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Production-level risk factors for syncytial hepatitis in farmed tilapia (*Oreochromis niloticus* L)

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Abstract

Syncytial hepatitis (SHT) is an emerging viral disease of tilapia characterized by significant morbidity and mortality. This study aimed to establish the production-level risk factors associated with presence and severity of SHT. Production factors were analysed during multiple outbreaks of SHT that occurred between 2011 and 2013 on a single tilapia farm in Ecuador and compared with the year 2010 before the SHT outbreaks. Relative risks, *t* tests, modified Poisson and forward stepwise linear regression analyses were performed using EPIINFO™. Compared to other strains, Chitralada had an elevated risk of SHT [RR = 2.1 (95%CI 1.8–2.4)]. Excessive mortality associated with the presence (and severity) of SHT increased by 611 (365), 6,814 (5,768) and 388 (340) deaths per 100,000 fry when stocking density, dissolved oxygen and pond production cycles were raised by 1 fish/m^2, 1 mg/L and 1 cycle, respectively. Excessive mortality associated with the presence (and severity) of SHT decreased by 337 (258) and 1,354 (1,025) deaths per 100,000 when stocking weight and water temperature increased by 1 g and 1°C, respectively. Time (season and stocking year) was not significantly associated with SHT. This study shows that some production factors increase the risk incidence and severity of SHTon a farm.

KEYWORDS
mortality, outbreaks, production-level, risk factors, syncytial hepatitis, tilapia

1 | INTRODUCTION

Lymphocystis, caused by an iridovirus, was for many years the only reported viral disease in tilapia populations (McGrogan, Ostland, Byrne, & Ferguson, 1998). Possibly due to the intensification of commercial tilapia aquaculture practices, or the increased movement of eggs, fry and farm products, trends show that a number of viruses previously known to target other aquaculture species are increasingly being reported in tilapia (Ariel & Owens, 1997). For instance, a betanodavirus caused high mortality of tilapia larvae (Bigarré et al., 2009), and a herpes-like virus, named tilapia larvae encephalitis virus (TLEV), was associated with high mortality among tilapia larvae raised in a laboratory (Shlapobersky et al., 2010).

More recently, an emerging viral disease of tilapia has been reported with differing clinical presentations depending on geographical location. In South America, the disease (called syncytial hepatitis of tilapia—SHT) was characterized by high mortality (greater than 80%), exophthalmos, distended abdomen and syncytial hepatitis (Ferguson et al., 2014). In Israel, by contrast, tilapia infected with the tilapia lake virus (TiLV) were characterized by similarly high mortality (between 80%–100%), but by buphthalmia and encephalitis, and limited hepatic lesions (Eyngor et al., 2014). Several outbreaks of TiLV with mortalities reaching 90% have been reported in Thailand (Dong et al., 2017). In Egypt, the disease has been reported to cause summer mortalities (5%–15%) in numerous farms (Fathi et al., 2017). The identity of the associated virus in all of these distinct pathological presentations was shown to be an RNA virus (Eyngor et al., 2014; Tsofack et al., 2016)-related genetically (Bacharach et al., 2016) and morphologically to the *Orthomyxoviridae* family (del-Pozo et al., 2017). There is now considerable information about the

transboundary distribution, clinical presentation, gross pathology, histopathology and ultrastructural pathology of the disease, as well as characterization of the associated Orthomyxo-like virus of tilapia (Bacharach et al., 2016; Dong et al., 2017; Eyngor et al., 2014; Ferguson et al., 2014; del-Pozo et al., 2017; Tsofack et al., 2016), but we are aware of no work on epidemiological characteristics associated with its incidence and distribution. Knowledge of the epidemiological factors for SHT may contribute to the development of prevention and control strategies that should minimize the spread of the associated virus. The purpose of this study was to determine the production-level risk factors that may lead to an increased incidence and severity of SHT in pregrowth ponds on a commercial tilapia farm.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted on a single farm that is located in Guayaquil, Ecuador, at Latitude −2°11′ 38.62″ S and Longitude −79°52′ 47.55″ W. Multiple outbreaks of SHT occurred on a 1,700-hectare farm (4,300 acres) where all-male populations of tilapia were intensively farmed in a closed recirculation culture system of ponds. Only 5%–8% new water was pumped each day (especially during the dry season) into the ponds from a nearby river to replace water lost through evaporation. At the time of the SHT outbreak, pregrowth (PC) ponds shared a common pump or recirculation system with the grow-out ponds, but this has since changed. Each PC pond measures about 1.0 ha and is 1.2 m deep. More than 95% (40/42) of the PC ponds were enclosed by nets to keep out any of the 67 fish-eating bird species. On average, each pond is stocked initially with 331,275 fry (*SD* 133,478), and they are transferred only at the end of the production cycle to the grow-out ponds. An all-male Chitralada population, bred on the farm by feeding fish on methyl testosterone, was the dominant strain on the farm. The other but less common strain was the genetically improved farm tilapia (GIFT). Later, in 2010, a genetically male tilapia (GMT) strain, purchased from another producer, was introduced to the farm. Each pond is stocked with one strain of tilapia. However, mixed shrimp–tilapia farming was practised at the time of SHT outbreak, with shrimp growing underneath tilapia as a biproduct.

2.2 | Data collection

Data were collected from multiple pond production cycles in a total of 42 PC ponds during multiple outbreaks of SHT in 2011, 2012 and 2013. These parameters included tilapia strains used (Chitralada, GMT and GIFT), stocking density (number of fish/m^2), individual weight of fry at transfer (g), weather pattern (dry or rainy), water temperature (°C), dissolved oxygen (DO_2) (mg/L), number of days spent preparing the pregrow-out (PC) pond, month of transfer to PC ponds, amount of feed per fish (g $fish^{-1}$ day^{-1}), number of pond production cycles per year, year of stocking, and mortality rate per production cycle (deaths/100,000 fry). Stocking density and mortality rate were determined at the beginning and at the end of a pond cycle, respectively. Temperature and DO_2 were measured once a week and the mean values of each pond production cycle recorded. The amount of feed per fish per day was also recorded as a mean value of the pond production cycle. Retrospective farm records for year 2010 were reviewed to obtain comparative data on similar parameters before the outbreak of SHT, and these were matched by month of collection.

A pond production cycle was defined as the time from which fry were transferred into the PC pond to the time they are transferred out of the PC pond to the grow-out ponds. Each pond production cycle on the farm constituted a unit of analysis, because the pond is considered a single living unit providing for the fish vital functions and biological processes that are similar to those performed by a single organism (Noga, 2000). Mortality in a pond production cycle was determined indirectly as a function of survival, that is, the difference between number of fry transferred into the PC ponds and after a period of growth, the actual number of fingerlings transferred out of PC ponds to grow-out ponds. Mortality rate was defined as the number of deaths in a PC pond with respect to that pond's population size per month and was expressed as deaths per 100,000 fry per production cycle.

For reverse transcriptase polymerase chain reaction (RT–PCR) studies, 59 clinically sick fish were collected from pond production cycles with excess mortality. Sixty-seven asymptomatic fish were also obtained from ponds without excess mortality. Specific RT–PCR assays were performed accordingly (del-Pozo et al., 2017).

2.3 | Statistical methods

Data obtained from document review were line listed with columns representing the variables of interest and rows representing pond production cycle identification. Pond production cycles for which data on the variables were missing were removed from the ensuing analysis. Statistical analyses were performed using EPIINFO™ version 7 (CDC, Atlanta Georgia) at a significance level $\alpha = 0.05$. Independent variables were analysed for collinearity using scatter graphs, where collinearity existed, the most suitable independent variable was selected. The selected independent variables used in the two final models were stocking density, weight of fry at the time of transfer, water temperature, DO_2 and number of pond production cycles per year.

The dependent variable was excess mortality associated with SHT, and it was calculated as the difference between the actual observed mortality and background mortality. Background mortality was defined as the usual mortality associated with parasites, bacteria, prey–predator relationships, fish-eating birds and trauma. It was defined as the average mortality of all pond production cycles during 2010 (before the SHT outbreak), and it was estimated to be 28,000 deaths per 100,000 fry per year. Data from 2008 and 2009 were not considered in the calculation of background mortality because they were associated with several outbreaks of bacterial infections.

During the outbreak, dead fish were removed from ponds with excess mortality and they were not replaced during the production cycle.

After assessing for normality of data, differences in means among the independent variables and mortality rates before (2010) and during (2011–2013) the SHT outbreaks were estimated using a student's *t* test. Relative risks (RR) were calculated to compare excess mortality associated with SHT in different tilapia strains and to establish the strength of association between excess mortality and SHT.

Presence of SHT was defined as excess mortality between 28,000 and 40,000 deaths per 100,000 fish per production cycle, weak to strong positive band signals on RT–PCR for the orthomyxo-like virus correlating with low (asymptomatic fish) to high viral loads (classical clinical signs), necrotizing hepatitis and syncytia giant cell formation (Bacharach et al., 2016; Ferguson et al., 2014; del-Pozo et al., 2017). A modified Poisson regression model was constructed and used to determine the production-level risk factors for the presence of SHT with the dependent variable, excess mortality being dichotomously categorized as a present or absent variable.

Severe SHT was defined as very high excess mortality ranging from 40,000 deaths per 100,000 fish to 80,000 deaths per 100,000 fish per production cycle, strong positive band signals on RT–PCR consistent with high viral load and the existence of typical classical signs, and pronounced pathological changes in infected fish (Bacharach et al., 2016; Ferguson et al., 2014; del-Pozo et al., 2017). After assessing linearity between the independent variables, a forward stepwise multiple linear regression model was also constructed to predict production-level risk factors for severity of SHT using only pond production cycles with excess mortality associated with SHT. Pond production cycles without excess mortality that was associated with SHT were not included in the linear regression model. The dependent variable, excess mortality of tilapia associated with SHT, was left as a continuous variable. Interaction and confounding between any two variables were assessed in the models.

3 | RESULTS

Only 79% (535/677) pond production cycles were considered in the study. While water quality parameters were found to be consistently within acceptable limits for farmed tilapia, statistically significant differences in means were observed in all three mortality rates, water temperature, DO_2, number of pond production cycles per year and number of fallowing days before and during the SHT outbreak. There were no significant differences in stocking weight, stocking density and amount of feed per fish per day before and during the SHT disease outbreak (Table 1). Dry or wet season and year of stocking the pond were not significant significantly associated with excess mortality due to SHT (data not shown).

The risk of excess mortality was about five times higher in tilapia infected with SHT [RR = 4.8, (95% CI 2.9–7.9)] when compared to non-infected tilapia. The risk of excess mortality was two times higher among the infected Chitralada strain [RR = 2.1, (95% CI 1.8–2.4)] when compared to the GMT and GIFT strains.

Before the SHT disease outbreak in 2010, excess mortality was recorded in 3% (4/135) of the pond production cycles. However, during the multiple SHT outbreaks between 2011 and 2013, excess mortality was recorded in 80% (320/400) of the production cycles. The number of pond production cycles was linearly related to number of days spent preparing (fallowing) the PC pond while the amount of feed per fish and weight of fish at time of transfer were related linearly, and thus, the number of pond production cycles and weight of fish were selected for inclusion in the modified Poisson regression and the forward stepwise multiple linear regression models. Excess tilapia mortality associated with the presence of SHT increased by 611, 6,814 and 387 deaths per 100,000 fry when stocking density, DO_2 and pond production cycles were raised by 1 fish/m^2, 1 mg/L and 1 pond production cycle per year, respectively. Excess tilapia mortality associated with the presence of SHT decreased by 337 and 1,354 deaths per 100,000 when the initial weight of the fish and water temperature increased by 1 g and 1°C, respectively (Table 2). These risk factors remained statistically significant in the degree of severity of SHT. The severity of mortality associated with SHT increased by 365, 5,768 and 340 deaths per 100,000 fry when stocking density, DO_2 and pond production cycles were raised by 1 fish/m^2, 1 mg/L and 1 pond production cycle per year, respectively. The severity of tilapia mortality associated with SHT decreased by 258 and 1,025 deaths per 100,000 fish when the initial weight of the fish and water temperature increased by 1 g and 1°C, respectively (Table 3). Results from the modified Poisson regression model and the forward stepwise multiple linear regression model were similar suggesting that presence of disease and its severity are comparable and, thus, either model can be used. Only 36% and 24% of the variation in mortality can be explained by water quality or production factors investigated in these models (Tables 2 and 3). Our diagnostics indicated that the linear regression model was a good fit to the excess mortality data observed in the ponds.

4 | DISCUSSION

Compared to open water systems which are characterized by high water turnover rates, supplying more oxygen and removing more waste including pathogens, closed culture systems allow for little or no new water replacement (in this case 5%–8%). It is possible that closed nature of the farm, probably due to increased exposure of fish to high pathogen loads, exacerbated mortality. Differences in severity of fish diseases and mortality rates between closed and open systems have been reported and can vary from subacute to chronic in open systems to acute in closed water systems (Conte, 2004). The fact that excess mortality was recorded in 3% of the production cycles in 2010 provides evidence that SHT was present on the farm before the first severe outbreak was observed in 2011. It is hypothesized that SHT was the consequence of introducing the GMT strain onto the farm.

TABLE 1 Comparison of water quality parameters in the ponds and husbandry practices before and during the disease outbreak associated with syncytial hepatitis viral infection in the tilapia population

	Before outbreak			During outbreak					
Parameter	***n***	**Mean**	**Std dev**	***n***	**Mean**	**Std dev**	***t* test**	***df***	***p*-Value**
Mortality $rate_{overall}$	135	28,000	11,358	400	52,676	19,808	−27.7	533	<.0001
Mortality $rate_{Chitra}$	131	31,512	11,364	191	60,531	14,041	−19.9	320	<.0001
Mortality $rate_{GMT}$	4	25,001	3,117	209	36,548	10,013	−2.3	211	.02
Temperature	135	27.40	1.61	400	22.81	10.15	5.0	533	<.001
Dissolved oxygen	135	3.32	0.73	400	3.61	1.60	2.0	533	.04
# of pond cycles	135	18.0	2.23	400	23.0	7.53	−7.6	533	<.001
Fry weight	135	0.85	0.54	362	0.69	0.89	1.9	491	.56
Stocking density	135	29.8	4.56	400	31.2	12.32	1.3	533	.19
# of fallowing days	135	14.4	7.8	400	11.3	11.3	5.2	533	<.001
Amount of feed	135	4.0	1.8	400	3.8	1.5	1.3	533	.20

#, number; *n*, number of ponds production cycles; std dev, standard deviation; *df*, degrees of freedom.

TABLE 2 Modified Poisson model for the presence of excess tilapia mortality associated with the syncytial hepatitis viral infection as function of production factors

Excess mortality	**Coefficient**	**Std. err**	***t***	***p*** **> ltl**	**(95% CI)**	
Stocking density	611.2164	68.10797	8.97	.000	477.4861	744.9468
Initial weight	−337.4236	50.06521	−6.74	.000	−435.7272	−239.1206
Temperature	−1,354.184	121.3601	−11.16	.000	−1592.475	−1,115.893
Dissolved oxygen	6,814.03	726.6416	9.38	.000	5,387.265	8,240.795
# of pond cycles	387.8346	92.33242	4.20	.000	206.5393	569.1298
Constant	−3,705.576	3,683.104	−1.01	.315	−10,937.37	3,526.22

Std. err, standard error; CI, confidence interval; Prob > F = 0.0000; R^2 = .3470; Adjusted R^2 = .3421; Chitra, Chitralada.

TABLE 3 Linear regression model for severity of excess tilapia mortality associated with syncytial hepatitis viral infection as function of production factors

Excess mortality	**Coefficient**	***SE***	***F* test**	***p*-Value**
Stocking density	365.651	59.599	37.6400	<.000001
Initial weight	−258.106	84.566	9.3154	.002405
Temperature	−1,025.331	122.099	70.5191	<.000001
Dissolved oxygen	5,768.980	749.898	59.1825	<.000001
# of pond cycles	340.179	82.853	16.8578	.000048
CONSTANT	−41,152.417	3,456.541	141.7449	<.000001

Correlation coefficient: r^2 = .24; no confounding or interaction was established in both models.

Weather patterns (dry or wet season) and month of transfer of fry were not significantly associated with excess tilapia mortality. By contrast, the strain of tilapia, stocking density, weight of fry at transfer, water temperature, DO_2 and the number of pond production cycles per year were statistically significant in predicting excess mortality associated with SHT.

The risk for infection with SHT was significantly increased in the Chitralada strains compared to the other strains of tilapia used in this farm, suggesting that Chitralada may have no or have lower concentrations of the Mx1 gene which has been reported to increase resistance to nodavirus infections in fish (Poisa-Beiro et al., 2008) and to orthomyxovirus infections in mice (Haller, Frese, Rost, Nuttall, & Kochs, 1995). However, differences in susceptibility of fish to viral infections have been reported, attributed to genetic resistance in fish strains or acquired immunity from previous exposure (Biacchesi et al., 2007; O'Brien & Evermann, 1988).

The significantly lower water temperatures during the disease outbreak may have lowered both cellular and humoral immunity of the fish (Avtalion, Wojdani, Malik, Shahrabani, & Duczyminer, 1973; Rijkers, Frederix-Wolters, & Van Muiswinkel, 1980). Higher water temperatures, and thus, lower DO_2, prior to the outbreak may have deterred replication of the syncytial hepatitis virus already present in the ponds. *In vitro* studies using E-11 and primary tilapia brain cells have shown the TiLV caused the highest cytopathic effect at 25°C but not at 27°C (Tsofack et al., 2016). The highest Koi mortality occurred at 23°C when fish were experimentally inoculated with the Koi herpesvirus, but modest to no viral-associated mortality of fish occured when temperatures were below 10°C or above 30°C (Gilad et al., 2003).

Both multivariate models show that excess mortality of tilapia due to the SHT increased when stocking density was increased. A previous study reported that stocking density of tilapia contributed to the presence of TiLV (Fathi et al., 2017). It has been reported that

stocking density is inversely proportional to the total body weight, daily weight gain and length of Nile tilapia fingerlings which impacts on the immunity of the fish (Breine, Nguenga, Teugels, & Ollevier, 1996; Sanudi, Jere, Mzengereza, & Chirwa, 2015). By contrast, excess mortality of tilapia with SHT reduced when the individual stocking weight of the fry increased at the time of transfer to PC ponds. This reduced mortality of fry might result from better immunity as the appearance of IgM is more dependent on weight and length of fish rather than age (Grøntvedt & Espelid, 2003). Fish housed at high density experienced severe acute disease, increased mortality rates, elevated viral loads and reduced body condition compared with fish held at low density (Gustafson, Ellis, & Bartlett, 2005; Inendino, Grant, Philipp, & Goldberg, 2005).

Due to increasing productivity and demand, fish ponds were used more frequently during the disease outbreak than before the disease outbreak, shortening the time between harvesting and restocking of the pond. This led to inadequate preparation (liming, fallowing) of the pond and failure to destroy all pathogens remaining from the previous production cycle. This may have permitted transgenerational accumulation and transmission of a range of pathogens including the syncytial hepatitis virus (Nylund et al., 2007) and may explain why excess tilapia mortality due to SHT rose with the increase in number of production cycles per pond per year.

These SHT outbreaks in farmed tilapia allowed diagnostic and epidemiological investigations of natural conditions of the disease. Our analysis suggests that SHT in tilapia populations leads to very high case fatality rates among infected fish. While these risk factors (either alone or in combination) can acutely or chronically stress fish or alter biochemical processes, increasing their susceptibility to infection by suppressing innate and adaptive immune responses, these production-level risk factors are preventable through management. Although increasing water temperature and lowering DO_2 was most associated with reducing risk of SHT, controlling the number of pond production cycles per year, improving weight of fish at time of transfer and lowering stocking density at the time of seeding the pond may be more practical approaches.

A limitation to this study is that our analysis did not control for dependencies in the pond production cycles. Another limitation is that dead fish were not followed up or replaced during the study.

5 | CONCLUSIONS

The production factors that increased the natural incidence and severity of SHT in farmed tilapia include being of the Chitralada strain, lower body weight at the time of transfer to on-growing ponds, lower water temperatures, increased DO_2, increased stocking density and increased numbers of pond production cycles per year.

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