UNIVERSIDADE DE BRASÍLIA
FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE ANIMAL

AVALIAÇÃO DOS RISCOS SANITÁRIOS QUE AFETAM A PRODUÇÃO DE TILÁPIA EM TANQUE-REDE E VIABILIDADE DE MEDIDAS DE PREVENÇÃO

MARINA KARINA DE VEIGA CABRAL DELPHINO

TESE DE DOUTORADO EM SAÚDE ANIMAL

BRASÍLIA/DF
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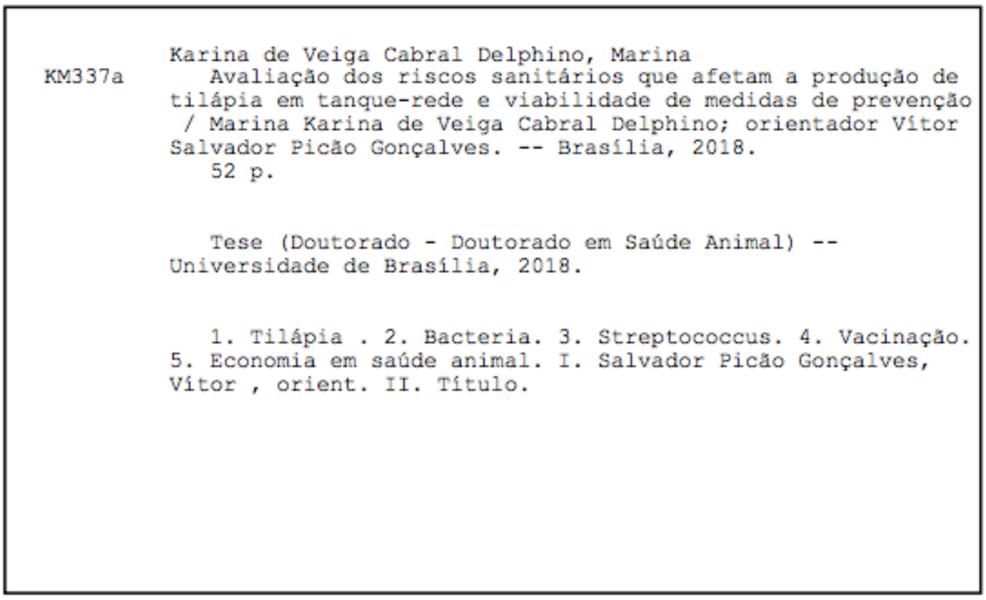

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RESUMO

A piscicultura cresce rapidamente no Brasil. Em 2016, foram produzidas 507 mil toneladas de peixes e a tilápia do Nilo (*Oreochromis niloticus*) foi a principal espécie cultivada, representando 47% da produção total de peixes. O cultivo dessa espécie é realizado, principalmente, por meio de um sistema intensivo realizado em tanques-rede instalados em grandes reservatórios nacionais. Entretanto, a presença de doenças desafia a sustentabilidade da cadeia produtiva, que detém pouco conhecimento sobre os problemas sanitários existentes. Este trabalho teve como objetivo identificar os principais riscos sanitários na criação de tilápias em reservatórios e caracterizar a dinâmica dos patógenos prevalentes, como base para o desenvolvimento de medidas efetivas de controle sanitário na tilapicultura. Para tanto, de agosto de 2015 a outubro de 2016, foi realizado um estudo longitudinal no município de Morada Nova de Minas, no reservatório de Três Marias, sudeste do Brasil. Dados diários e mensais foram coletados em seis das 32 fazendas de peixes existentes, incluindo amostras de peixes, mortalidade, temperatura e parâmetros de qualidade da água. As principais bactérias detectadas foram *Streptococcus agalactiae*, infectando principalmente tilápias adultas ao longo de todo o ano, com maior frequência à medida que a temperatura da água aumenta, e a *Francisella noatunensis* subsp. *orientalis* (Fno), infectando principalmente tilápia jovens, somente durante os meses mais frios. A detecção de Fno em uma fazenda em dois invernos consecutivos, sem evidência de reintrodução, reforça a necessidade de investigação sobre a capacidade desse patógeno sobreviver e infectar novos animais causando novos surtos. Além disso, um modelo de orçamento parcial simples e flexível foi desenvolvido para avaliar o retorno econômico da vacinação contra *S. agalactiae* em tilápias do Nilo cultivadas em tanques-rede. Essa bactéria é considerada um grande obstáculo para a expansão da aquicultura brasileira devido, principalmente, a alta prevalência e significativas perdas econômicas. A vacinação é considerada um método eficaz para prevenir doenças importantes na aquicultura. Foram avaliados três cenários de mortalidade cumulativa devido a *S. agalactiae* (5%, 10% e 20%, por ciclo de produção) em uma fazenda não vacinada. Para cada cenário, calculou-se o retorno líquido (benefícios - custos) da vacinação, dada uma combinação de valores referentes a eficácia da vacina e a um possível ganho na conversão alimentar, de forma a modelar a incerteza sobre o verdadeiro valor de tais parâmetros. Os resultados indicam que a vacinação contra *S. agalactiae* é provavelmente lucrativa em cultivos de tilápia, embora em cenários onde a mortalidade cumulativa é menor que 10%, a lucratividade da vacinação seria mais dependente de maior eficácia vacinal e/ou melhor conversão alimentar.

Palavras-chave: tilápia do Nilo; *Francisella*, *Streptococcus*, vacinação, orçamento parcial.

ABSTRACT

Fish aquaculture is rapidly growing in Brazil and, in 2016, 507 thousand tons of fish were produced. Nile tilapia (*Oreochromis niloticus*) is the most cultivated species, representing over 47% of the total production of fish, mainly through an intensive system carried out in floating cages installed in large reservoirs. However, fish pathogens pose a major challenge to production chain sustainability, and tilapia farmers often have limited knowledge of prevailing health problems. This study aimed to identify the key disease risks of tilapia farming in a tropical reservoir and characterize the dynamics of the prevalent pathogens, as a basis for development of effective control measures for tilapia health and surveillance programs. From August 2015 to October 2016, a longitudinal study was carried out at the, Três Marias reservoir, in the, municipality of Morada Nova de Minas in the southeast of Brazil. Daily and monthly data were collected from six out of 32 existing fish farms, including fish samples, mortality, temperature and water quality parameters. The main bacteria detected were *Streptococcus agalactiae,* infecting mostly adult tilapia throughout the period, with higher frequency as the average temperature increased, and *Francisella noatunensis* subsp. *orientalis (*Fno), infecting mainly younger tilapia, only during the cooler months. The detection of Fno in one farm in two consecutive winters, without evidence of sustained introduction of infected stock, strengthens the case for investigating if this pathogen can survive and remain infective causing new outbreaks. Furthermore, we developed a simple and flexible partial-budget model to undertake an economic appraisal of the vaccination against *S. agalactiae* in Nile tilapia farmed in net cages in large reservoirs. This bacterium is considered a major obstacle to the expansion of Brazilian aquaculture because its high prevalence and economic losses. Vaccination is an effective method to prevent important diseases in aquaculture. We analyzed three epidemiological scenarios of cumulative mortality due to *S. agalactiae* (5%, 10% and 20%, per production cycle) in a non-vaccinated farm. For each scenario, we calculated the net return (benefits – costs) of vaccination, given a combination of values of "vaccine efficacy" and "gain in feed conversion ratio", in order to model uncertainty about the true value of such parameters. Results indicate that vaccination against *S. agalactiae* is likely be profitable in Nile tilapia farms, although in scenarios where cumulative mortality is lower than 10%, the profitability of vaccination would be more dependent on higher vaccine efficacy and/or better feed conversion rate.

Key-words: Nile tilapia, *Francisella*, *Streptococcus*; vaccine; partial budget.

LISTA DE TABELAS

Página

Capítulo 1

Capítulo 2

LISTA DE FIGURAS

Página

Capítulo 1

LISTA DE ABREVIATURAS E SIGLAS

AQUACEN	Laboratório oficial central da Rede Nacional de Laboratórios da Pesca e Aquicultura
C_{Atb}	*Cost of treatment per kilogram*
$C_{\text{Atb-Aquaflor}}$	*Cost of treatment by florfenicol*
$C_{\text{Atb-TM700}}$	*Cost of treatment by oxytetracycline*
$C_{\text{Hired-Labour}}$	*Cost of vaccine labour per fish*
C_{Vaccine}	*Cost of vaccine dose per fish*
CEUA	Comissão de Ética no Uso Animal da UnB
CNA	Confederação da Agricultura e Pecuária do Brasil
CODEVASF	Companhia de Desenvolvimento dos Vales do São Francisco e do Parnaíba
CONAMA	Conselho Nacional do Meio Ambiente
FCR	*Feed Conversion Ratio*
$\text{Feed}_{\text{Price}}$	*Average feed price*
Feed_{VAC}	*Total feed consumption in a vaccinated farm*
$\text{Feed}_{\text{N-VAC}}$	*Total feed consumption in a non-vaccinated farm*
$\text{Fish}_{\text{Price}}$	*Average fish market price*
FNO	*Francisella noatunensis* subsp. *orientalis*
M_{Strep}	*Mortality due to S. agalactiae without vaccination*
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
$\text{Mortality}_{\text{Vacc}}$	*Total mortality due to disease in a vaccinated farm*
$\text{Mortality}_{\text{Non-Vacc}}$	*Total mortality due to disease in a non-vaccinated farm*
MG	Minas Gerais
MNM	Morada Nova de Minas
MR_{D}	*Daily mortality rate*

N_{Fish}	*Batch size in number of fish*
n_{mort_day}	*Number of dead fish per day*
N_{Risk}	*Farm population at risk per day*
RENAQUA	Rede Nacional de Laboratórios da Pesca e Aquicultura
RPS	*Relative Percent Survival*
$Treat_{Strep}$	*Expected % of fish to be treated with antibiotics*
UFMG	Universidade Federal de Minas Gerais
UNB	Universidade de Brasília
VBNC	*Viable but non-culturable*
$W_{Harvested}$	*Fish weight at harvest*
$W_{Treated}$	*Average weight of treated fish*

SUMÁRIO

ANEXOS

INTRODUÇÃO

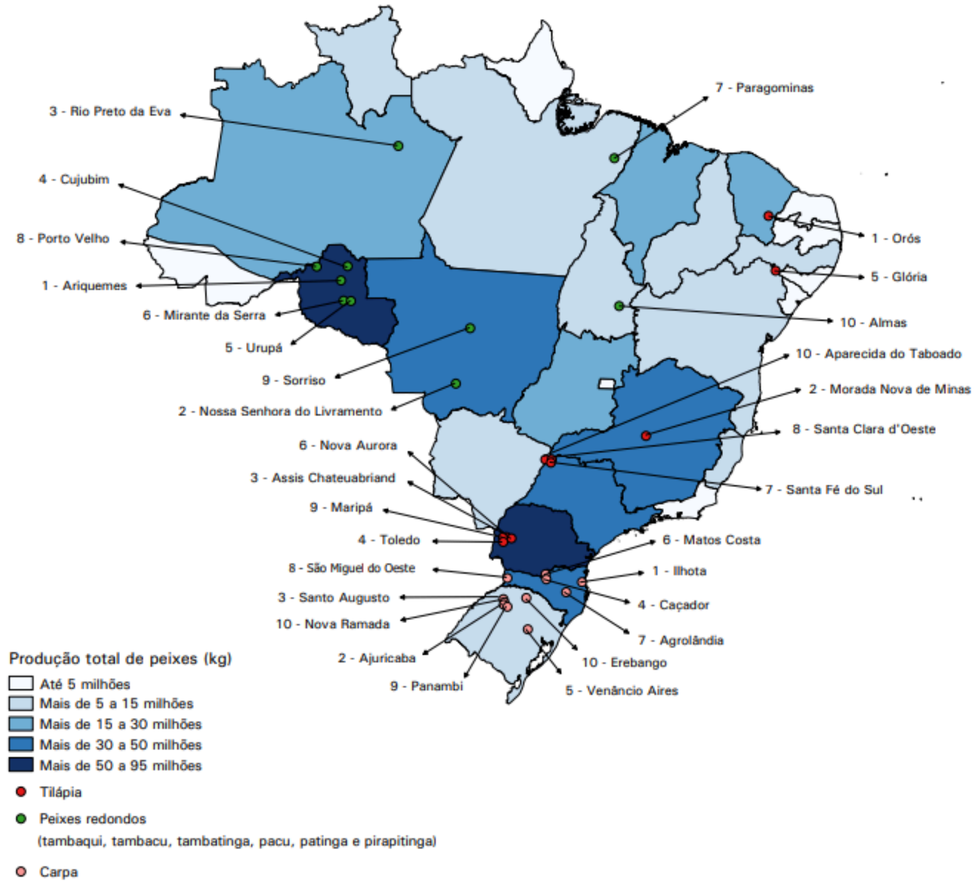
A aquicultura é o setor da agropecuária brasileira com maior crescimento nos últimos anos. Apesar disso, em 2016, o Brasil foi apenas o 14º maior produtor de pescados, com uma produção de pouco mais de meio milhão de toneladas (FAO, 2017), enquanto que a China é o maior produtor mundial, com aproximadamente 45 milhões de toneladas ao ano.

No cenário nacional, a piscicultura tem se destacado. Em 2016, foram produzidas 507 mil toneladas de peixes, sendo que a tilápia do Nilo (*Oreochromis niloticus*) foi a principal espécie cultivada, representando mais de 47% da produção total de peixes (IBGE, 2016). A piscicultura brasileira vem se especializando na criação e na exploração da tilápia devido, dentre outros motivos, às condições de boa adaptação a diferentes ambientes e pela grande aceitação comercial, tanto no mercado interno quanto externo.

Até o final da década de 1990, o cultivo de tilápia seguia um modelo semi-intensivo. Com a difusão da tecnologia de reversão sexual, a produção começou a ser incrementada e o pioneirismo coube ao Paraná, estado que iniciou a tilapicultura com foco industrial (SCHULTER & VIEIRA FILHO, 2017). Entretanto, foi a partir do ano 2000, com a Política Nacional de Recursos Hídricos (BUENO et al., 2015), que possibilitou o uso múltiplo dos reservatórios das hidroelétricas, que a tilapicultura consolidou-se como atividade comercial (Figura 1).

Desde então, o modelo intensivo de produção tem se desenvolvido no país, principalmente, por meio de tanques-rede (gaiolas) instalados em águas públicas federais e estaduais oriundas de usinas hidroelétricas, onde os peixes são confinados e alimentados com ração. Dentre as vantagens desse sistema de produção destacam-se a renovação contínua da água (sistema semi-aberto), menor variação dos parâmetros físico-químicos da água durante o cultivo, facilidade no manejo (despesca e controle da alimentação, por exemplo), produção escalonada num mesmo corpo d’água e produção em altas densidades, permitindo uma produtividade elevada (CARRIÇO et al., 2008). Por outro lado, levou a um aumento da condição de estresse dos animais (elevada densidade de estocagem e manejo intensivo), que resultou no aumento significativo da mortalidade de peixes e no surgimento de doenças emergentes, comprometendo o desenvolvimento sustentável da atividade (MENNERAT et al., 2010; PEELER & TAYLOR, 2011).

Em produções intensivas, os peixes podem ser cultivados em densidades até 1000 vezes superior do que em condições naturais, que significa um aumento no número de hospedeiros suscetíveis disponíveis e a maior probabilidade de um contato entre estes e hospedeiros infectados (CONTE, 2004; SHOEMAKER et al., 2000). Além disso, cria condições drasticamente diferentes daquelas que os patógenos experimentam em populações de hospedeiros selvagens, podendo alterar diversas características, inclusive a virulência (PULKKINEN et al., 2010).



Fonte: IBGE, Diretoria de Pesquisas, Coordenação de Agropecuária, Pesquisa da Pecuária Municipal 2016.

Figura 1. Principais municípios produtores de peixe no Brasil.

Apesar da escassez de dados sobre a frequência de doenças em tilápias, sabe-se que os principais problemas sanitários em fazendas brasileiras de tilápia do Nilo estão associados a infeções por bactérias. Dentre estas, destaque para as infecções por *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus iniae, Flavobacterium columnare, Aeromonas hydrophila, e Francisella noatunensis subsp. orientalis* (FIGUEIREDO et al., 2005; MIAN et al., 2009; CARVALHO-CASTRO et al., 2010; NETTO et al., 2011; LEAL et al., 2014; LEIRA et al., 2016; CHIDEROLI et al., 2017). Em todo o mundo, enfermidades bacterianas são motivo de constante preocupação, pois são responsáveis por elevadas taxas de mortalidade de peixes. Quando não levam à morte, provocam lesões que inviabilizam sua comercialização, causando grandes prejuízos econômicos. Contudo, casos de infecções múltiplas por diferentes patógenos, como bactérias, vírus e parasitas, são cada vez mais comuns em sistemas de produção intensivos e podem alterar o curso e a gravidade de diferentes doenças dos peixes (KOTOB et al., 2017; ASSIS et al., 2017), dificultando ainda mais o entendimento da dinâmica das doenças no ambiente produtivo. No que se refere às principais infecções virais que impactam a tilapicultura mundial, até o momento, não há relatos da ocorrência no Brasil.

Na aquicultura, a movimentação de animais vivos é considerada a principal via de introdução de patógenos, sendo que a maioria dos agentes responsáveis por doenças emergentes demonstraram ser originários de outra área geográfica ou de outra espécie hospedeira (OIDTMANN et al., 2011). No entanto, micro-organismos oportunistas também podem causar surtos inesperados. É importante destacar que muitos desses patógenos fazem parte do ambiente aquático e na presença de fatores predisponentes que aumentam a susceptibilidade dos animais aos agentes, condições típicas da aquicultura intensiva, podem causar doenças. A forma como os fatores ecológicos afetam a dinâmica populacional dos patógenos e contribuem para o surto de doenças precisa ser melhor investigada.

Nota-se que, apesar da rápida expansão da piscicultura no Brasil, a atenção aos aspectos sanitários não acompanhou essa evolução. Medidas mínimas de biosseguridade são adotadas e pouco esforço é feito no sentido de prevenir a introdução e disseminação de doenças. A maioria dos produtores de tilápia ainda não considera a presença de patógenos um fator relevante para o sistema de produção e acredita que a temperatura e a qualidade da água são as principais causas da mortalidade observada nas pisciculturas (RORIZ et al., 2017).

São muitos e urgentes os desafios para a melhor compreensão da interação patógeno-hospedeiro-ambiente no ambiente aquático, especialmente nos sistemas de produção intensivo. Para tanto, torna-se necessário avançar o conhecimento da ocorrência dos patógenos no sistema produtivo, entender quais fatores estão envolvidos no desenvolvimento de doenças e na interação entre diferentes patógenos, bem como identificar medidas de prevenção/controle. Isso ajudará na definição de ações que sejam eficientes e economicamente viáveis para a melhoria da saúde das pisciculturas, especialmente em cenários nos quais os recursos são escassos.

Os objetivos deste trabalho foram, então: (1) identificar os principais riscos sanitários que afetam cultivos de tilápia do Nilo localizados em reservatórios; (2) avaliar a viabilidade econômica da vacinação contra *S. agalactiae* em tilápias cultivadas em tanque-rede. Em virtude dos numerosos reservatórios existentes se encontrarem espalhados por vários estados brasileiros, o projeto foi realizado em apenas um reservatório, Três Marias, localizado no estado de Minas Gerais, por uma questão de logística e exequibilidade da proposta. Dentre os oito municípios do entorno desse reservatório, destaca-se Morada Nova de Minas, que, em 2016, apareceu em segundo lugar entre os maiores produtores de tilápia do país, com 8,74 mil toneladas, somente atrás do município de Orós, Ceará, na produção da espécie (IBGE, 2016). Os números de 2016 também colocaram Morada Nova de Minas no quarto lugar entre os maiores criadores de peixe do Brasil.

Portanto, no capítulo 1, será apresentado o estudo observacional longitudinal conduzido entre agosto de 2015 e outubro de 2016, no município de Morada Nova de Minas, e que teve como objetivo identificar as principais bactérias patogênicas que afetam o cultivo de tilápias em tanques-rede localizados em reservatórios, bem como caracterizar a dinâmica desses patógenos no sistema produtivo. Durante o acompanhamento, foram realizadas coletas mensais de peixes moribundos em seis das 32 pisciculturas existentes no município. Além disso, parâmetros relacionados à qualidade da água nas pisciculturas foram coletados mensalmente e dados de mortalidade e temperatura da água foram coletados diariamente pelos produtores, com o objetivo de explorar uma possível relação entre variáveis produtivas e ambientais com o aumento da incidência dos patógenos ao longo do *follow up*. No capítulo 2, é apresentado um modelo de orçamento parcial para avaliar o retorno econômico da vacinação contra *S. agalactiae*, em cultivos de tilápias em tanques-rede. Essa bactéria é considerada a enfermidade de maior impacto econômico nas criações comerciais de tilápia e a vacinação tem sido considerada um importante método na prevenção e controle da mortalidade. Para tanto, o modelo considerou três cenários epidemiológicos distintos (5%, 10% e 20% de mortalidade pré-vacinação). Em cada cenário, o resultado líquido da vacinação foi calculado considerando valores relacionados à eficácia da vacina e possível incremento na taxa de conversão alimentar.

CAPÍTULO 1

DYNAMICS OF BACTERIAL PATHOGENS OF NILE TILAPIA FARMED IN A BRAZILIAN RESERVOIR

Abstract

Fish aquaculture is rapidly growing in Brazil. Nile tilapia is the most cultivated species, mainly through an intensive system carried out in floating cages installed in large reservoirs. However, fish pathogens pose a major challenge to production chain sustainability, and tilapia farmers often have limited knowledge of prevailing health problems and rarely implement biosecurity practices to prevent introduction of economically important infectious agents. This study aimed to identify the key disease risks of tilapia farming in a tropical reservoir and characterize the dynamics of the prevalent pathogens, as a basis for development of effective control measures for tilapia health and surveillance programs. From August 2015 to October 2016, a longitudinal study was carried out at the, Três Marias reservoir, in the, municipality of Morada Nova de Minas in the southeast of Brazil. Daily and monthly data were collected from six out of 32 existing fish farms, including fish samples, mortality, temperature and water quality parameters. The main bacteria detected were *Streptococcus agalactiae,* infecting mostly adult tilapia throughout the period, with higher frequency as the average temperature increased, and *Francisella noatunensis* subsp. *Orientalis (*Fno), infecting mainly younger tilapia, only during the cooler months. Coinfections with multiple pathogens were detected in eight fish. The detection of Fno in one farm in two consecutive winters, after months of unfavorable water temperature conditions and without evidence of sustained introduction of infected stock, strengthens the case for investigating if this pathogen can survive and remain infective causing new outbreaks. Furthermore, variation in mortality was likely associated with the dynamics of the studied pathogens.

Keywords: Nile tilapia, *Francisella*, *Streptococcus*, daily mortality, observational study

Introduction

Aquaculture is the fastest growing food producing sector in the world and an important component in many poverty alleviation and food security programmes (FAO, 2017a). Tilapia aquaculture has become the second largest (by weight) farmed fish crop after the carps and are farmed in over 100 countries with the widest distribution of any farmed seafood (FAO, 2017b).

In Brazil, during the last 10 years, national aquaculture production (particularly tilapia) has grown 14% a year, emerging in the national animal protein production chain (IBGE, 2016). In 2015, Brazil was the 13th largest producer of fish from aquaculture, with a production of just over half a million tons (FAO, 2017b). Nile tilapia (*Oreochromis niloticus*) is the most cultivated fish species, with an annual production of 239,090 metric tons in 2016, accounting for almost 50% of national aquaculture production.

The growth of tilapia farming has been achieved due to the intensification of production systems and investments in the sector. The Brazilian federal government has given incentives for the installation of fish farming sites in reservoirs of hydroelectric power plants (5.5 million hectares of waters impounded in lakes and reservoirs), as a means to increase food production and promote social development (BUENO et al., 2015). There is a potential for at least 210 aquaculture parks, but only 36 are currently active (personal communication), including Três Marias reservoir, in the southeast of Brazil, where aquaculture started in 2001.

The municipality where the present study was conducted, Morada Nova de Minas, surrounds the Três Marias reservoir and is the second largest tilapia producer in the country (IBGE, 2016). In a recent study, RORIZ et al. (2017) described Três Marias reservoir as an emerging value-chain of small-scale farmers, with limited production infrastructure and investment. Local fish farmers did not apply effective biosecurity measures and were not able to identify the signs of specific diseases or to attribute a cause to them. The lack of technical assistance, undiagnosed diseases and the uncontrolled use of antibiotics were important issues that may impact on fish productivity and public health.

Fish diseases constrain production, with resultant socio-economic impacts for individuals, communities and economies which rely on aquaculture (ADAM & GUNN, 2017). Some important pathogens have been associated with disease outbreaks in farmed Nile tilapia in Brazil. Among the most important from an economic perspective are *Streptococcus spp.* (NETO et al., 2011; FIGUEIREDO et al., 2012*), Francisella noatunensis* subpsp. *orientalis*

(LEAL et al., 2014) and *Flavobacterium columnare* (FIGUEIREDO et al., 2005; BARONY et al., 2015).

Like others farming sectors, the likelihood of new, emerging and re-emerging diseases increases as aquaculture activities intensify and expand (BONDAD-REANTASO et al., 2005; WALKER & WINTON, 2010; OIDTMANN et al., 2011). One way to improve health management is to understand the production system events, possible risks and pathways for pathogen transmission, and to identify interventions that may lead to improvements in the health status of fish farms (BONDAD-REANTASO et al., 2005). Therefore, the objectives of this study were to: (1) identify the key health risks of tilapia farming in a tropical reservoir; and (2) characterize the dynamics of the prevalent pathogenic bacteria, in order to gather knowledge that can guide the development of effective disease control measures and surveillance programs.

Materials and methods

Study area

This study was carried out in the Três Marias reservoir, one of the largest in Brazil (1040 km^2 and volume of 21 billion m^3), and which offers good conditions for fish production (CODEVASF, 2015). It is located in the São Francisco River, in the state of Minas Gerais, and has boundaries with eight municipalities (18.212208, −45.262348). Morada Nova de Minas (MNM) is one of these eight municipalities and is considered the main tilapia floating cage producer in the state and the second largest in Brazil, with 8.74 thousand tons in 2016 (IBGE, 2016). In addition, it is the only municipality in the surrounding area hosting an office of the Company for the Development of the San Francisco and Parnaiba Rivers (CODEVASF), which made it possible to enlist the Company's personnel for logistic and operational support throughout the study period.

Based on a list provided by CODEVASF in 2015, there were 32 fish farms registered in their database operating in MNM, wherein all were rearing Nile tilapia in commercial floating cages. This study enrolled six out of 32 existing fish farms given the budget and logistic constraints for collecting and sending fish to the laboratory every month. Farms were selected based on results of a survey on producers' perceptions and attitudes towards health risks (Roriz et al., 2017). The choice of farms took into account the production scale (1 small, 4 medium and 1 large-scale farm), the geographical spread across the reservoir and the willingness of farmers to participate (Table 1). Figure 1 displays the location of the selected farms.

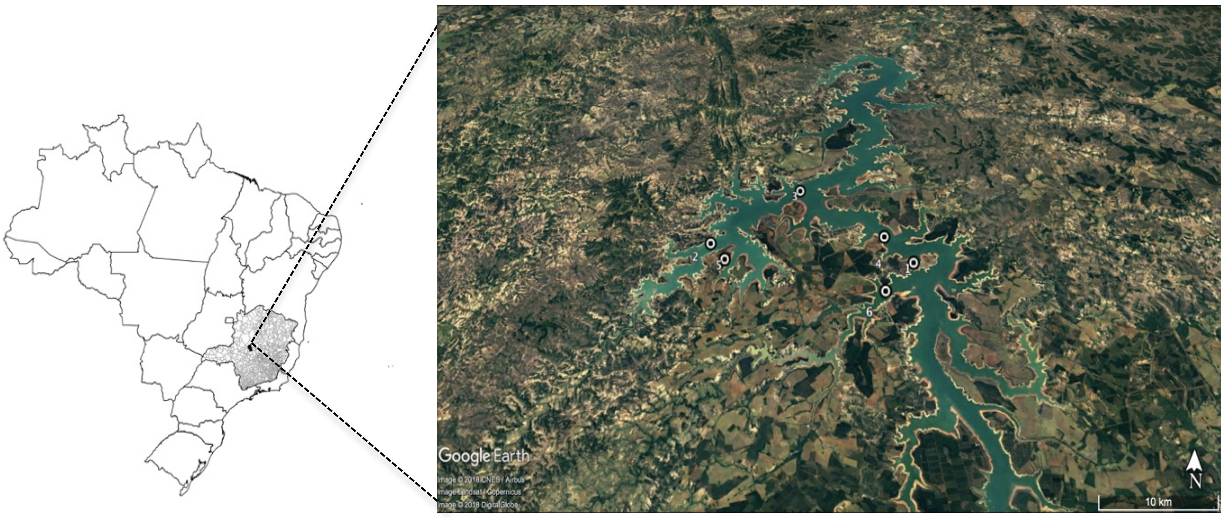


Figure 1. Location of the studied tilapia farms in Três Marias reservoir.

Table 1. Selected farms characteristics.

Farm	Number of cages (m x m x m)	Stocking density	Stocking (number of fish)	Harvest (kilograns)
1	6 (2x2x1.5) 60 (3x3x2)	84 fish/m^3 56 fish/m^3	40000	35000
2	185 (2x2x1.5) 36 (6x3x4)	92 fish/m^3 67 fish/m^3	45000	45000
3	60 (2x2x1.5) 11 (6x3x4)	84 fish/m^3 42 fish/m^3	6000	5000
4	51 (2x2x2) 251 (3x3x2)	69 fish/m^3 67 fish/m^3	60000	55000
5	39 (2,5x2,5x2) 42 (3x3x2) 21 (6x3x4)	88 fish/m^3 84 fish/m^3 70 fish/m^3	34000	30000
6	450 (2x2x1.5) 80 (3x3x2) 110 (6x3x4)	100 fish/m^3 84 fish/m^3 70 fish/m^3	140000	10000

Data collection

We conducted a longitudinal study from August 2015 to October 2016. The production site (fish farm) was considered the study unit because farmers operated a continuous system, introducing new batches of fingerlings and harvesting throughout the year. All sites produced tilapia using a continuous stocking system with multiple age classes on each site (typically, 1 to 8 months).

During the follow-up period farmers were responsible for daily collection of water temperature and mortality data. All data were collected at the farm level, since farmers did not record cage-level data. Furthermore, once a month, fish samples and water quality data were collected by our research team. At each sampling site, water quality data were determined *in situ* using a calibrated Horiba water quality multi-parameter (Horiba Instruments Inc., Irvine, CA, USA), which included reservoir depth, water pH, water turbidity, dissolved oxygen, total dissolved solids and redox potential, measured at one sampling point surrounding the cages, at a 1m depth. Only "fish weight" and "bacteria detected" were recorded at the individual level for every fish at each sampling round.

Temperature data

Daily temperature was registered by farm workers who used their own mercury thermometers. Each site had a different frequency of collecting temperature data. So, we used the average daily temperature for those who checked more than once. In addition, we calculated the average monthly temperature as well as the maximum and minimum recorded.

Mortality data

Mortality data were collected daily and per farm, not per cage. Although the fish population in this production system might be considered open, we decided to calculate the cumulative mortality (reported as percentage), since fluctuation of number of fish is minimal and does not significantly alter the population-at-risk over one month (DOHOO et al., 2003). The daily mortality rate (MR_D) was calculated as mortality per 100 000 fish days (HAMMELL & DOHOO, 2005). The numerator is the number of dead fish per day (n_{mort_day}) and the denominator is the farm population at risk per day (N_{risk}). Therefore, farm population at risk per day refers to the total number of fish kept per month as indicated by each farm. All mortality rates were expressed on a daily basis.

$$MR_D = n_{mort\_day} \text{ x } N^{-1}_{risk\ per\ day} \text{ x } 100\ 000d^{-1}$$

Two different types of information were found in the database for the 6 farms: entries with zero and missing records for some of the days. The former indicated that the site had been inspected by the farmer and no dead fish were found, whereas the latter was treated as missing data because there had been no inspection on that day.

Fish sampling

The population within the farm was considered to be homogenous with regard to the risk of infection. The sample size comprised at least 30 fish per farm to allow for the detection of at least one positive individual with 95% confidence, should a pathogen be present in at least 10% of the population and assuming perfect sensitivity of tests used (Cameron & Baldock, 1998). Sampling was targeted at moribund fish and those with clinical signs of infectious diseases (melanosis, exophthalmia, erratic swimming, skin ulcers, gill palor and others), with a view to increase the sensitivity of detection. Animals were euthanized according to a method approved by the Ethics Committee on Animal Use (CEUA) of the University of Brasilia, UnBDoC n. 52858/2016, and specimens were sent to the AQUACEN laboratory on ice within 48h of collections.

Diagnostic methods

All laboratory testing was carried out at AQUACEN, the national reference laboratory for the Official Veterinary Services, located at the Federal University of Minas Gerais (UFMG).

Bacterial isolation

Immediately after the arrival of the fish in the laboratory, each fish was weighed, washed with neutral detergent to remove the surface mucus and submitted to surface disinfection with 2% iodine in 70% ethanol solution. For bacteriology broad isolation, swabs of brain and kidney were aseptically sampled and streaked onto 5% sheep blood agar (SBA). Similarly, samples of kidney and spleen were aseptically sampled and streaked onto cysteine heart agar supplemented with 2% bovine hemoglobin (CHAH; BD Biosciences, Bedford, MA, USA) for *Francisella noatunensis* subsp. *orientalis* (Fno) isolation. All plates were incubated at 28°C for 2 and 7 days, respectively.

Bacterial identification

A fish was considered positive when the bacteria were detected in at least one of the organs. Colonies were identified by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry with a Bruker Microflex MALDI Biotyper 2.0 (Bruker Daltonics) as previously described (ASSIS et al., 2017a). Briefly, a fresh single colony of each bacterial strain was spotted using a toothpick into a target steel plate and applied 1 µL of formic acid (70%) and 1 μL of MALDI-TOF MS matrix (α-cyano-4-hydroxycinnamic acid) (Bruker Daltonics, Bremen, Germany). After air-dry, the plate was placed into the Microflex and the vaccum was established. Spectra were acquired using the FlexControl MicroFlex LT mass spectrometer (Bruker Daltonics). The device parameters were in the standard format. Prior to measurements, calibration was preceded with a bacterial test standard (*E. coli* DH5 alpha; Bruker Daltonics). The Real Time (RT) identification score criteria used were those recommended by the manufacturer: score>=2.000 indicates a species-level identification, score>=1.700 and <2.000 indicates a genus-level identification, and a score<1.700 indicates no reliable identification.

Data analysis

Data registered on paper records were stored and verified in an Excel spreadsheet (Microsoft Corporation, Redmond, Washington). Descriptive statistics were generated to describe mortality patterns, water temperature, water quality and bacteria detected throughout the follow-up period. Scatter plots were used to explore the relationship between the response ("mortality") and explanatory variables. The chi-square test was applied to explore the relationship between the variables "fish weight" and "bacteria isolated". The statistical analysis was performed using Stata®version 14 (StataCorp 2015, College Station, TX, USA).

Results

Database description

Although the follow-up period covered 15 months (from August 2015 to October 2016), fish collection and water quality parameters were carried out only in 12 months. The author responsible for the collection (Delphino) was unable to go to MNM in February, May and September 2016. In addition, in August 2015, only farms 1 and 5 were able to provide fish samples. In spite of this limitation, we decided to present all findings regarding detection of fish pathogens. However, with respect to data collected daily by the producers, we have excluded Farms 1 and 5 from the analysis. Farm 1 did not provide any data and Farm 5 provided data only for four months.

Water quality parameters

Water quality parameters are presented in Table 2. The recorded values in all farms were within the normal standards for tilapia culture, as stated in the Resolution CONAMA (National

Council for the Environment in Brazil) no. 357 of 2005, throughout the follow-up period. Therefore, we did not carry out an analysis to investigate the association between mortality and the water quality parameters.

Although most farmers believe that environmental factors are the main cause of fish mortality, rather than infectious agents (RORIZ et al., 2017), these results rule out water quality as an important factor for fish mortality in the studied farms.

Table 2. Mean ± SD of water quality parameters throughout the follow-up period.

Parameters	**Farm 2**	**Farm 3**	**Farm 4**	**Farm 6**
pH	6.39±0.56	6.48±0.60	6.50±0.70	6.33±0.58
Dissolved oxygen (mg/L)	7.11±0.68	7.10±0.72	7.03±0.75	7.45±0.61
Depth (m)	14.45±3.67	13.98±4.25	8.79±3.82	15.18±3.98
O.R.P.[a] (mV)	300.9±79.5	319.4±68.1	307.4 ± 65.1	315.6 ± 81.5
T.D.S[b] (g/L)	0.051 ± 0.002	0.054 ± 0.002	0.055 ± 0.003	0.054 ± 0.003

[a]Oxidation Reduction Potential. [b]Total dissolved solids.

Temperature

MNM has a tropical climate with rainy summers and dry winters. Variation in temperature followed the same pattern at all farming sites during the follow-up period (Fig. 2), as expected (https://en.climate-data.org/). The warmest period was registered from October to April, when the mean water temperature was 28ºC or above, ranging from a minimum of 25ºC to a maximum of 32ºC (Table 3). February was the hottest month. The coldest period was registered from May to August, when the mean water temperature was less than 26ºC, ranging from a minimum of 22ºC to a maximum of 28ºC. September had the widest range of temperature measurements.

Table 3. Overall mean, minimum and maximum daily temperature (ºC) over the follow-up period.

	2015				2016									
	Sep	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Mean	26.4	28.2	28.8	28.7	28.1	29.1	28.9	28.4	25.9	23.7	22.8	23.3	26.4	28.0
Min	23	25	26	26	26	26	26	26	24	22	22	22	23	26
Max	31	31	31	30	30	32	31	30	28	26	24	25	32	31

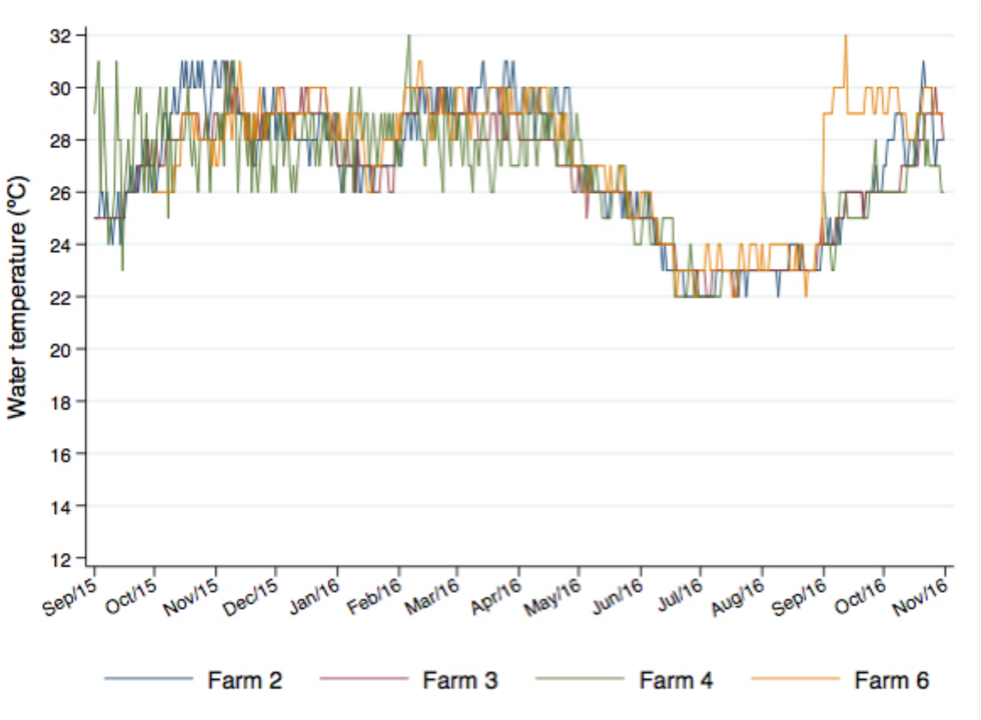


Figure 2. Mean daily water temperature from September 2015 to November 2016 for each farm.

Frequency, seasonal and age patterns of isolated bacteria

A total of 2233 fish were sampled throughout the follow-up period, from the six farms. Sampled fish usually exhibited clinical signs consistent with bacterial infections but not specific to any single infection. The main clinical signs were lethargy, corneal opacity, exophthalmia, erratic swimming, melanosis and ascites. A total of 504 (22.6%) were culture-positive for at least one bacteria. Table 4 shows that the most frequent were *Streptococcus spp.* and *Francisella noatunensis* subsp. *orientalis* (Fno). It is worth noting that we detected 33 cases of coinfection by two or three bacteria (Table 5).

The most important pathogen of Nile tilapia in Brazil, *S. agalactiae*, was isolated throughout the follow-up, though with higher frequency during the warmer season, when water temperature was above 28°C. In contrast, Fno was isolated only during the colder season, when water temperature was below 26°C. *S. agalactiae* was isolated from all six farms throughout the year, whereas only three fish farms were positive for Fno (Fig. 3).

Table 4. Species, number and frequency (%) of isolated bacteria and total number of sampled fish during the follow-up.

Bacteria	**Farm 1 (n=353)**		**Farm 2 (n=377)**		**Farm 3 (n=384)**		**Farm 4 (n=356)**		**Farm 5 (n=425)**		**Farm 6 (n=338)**		**Total (n=2233)**	
	n.	**%**	**n.**	**%**	**n.**	**%**	**n.**	**%**	**n.**	**%**	**n.**	**%**	**n.**	**%**
S. agalactiae[a]	42	12	71	19	60	16	58	16	35	8	57	17	323	15
FNO[b]	0	0	15	4	0	0	10	3	46	11	0	0	71	3
S. iniae[c]	8	2	14	4	3	<1	6	2	8	2	4	1	43	2
A. hydrophila[d]	1	<1	11	3	0	0	3	<1	10	2	4	1	29	1
P. shiguelloides[e]	3	<1	2	<1	4	<1	6	2	3	<1	9	3	27	1
E. tarda[f]	7	2	3	<1	6	2	1	<1	3	<1	3	<1	23	1

[a]*Streptococcus agalactiae*, [b]*Francisella noatunensis subsp. orientalis*, [c]*Streptococcus iniae*, [d]*Aeromonas hydrophila*, [e]*Pleisiomonas shiguelloides*, [f]*Edwardsiella tarda*.

Table 5. Number of coinfections during the follow-up period.

Coinfections	**Number**	**Farm source**
S. agalactaie and *S. iniae*	8	1, 2, 2, 2, 4, 4, 6, 6
S. agalactiae and *Fno*	1	5
S. agalactiae and *E. tarda*	2	2, 3
S. agalactiae and *A. hydrophila*	2	6, 6
S. agalactiae and *P. shiguelloides*	2	3, 3
S. iniae and *A. hydrophila*	4	2, 5, 5, 6
S. iniae and *Fno*	2	2, 2
S. iniae and *E. tarda*	1	1
E. tarda and *P. shiguelloides*	2	5, 6
S. agalactiae and *A. hydrophila*	1	4
S. iniae and Fno and *A. hydrophila*	1	2

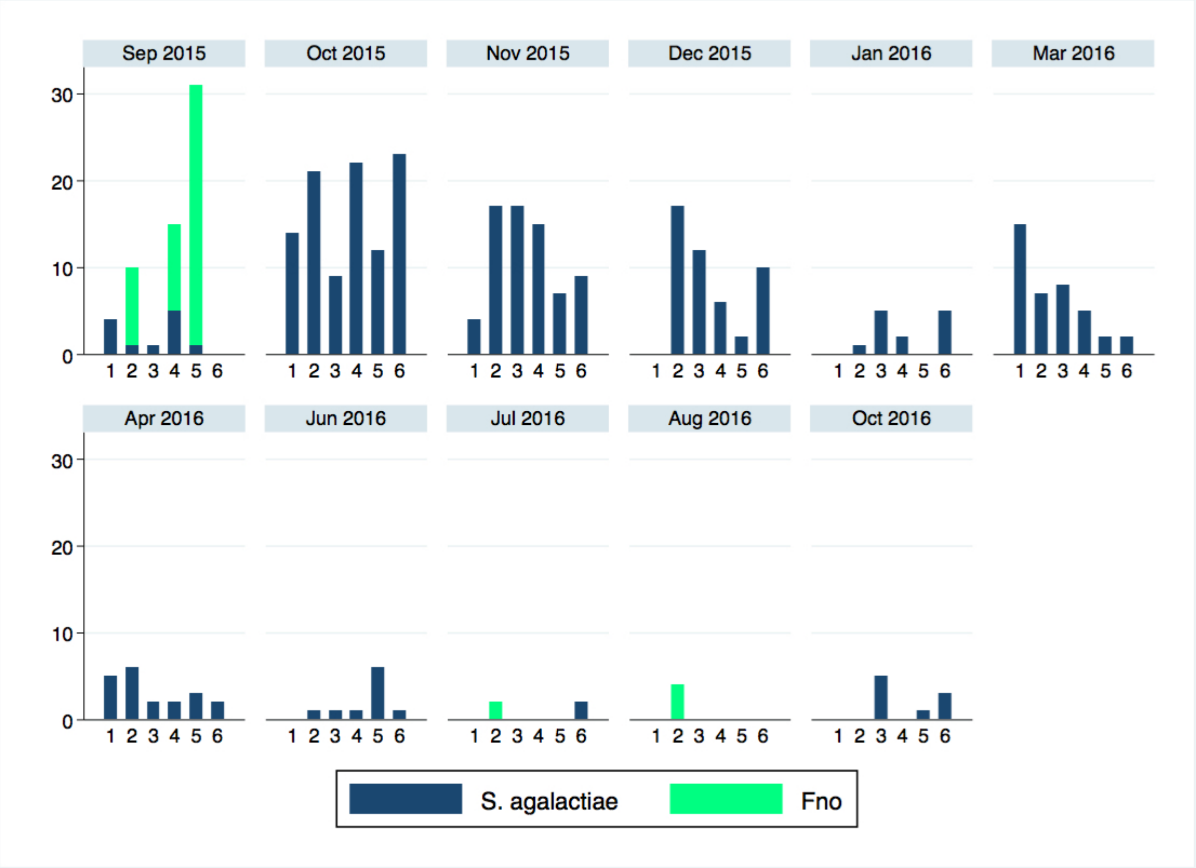


Figure 3. Number of isolates of the two most frequent bacterial species over the follow-up period.

We used fish weight as a proxy variable for age in order to investigate its association with the isolated pathogens, notably *S. agalactiae* and Fno. The continuous variable "weight" was transformed into a categorical variable and then tested for each detected species of bacteria. A significant statistical association between “species” and “weight” was found only for Fno and *S. agalactiae* (Table 6).

These results suggest that *S. agalactiae* infection involves mainly adults (>100g), although the body weight of infected fish ranged from 14g to 1126g. The frequency of Fno infection was higher in younger (up to 100g) stock, ranging from 5g to 1338g.

Table 6. Number (%) within each category of fish weight, using χ^2.

Variables	***S. agalactiae***			**Fno**		
	Negative	**Positive**	**χ^2 value (p-value)**	**Negative**	**Positive**	**χ^2 value (p-value)**
Weight						
≤100g	757 (90.2)	82 (9.8)	35.2 (<0.0001)	792 (94.4)	47 (5.6)	18.9 (<0.0001)
>100g	962 (80.6)	232 (19.4)		1170 (98.0)	24 (2.0)	

Mortality data

Because of the high frequency of missing data, mortality data from Farm 1 and Farm 5 were excluded from the analysis. In addition, was not possible to calculate mortality per production cycle, since data were collected at a farm level and not at cage level.

Fish mortality

Table 7 displays the calculated daily mortality rates, expressed as mean, median, percentiles and peak, for all farms over the whole follow-up period. The mean and median daily mortality rate (MR_D) were 52 and 31 deaths per 100 000 fish, respectively.

Among the farms, Farm 2 presented the highest mean MR_D, while Farm 6 had the highest median MR_D. These results suggest a larger overall mortality in Farm 6, compared to others farms, and a high mortality variability in Farm 2, probably influenced by the existence of days with exceptionally high mortality. Farm 3 and Farm 4 presented median and mean daily mortality below the overall values.

Mortality was higher from October to December, coinciding with the beginning of the hot and rainy season (Fig. 4). Conversely, the mean and median MR_D were 94 and 84 deaths per 100 000 fish, respectively. The lowest mortality rates were observed during colder months, from May through August 2016, when mean and median MR_D were 51 and 21 deaths per 100 000 fish, respectively.

Figures 5 and 6 display the mortality peaks on Farm 6 (hot season) and Farm 2 (cold season). The latter reached 2278 deaths in a single day.

Table 7. Farm-level daily mortality rates per 100 000 fish, for all farms over the whole follow-up period.

Farm	**Percentiles**					**Mean**	**Peak**	**Number of observations**
	25%	**50%**	**75%**	**90%**	**99%**			
2	15	32	85	143	591	71	2278	427
3	9	23	48	111	291	42	426	427
4	7	16	37	53	158	28	210	385
6	34	53	77	99	447	64	879	397
Overall	**13**	**31**	**59**	**105**	**238**	**52**	**2278**	**1636**

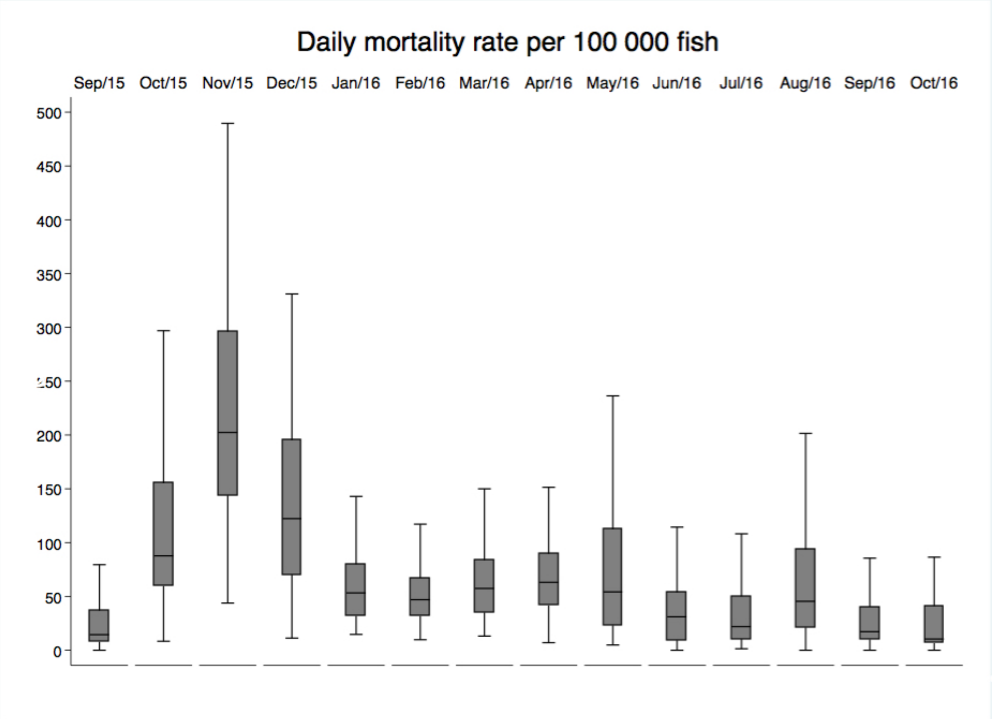


Figure 4. Overall daily mortality rate throughout the follow-up. Outliers were not plotted.

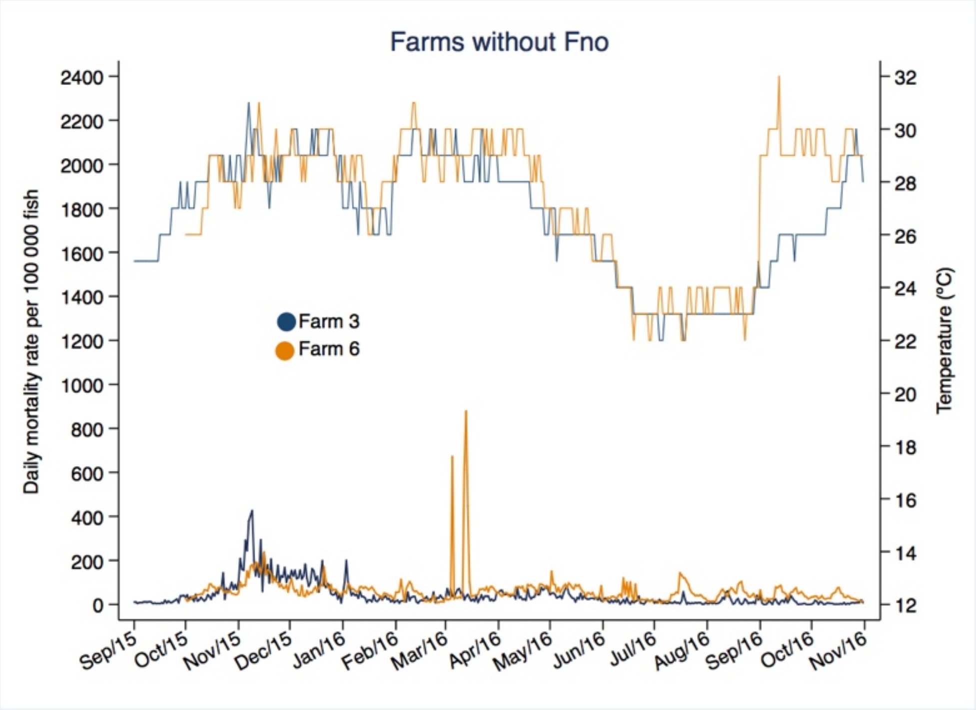


Figure 5. Daily mortality rate and water temperature (ºC) in farms without Fno over the folllow-up period.

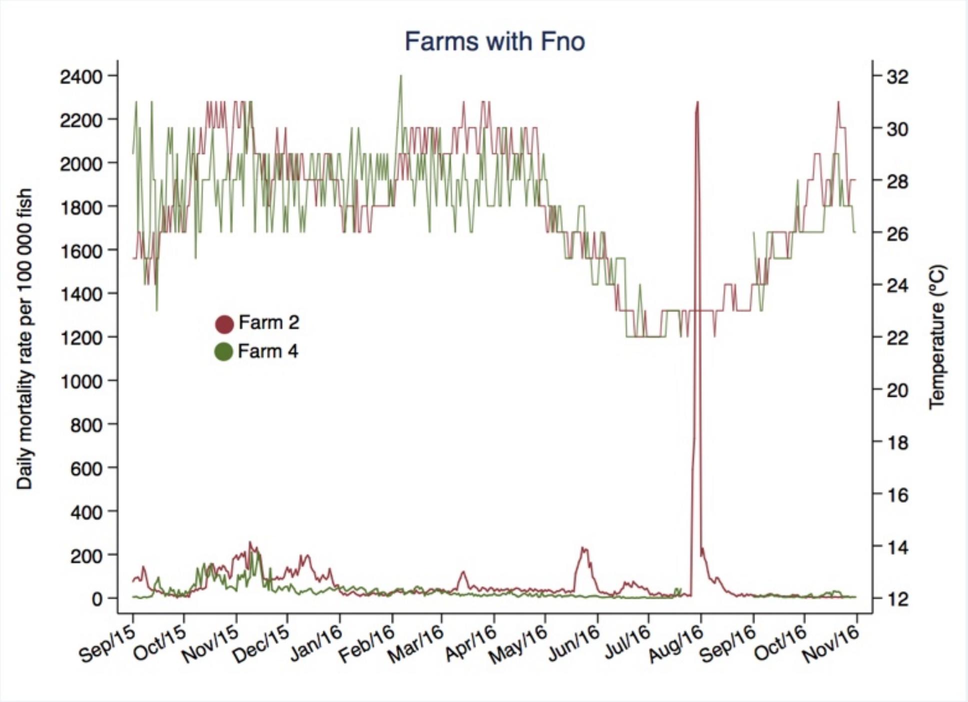


Figure 6. Daily mortality rate and water temperature (ºC) in farms with Fno reports over the folllow-up period.

Discussion

The majority of fish farmers in MNM do not keep regular and reliable fish mortality records (RORIZ et al., 2017), even though the identification of unusual mortality losses may help fish producers identify and control health and management problems or as a warning sign in surveillance programs (SOARES et al., 2012; JIA et al., 2018).

Although there are several reports describing mortality rates during disease outbreaks (SUANYUK et al., 2008; MIAN et al., 2009; ASSIS et al., 2017a; CHIDEROLI et al., 2017), to the best of our knowledge, this is the first longitudinal study describing mortality data on Nile tilapia farms in Brazil. There is a case for future studies that can identify "abnormal mortality rates", especially at cage-level, that can be used as alerts for surveillance and disease control.

We detected several bacteria known to be pathogenic to Nile tilapia, such as *A. hydrophyla, A. jandaei*, *A. veronii, A. caviae*, *E. tarda* and *S. iniae* (PARK et al., 2012; DONG et al., 2017) but the species isolated with higher frequency were the Gram-positive *S. agalactiae* (64% of the isolates) and the Gram-negative *F. noatunensis* subsp. *orientalis* (14% of the isolates). These bacteria are considered the major health constraints to farmed Nile tilapia, contributing to severe economic losses worldwide (SOTO et al., 2009; SOTO et al., 2011a; COLQUHOUN & DUODU, 2011; CHEN et al., 2012). In Brazil, *S. agalactiae* is considered endemic in Nile tilapia farms and has been described since 2006 (FIGUEIREDO et al., 2006). Fno is an emerging bacterial pathogen still restricted to certain geographic regions (Southeast and South of Brazil), infecting tilapia floating cages farms since 2012 (LEAL et al., 2014; SEBASTIÃO et al., 2017). While much is known about fish diseases caused by the pathogens found in this study, there is a need to gain insights into the seasonal patterns of the occurrence of such bacteria in specific environments.

We also detected 33 cases of coinfection, including two adult fish coinfected by *S. iniae* (384g and 422g) and Fno and one adult fish coinfected by *S. iniae*, Fno and *A. hydrophila* (849g). This is the first report of natural coinfection by these pathogens in farm-raised Nile tilapia. Coinfections might have been triggerred by a combination of distinct predisposing factors, such as water temperature and stress caused by high stocking density and frequent handling, although our exploratory study does not allow for the investigation of such causal hypotheses.

The species *S. agalactiae* was isolated from all six farms throughout the year, affecting mainly adult fish, with higher frequency in warmer months, when mean water temperature was above 28°C. Similarly, the highest cumulative mortality was observed from October to December 2015, the beginning of the hot and wet season in MNM. This is in agreement with previous studies that reported increased temperature associated with *S. agalactiae* outbreaks (BROMAGE & OWENS, 2009; MIA et al., 2009; KAYANSAMRUAJ et al., 2014; AMAL et al., 2015). There were mortality peaks (e.g., Farm 6), possibly related to the presence of *S. agalactiae* under stress conditions, and the mortality pattern observed throughout the year, persistent and ranging from low to high, is consistent with an endemic status of *S. agalactiae*.

In October 2016, the frequency of *Streptococcus* isolates was much lower. This may have resulted from the use of a commercial vaccine, AQUAVAC®Strep Sa (MSD Animal Health, Brazil), in Farms 2 (started in March 2016), 4 (started in September 2016) and 6 (started in June 2016). This product is an inactivated oil-adjuvanted vaccine administrated by intraperitoneal (IP) injection that provides specific protection against the serotype Ib strains of *S. agalactiae*, the most prevalent *Streptococcus* strain in Brazil. The use of a vaccination plan should be an important alternative to be considered by the producers. Some studies have documented positive results using vaccines against endemic streptococcosis (LIU et al., 2016; MUNANG'ANDU et al., 2016; ISMAIL et al., 2017), but its financial worth should be assessed. The efficacy of *S. agalactiae* vaccine to Nile tilapia seems to be linked to strain specificity and Brazilian strains of *S. agalactiae* were shown to be heterogeneous in their genome sequences, as well as distributed in different regions of the country. Further studies should assess the genetic diversity of isolates from Morada Nova de Minas, in order to tailor strategic control measures (BARONY et al., 2017).

Fno was detected only in cooler months, when mean water temperature was below 25ºC, affecting mainly fingerlings and juveniles (up to 100g). Our results are consistent with previous studies that associated lower water temperature with the occurrence of francisellosis in Nile tilapia (SOTO et al., 2012; LEAL et al., 2014). Only three out of six studied farms were positive for Fno (Farms 2, 4 and 5) and only Farm 2 was positive for Fno in the consecutive winters of 2015 and 2016. However, these results should be interpreted with caution because Fno may have present in other farms throughout the follow-up period, albeit at rates that could be lower than the detectable threshold prevalence. It should be stressed, however, that Farm 2 had an outbreak in the winter of 2016, probably related to the presence of Fno, whereas the other two showed no mortality peaks in the winter of 2016. This may have happened because: (1) Fno is regularly introduced into the farms; or (2) Fno persisted in the fish or the environment without being detected by our sampling scheme and testing procedures.

Although the most important pathway of pathogen introduction into freshwater fish farms is via introduction of infected live fish (subclinical) onto the farm (OIDTMANN et al., 2011; OIDTMANN et al., 2013), the first option is not very likely because the two hatcheries operating in MNM tested negative for Fno throughout the follow-up period, according to data of the hatcheries' bacteriological monitoring program carried out by the Ministry of Agriculture, Livestock and Food Supply (MAPA). Notwithstanding, ASSIS et al. (2017b) found that direct qPCR using fresh or ethanol-fixed tissues (technique used by MAPA) has a sensitivity of about 50% to detect Fno in subclinical tilapia. Therefore, it is not possible to rule out the introduction of a batch of Fno-false negative fingerlings.

Transmission between cultivated tilapia and wild species cannot be ruled out either (TAMARU, et al., 2011). The efficacy of *S. agalactiae* vaccine to Nile tilapia seems to be linked to strain specificity and Brazilian strains of *S. agalactiae* were shown to be heterogeneous in their genome sequences, as well as distributed in different regions of the country. Further studies should assess the genetic diversity of isolates from Morada Nova de Minas, in order to tailor strategic control measures (BARONY et al., 2017).

The persistence of Fno in fish or in the environment, is enhanced by the ability of this bacterium to undergo and remain in a "viable but non-culturable" (VBNC) state after a period in unfavorable environments (SOTO & REVAN, 2012), as well as the possibility of forming biofilm (SOTO et al., 2015). Other species from this genus (e.g *Francisella tularensis* and *F. noatunensis* subsp. *noatunensis*) have been found to enter viable but non-culturable states (FORSMAN et al., 2000; DUODU & COLQUHOUN 2010). Under these states, Fno culturability decreases rapidly and bacteriological culture performance may be lower (ASSIS et al., 2017b). This would be consistent with Fno being present but not detected by culture in the warmer months. Moreover, despite SOTO & REVAN (2012) reporting that pathogenic properties of the Fno suspended in water microcosms appear to decrease after only 24h and become non-infective after two days in the absence of the fish host, continuously presence of susceptible animals can be favorable to the disease persistence. Although this seems like a relatively short period of time, the high pathogenic capacity of this bacteria makes this short period of time sufficient to colonize, infect and cause high levels of morbidity and mortality.

Even more surprising, was the possibility of Fno to remain infective and lead to new outbreaks after long periods under unfavorable conditions, even though DUODU & COLQUHOUN (2010) reported VBNC fish pathogenic bacteria as non-virulent cells, at least in experimental conditions. Thus, we should not ignore the hypothesis of this pathogen to enter and recover from VBNC state, when provided with the necessary nutrients and environmental conditions, and to regain its virulence under suitable conditions. Further research into the persistence of viable but non-culturable bacteria and the virulence of Fno biofilms is warranted.

Our results highlight the importance of buying fingerlings from accredited hatcheries and the need for of a strict control of the movement of live aquatic animals. With regard to reservoirs, which involve public money and public waters, the fingerlings monitoring program should become a high priority action for aquatic animal health programs. No commercial vaccine against fish francisellosis is currently available. However, attempts using attenuated mutant strains of *F. noatunensis* subsp. *orientalis* have provided promising results in tilapia (SOTO et al., 2011b). Establishing a fallow period between fish production cycles may be considered an alternative strategy to reduce or eliminate pathogenic organisms. However, the effectiveness of this practice for controlling tilapia diseases has not been assessed and the costs and benefits of such practice would need to be appraised before recommending it in specific production environments.

Conclusion

Several pathogenic bacteria were isolated from Nile tilapia raised in the Três Marias reservoir, including cases of single and multiple infection. Not surprisingly, temperature appeared to have an important role in the development of clinical cases of *S. agalactiae* and Fno, the main pathogenic bacteria isolated. *S. agalactaie* occurred with higher frequency in warmer months and the latter was more likely to be isolated when water temperature is lower. While *S. agalactiae* was endemic and widespread in the studied farms, Fno seemed to have been limited to a few farms. This was the first observational study that described the seasonal dynamics of *F. noatunensis* subsp. *orientalis*. There is a case for further studies into the mechanisms of Fno maintenance and spread, its real impact on production and how to prevent it from causing disease.

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CAPÍTULO 2

ECONOMIC EVALUATION OF VACCINATION AGAINST *STREPTOCCOCUS AGALACTIAE* IN NILE TILAPIA FARMS

Abstract

Streptococcus agalactiae causes mortality and major economic losses in Nile tilapia (*Oreochromis niloticus*) farming worldwide. In Brazil, serotype strains Ia, Ib and III have been isolated in streptococcosis outbreaks, but serotype Ib is the most frequent. Vaccination is considered an effective method to prevent economically-important diseases in aquaculture and has been associated with decreased usage of antibiotics and improvements in fish survival. We developed a simple and flexible partial-budget model to undertake an economic appraisal of the vaccination against *Streptococcus agalactiae* in Nile tilapia farmed in net cages in large reservoirs. The model considers the benefits and costs that are likely to occur in one production cycle (time for fish to reach the marketable size), because of the proposed intervention. We analyzed three epidemiological scenarios of cumulative mortality due to *S. agalactiae* (5%, 10% and 20%, per production cycle) in a non-vaccinated farm. For each scenario, we calculated the net return (benefits – costs) of vaccination, given a combination of values of "vaccine efficacy" and "gain in feed conversion ratio", in order to model uncertainty about the true value of such parameters. Results indicate that vaccination against *S. agalactiae* is likely be profitable in Nile tilapia farms, although in scenarios where cumulative mortality is lower than 10%, the profitability of vaccination would be more dependent on higher vaccine efficacy and/or better feed conversion rate.

Keywords: Nile tilapia; Streptococcus agalactiae; vaccine; economics; partial budget.

Introduction

Streptococosis, caused by *Streptococcus agalactiae* (Lancefield's group B Streptococcus, GBS) frequently leads to high mortality and economic losses to tilapia farming worldwide. Inapparent *S. agalactiae* infections in adult/commercial tilapia have been reported causing consistently low death rates that eventually lead to substantial cumulative mortality (SUN et al., 2016). This disease is a major constraint to the productivity of Brazilian aquaculture, affecting Nile tilapia (*Oreochromis niloticus*), the most frequently farmed fish in the country.

Although there are 10 serotypes described for this pathogen, the serotypes Ib, Ia and III are the most common fish pathogens worldwide. In Brazil, serotype Ib is the most frequent, although strains of serotype Ia and, more recently, serotype III have been isolated in streptococcosis outbreaks (CHIDEROLI et al., 2017). Knowledge of circulating *S. agalactiae* serotypes in a country can inform control measures to avoid the spread of specific bacterial lineages (BARONY et al., 2017).

Vaccination is considered a suitable method to control streptococcosis in fish and has been associated with decreased usage of antibiotics (BRUDESETH et al., 2013) and improvements in fish survival rate (LIU et al., 2016; ISMAIL et al., 2017). It also plays an important role in large-scale commercial fish farming and has played a key role in the success of salmon cultivation (SOMMERSET et al., 2005). In Brazil, since 2011, a commercial adjuvant based inactivated vaccine for the control of *S. agalactiae* is available.

The financial evaluation of a disease control measures, such as vaccination, allows farmers to weigh the losses against the costs of the intervention. However, economic analysis of fish health interventions remains a relatively neglected field. The objective of this study was to provide farmers with a simple and flexible method to decide on the economic worth of vaccination against *Streptoccocus agalactiae* in Nile tilapia farmed in net cages in large reservoirs in Brazil.

Methods

We used a deterministic model to undertake a partial budget analysis of the vaccination against *Streptococcus agalactiae* in Nile tilapia. The vaccine considered in this study is AQUAVAC® Strep Sa (MSD Animal Health, Brazil), the only commercial vaccine available for streptococcosis control in Brazil. It is a vaccine made of inactivated *S. agalatiae* Serotype Ib and administrated to fish by intraperitoneal injection. The model does not take time into account and considers the benefits and costs that are likely to occur in the new steady-state, as a result of the proposed intervention.

The partial budget model was built using an Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) and divided into four sections as proposed by RUSHTON (2009): (a) new revenues, (b) costs saved, (c) revenue foregone, and (d) new costs. To assess the financial worth of such investment, we calculated the difference between benefits (a + b) and costs (c + d). If the marginal benefits exceed the marginal costs, then it would be advantageous for the farmer.

The costs and benefits of vaccination were calculated at the farm level for one production cycle. A small-scale Nile tilapia farm was used as an example. In this farm, fish are raised in floating cages year-round and a batch of 20 thousand fingerlings is introduced every month. The average duration of the production cycle (time for fish to reach the marketable size) is 6-7 months and the annual production is about 170 tonnes.

We analyzed three epidemiological scenarios of cumulative mortality attributable to *S. agalactiae* (5%, 10% and 20%, per production cycle) in a non-vaccinated farm. For each scenario, we calculated the impact of a combination of changes in “vaccine efficacy” and “gain in feed conversion ratio”. The values assigned to all modeled variables were based on information provided by Brazilian Confederation of Agriculture and Livestock (CNA), vaccine resellers, published manuscripts and personal communication with experts (Table 1 and Table 2). Vaccine efficacy was estimated using previous reports (LIU et al., 2016; MUNANG’ANDU et al., 2016).

Table 1. Value of inputs used in the economic model of vaccination of tilapia against *S. agalactiae*.

Input	**Non-vaccinated**	**Vaccinated**
Batch size in number of fish (N_{Fish})	20000	20000
Mortality due to *S. agalactiae* without vaccination (M_{Strep})	10%	-
Relative Percent Survival (*RPS*) of vaccinated fish	-	80%
Mortality due to *S. agalactiae* after vaccination in number	2000	400
Number of fish survival to market	18000	19600
Average feed conversion ratio (*FCR*)	1,6	1,52
FCR gain in a vaccinated farm	-	5%
Fish weight at vaccination time in kilogram	0,03	0,03
Fish weight at harvest in kilogram ($W_{Harvested}$)	0,85	0,85
Total weight gain in kilogram	14769	16072
Average weight of dead fish in kilogram	0.25	0.25
Total feed consumption in kilogram	24480	24594
Average weight of treated fish by TM-700 in kilogram ($\mathrm{W}_{\mathrm{Treated}}$)	0.25	0.25
Average feed price per kilogram ($Feed_{Price}$)	R$2.50	R$2.50
Average fish market price ($Fish_{Price}$)	R$5.20	R$5.20
Cost of oxytetracycline[a] per kilogram ($C_{Atb\text{-}TM700}$)	R$0.10	
Cost of florfenicol[b] per kilogram ($C_{Atb\text{-}Aquaflor}$)	R$0.38	
Cost of vaccine dose per fish ($C_{Vaccine}$)		R$0.11
Cost of vaccine labour per fish ($C_{Hired\text{-}Labour}$)[c]		R$0.05

[a] TM-700, Phibro® (Treatment: 10mg/kg per 10 days), [b] Aquaflor®, Merck Animal Health, Summit, NJ, US (Treatment: 100mg/kg per 10 days), [c] Includes vaccination equipment and anaesthesia.

Description of the partial budget model

New costs

Vaccination-related costs comprise the unit price of the vaccine, hired labour, antibiotic usage (preventively) and additional feed costs. The price of the vaccine is the average market value in Brazil, as reported by the major vaccine resellers. We considered the value of labour hired for vaccination. This practice is already common in some regions of the country and includes equipment and anaesthesia costs. The value of hired labour can be taken as the opportunity cost for fish farmers that assign permanent labour to this additional activity. The antibiotic (usually oxytetracycline) is used preventively five to seven days prior to vaccination. We also took into account the difference in feed intake with and without vaccination, as well as the estimated feed consumed by fish prior to death. The average weight considered for this calculation was 250g (DELPHINO, unpublished data). The extra cost of vaccination was derived as:

$$\text{Extra costs} = (C_{Vaccine} * N_{Fish}) + (C_{Hired\text{-}Labour} * N_{Fish}) + (N_{Fish} * W_{Vaccination} * C_{Atb} * D_{Treat}) + [(Feed_{VAC} - Feed_{N\text{-}VAC}) * Feed_{Price}]$$

where N_{Fish} = batch size in number of fish, $C_{Vaccine}$ = cost of vaccine dose per fish vaccinated, $C_{Hired\text{-}Labour}$ = cost of vaccine hired labour per fish (including vaccination equipment and anaesthesia), $W_{Vaccination}$ = fish weight at vaccination time in kilogram, C_{Atb} = cost of antibiotic per kilogram, D_{Treat} = number of days of treatment, $Feed_{VAC}$ = total feed consumption in a vaccinated farm in kilograms, $Feed_{N\text{-}VAC}$ = total feed consumption in a non-vaccinated farm in kilograms and $Feed_{Price}$ = average feed price per kilogram.

Revenue foregone

There was no revenue foregone as a consequence of vaccination.

Costs saved

The expenditure with antibiotics prior to vaccination was considered the only cost saved with vaccination, since vaccines against bacterial diseases typically reduce antibiotics' dependency compared to non-vaccinated fish (BRUDESETH et al., 2013). We assumed that no antibiotics would be used in vaccinated fish. The savings in antibiotics costs were calculated as follows.

$$\text{Costs saved} = [N_{Fish} * (Treat_{Strep} / 100) * W_{Treated}] * C_{Atb} * D_{Treat}$$

where $Treat_{Strep}$ = expected % of fish to be treated with antibiotics and $W_{Treated}$ = average weight of treated fish in kilogram. In this model, the $Treat_{Stre}$ was 90%, assuming the whole fish batch would be medicated after 10% cumulative losses. Antibacterial treatment was considered using oxytetracycline (100 mg/kg) for ten days, once in a cycle, although some producers report, in some cases, re-treatment or alternate between two antibiotics (oxytetracycline and florfenicol) (Chideroli et al., 2017).

New revenue

An increase in fish survival is the only consequence of the vaccination that yields an additional return. It was calculated as:

$$\text{New revenue} = [(Mortality_{Non\text{-}Vacc} - Mortality_{Vacc)}) * W_{Harvested} * Fish_{Price}]$$

$$\text{Considering: } Mortality_{Non\text{-}Vacc} = [N_{Fish} - (N_{Fish} * M_{Strep} / 100)] \text{ and}$$

$$Mortality_{Vacc} = Mortality_{Non\text{-}Vacc} * [1 - (RPS / 100)]$$

where $Mortality_{Non\text{-}Vacc}$ = total mortality due to disease in a non-vaccinated farm (number of fish), $Mortality_{Vacc}$ = total mortality due to disease in a vaccinated farm (number of fish), M_{Strep} = expected mortality due to *S. agalactiae* if fish are not vaccinated (in %), *RPS* = relative percent survival provided by the vaccine (in %), $W_{Harvested}$ = fish weight during harvesting and $Fish_{Price}$ = fish market price.

Results and Discussion

Table 2 displays the layout of the partial budget model used to estimate profitability of *S. agalactiae* vaccination in a small-scale tilapia farm, for one production cycle starting with 20,000 fingerlings, 20% mortality over the whole period, 5% improvement in FCR and a RPS of 60%. The net change in income (benefits – costs) was R$0.26 per fingerling vaccinated.

Table 2. Vaccination against *S. agalactiae* - partial budget for one production cycle, starting with 20,000 fingerlings, 20% mortality over the whole period, 5% improvement in FCR and a RPS of 60%.

COSTS				**BENEFITS**			
	No.	Cost/unit	Total		No.	Cost/unit	Total
New costs				**Costs saved**			
Vaccine dose	20000	R$0.11	R$2,200.00	Reduction in antibiotic	4500	R$0.01	R$450.00
Vaccine labour	20000	R$0.05	R$1,000.00				
Antibiotic (preventive)	7000	R$0.01	R$70,00				
Feed consumption	1069	R$2.50	R$2,672.00				
Revenue foregone				**New revenue**			
				Extra fish sales	2040	R$5.20	R$10,608.00
		Total costs	R$5,942.00			**Total benefits**	R$11,058.00
						Change in income due to vaccination	**R$5,116.00**
						Change in income per fingerling	**R$0.26**

The results for different scenarios where we considered various combinations of key inputs (cumulative mortality, relative percent survival (RPS) and improved FCR) are shown in Table 3, as net result per fingerling vaccinated. In the 20% cumulative mortality scenario, the results indicate that vaccination is profitable, even in the absence of any improvement in feed conversion, assuming a 50% RPS associated vaccine. The profitability of vaccination is lower when cumulative mortality without vaccination is lower, in which case it would be more dependent on higher vaccine efficacy and/or improvements in feed conversion rate. Although the vaccine manufacturer indicates that FRC in a vaccinated farm goes up to 10% (MSD Animal Health, 2015), an improvement of 5% in FCR would be sufficient to justify the intervention in a low mortality scenario.

The minimum RPS value (50%) that was assumed during the analysis can be considered conservative, since there are reports of up to 90% RPS of fish immunized with injectable vaccines against *S. agalactiae* (LIU et al., 2016; MUNANG'ANDU et al., 2016). The vaccine available in Brazil is composed of an inactivated strain of *S. agalactiae* of serotype Ib, the most frequent in the country. Nevertheless, genetic diversity within the same serotype has been identified in Brazil (BARONY et al., 2017). There is also evidence that the protective immunity of *S. agalactiae* from tilapia was not only associated with its serotypes, but also differs among prevalent strains with different genotypes of the same serotype (CHEN et al., 2012). Thus, changes in vaccine efficacy may occur where the prevalent strain does not match the serotype/genotype strain of the vaccine. CHIDEROLI et al. (2017) reported a complete failure of the vaccine coverage after the emergence of serotype III in the northeast of Brazil, associated to *S. agalactiae* outbreaks in 100% vaccinated farms. Therefore, it would be desirable to carry out a robust serotyping scheme at the major tilapia farming regions.

Although our results indicate the vaccination is likely to bring about economic gains, tilapia farmers should be aware that profitability of the intervention depends on a combination of inputs and, therefore, the partial budget must be updated to reflect changes in economic or biological factors. It is noted that the net result is more sensitive to variations in cumulative mortality pre-vaccination and, within each epidemiological scenario, improvement in feed conversion seems to have greater impact than vaccine efficacy on the net return. It should be stressed that cumulative mortality is the only parameter that can be assessed by the farmer before deciding to vaccinate. Both the RPS and the FCR are traits of the vaccine and are included in our decision-support model to take their variability and uncertainty into account.

There is a case for undertaking applied research into these parameters with a view to reduce the risk of vaccination from a farmer's perspective.

Finally, our model considers marginal benefits and costs that are directly associated with vaccination and was not designed to be a farm budget. This makes it readily adaptable to specific field conditions, whenever adequate knowledge of economic and biological factors is available.

Table 3. Net result (benefits – costs) per fingerling vaccinated for a combination of model inputs.

	Mortality[a]	**5%**			**10%**			**20%**		
	RPS	*50*	*60*	*80*	*50*	*60*	*80*	*50*	*60*	*80*
FCR[b]	*Null*	- R$0.09	-R$0.08	-R$0.06	-R$0.04	-R$0.02	R$0.02	R$0.06	R$0.10	R$0.19
	1,00%	-R$0.05	-R$0.04	-R$0.02	R$0.00	R$0.02	R$0.06	R$0.09	R$0.14	R$0.22
	5,00%	R$0.07	R$0.08	R$0.10	R$0.12	R$0.14	R$0.19	R$0.22	R$0.26	R$0.34
	10,00%	R$0.24	R$0.25	R$0.27	R$0.28	R$0.30	R$0.35	R$0.37	R$0.41	R$0.50

[a] Mortality pre-vaccination, [b]Improvement in FCR.

Conclusion

The results indicate that vaccination against *S. agalactiae* is likely to be profitable in Nile tilapia farms where streptococosis is a production constraint. The higher the pre-vaccination mortality, the greater the financial net return. However, on farms where cumulative mortality pre-vaccination is low, the profitability of vaccination would be more dependent on higher vaccine efficacy and/or better feed conversion rates. Although considering the vaccine efficacy less than 80% is a very pessimistic scenario.

Farmers can use this simple and flexible model to make informed decisions regarding the adoption of a vaccination program. The model should be updated considering the variability arising from different epidemiological and economic scenarios (average feed costs and/or market prices), as well as new knowledge on uncertain parameters, like vaccine efficacy and feed conversion gains associated with vaccination.

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ANEXO

Universidade de Brasília
Instituto de Ciências Biológicas
Comissão de Ética no Uso Animal

Brasília, 7 de junho de 2017.

DECLARAÇÃO

Declaramos que o projeto intitulado **"RISCOS SANITÁRIOS NA AQUICULTURA COM TANQUES-REDE EM RESERVATÓRIOS PÚBLICOS."**, UnBDoC n.º 52858/2016, sob responsabilidade do Professor Vitor Salvador Picão Gonçalves foi avaliado e aprovado pela Comissão de Ética no Uso Animal (CEUA) da Universidade de Brasília. Este projeto foi aprovado para utilização de: *OREOCHROMIS NILOTICUS* (2160). A presente aprovação é válida pelo período de 10/05/2016 a 10/04/2017.

Profa. Dra. Paula Diniz Galera
Coordenadora da CEUA – UnB

*Este documento se restringe à avaliação ética do projeto supracitado e não substitui outras licenças e permissões que porventura se façam necessárias.