of infection remains unknown. Additionally, infectious spleen and kidney necrosis virus (ISKNV) has been associated with gross signs of lethargy, gill pallor and distension of the coelomic cavity (Subramaniam *et al*. 2016), and recently detection of infectious pancreatic necrosis virus (IPNV) was described in farmed tilapia in Kenya, although no clinical abnormalities were reported in association with the infection (Mulei, *et al*., 2018).

The fourth component of the World Bank West Africa Regional Fisheries Program is aquaculture development. This component aims to set the framework for increased investment in inland aquaculture, and includes developing the aquaculture policy and legal framework, improving the genetic quality of tilapia fingerlings and brood stock, catalysing aquaculture development for medium- and large-scale enterprises and supporting small-scale aquaculture development.

Against this background, the World Bank West Africa Regional Fisheries Program requested support to investigate the current outbreaks of tilapia mortality in Ghana. The specific aims were to i) identify the cause(s) of mortality, ii) assess the environmental, social and economic consequences of the outbreaks, iii) assess risk for further spread of the disease within the country and neighbouring countries, and iv) propose management options for the containment of the outbreaks.

Field visits

One key task for the mission was to gather available data and information for descriptive analyses of tilapia mortality events. In addition, samples were to be collected in order to attempt to establish the cause of the observed mortalities. The majority of Ghana's tilapia production takes place in the southern part of Lake Volta and the Fisheries Commission has an office in Akosombo staffed by a Professional

Officer that has received fish health training in Norway through the Norway Ghana Tilapia Initiative (NORGHATI) led by the Norwegian Veterinary Institute. As a result, a convenience sample of farms and hatcheries under the inspectorate of the Fisheries Commission Akosombo office was selected. The visited units included hatcheries, as well as large- and medium-sized grow-out farms with African -, Asian - and European ownerships. The two visited hatcheries were assigned identifiers "D" and "G", while the visited grow-out farms were assigned identifiers "A", "B", "C", "E" and "F".

A planned two-day workshop with representatives from a wide range of stakeholders (fish farmers, hatchery owners, Fish Farmers Association, Aquaculture Advisory Board, feed millers, veterinary services, Water Research Institute, Water Resources Commission, Environmental Protection Agency, Volta River Authority, District Assemblies, Fisheries Commission and universities) was cancelled at the last minute by the Ministry of Fisheries and Aquaculture Development. As a result, it was not possible to ascertain the opinion of these stakeholders on disease occurrence, aquatic animal health issues and/or related intervention measures within the time-frame allowed for this mission.

Farm manager interviews

Farm manager interviews were conducted in order to gather data related to disease outbreaks and mortality events in farmed tilapia (hatcheries and grow-out farms). At the time of the mission there was no available, collated information on mortality levels and frequency of adverse events in tilapia farming operations in Ghana.

A total of seven farm managers (five for grow-out farms, two for hatcheries) were interviewed using a questionnaire jointly developed by the Norwegian Veterinary Institute and WorldFish to assess tilapia health and impacts of disease occurrence. The questionnaire included questions relating to operational procedures, stock details, production data, markets, production costs, biosecurity measures, mortalities, disease investigations and economic losses and impacts as a result of mortality events.

Sample collection and analyses

In an attempt to determine the cause(s) of mortality samples for diagnostic investigation was collected from all visited grow-out farms and hatcheries. Samples were collected on RNAlater (PCR analyses for TiLV-presence), formalin (histopathology for TiLV-related- and other pathology) and bacteriology (blood agar for general bacteriology, CHAB medium for *Francisella* sp., Ordal medium for *Flavobacterium* sp. and swabs for bacterial back up).

The number of samples collected per unit (grow-out farm or hatchery) was limited due to financial- and time constraints. In order to increase the probability of detecting causative agents risk-based sampling of moribund fish was performed where possible. For hatcheries all available life stages were sampled for PCR analyses. As one primary aim of the investigation was to assess the presence of TiLV at visited units, samples for PCR-analyses was prioritized in terms of resource allocation. All sampled fish had tissues collected for PCR-analyses, with some individuals also sampled for histopathology and bacteriology (risk-based where gross lesions were identified, otherwise from randomly selected individuals). The total number of individuals sampled for PCR analyses per unit were: "A" - 30 fish, "B"- 15 fish, "C" - 25 fish, "D" - 25 samples (3 eggs, 3 fries, 15 fingerlings, 4 broodfish), "E" - 15 fish, "F" - 5 fish and "G" - 15 samples (1 sample with pooled eggs, 2 samples with pooled fries, 4 fingerlings, 6 broodfish). The exact number of samples collected for histopathology and bacteriology varied between sites, depending on gross findings and available time.

Brief summary of farm manager interview results

Interviews with hatchery- and farm managers were conducted in order to gain the knowledge required for completion of the mission aims.

Hatcheries

The hatchery managers of both selected hatcheries consented to being interviewed.
In combination, the two hatcheries supplied mixed sex multipliers and monosex grow-out stock. Demand generally matched or exceeded available supply, and stock was supplied to both local- and nation-wide units. One hatchery additionally exported some fingerlings to other countries within the West-African region.

Stock were not accompanied by any form of health certification, however, this will be implemented in the future as a result of the new Aquatic Animal Health Policy. The introduction of this new policy was perceived as a positive requirement. One hatchery used a questionnaire to ascertain the absence of clinical abnormalities in the delivered stock, and both hatcheries reported close collaboration with the supplied units regarding feed-back on the performance of the delivered stocks. No fish were vaccinated prior to leaving the hatcheries.

There were no abnormal mortality levels at the time of the visits and no previous, large mortality events were reported. On average, both hatcheries reported around 70% survival from hatching to fingerling sales. Generally, the disease burden was reported to be low, however Saprolegnia-infection occurred as an intermittent problem. No, or very low levels of, antibiotics were reported used (no detailed information available), while some salt treatments were administered against parasites.

The potential introduction of an aquatic animal health service was seen as an important contribution to improving the health management and production performance of the hatcheries. Regular visits, combined with facilities for rapid disease investigations, were requested. Both hatchery managers reported a willingness to pay for supplied health services and disease investigations.

Grow-out farms

All farm managers at the selected grow-out farms consented to being interviewed.
The visited farms produced tilapia for the food-fish market, either distributed through their own marketing channels or for sale to middlemen and retailers. The fish were destined for the local-, regional- and/or national market. The majority of visited farms stocked Nile tilapia of the local, improved Akosombo strain, while one farm stocked fish originating from wild-caught stock from Lake Volta.

All farms reported having a continuous production of fish all year round. Any fallowing of the site was not applied. Some farms produced some of their own replacement stocks while the majority purchased the required fingerlings. Some farms reported quarantining imported stock in ponds for a short period prior to cage stocking. In general, there was a total absence of other biosecurity measures at the visited farms. One farm had a visitor's book and one farm manager specifically wanted to implement footbaths and vehicle disinfection sprays on entry and exit once the required funds became available. Dead fish were collected frequently, from almost daily to several times per day. Dead fish were usually buried on farmland but one farm sold dead fish for animal feed. Two farms reported problems with locals and/or staff trying to access dead fish in order to acquire free food.

Mortality levels varied amongst the visited farms, and most farm managers reported seasonal variations in mortality levels. In general, mortality levels of 10 to 20% were common, with mortality as high as 40% sometimes seen in unvaccinated stocks. Several farms reported mortality patters indicative of an infectious cause (cage-to-cage spread, gradual increase in mortality), but no current or historical obvious mass mortality events of unexplained cause were described. Mortality could affect all sizes of fish, but were commonly reported to affect larger sized fish approaching market size.

While general fish health services are absent, feed companies and international actors supply some degree of diagnostic support and the production of autogenous vaccines against bacteria (mainly Streptococcus spp.) at that particular farm. The costs associated with such services are either borne by the supplier of the service or shared between several producers.

All farmers expressed an interest in paying for regular visits and investigations by a fish health service such service should become available. In general, a routine visit frequency of once every one to two months was commonly mentioned. When asked about current obstacles to the implementation of such services, factors such as the unavailability of qualified fish health personnel and the lack of available diagnostic services was consistently mentioned. The current lack of feedback from government agencies when they conduct samplings was also noted.

Results from laboratory analyses

The mission was requested to attempt to identify the cause(s) of mortality at the visited units. Due to a lack of the required diagnostic facilities within Ghana, or in the West-African region, all laboratory analyses were conducted in Norway, either at the Norwegian University of Life Sciences (TiLV PCR according to the protocol described by Mugimba *et al*., 2018) or the Norwegian Veterinary Institute (bacteriology and histopathology, in line with standard operating procedures). Due to a lack of functional incubation facilities both in the Akosombo-region and in Accra the bacteriology plates could not be incubated while in Ghana. As a result, they were kept chilled until arrival at the Norwegian Veterinary Institute and subsequently incubated. The late incubation resulted in a degree of non-specific growth and extensive re-seeding were required. An underestimation of the number of positive samples on bacteriology can therefore not be ruled out. Detailed reports from bacteriological and histopathological analyses can be found in Appendix 3 and 4, respectively.

Hatcheries

There were low levels of mortality at the hatcheries at time of the visits and convenience sample of apparently normal /healthy individuals (eggs, fry, fingerlings and/or broodfish) were collected.

No TiLV genome-products were detected from any of the collected samples and no pathogenic bacteria were isolated. Histopathological examination revealed no significant pathology.

Grow-out farms

Risk-based sampling of moribund tilapia was performed from all grow-out farms (where possible) in order to increase the probability of detecting TiLV if it was present. No TiLV genome-products were detected from any of the collected samples.

There were significant bacterial findings from three sites (sites "B", "C", and "E"). *Streptococcus agalactiae* was detected in samples from three fish from farm "B" and one fish from farm "E" and *Streptococcus iniae* was detected in a sample from one fish with gross skin lesions on farm "E". Farm "B" had one further positive sample, with *Aeromonas veronii* detected in a sample from a fish with gross skin lesions. In addition, one fish from farm "C", with gross abnormalities in the liver on visual inspection and bacterial sepsis on histopathology, was found to be positive for *Vibrio albensis* (*Vibrio cholerae* biovar *albensis*).

Histopathological examination revealed sepsis, meningitis, hepatitis and peritonitis associated with bacterial infections in fish from farms "A", "B", "C" and "E". In addition, some minor gill infections by epitheliocystis were detected in the gills of fish from farms "A", "B", "E" and "F" and limited parasitic infections by *Trichodina* sp. and microsporidian-like organisms in samples from farms "A", "B" and "E". Based on the combined histopathology and bacteriology results immunohistochemistry analysis for *Francisella* sp. and nodavirus should be performed on the specific samples. This cannot be done for this report because of time constraints.

Likely epidemiological scenarios of detected agents

The results from the analyses of the samples collected on this mission suggest that the major agent of interest at the current time, TiLV, is not present in the sampled farms. Due to limited resources (time and finances for sample analyses) a restricted number of samples were collected from each farm. From farm "A", where 30 samples were collected, there is a 95% probability that the farm result is truly correct (the sampling of moribund fish is assumed to counteract the non-valid assumption of perfect specificity and sensitivity of the PCR test). For sites where a lower sample number was collected there is a larger uncertainty at farm level in the obtained result (e.g. 15 samples gives a >80% probability that the farm result is truly correct under the same assumptions as the above estimate).

With the given uncertainty, we may conclude from our results that there is a high probability TiLV infection is not present in tilapia hatcheries and grow-out farms the Eastern Province-part of Lake Volta. Whether the results obtained from this mission is representative for the status within hatcheries and farms in other parts of Lake Volta or in tilapia ponds in the rest of Ghana is currently unknown. The absence of detected TiLV in the hatchery samples is a very important finding for the industry in Lake Volta as such facilities have the potential to quickly spread agents like TiLV and other pathogens, to the whole region.

The risk of TiLV-spread within the Lake Volta in the case of introduction, is significant and measures to further assure Ghana's assumed free-status and to minimise the risk of introduction from affected countries should be highly prioritised (see sections 9 (Surveillance strategies) and 10 (Summary of recommendations).

The detection of *Streptococcus agalactiae*, *Streptococcus iniae* and *Aeromonas veronii* are in line with common findings from a range of tilapia-producing countries. These bacteria are common in the aquatic environment and entries into hatcheries are therefore difficult to prevent unless very stringent biosecurity measures are introduced. The feasibility of preventing entry in open-system grow-out farms is non-existent and other management options are needed. Bacterial infections in aquaculture are commonly controlled by vaccination and all interviewed farm manager used autogenous vaccines in order to reduce the impact of these agents. One farm manager had ceased vaccination after it successfully reduced the impact of bacterial infections at the farm. Information on the biology of these agents and the importance of maintaining high vaccination coverage should be prioritized in order to limit the effect of bacterial infections on the production. Even a good vaccine may lose its efficacy at population level with low vaccine coverage and stressful environmental conditions.

The significance of the detection of *Vibrio cholerae* biovar *albensis* in one fish with both gross- and histopathological lesions remain undetermined. While this agent has previously been isolated from clinically diseased fish with mixed bacterial infections in e.g. Thailand its significance remains unknown. There was only one fish from farm "C" that were tested for bacteriology and it is therefore impossible to ascertain whether this is an incidental finding of an environmental, non-pathogenic serovar of *Vibrio Cholerae* or whether it is a previously unrecognized pathogenic variant. Future bacteriological investigations of this farm and other farms in vicinity may shed further light on this matter.

Some farm managers expressed concern regarding gill health and its possible association with increased mortality. No severe gill disease were detected by histopathology, but hyperplasia, mucus/debris, *Trichodina* sp. and a few epitheliocystis were seen on histopathological examination, without any direct link being made to potential reduced gill capacity. None of the other histopathological findings suggests a high risk of spread of important pathogenic organisms.

Consequences of mortality events

In contrast to terrestrial farming there is very little information available in relation to the impact of mortality events in aquaculture. In 1997, one study estimated the yearly economic loss in aquaculture due to infection with Streptococcus was in the order of $150 million (Shoemaker & Klesius 1997).

Mortalities and known disease events (bacterial infections) were primarily a problem within the grow-out farms. While a wide range of fish sizes could be affected by mortality, mortalities in larger, almost market size fish had the largest impact due to investments made, particularly in terms of feed and labour costs. No exact figures for the incurred losses were available to the interviewed farm managers. Farm-gate sales prices per kg varied with fish size but were frequently reported to be in the range of 9 to 11,5 GHS/kg (10 GHS ≈ 2 USD). As a simple example, the farm-gate loss associated with 15% mortality in a cage with 100 000 fish of 250g size would give a biomass loss of 3750 kg, with a potential farm-gate value of around 37 500 GHS (without accounting for other variables).

The farms varied widely in size and production volume which was reflected in the size of the employed labour force. The visited grow-out farms employed between 40 and 650 staff, with most farms employing both permanent - and part-time staff. The majority of permanent staff were male although many farms also employed a significant number of female full-time staff, particularly at slaughter and processing lines. Part-time staff usually had a higher relative proportion of women than the permanent staff. While no direct estimate of the effect of mass mortalities of the employees can be generated from the available information, the general high unemployment rate and the lack of alternative opportunities suggest that mass mortalities are likely to affect employees and the surrounding communities significantly.

Both hatcheries and grow-out farms indicated that the current disease situation had a low to moderate impact on the reputation and future trade. No subsistence or small-scale units were visited and consequently the impact of lost opportunities as a consequence of mortality events could not be assessed.

A detailed economic impact assessment as results of disease outbreaks was not achievable within the limits of the current mission.

Management strategies

Health management and disease surveillance

There are currently no systematic heath management in place, passive- or active surveillance programmes or other strategies in place in the lake or in Ghanaian aquaculture. This puts the country at great risk for emerging diseases to occur which then may spread before any containment strategies and controls can be implemented. The baseline disease situation should be monitored both for listed and production related diseases, and due to the global threat of TiLV, an early detection surveillance program for TiLV should be urgently prioritised.

Effective surveillance requires adequate levels of competent personnel, laboratory facilities and financial resources. Observations during the mission indicate that the availability of all of these components is severely limited. As a result, it may be difficult to foresee launching large-scale surveillance systems within the short term unless resources (money and competence) are made available. More efficiently even, a health service team that visits farms on regular basis should be established and encouraged to monitor and investigate fish health and mortalities in farms. This team should especially be alerted when high mortality events occurs to perform targeted surveillance.

To facilitate the work of such a team, it should be evaluated, with respect to biosecurity, if a patrol boat could be acquired to help reduce significantly the time used in visiting and monitoring farms.

In order to achieve proper surveillance there is a need for an efficient reporting system from farmers to the competent authority with subsequent sample collection and sample analyses. Currently there is no available list of all fish farming units (hatcheries and grow-out farms). While all larger farms need a license to operate and therefore should be licenced and registered, many smaller units (both cage farms and ponds farms) remain unregistered. Efforts to achieve a higher coverage of registrations should be encouraged. In the absence of any regular fish health services that can conduct surveillance activities the inspections conducted by the regional officers from the Fisheries Commission constitute the backbone and should be trained and authorized to perform at least this passive surveillance.

Until the absence of TiLV can be ascertained an increased vigilance for the possible presence of disease associated with TiLV infection should be maintained. Based on the currently available scientific literature the following case definitions could serve as a baseline:
A suspected case of TiLV infection may have one or more of the following characteristics: (i) A pond/cage of
tilapia fingerlings or juveniles with increased abnormal mortality during early period of cultivation (1-4 weeks after stocking) in the absence of obvious non-infectious causes or (ii) A pond/cage of tilapia subadults/adults with increased abnormal mortality in the absence of obvious non-infectious causes or (iii) A pond/cage where the tilapia show one or more of the following clinical signs: behavioural changes, exophthalmia or other ocular lesions, skin erosions, discolouration, skin haemorrhage, scale protrusion and/or abdominal swelling or (iv) A pond/cage where at least one tested tilapia show histopathological feature of syncytial hepatitis.
A confirmed case of TiLV infection has a positive PCR analysis for TiLV with subsequent sequencing of the representative PCR product showing TiLV presence.

If any health management or surveillance strategy is to succeed, there is a need to build epidemiological and diagnostic capacity and competence in Ghana.

Vaccination

The sampled farms showed a range of bacterial infections typical of that found in tilapia farms. The number of positive fish was relatively low and may be due to the high vaccination coverage by autogenous

vaccines in the sampled farms (or delayed cultivation). The efficacy of the vaccine in the field was, however, not documented. One farmer had ceased vaccination after the disease problem was "eradicated" due to a period of vaccination and the perceived need for further vaccination was thereby absent. This view may be representative also for other farmers. Information campaigns aimed at increasing the understanding of the interaction between environment, host, and agents, and how vaccines work should be initiated. Experience from non-tilapia aquaculture industries has proven the importance of good environment and continuous high vaccination coverage in combating bacterial diseases. Reduced vaccine coverage in an area will increase the environmental burden of bacteria thereby increasing the risk of bacterial infections and subsequent significant clinical effects for all farms in an area. If the regional burden of bacteria exceeds the vaccine capacity, the effect of vaccination may drastically be reduced also for those who still vaccinate. Better understanding of vaccination and a more coordinated vaccine strategy should be encouraged.

Fallowing and regular monitoring the floor underneath the cages could be important measures to keep-up a good water environment, improve the conditions for the fish, reduce bacterial burden and increase the environmentally sustainability of the sites.

Competent authority, fish health services and diagnostic capacity

The nation-wide ban on the import of all ornamental fish species and tilapia (live, dead and reproductive material) by the Ministry of Fisheries and Aquaculture Development that comes in effect from 1.7.2018 is a crucial measure while ascertaining the assumed TiLV-free status of Ghana.

Competent authority represented by an officer from the Fishery Commission, has a good and professional relation to the farmers. However, lack of information access and transparency regarding health and production issues, makes farm visits less constructive and efficient than should be. Better communication to central office should also be highly encouraged.

The regional arm of the competent authority has the potential to play an essential role in the monitoring of the health status of the aquaculture units. The regional offices of the Fish Health Unit need to be adequately equipped to be able to respond rapidly to notifications of adverse events at tilapia hatcheries and grow-out farms in their region. Logistical challenges need to be minimised so that frequent inspections can be facilitated and rapid response ensured. An efficient system for communication between regional offices and the main office should ensure that information on emerging situations on one region is notified to other regions. In the absence of commercial, independent fish health services it is essential that the Fisheries commission regional offices fill this role in order to monitor fish health and act as an early warning system for emerging threats.

Diagnostic facilities need to be developed, both for routine investigations and as a part of an emergency preparedness strategy. A more advanced laboratory can be established in connection to the veterinary institutions in Accra. This laboratory can develop competence in collaboration with a "parent" laboratory which can train staff and be supportive on non-routine investigations. A more simple diagnostic facility (e.g. primary autopsy and bacteriology) may be put in place close to the fish farming areas, for example in conjunction with Fisheries Commission regional offices in order to avoid sample deterioration between collection and processing.

To execute good service for the industry it is important that the officers from the Fishery Commission have access to any farm and its sub-units and to necessary documentation related to stocks, production and health.

Biosecurity

Biosecurity is a concept with many layers and should be tailored according to the needs of the nation, the region, the industry down to each production facility. The lack of biosecurity at national and regional level is a responsibility for the authorities.

For the tilapia industry, there should be a joint effort in producing information material on basic biosecurity that can be distributed to workers and displayed at all critical points at the farm. Such material should take into account the potential range in literacy level amongst permanent- and temporary staff and visitors, and be designed for optimal ease of understanding. Even with extensive education programs group-level incentives may be required to achieve the required biosecurity level.

None of the visited farms had any visible biosecurity measures in place, which was subsequently confirmed by the farm managers during the interviews. Most farm managers stated their awareness of the lack of biosecurity and expressed a wish for implementing biosecurity measures, although one farm manager seemed unaware of the importance of biosecurity. Even where the farm managers were interested in implementing biosecurity measures it is likely to take some time before biosecurity measures become effective.

A field health service could play an important role to help developing and implementing biosecurity plans at industry/farm level.

Hatcheries

The general lack of biosecurity measures places the hatcheries at an unnecessary high risk of inadvertent events related to biosecurity failures. As the presence of an infection in fish groups supplied from a hatchery – the top of the supplier pyramid - will have wide-ranging effects on the spread of disease agents within the grow-out sector. Hatcheries are the most important units for biosecurity implementation. While it is clear that it will be very difficult to make the hatcheries fully biosecure, there is ample opportunity to improve biosecurity at critical points. The Fisheries Commission regional units should be in a good position to facilitate biosecurity training for hatchery operators and evaluate the progress made. It is, however, important that biosecurity training is at a level suitable for practical implementation in the hatcheries. The implementation of improved biosecurity will most likely have to be a multi-stage process until the desired level of biosecurity for each hatchery can be achieved.

Grow-out farms

It is unlikely that any of the grow-out farms can be fully biosecure given their exposure to the open environment. Necessary biosecurity measures should nevertheless be implemented at critical units and at critical points in the production. Due to the large number of workers, particularly at larger operations, the challenge will be to ensure a common understanding and implement efficient collective action. The Fisheries Commission regional units should be in a good position to facilitate biosecurity training for farm managers and to evaluate the progress made. As farms vary widely in their biosecurity requirements and opportunities for action individual guidance is likely to need to be higher than within the hatchery sector.

Summary of recommendations

The following points are recommended;

- The development and enforcement of a national biosecurity plan

- The establishment of a field fish health service for regular monitoring of all farms
- The establishment of diagnostic and laboratory capacity to undertake primary and more advanced analyses (incl PCR-analyses) – initially under a parent institutional guided training program.
- The establishment of good baseline knowledge of the disease situation (also "production" related diseases) and the implementation of risk based surveillance programs.
- The development and implementation of an overall vaccination strategy for Lake Volta

Appendix 1

References

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

Amal MNA, Koh CB, Nurliyana M, Suhaiba M, Nor-Amalina Z, Santha S et al. (2018) A case of natural co-infection of tilapia lake virus and *Aeromonas veronii* in a Malaysian red hybrid tilapia (*Oreochromis niloticus* * *O. mossambicus*) farm experiencing high mortality. Aquaculture 485: 12-16.

Assis GBN, Tavares GC, Pereira FL, Figueiredo HCP, Leal CG (2017) Natural coinfection by *Streptococcus agalactiae* and *Francisella noatunensis subsp. orientalis* in farmed Nile tilapia (*Oreochromis niloticus* L.). Journal of Fish Diseases, 40, 51-63.

Bigarré, L., Cabon, J., Baud, M., Heimann, M., Body, A., Lieffrig, F., Castric, J. (2009) Outbreak of betanodavirus infection in tilapia, *Oreochromis niloticus* (L.), in fresh water. Journal of Fish Diseases, 32, 667-673.

CGIAR Research Program on Fish Agri-food Systems (2017) Tilapia lake virus (TiLV): What to know and do? Penang, Malaysia: CGIAR Research Program on Fish Agri-food Systems. Factsheet: FISH-2017-03. Available from http://fish.cgiar.org/publications/tilapia-lake-virus-tilvwhat-know-and-do

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

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

Dong HT, Techatanakitarnan C, Jindakittikul P, Thaiprayoon A, Taengphu S, Charoensapsri W et al. (2017) *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in Nile tilapia (*Oreochromis niloticus*). Journal of Fish Diseases, 40, 1395-1403.

Fathi M, Dickson C, Dickson M, Leschen W, Baily J, Muir F et al. (2017) Identification of tilapia lake virus in Egypt in Nile tilapia affected by 'summer mortality' syndrome. Aquaculture, 473, 430-432.

Figueiredo HC, Klesius PH, Arias CR, Evans J, Shoemaker CA, Pereira DJ Jr et al. (2005) Isolation and characterization of strains of Flavobacterium columnare from Brazil. Journal of Fish Diseases, 28, 199-204.

Food and Agriculture Organization of the United Nations (FAO) (2017a) Fishery and Aquaculture Statistics. Global aquaculture production 1950-2015 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Available at www.fao.org/fishery/statistics/software/fishstatj/en

Food and Agriculture Organization of the United Nations (FAO) (2017b) Outbreaks of Tilapia Lake Virus (TiLV) Threaten the Livelihoods and Food Security of Millions of People Dependent on Tilapia Farming. FAO, Rome. Global Information and Early Warning System (GIEWS) Special Alert No. 338 - Global, 26 May 2017. Available from http://www.fao.org/documents/card/en/c/3ce1da5b-1529-4e7c-8b88-7adfef8d138c/

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

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

Li Y, Cai SH (2011) Identification and pathogenicity of *Aeromonas sobria* on tail-rot disease in juvenile tilapia *Oreochromis niloticus*. Current Microbiology, 62, 623-627.

Ministry of Fisheries and Aquaculture Development (MoFAD) (2018) Press release on Tilapia Lake Virus (TiLV) - Ban on tilapia and ornamental imports, Press release dated 18th June 2018.

Mugimba KK, Chengula AA, Wamala S, Mwega ED, Kasanga CJ, Byarugaba DK et al. (2018) Detection of tilapia lake virus (TiLV) infection by PCR in farmed and wild Nile tilapia (Oreochromis niloticus) from Lake Victoria. Journal of Fish Diseases, https://doi.org/10.1111/jfd.12790

Mulei, I.R., Nyaga, P.N., Mbuthia, P.G., Waruiru, R.M., Njagi, L.W., Mwihia, E.W., Gamil, A.A.A., Evensen, Ø, Mutoloki, S. (2018) Infectious pancreatic necrosis virus isolated from farmed rainbow trout and tilapia in Kenya is identical to European isolates. Journal of Fish Diseases, doi: 10.1111/jfd.12807.

Network of Aquaculture Centres in Asia-Pacific (NACA) (2017) Urgent Update on Possible Worldwide Spread of Tilapia Lake Virus (TiLV). Available from https://enaca.org/?id=870&title=urgent-update-on-possible-worldwide-spread-of-tilapia-lake-virus-tilv

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

Nicholson P, Fathi MA, Fischer A, Mohan C, Schieck E, Mishra N et al. (2017) Detection of tilapia lake virus in Egyptian fish farms experiencing high mortalities in 2015. Journal of Fish Diseases, 40, 1925-1928.

Prihartini, N.C., Yanuhar, U., Maftuch (2015) Betanodavirus Infections in Tilapia seed (Oreochromis sp.), in Indonesia, Journal of Life Science and Biomedicine, 5, 106-109.

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

Shoemaker, C., Klesius, P. (1997) Streptococcal Disease Problems and Control: A Review, International Symposium on Talipia in Aquaculture. Available from https://www.ars.usda.gov/research/publications/publication/?seqNo115=82994

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

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

Subramaniam K, Gotesman M, Smith CE, Steckler NK, Kelley KL, Groff JM et al. (2016) Megalocytivirus infection in cultured Nile tilapia *Oreochromis niloticus*. Diseases of Aquatic Organisms, 119, 253-258.

Surachetpong W, Janetanakit T, Nonthabenjawan N, Tattiyapong P, Sirikanchana K, Amonsin A (2017) Outbreaks of tilapia lake virus infection, Thailand, 2015-2016. Emerging Infectious Diseases, 23, 1031-1033.

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

World Organisation for Animal Health (OIE) (2017) Tilapia Lake Virus (TiLV) - A Novel Orthomyxo-Like Virus. OIE technical disease card. Available from http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/A_TiLV_disease_card.pdf

Appendix 2

PCR analyses results

FARM ID	NVI ID#	Analysis	Date analysis	Final Result
FARM-A	A1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A4	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A5	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A6	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A7	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A8	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A9	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A10	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A11	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A12	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A13	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A14	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A15	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A16	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A17	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A18	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A19	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A20	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A21	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A22	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A23	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A24	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A25	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A26	PCR-TiLV-Segment-2	14-24/June-2018	Negative

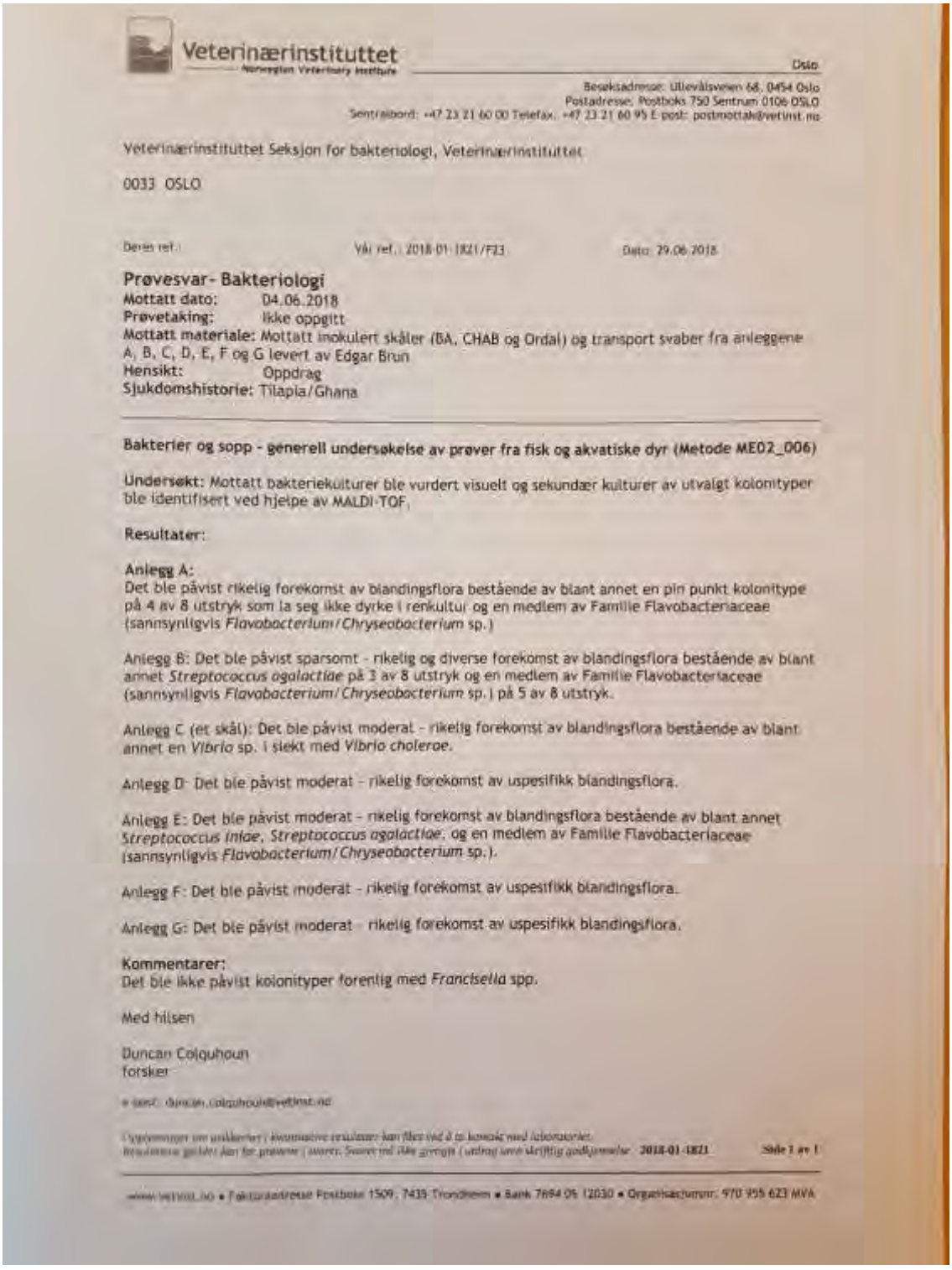
FARM-A	A27	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A28	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A29	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-A	A30	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B4	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B5	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B6	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B7	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B8	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B9	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B10	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B11	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B12	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B13	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B14	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-B	B15	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C4	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C5	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C6	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C7	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C8	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C9	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C10	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C11	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C12	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C13	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C14	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C15	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C16	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C17	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C18	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C19	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C20	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C21	PCR-TiLV-Segment-2	14-24/June-2018	Negative

FARM-C	C22	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C23	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C24	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-C	C25	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D1-1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D1-2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D1-3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D2-1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D2-2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D2-3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-4	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-5	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-6	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-7	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-8	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-9	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-10	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-11	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-12	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-13	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-14	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D3-15	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D4	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D5	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D6	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-D	D7	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E4	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E5	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E6	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E7	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E8	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E9	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E10	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E11	PCR-TiLV-Segment-2	14-24/June-2018	Negative

FARM-E	E12	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E13	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E14	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-E	E15	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-F	F1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-F	F2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-F	F3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-F	F4	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-F	F5	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G1	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G2	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G3	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G4	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G5	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G6	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G7	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G9	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G10	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G11	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G12	PCR-TiLV-Segment-2	14-24/June-2018	Negative
FARM-G	G13	PCR-TiLV-Segment-2	14-24/June-2018	Negative

Appendix 3

Bacteriology analyses results



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0033 OSLO

Deres ref.: Vår ref.: 2018-01-1821/F23 Dato: 29.06.2018

Prøvesvar- Bakteriologi
Mottatt dato: 04.06.2018
Prøvetaking: Ikke oppgitt
Mottatt materiale: Mottatt inokulert skåler (BA, CHAB og Ordal) og transport svaber fra anleggene A, B, C, D, E, F og G levert av Edgar Brun
Hensikt: Oppdrag
Sjukdomshistorie: Tilapia/Ghana

Bakterier og sopp - generell undersøkelse av prøver fra fisk og akvatiske dyr (Metode ME02_006)

Undersøkt: Mottatt bakteriekulturer ble vurdert visuelt og sekundær kulturer av utvalgt kolonityper ble identifisert ved hjelpe av MALDI-TOF.

Resultater:

Anlegg A:
Det ble påvist rikelig forekomst av blandingsflora bestående av blant annet en pin punkt kolonitype på 4 av 8 utstryk som la seg ikke dyrke i renkultur og en medlem av Familie Flavobacteriaceae (sannsynligvis *Flavobacterium/Chryseobacterium* sp.)

Anlegg B: Det ble påvist sparsomt - rikelig og diverse forekomst av blandingsflora bestående av blant annet *Streptococcus agalactiae* på 3 av 8 utstryk og en medlem av Familie Flavobacteriaceae (sannsynligvis *Flavobacterium/Chryseobacterium* sp.) på 5 av 8 utstryk.

Anlegg C (et skål): Det ble påvist moderat - rikelig forekomst av blandingsflora bestående av blant annet en *Vibrio* sp. i slekt med *Vibrio cholerae*.

Anlegg D: Det ble påvist moderat - rikelig forekomst av uspesifikk blandingsflora.

Anlegg E: Det ble påvist moderat - rikelig forekomst av blandingsflora bestående av blant annet *Streptococcus iniae*, *Streptococcus agalactiae*, og en medlem av Familie Flavobacteriaceae (sannsynligvis *Flavobacterium/Chryseobacterium* sp.).

Anlegg F: Det ble påvist moderat - rikelig forekomst av uspesifikk blandingsflora.

Anlegg G: Det ble påvist moderat - rikelig forekomst av uspesifikk blandingsflora.

Kommentarer:
Det ble ikke påvist kolonityper forenlig med *Francisella* spp.

Med hilsen

Duncan Colquhoun
forsker

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Opplysninger om usikkerhet i kvantitative resultater kan fåes ved å ta kontakt med laboratoriet.
Resultatene gjelder kun for prøvene i svaret. Svaret må ikke gjengis i utdrag uten skriftlig godkjennelse. 2018-01-1821 Side 1 av 1

Appendix 4

Histopathology analyses results

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Deres ref.: Vår ref.: 2018-04-17553 / F98 Dato: 26.06.2018

Prøvesvar - KLADD
Received date: 06.06.2018
Sampling date: unknown
Material: Formalin-fixed tissues from xx Tilapia
Purpose: Ghana project no:
Sjukdomshistorie:

Farm A; mixed lot, mostly normal?
Farm B; mainly moribune
Anlegg C; apparently healthy
Anlegg D; Brood fish stamfisk – apparently healthy
Anlegg E ; mainly moribune
Anlegg F; apparently healthy
Anlegg G; Brood fish stamfisk – apparently healthy

Histopatologi (Metode ME01_002)

A complete overview of histological findings and bacteriology is given in an excel spreadsheet attached MÅ OPPDATERES MED DAGENS FUNN

Significant histopathology was found in the following fish:

A- Group gills: some epithelial lifting (sloughing) in the higher numbered fish which is most probably a sampling artefact. A11 had some epithelial hyperplasia and high number of ECG/mast cells, mucus/debris on the surface along with a few *Trichodina* sp. A10, 14, 16, 18 – also a few Ephitheliocysts. A18 is very autolytic and can not be evaluated further. A 21 has a few metazoans, possibly trematodes in between gill filaments - no apparent pathology, some epithelial hyperplasia other places.

A10: Bacterial sepsis: high numbers of cocci-like organisms in macrophage-like cells in spleen. . and also on the inflamed surface of liver - probably reflecting severe peritonitis.
A14: increased number of inflammatory cells in the hepatopancreas, the intestines are very autolytic, but apparently with bacterial microcolonies in both epithelium and lamina propria, morphologically quite similar to the epitheliocysts in the gills.
A18: some small granulomas in liver, well demarcated (old lesions?)

B- group:

B1; a few epitheliocysts, one with an advanced multilobular organization within a circular capsule indicating rather parasitic (microsporidian?) than bacterial nature. Hepatitis and numerous bacteria in necrotic ellipsoids of the spleen. Str agalactia isolated.
B2; Str agalactia isolated - but no significant pathology, possibly some changes (lysis-clearing of granules) in the macrophage centers of the spleen.
B3 Gill - autolytic, one multilobular (microsporidian?) epitheliocyst, severe bacterial peritonitis.

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degenerative changes spleen
B4 Str agalactia isolated - but no significant pathology internal organs (no intestine included here)
Gills -sparse epithelial hyperplasia, mucus/debris on the surface along with some *Trichodina* sp.
B6 similar to B4, but spleen has visible bacteria in many melanomacrophages
B7 - only gills sampled, three filaments necrotic distal part, thrombi and inflammatory cells proximally – reflecting sepsis - not gill inflammation? (lack internal organs to decied)

C group, one fish only: bacterial sepsis - Vibrio albensis isolated

D- group, no significant histopathology, one epitheliocyst and one trematode-like parasite in gills.

E- group, Findings in sections of brain included: E1 few, small foci of possible gliosis. E2: severe granulomatous meningitis with bacteria-like organisms - well demarcated granulomas.
E4 - Str iniae isolated, gill inflammation with moderate hyperplasia, *Trichodina* sp. and small, simple epitheliocysts, no significant pathology internal organs. (intestines not sampled)

F-group, no significant pathology, possibly except an unusal distribution of ECG/mast cell in F2 medulla oblongata and one encapsulated parasite (calcified?) in brain of F3, minor hepatitis in F4, sparse gliosis in F5 brain

G-group, no significant pathology, possibly except: G8 small fish, one small parasite (pleistophora-like?) encysted in muscle of, strange (to us) feed in the gut - no pathology. G9: moderate number of granulomas in gill filaments, with parasite cells(?), another parasite (Zschokkella-like) in bile duct in liver, G11 gills- very few granulomas as in G9, brain some hypercellularity and ECG/mast cells in brain tissue close to the meningeal surface. G13 brain - possible gliosis.

Comments:
Gills - no severe gill disease, but often some hyperplasia, mucus/debris and *Trichodina* sp. and a few epitheliocysts that have not incited inflammation (may upon rupture into gill tissue). Sampling may also have affected the epithelium here: lifting/sloughing are most probably artefacts
Brain/liver - no clear signs indicating TILV diseases.

Apart from fish with bacterial sepsis - mostly minor findings of variable nature - weak fish / no specific disease?

Med hilsen

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Adam Zerihun

DETTE DOKUMENTET ER ELEKTRONISK SIGNERT