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

TESE DE DOUTORADO

CARACTERIZAÇÃO PRODUTIVA, SANITÁRIA E EPIDEMIOLÓGICA DA TILAPICULTURA COMERCIAL DO DISTRITO FEDERAL (2021-2023)

RICARDO DA SILVA RAPOSO

BRASÍLIA - DF

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PRODUCTIVE, SANITARY AND EPIDEMIOLOGICAL CHARACTERIZATION OF COMMERCIAL TILAPIA FARMING IN THE DISTRITO FEDERAL, BRAZIL (2021-2023)

RICARDO DA SILVA RAPOSO

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TESE DE DOUTORADO EM SAÚDE ANIMAL

ÁREA DE CONCENTRAÇÃO: MEDICINA PREVENTIVA E PATOLOGIA VETERINÁRIA

LINHA DE PESQUISA: EPIDEMIOLOGIA, PREVENÇÃO E CONTROLE DE DOENÇAS DOS ANIMAIS E GESTÃO DOS RISCOS PARA A SAÚDE PÚBLICA

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TESE DE DOUTORADO SUBMETIDA AO PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE ANIMAL, COMO PARTE DOS REQUISITOS NECESSÁRIOS À OBTENÇÃO DO GRAU DE DOUTOR EM SAÚDE ANIMAL.

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“Peace, love, empathy.”

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RESUMO

A indústria global da tilápia emergiu como um importante segmento de proteína animal, com produção estimada em 6,2 milhões de toneladas, tornando-se o segundo peixe mais cultivado do mundo. No Brasil, a produção foi estimada em 579 mil toneladas no ano de 2023, com volume de negócios superior a 5 bilhões de reais. No Distrito Federal, a produção de tilápia está estimada em aproximadamente 1.800 t/ano, sendo importante fonte de renda para agricultores locais e servindo como fonte de proteína para o mercado consumidor de Brasília, terceiro maior do país em consumo de pescados. O objetivo geral desse trabalho foi realizar a caracterização da produção comercial de tilápias do DF quanto aos aspectos produtivos, sanitários e epidemiológicos. A tese foi subdividida em 5 capítulos e anexos, sendo o Capítulo I composto de uma introdução e cada uma das demais seções representando um artigo científico que será submetido a um periódico específico. O capítulo II apresenta uma revisão de literatura sobre a vigilância de doenças da tilápia e os planos amostrais utilizados em monitoramentos e inquéritos epidemiológicos de doenças de peixes. No capítulo III, abordou-se a caracterização produtiva e sanitária da tilapicultura comercial do DF no período entre 2021 e 2022 a partir de um questionário semi-estruturado aplicado junto a 112 produtores. Os resultados apontaram que as fazendas de tilápia do DF dispõem, no geral, de uma boa biosseguridade de forma que 82,1% das pisciculturas foram classificadas com nível de biosseguridade B (risco baixo) ou C (risco moderado) na avaliação quanto ao grau de vulnerabilidade para introdução e disseminação de patógenos. Esse estudo também verificou diferenças significativas ($p<0,05$) entre alguns estratos comerciais, com destaque para o grupo de engorda de sistema fechado que apresentou superioridade nos escores em relação aos grupos de fazendas de engorda de sistema semi-fechado, fornecedores de alevinos e pesque pagues. No capítulo IV, é apresentado o resultado de estudo epidemiológico utilizando um modelo de inquérito que incluiu dois componentes epidemiológicos complementares (um estudo baseado em amostragem direcionada e outro em amostragem aleatória em estabelecimento de maior risco). Verificou-se que é bastante baixa a frequência de doenças de notificação obrigatória nas tilapiculturas do DF. No capítulo V, avaliou-se a funcionalidade de um sistema de monitoramento e comunicação instantânea por aplicativos de mensagens para smartphones como componente de um programa de vigilância de doenças de notificação obrigatória de peixes. Esse método demonstrou-se superior ao sistema tradicional de vigilância passiva, sendo capaz de detectar pela primeira vez a presença de uma doença viral no território do DF.

Palavras-chave: tilápia; biosseguridade; vigilância de doenças; monitoramento; inquérito epidemiológico, classificação de risco.

ABSTRACT

The global tilapia industry has emerged as an important animal protein segment, with production estimated at 6.2 million tons, making it the second most cultivated fish in the world. In Brazil, production is estimated at 579 thousand tons in 2023, with a turnover of more than USD 1,2 billion. In the Distrito Federal (DF), tilapia production is estimated at approximately 1,800 tons per year, which is an important source of income for local farmers and serves as a source of protein for the consumer market in Brasília, the third largest in the country in terms of fish consumption. The general objective of this work was to characterize the commercial production of tilapia in the DF in terms of production, sanitary and epidemiological aspects. The thesis was subdivided into 5 chapters and annexes, with Chapter I consisting of an introduction and each of the other sections representing a paper that will be submitted to a specific scientific journal. Chapter II presents a literature review on tilapia disease surveillance and the sampling plans used in fish disease monitoring and epidemiological surveys. Chapter III deals with the production and sanitary characterization of commercial tilapia farming in the Distrito Federal between 2021 and 2022, based on a semi-structured questionnaire applied to 112 farmers. The results showed that tilapia farms in the DF generally have a good biosecurity, as 82.1% of the fish farms were classified as having a biosecurity level of B (low risk) or C (moderate risk) when assessing the degree of vulnerability to the introduction and spread of pathogens. This study also found significant differences ($p<0.05$) between some commercial strata, with the closed system fattening group standing out as having superior scores compared to the semi-closed system fattening, young form fish and pay-to-fish farms. Chapter IV presents the results of an epidemiological study using a survey model that included two complementary epidemiological components (a study based on targeted sampling and another on random sampling in an establishment at risk). It was found that the frequency of notifiable diseases in tilapia farms in the Distrito Federal is quite low. Chapter V evaluates the functionality of a monitoring and instant messaging system for smartphones as part of a surveillance program for notifiable fish diseases. This method proved to be superior to the traditional passive surveillance system and was able to detect the presence of a viral disease in the DF for the first time.

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Keywords: tilapia; biosecurity; disease surveillance; monitoring; epidemiological surveys, risk ranking.

LISTA DE ABREVIATURAS E SIGLAS

ABPA	Associação Brasileira de Proteína Animal
ABRAFRIGO	Associação Brasileira de Frigoríficos
AHE	*Atypical health events*
APHIS	*Animal and Plant Health Inspection Service*
BKD	*Bacterial Kidney Disease*
BL	*Biosecurity Level*
DF	Distrito Federal
FAO	*Food and Agriculture Organization*
FHP	*Fish Health Personnel of Norway*
FO	*Francisella orientalis*
IBGE	Instituto Brasileiro de Geografia e Estatística
IHN	*Infectious haematopoietic necrosis*
ISA	*Infectious Salmon Anemia*
ISA HPR-del	*Infectious Salmon Anemia - pathogenic highly polymorphic region*
ISA HPR-0	*Infectious Salmon Anemia - non-pathogenic highly polymorphic region*
ISKNV	*Infectious Spleen and Kidney Necrosis Virus*
MAPA	Ministério da Agricultura e Pecuária do Brasil
MCS	*Monitoring and communication system*
n	Tamanho amostral (*sample size*)
n_{MCS}	*Number of notifications received in the MCS*
n_{PS}	*Number of notifications received in the PS*
NRF	*Notification rate per farm*
p	*Statistical p-value*
p_{MCS}	*Universe of farm in the MCS*
$p_{overall}$	*Overall universe of farm*
ppm	*parts per million*
PCR	*Polymerase Chain Reaction*
PS	*Passive surveillance system*
NFSA	*Norwegian Food Safety Authority*
NVI	*Norwegian Veterinary Institute*
PEIXE-BR	Associação Brasileira da Piscicultura

pH	Potencial hidrogeniônico
RBS	*Risk-based surveillance*
RT-PCR	*Reverse transcription polymerase chain reaction*
RT-qPCR	*Reverse transcription real-time polymerase chain reaction*
SA	*Streptococcus agalactiae*
SD	*Standard Deviation* (desvio padrão)
SEAGRI	Secretaria de Agricultura, Abastecimento e Des. Rural do Distrito Federal
OVS	*Official Veterinary Service* (Serviço Veterinário Oficial)
RD	*Risk of dissemination*
R_{MMN}	*Ratio of mean monthly notifications*
TiLV	*Tilapia Lake Virus*
TiPV	*Tilapia parvovirus*
VNN	*Viral Necrosis Virus*
USDA	*Department of Agriculture of United States*
UFMG	Universidade Federal de Minas Gerais
UnB	Universidade de Brasília
VHS	*Viral Hemorrhagic Septicemia*
VL	*Vulnerability Level*
$\bar{X}$	*Mean*
WOAH	*World Organisation for Animal Health*

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SUMÁRIO

CAPÍTULO I

INTRODUÇÃO

A indústria da tilápia emergiu como um importante segmento de proteína animal e tem se expandido cada vez mais por todos os continentes habitados (FAO, 2023). De acordo com dados divulgados pela FAO (2023), a produção global de tilápia (*Oreochromis* sp.) atingiu 6,5 milhões de toneladas, sendo um dos peixes mais cultivados do mundo.

Entre os motivos para o sucesso da indústria da tilápia, estão as características nutricionais dessa espécie, que constitui uma fonte rica de proteína de alto valor biológico, e os aspectos comerciais, sendo um dos peixes de cultivo de maior valor de mercado, com volumes expressivos de negócios em todo o mundo e grande aceitação em mercados nacionais e internacionais (RAJE, 2023).

Atualmente, a *commodity* tilápia detém importante fatia no mercado internacional de pescados e muito se deve à sua carne branca, suave e de textura firme, que é considerada saborosa e versátil para preparações culinárias (JORY et al., 2000), facilidade de cultivo devido à alta taxa de conversão alimentar, boa resistência e adaptabilidade a diferentes condições de temperatura e salinidade da água (AVNIMELECH, 2007) e à precocidade sexual que possibilita a produção em ciclos relativamente curtos, entre 5 e 8 meses (KUBITZA; KUBITZA, 2013; VIEIRA-FILHO, FISHLOW, 2017).

Indústria brasileira da tilápia: uma *commodity* em franca expansão

No Brasil, a produção de tilápia foi a atividade pecuária que apresentou o maior crescimento acumulado nos últimos dez anos, acima de 100%, consolidando-se como principal segmento da aquicultura no país, com participação de 65% da produção de peixes (BRASIL, 2022; PEIXE-BR, 2024). O volume de negócios dessa indústria movimenta valores superiores aos R$ 6 bilhões, com produção estimada em 579 mil toneladas em 2023 (PEIXE-BR, 2024).

A tilapicultura nacional é caracterizada por uma produção diversificada, que abrange desde pequenas propriedades rurais de agricultura familiar que ajudam a abastecer mercados locais até grandes empreendimentos comerciais representados por cooperativas agroindustriais, empresas especializadas em produção verticalizada a partir de cultivos próprios, ou ainda de grandes produtores independentes, voltados para o abastecimento de grandes mercados consumidores internos e externos (PEIXE-BR, 2024; SEAGRI, 2023; BASSO, 2023).

De acordo com dados do Anuário da Piscicultura (PEIXE-BR, 2024), a produção de tilápia no país tem experimentado um crescimento constante nas exportações. A *commodity* tilápia representa

95% do total de pescados provenientes de aquicultura que são comercializados com outros países, sendo os Estados Unidos o principal comprador, gerando um lucro aproximado de R$ 124 milhões.

Apesar do crescente aumento do volume de exportação, os valores ainda estão muito distantes de outras cadeias nacionais de proteína animal mais consolidadas, como as indústrias da carne bovina, suína e do frango. A produção brasileira de tilápia ainda é muito voltada para o enorme mercado interno, onde o consumo algumas vezes é maior do que a oferta (PEIXE-BR, 2024). Entre os pescados, considerando o montante oriundo de aquicultura e recursos pesqueiros, a tilápia se consolidou como a espécie mais consumida do país, representando quase 1/3 do volume consumido no geral e aproximadamente 60% dos produtos oriundos de cultivo. Por isso, de forma oportuna, a indústria da tilápia vem aumentando sua participação no cenário nacional de proteína animal deixando para trás outras proteínas tradicionais como as carnes de peru, pato, ovinos e caprinos.

A Tabela 1 compara os dados de produção entre as principais cadeias de carne do Brasil no período entre 2022 e 2023, enquanto a Tab. 2 ilustra a participação das proteínas no cenário de exportação.

Tabela 1. Comparação da tilápia com as principais cadeias nacionais de carne entre 2022 e 2023, quanto aos dados de produção, movimentação de negócios, variação em relação ao ano anterior, consumo per capita anual e posição no ranking global de maiores produtores.

Tipo de carne	Produção (milhões de t)	Movimentação de negócios (estimativa em bilhões de R$)	Variação da produção em relação ao ano anterior (%)	Consumo per capita (kg/hab/ano)	Posição global de produção
Frango	14,52	112,0[a]	1,39%	45,0	2º
Bovina	10,35	400,0	10,65%	32,0	2º
Suína	4,98	32,0[a]	5,99%	18,0	4º
Aquicultura	1,02	13,0	2,26%	4,5[b]	13º
Tilápia	**0,58**	**6,0**	**5,28%**	**3,0**	**4º**
Peru	0,16	1,0[a]	3,31%	0,5	3º
Ovina	0,10	1,0	2,50%	0,5	23º

Fonte: Adaptado de IBGE, 2023; Peixe-BR, 2024; ABCC, 2023; ABPA, 2023; Abrafrigo, 2024; FAO, 2022; MAPA e Secex, Brasil. [a]Montantes que envolvem somente o faturamento estimado com comercialização da carne, sem incluir os negócios movimentados por todo o segmento. [b]Consumo per capita de pescados é de 10 kg/hab/ano somando produtos de pesca e aquicultura.

Tabela 2. Comparação da tilápia com as principais cadeias nacionais de carne no período entre 2022 e 2023, quanto aos dados de exportação: volume, faturamento e posição no ranking global de maiores exportadores.

Tipo de carne	Volume de Exportação (bilhões de t)	Receita de Exportação (bilhões de US$)	Posição global de exportação
Frango	4,822	9,700	1º
Bovina	2,536	10,845	1º
Suína	1,120	2,500	4º
Tilápia	**0,007**	**0,025**	**8º**
Peru	0,059	0,189	3º
Ovina	0,001	0,006	S/I

Fonte: Adaptado de IBGE, 2023; PEIXE-BR, 2023, ABPA, 2023; Abrafrigo, 2024; FAO, 2023; MAPA e Secex, Brasil. S/I = Sem informação disponível.

Empresas que atuam há décadas em outros setores de proteína animal, como as cooperativas agroindustriais, geralmente voltadas para produção de frangos, suínos e produtos lácteos, estão investindo na cadeia da tilápia, como forma de diversificar seus investimentos e aproveitar a alta lucratividade desse segmento (BASSO, 2023; PEIXE-BR, 2024). Na região oeste do Paraná, maior polo nacional de produção de tilápia, algumas cooperativas já são grandes produtoras desse peixe, operando no mesmo sistema de integração vertical utilizado para a produção de aves, suínos e bovinos de leite. Essas empresas utilizam toda expertise em frigorificação e produção de larga escala para comercializar grandes volumes de carne de tilápia nos mercados interno e externo (BASSO, 2023).

Desafios sanitários da tilapicultura brasileira

Apesar de ter despontado como uma cadeia de grande potencial econômico no Brasil devido às condições climáticas e à grande disponibilidade de água, a indústria nacional da tilápia enfrenta importantes desafios, entre eles os efeitos causados pelas doenças e a falta de estrutura para o seu diagnóstico (BONDAD-REANTASO et al., 2005; FIGUEIREDO; LEAL, 2008; PÁDUA, 2017). Alguns microrganismos podem ser extremamente patogênicos para os peixes, levando a perdas produtivas e gerando impactos socioeconômicos para indivíduos, comunidades e economias (ADAM; GUNN, 2017).

A biosseguridade aplicada à tilapicultura pode ser entendida como o conjunto de medidas e práticas adotadas de forma proativa para prevenir a introdução e disseminação de microrganismos não desejáveis no ambiente em que os animais estão inseridos. Na produção animal intensiva, os melhores índices de eficiência produtiva são alcançados investindo mais em prevenção e profilaxia do que no controle de doenças já estabelecidas (YANONG; ERLACHER-REID, 2012). A chance de sucesso aumenta quando os procedimentos de biosseguridade são aplicados em conjunto com medidas de boas práticas em aquicultura (PEIXE-BR, 2022).

Entretanto, uma grande parcela de tilapicultores ainda desconhece o real impacto provocado pelos desafios sanitários, atribuindo às perdas na produção, sobretudo, à temperatura e condições físico-químicas da água de cultivo inadequadas (RORIZ et al., 2017). Sabe-se que esse impacto negativo é motivado por um conjunto de fatores dos quais se destacam a estrutura inapropriada, baixo nível de biosseguridade e falhas de manejo (RAPOSO et al., 2021), ausência de boas condições ambientais (MEYER; BARCLAY, 2009), de bem-estar animal (VOLPATO et al., 2007) e presença de patógenos no sistema de produção (STENTIFORD et al., 2012). Os prejuízos decorrentes dos aspectos sanitários são percebidos pelas frequentes mortalidades atípicas que acometem os ciclos produtivos (DELPHINO et al., 2019) e pelo baixo desempenho produtivo caracterizado pelo crescimento lento e conversão alimentar ineficiente (KUBITZA; KUBITZA, 2013).

Em vias gerais, quando nos referimos ao nível de biosseguridade, a aquicultura brasileira ainda se mantém distante da realidade de outras cadeias de proteína animal como a avicultura e suinocultura industrial. Embora o princípio seja o mesmo, de produção animal em sistemas intensivos e superintensivos, caracterizados por condições estressantes e de grande suscetibilidade aos patógenos (JORDAN et al., 2011), existem grandes diferenças entre esses setores especialmente na importância que cada cadeia dá à sanidade do plantel e à biosseguridade das unidades produtivas. Enquanto na avicultura e suinocultura industrial os padrões sanitários para profilaxia de doenças são sistematicamente definidos por um arcabouço legislativo bastante robusto, visando o impedimento da introdução dos patógenos, que envolvem desde a estrutura das granjas até a obrigatoriedade de vacinas (Programa Nacional de Sanidade Avícola; Programa Nacional de Sanidade Suídea; MORES et al., 2017; BANDEIRA; SANCHES, 2022), na aquicultura, incluindo a produção de tilápia, o exercício sistemático das medidas de biosseguridade é adotado por um pequeno nicho de propriedades e agroindustrias, como as empresas verticalizadas e cooperativas que realizam a produção de formas jovens e o cultivo de engorda em viveiros escavados (PEIXE-BR, 2022).

Objetivos

Por todo exposto, a presente tese tem como objetivos:

a) caracterizar a tilapicultura comercial do DF quanto aos aspectos produtivos e sanitários;

b) determinar a frequência e distribuição dos principais patógenos de interesse econômico da tilapicultura do DF; e

c) avaliar a eficácia da utilização de aplicativos de mídia social para smartphones como componente de sistema de vigilância de doenças de tilápia.

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CAPÍTULO II

TILAPIA DISEASE SURVEILLANCE AND SAMPLING PLANS FOR EPIDEMIOLOGICAL SURVEYS AND MONITORING: A REVIEW

Artigo para submissão ao periódico ***Pesquisa Veterinária Brasileira (Brazilian Journal of Veterinary Research***)

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ABSTRACT. The tilapia industry has been expanding more and more among countries on all continents, representing a major international commodity and an important source of animal protein for the global population. However, this industry has been threatened by emerging pathogens that take advantage of the highly vulnerable situation of tilapia cultures, which are increasingly subjected to super-intensive management with a high density of fish, favoring the rapid multiplication and dispersal of these agents between farms and in the environment. The aim of this study was to carry out a literature review on the general aspects of tilapia disease surveillance and the sampling plans used for epidemiological surveys and monitoring conducted in Brazil and neighboring countries, discussing the differences to the sampling plan for the Distrito Federal (Brazil). A wide variety of designs and sample sizes were observed between the different health plans and programs carried out in Brazil and other countries, with a very strong tendency to use risk-based strategies and targeted sampling. It can be concluded that there is a very wide variety of designs and sample sizes between the different health plans and programs for diseases of tilapia and other fish species. In the Distrito Federal, the only federal unit in Brazil that has a surveillance plan for tilapia diseases, the models applied were defined based on risk-based surveillance strategies and sampling of symptomatic animals to adjust logistical/laboratory costs and increase the sensitivity of epidemiological surveys.

INDEX TERMS: Tilapia; aquatic animals, disease surveillance; sampling plan, epidemiological surveys.

RESUMO. A indústria da tilápia tem se expandido cada vez mais entre os países de todos os continentes, representando uma commodity internacional de destaque e importante fonte de proteína animal para população global. Contudo, essa indústria vem sendo ameaçada por patógenos emergentes que se aproveitam da situação de alta vulnerabilidade dos cultivos, que são submetidos cada vez mais a manejos superintensivos com alta densidade de peixes, favorecendo a rápida multiplicação e dispersão desses agentes entre as fazendas e no meio ambiente. O objetivo desse estudo foi realizar uma revisão de literatura sobre os aspectos gerais da vigilância de doenças da tilápia e os planos amostrais utilizados para inquéritos e monitoramentos epidemiológicos conduzidos no Brasil e países vizinhos, discutindo as diferenças para o plano existente no Distrito Federal. Observou-se uma grande variedade de desenhos e tamanhos de amostra entre os diferentes planos e programas sanitários executados no Brasil e demais países, sendo observada uma tendência muito forte de utilização de estratégias baseadas em risco e amostragem direcionada. Conclui-se que existe uma variedade muito grande de desenhos e tamanhos de amostra entre os diferentes planos e programas sanitários para doenças de tilápia e outras espécies de peixes. No DF, única unidade da federação do Brasil que possui plano de vigilância para doenças da tilápia, os modelos aplicados foram definidos com base em estratégias de vigilância baseada em risco e amostragem de animais sintomáticos para adequação de custos logísticos/laboratoriais e aumento da sensibilidade dos inquéritos epidemiológicos.

TERMOS DE INDEXAÇÃO: Tilápia; animais aquáticos, vigilância de doenças; plano amostral, estudos epidemiológicos.

INTRODUCTION

The tilapia industry has been expanding more and more among countries on all continents, with global production estimated at 6.5 million tons in 2021, making it the second most cultivated fish in the world (FAO, 2023). Among the reasons for this success are the nutritional characteristics of this species, which is a rich source of protein of high biological value, and the commercial aspects, being one of the fish cultures most in demand by national and international markets (Raje, 2023).

Like any animal protein industry, the global tilapia industry has been threatened by emerging pathogens that take advantage of the highly vulnerable situation of tilapia cultures, which are increasingly subjected to super-intensive management with high fish density, favoring the rapid multiplication and dispersion of these agents between farms and in the environment (Figueiredo; Leal, 2008; FAO, 2017a). In order for health challenges to be less felt in production, it is essential that animal health authorities participate in the construction of public health policies aimed at mitigating the risks and negative impact caused by diseases (Gregg et al., 1996).

Therefore, the aim of this study was to carry out a literature review on sampling plans used for surveys, monitoring and epidemiological studies of tilapia diseases, discussing the differences that exist for the sampling plan conducted in the Distrito Federal (DF), Brazil, which is currently the only federal unit that carries out epidemiological surveys and monitoring on an ongoing basis.

GENERAL ASPECTS OF DISEASE SURVEILLANCE

The WOAH (2015) defines surveillance as the systematic and continuous collection of data and information related to animal health, with timely disclosure of information to those who need to know, so that measures can be taken to control or eradicate diseases. Maintaining active surveillance systems for certain diseases has a strong impact on economic and social aspects, since the absence of a surveillance system in a state or country can restrict the productive and economic growth of an activity with great potential. The confidence of commercial partners lies in the results of national reports on the state of health of a country, zone or compartment (Corsin et al., 2009).

According to Cameron et al. (2020), there are four possible categories of surveillance purposes. For diseases that are present in the population, surveillance can be aimed at estimating the amount of disease through epidemiological methods (e.g. prevalence or incidence) in order to compare over time, space or other factors; or aimed at supporting case detection in order to respond to individual cases, for example as part of a disease control or eradication program. For diseases that are currently absent in the population, surveillance can aim to demonstrate the absence of the disease or infection in order to facilitate safe trade or to confirm successful eradication; or to carry out early detection to enable elimination of the pathogen before it spreads to the population.

Methods and components of surveillance systems

Traditional surveillance programs can be carried out in various ways, using different components (Salman, 2003; WOAH, 2019; WOAH, 2023; Tan et al, 2023). The main components of fish disease surveillance systems are shown in Fig. 1.

There are various surveillance methods such as active surveillance based on routine clinical inspections; passive surveillance (disease reporting systems); epidemiological surveys; syndromic surveillance; ante-mortem and post-mortem inspections; risk-based surveillance; surveillance in sentinel units and others that can be consulted in the World Organization for Animal Health's terrestrial (WOAH, 2023) and aquatic (WOAH, 2019) animal surveillance guides, such as monitoring in free-living wild animal populations, disease monitoring carried out based on communications of results carried out by professionals and private laboratories or universities.

Surveillance methods can be used to sample randomly selected production units or in the form of a census (WOAH, 2023). If it is a sample of the population, surveillance can be accompanied by the collection of samples for laboratory testing in quantities estimated using probabilistic/statistical methods, so that the results can be extrapolated to that particular population at that particular time (WOAH, 2019).

Fig.1. Possible surveillance components for fish disease surveillance systems.

Active surveillance can be carried out with visits to inspect animals and ponds, clinical inspection of individuals accompanied or not by sample collection and laboratory monitoring, while the passive surveillance method is based on clinical, epidemiological and laboratory investigations of suspect animals, and is the most sensitive component of surveillance systems (WOAH, 2019).

Syndromic surveillance, on the other hand, is characterized by the use of pre-diagnostic data and near real-time statistical tools to detect and characterize unusual activities for future "notifiable disease" investigations (Zelicoff, May, 2011). Also known as data-driven surveillance, this type of surveillance has been used in animal health to help with disease prevention, detection and control strategies (Dórea, Revie, 2021).

Another surveillance method that has been widely used in aquatic and terrestrial animal health programs over the last decade is risk-based surveillance (RBS) (Diserens et al., 2013; Oidtmann et al., 2013; Oidtmann et al., 2014; Diserens et al., 2017; Brasil, 2020; Seagri, 2023). The fundamental principle of RBS is the targeting of resources to populations in zones, groups or farms with the highest health risk (Stärk et al., 2006). RBS is based on the main risk factors for a given disease and population to categorize groups of animals and production units with the highest probability of the pathogen occurring (Oidtmann et al., 2011; Oidtmann et al., 2013). For European Union countries, legislation requires fish farms to be individually classified with regard to the risks of introducing and spreading diseases, to enable the execution of RBS activities (EC Directive 2006/88; EU, 2006, Diserens et al., 2013). The main advantage of using RBS is the increase in efficiency (higher probability of detection), despite the initial costs related to obtaining data from the farms for their characterization (Oidtmann ET AL., 2011; Cameron et al. 2020).

Implementation of the surveillance system for fish diseases

Surveillance of aquatic animal diseases is generally planned and carried out by official veterinary services (WOAH, 2019), but can also be carried out or complemented by actions of other actors in the production chain such as aquaculture professionals, private companies and laboratories, universities, etc. through self-control/monitoring programs or public-private partnerships (Bisson et al., 2019; Poupaud et al., 2019).

A 12-point checklist can be applied to facilitate the process of setting up a surveillance system for fish diseases (Bondad-Reantaso et al., 2021): (1) scenario setting; (2) definition of the surveillance objective; (3) defining populations; (4) clustering of disease; (5) case definition; (6) availability and validation of diagnostic tests; (7) study design and sampling; (8) data collection and management; (9) data analysis; (10) validation and quality assurance; (11) human resources, financial and logistical requirements; and (12) surveillance in bigger picture (Bondad-Reantaso et al. , 2021).

During the implementation of the system, the surveillance methodology for a given group of diseases may involve more than one activity or component with the aim of generating information on the population of susceptible animals (Oidtmann et al, 2013; WOAH, 2023). For this reason, surveillance plans or programs usually integrate more than one component.

DISEASES OF ECONOMIC IMPORTANCE TO THE TILAPIA INDUSTRY

Disease is the result of the interaction between the agent, the susceptible host and the environment where causality is associated with the loss of balance in the epidemiological triad between host, agent and environment (Gordis, 1996), resulting in compromised fish health, low production performance and mortality. Although tilapia are recognized for their great adaptability and resistance (Avnimelech, 2007), factors such as low water quality and high stocking density generate high levels of physiological stress in fish, making them highly susceptible to disease (Kubitza; Kubitza, 2013; Adam; Gunn, 2017).

In tilapia farming, there are some diseases that have greater clinical and epidemiological importance due to their high degree of pathogenicity, power of spread and ability to cause negative impacts on the production chain, such as *Streptococcus agalactiae* (*SA*), *Francisella orientalis* (*FO*), Tilapia Lake Virus (TiLV), Infectious Spleen and Kidney Necrosis Virus (ISKNV), Viral Nervous Necrosis Virus (VNN) and Tilapia Parvovirus (TiPV) (HE et al., 2002; Leal et al., 2018; Leal;

Figueiredo, 2018; Machimbirike et al., 2019; DU et al., 2019; Kembou-Ringert et al., 2023). In addition to these, there are also emerging diseases, which are those that appear suddenly causing mortality and economic losses, resulting from the emergence or introduction of exotic pathogens or the modification of behavior and pathogenicity of a particular agent due to genetic, environmental, anthropic factors, among others (Morse, 1995).

Among the diseases of greatest economic importance, we highlight the TiLV, which has emerged as the main threat to global tilapia stocks (FAO, 2017b; Jansen et al., 2019) due to its high capacity for transboundary spread (Kenne et al., 2021; Aich et al., 2022), whether by horizontal (Eyngor et al., 2014; Liamnimitr, et al., 2018) or vertical transmission (Dong et al., 2020). In Brazil, there are no records to date of the presence of TiLV. Between 2017 and 2021, some countries launched surveillance and/or emergency plans aimed at early detection and contingency of the virus, such as Peru (Sanipes, Peru, 2017), Colombia (ICA, Rodriguez, 2021) and the United States (USDA, USA, 2021). In 2022, the disease was included in the list of diseases requiring immediate notification to the WOAH (WOAH, 2022). Figure 2 illustrates the dynamics of the TiLV virus with the year of record of outbreaks until January 2024, while Table 1 presents the general overview of the main tilapia diseases subject to surveillance programs.

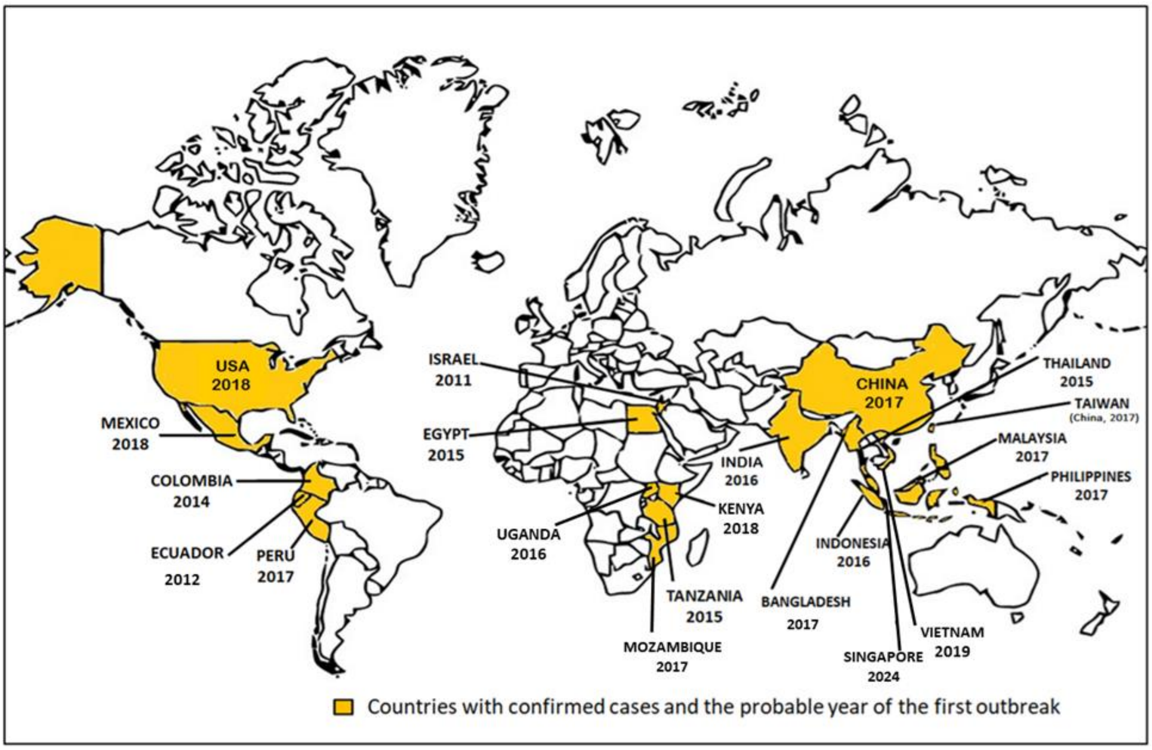

Fig.2. Global distribution of countries with TiLV outbreaks and the probable year of introduction. Source: EYNGOR et al. (2014), FERGUSON et al. (2014), TSOFACK et al. (2017), DONG et al. (2017), FATHI et al. (2017), MUGIMBA et al. (2018), BEHERA et al. (2018), KOESHARYANI et al. (2018), AMAL et al. (2018), PULIDO et al. (2019), CHAPUT et al. (2020), AHASAN et al. (2020), HE et al. (2023), WAHIS/WOAH (2024).

Table 1. Overview of the diseases of greatest economic importance to the tilapia industry, for which surveillance programs are recommended.

Disease / etiology	Geographic distribution[a]	Zoonosis	Main clinical signs and findings	Importance of the disease[b]	Notifiable disease in Distrito Federal (Ordinance No. 75/2022)	Notifiable disease in Brazil[c]	Notifiable disease for WOAH (WOAH list)	References
Tilapia Lake Virus Disease *Tilapia tilapinevirus* (TiLV)	Asia, Africa, North and South America	No	Neurological signs (irregular swimming, loss of balance) and non-specific signs, mortality between 10% and 90%, syncytial hepatitis and hepatocellular necrosis with intracytoplasmic and eosinophilic inclusion bodies in the liver	High	Yes	Yes	Yes	EYNGOR et al., 2014 DONG et al., 2017 AICH et al., 2022 WOAH, 2022 KEMBOU-RINGERT et al. (2023) HE et al. (2023)
Infectious spleen and kidney necrosis virus (ISKNV) Iridovirus (Megalocytivirus)	Africa, Asia, North and South America	No	High mortality of young forms, immunosuppression, splenic hypoplasia, nonspecific signs (secondary bacterial diseases)	Medium	Yes	Yes	No	McGROGAN et al., 1998 HOWELL, 2019 FIGUEIREDO et al., 2020 ALATHARI et al., 2023
Tilapia parvovirus (TiPV)	Asia	No	Mortality between 60 and 70%, neurological signs, swimming in circles, nonspecific signs	Medium	No	Yes	No	DU et al., 2019; LIU et al., 2020 YAMKASEM et al., 2021a
Viral nervous necrosis Nervous necrosis virus (NNV) Betanodavirus	Africa, Asia and Europe	No	High mortality in larvicultures, neurological signs	Medium	No	Yes	No	HODNELAND et al., 2011 SHETTY et al., 2012 MACHIMBIRIKE et al., 2019
Franciselosis *Francisella orientalis*	Asia, Europe, and Central and North America	No	Granulomatous lesions in the spleen, kidney and other tissues, exophthalmos, ascites, high mortality in young forms	Low	Yes	Yes	No	LEAL et al., 2014 ASSIS et al., 2017 CARREON et al., 2021
Streptococcosis *S. agalactiae* Ia ST7	Asia, Central and South America	Yes	Septicemia, exophthalmos, ascites, meningoencephalitis and neurological symptoms	Low	Yes	Yes	No	KAYANSAMRUAJ et al. 2014 KAYANSAMRUAJ et al. 2019 HALPIN, 2023
Streptococcosis *S. agalactiae* Ib	Worldwide distribution	No	Septicemia, exophthalmos, ascites, meningoencephalitis and neurological symptoms	Low	Yes	No	No	ASSIS et al., 2017 ABU-ELALA et al., 2016 LEAL; FIGUEIREDO, 2018
Streptococcosis *S. agalactiae* III subtype 4 ST283	Asia and South America	Yes	Septicemia, exophthalmos, ascites, meningoencephalitis and neurological symptoms	Medium	Yes	No	No	RAJENDRAM et al., 2016 KAYANSAMRUAJ et al. 2019 BARKHAM et al., 2019
Lactococcosis *L. garvieae and L. petauri*	Worldwide distribution	Yes	Septicemia and bacterial meningoencephalitis, exophthalmos, ascites and high mortality	Low	No	No	No	EVANS et al., 2006 GOODMAN et al., 2017 EGGER et al., 2023

[a] Geographical distribution based on known cases of infection in the tilapia species. [b] Importance of the disease on the international scene based on the pathogen's ability to spread across borders and on lists and reports from the WOAH, FAO and health authorities in member countries. [c] The list of notifiable diseases in aquatic animals (MPA Ordinance No. 19/2015) is under revision. Exotic diseases in Brazil (such as TiLV, TiPV and others) and emerging diseases require notification to the official service in accordance with MPA Normative Instruction No. 04/2015.

SURVEILLANCE AND SAMPLING PLANS

Tilapia disease surveillance and sampling plans used in Brazil

In Brazil, MAPA and the state agricultural defence agencies are the health authorities responsible for surveillance and measures to prevent and control notifiable diseases in tilapia. The current animal health legislation – MPA Normative Instruction nº 4 of 2015 (Brasil, 2015) – also considers any exotic or emerging disease that presents a significant morbidity or mortality rate, or even repercussion for public health, as an official control disease. MAPA currently monitors three tilapia diseases based on the investigation of suspected cases: TiLV, ISKNV and FO. The state agencies are responsible for investigating suspected cases and taking samples that are sent to the Federal Agricultural Defence Laboratory of MAPA (FADL) for official diagnosis (Brasil, 2022b). However, there is still no national plan for standardised surveillance components for tilapia and other aquatic animal diseases in the same way as the national poultry, swine and foot-and-mouth disease programmes (ruminants and pigs) (Brasil, 2020; Brasil, 2022c; Brasil, 2023).

Epidemiological studies of notifiable fish diseases are scarce in Brazil and, except for the DF, the few surveys that have been carried out have been limited to farms in certain regions or reservoirs. Among the state agencies, there is notable difficulty in carrying out surveys aimed at monitoring fish and aquatic animal diseases, probably due to limited financial, logistical, laboratory and human resources (professionals specialised in aquatic animals).

Epidemiological survey by the Paraná Agricultural Defence Agency. In 2014, an epidemiological study of diseases in tilapia hatcheries in the north and west of Paraná state was conducted to determine the most frequent diseases. Around 5,000 fry/fingerlings (5 to 7 fish per pond) from the 34 fish farms registered with the agency were subjected to bacterial and viral isolation tests, using design prevalence (DP) =50%, assumed sensitivity(Se)/ specificity(Sp) = 100% and CI = 95% as parameters (Adapar, 2014).

It should be noted that the state of Paraná is the largest producer of tilapia in Brazil. The impossibility of applying a sample study to the entire state and all production typologies possibly influenced the definition of the design and target population. Because of the greater risk of disease spread, only hatcheries farms in the state's two main tilapia producing regions were sampled. At the time, the model used made it possible to verify the high presence of various bacterial and parasitic pathogens and the absence of viral infections in the state's major production centres (Adapar, 2014).

Epidemiological study at the Morada Nova de Minas production centre. Between 2015 and 2016, a group of researchers conducted a longitudinal study on tilapia farms located in the Três

Marias reservoir, in the state of Minas Gerais, Brazil. They sampled 6/32 semi-open system farms (cages production), using a minimum of 30 fish per month (targeted sampling of moribund tilapia) for 12 months in order to characterise the dynamics of bacterial pathogens of major economic interest (DELPHINO et al, 2019).

The laboratory analyses used bacterial culture techniques. At the time, there was still no evidence of the presence of viral pathogens circulating in Brazil. According to the authors, this sampling would be sufficient to detect at least one positive individual with 95% confidence if the pathogen was present in at least 10% of the population, assuming perfect sensitivity of the tests used (Cameron & Baldock, 1998; Delphino et al., 2019).

Other epidemiological studies. In addition to these, other epidemiological studies carried out in different regions have been published, but all of them have had a regional scope, such as the epidemiological monitoring of francisellosis conducted in the state of São Paulo on 6 semi-open culture farms from three different reservoirs (Paraná, Paranapanema and Tietê rivers), which used 30 tilapia/farm to assess the prevalence of the disease in this population (Rodrigues et al., 2018) and the epidemiological study carried out on 8 farms in the state of Pernambuco (n=73 and targeted samples) to check the frequency of bacterial diseases that are not notifiable (Meirelles, 2010).

Surveillance plan for tilapia diseases in the Distrito Federal. The DF is a federative unit made up of a single municipality, Brasília, which is among the cities with the largest number of tilapia farms in the country (Brasil, 2022a), with more than 660 fish farms raising tilapia (Seagri, 2023). With regard to tilapia disease surveillance, the Distrito Federal has its own list of officially controlled diseases (Seagri Ordinance No. 75/2022) which includes, among others, *S. agalactiae* infection, which is not on the MAPA national list, and which provides for the sanitation (elimination and decontamination) of farms positive for the listed diseases. The Secretariat of Agriculture, Supply and Rural Development (Seagri), which is the DF's animal health authority, has a voluntary health certification programme for biosecure establishments free of TiLV and monitored for ISKNV, *FO* and *Streptococcus* sp., regulated by Seagri Ordinances No. 75 of 2022 and No. 88 of 2023, and aimed especially at hatcheries and farms that sell young fish.

In 2023, Seagri launched the District Plan for Disease Surveillance and Best Practices in Aquaculture, made up of four surveillance components and one component for best practices, prophylaxis and biosecurity (Seagri, 2023), whose actions are aimed especially at the tilapia chain, as it represents 88% of aquaculture in the DF. However, two of these components have already been applied in the DF since 2018, such as active surveillance visits to fish farms for observation and clinical inspection, and visits to attend to suspected diseases following notification by the person responsible for the farm (passive surveillance).

Sampling plans and monitoring carried out in the Distrito Federal. The first epidemiological monitoring carried out in the DF took place between 2021 and 2022 using targeted sampling. A 12-month period was established for collecting samples (n = 5 to 20 moribund fish) based on atypical events reported by farms of fattening, pay-to-fish and hatchery tilapia who had previously been sensitized by the local veterinary service (Raposo, 2024). With the implementation of the surveillance plan (SEAGRI, 2023), since 2023 the DF has been carrying out annual epidemiological monitoring (detection studies) of TiLV, ISKNV, Francisella orientalis and Streptococcus sp. on farms with a higher health risk, such as hatcheries and the fry trade, with the samples being processed at the LFDA/MAPA and the Veterinary Medical Microbiology Laboratory at FAV/UnB.

The current plan aims to monitor the prevalence of pathogens that are present in Brazil in order to assess the effectiveness of the strategic control measures employed by the official service and to carry out early detection of TiLV. The samples used for TiLV research in 2023 used a sample size of 156 fish, selected at random, plus moribund tilapia seen at the time of the visit. To sample the other diseases with a registered presence in Brazil, an n of 30 random individuals was used. For this scenario, the values of Se=95%, Sp=100%, CI 95% and DP=2% and 10% were assumed for TiLV and the other diseases, respectively. In all cases, pools of a maximum of 3 individuals per molecular test are adopted by the laboratory where this protocol was validated, using fragments of the brain, spleen, kidney, liver and ovaries (in the case of broodstock) from fish over 4cm in length and whole fry. Additional information on both surveys is described by Raposo (2024).

Sampling plans and monitoring of fish diseases in other countries

In this subchapter, the sampling plans used for studies and epidemiological monitoring carried out in countries on the American continent that stand out in the production of farmed fish were discussed, as well as countries in other parts of the world considered as a reference in fish disease surveillance.

Colombia. The second largest producer of tilapia in the Americas (second only to Brazil) and the largest exporter of tilapia fillets on the continent, Colombia conducted an active surveillance survey through the Colombian Agricultural Institute (ICA) to assess the prevalence of TiLV in four Colombian departments since the disease has been present in the country since 2016 (Kembou-Tsofack et al., 2017). To make the epidemiological study feasible, ICA sampled only the hatcheries and fry establishments in these regions, which totaled 33 establishments, with 60 individuals being collected per farm (Sp=95%, Se=100%; WOAH, 2019). And to reduce the high laboratory costs, the samples of choice were grouped into 12 pools of 5 alevins over 15g or 12 pools of 10 alevin under 15g for RT-PCR testing (Rodríguez, 2021). Whilst on the one hand the study served to assess the prevalence of TiLV in the most epidemiologically important stratum, which are the suppliers of genetic material, on the other it did not make it possible to assess the presence of the virus in other important production strata.

Foreseeing this limitation, a group of Colombian researchers carried out an epidemiological study using samples of dying tilapia (n of 5 to 10 fish per farm/lot; sampling completed with asymptomatic fish) in 13 departments of the country between 2016 and 2018. Unlike the ICA study which sampled young form establishments, all segments of the tilapia chain were sampled including 283 alevins, 57 matrices/broodstock, 49 larvae and 44 fattening fish. Suspect fish were sent live to the laboratory for molecular (RT-qPCR), histopathological, microbiological and genomic sequencing analyses. The epidemiological assessments found 109 positive cases of TiLV in 463 samples sent (23%), representing 25 positive farms, 21 districts and 13 departments. The study concluded that the virus was widespread and endemic in Colombia, with positivity in 72.4% of the municipalities sampled. The authors highlighted the importance of including not only fingerlings, but also fish from other stages of production in surveillance programs (Barato et al., 2022).

Peru. Peru, a country that also has recent records of TiLV, used an Emergency Plan for TiLV published by the National Fisheries Health Agency (Sanipes, Ministry of Production) to control the virus and strengthen early detection with a sampling design that varied according to the expected prevalence in each department. The size of the pools of individuals was 3 for broodstock, 5 for alevins over 15g, 10 for alevins under 15g and 100 for try or larvae under 1g live weight. In 2020, Sanipes launched an official surveillance plan for the period 2020 and 2021 that included surveillance for TiLV, *FO* and *S. agalactiae* in Peruvian tilapia farming. The sampling design was segmented by epidemiological unit, district and department, with different sample sizes based on the expected prevalence of each segment. The model used pools of 5 individuals and collections at two points in time (May/June and October/November) in order to evaluate different fish cycles and seasons (Sanipes; Peru, 2020). Due to the lack of published results, it is not possible to analyze the effectiveness of the plan, but among all the Latin American countries, the Peruvian government's plan is certainly the one with the most detailed actions.

Chile. The country, whose Atlantic salmon industry is one of its main commodities, went through a severe health and economic crisis between 2007 and 2011 due to the strong impact caused by the Infectious Salmon Anemia (ISA) virus (Godoy et al., 2013). Since 2011, the country has had a health surveillance program for ISA called the Specific Health Program for the Surveillance and Control of Infectious Salmon Anemia (PSEVC-ISA), revised in 2019 (Resolución 1577 Exenta, updated by resolutions 228 and 3610 exentas; Sernapesca; Chile, 2011), which establishes a quarterly protocol for collecting 150 individuals from fish farms with fingerlings and 30 fish from fattening cages on the high seas for diagnosis by specific RT-PCR for the ISA HPR virus, always opting for the ponds/cages with the highest mortality and carrying out at least one pool per pond/cage. With this surveillance method for early detection and a series of measures that included the regionalization of production in "barrios", Chile has managed to establish control of this virus after the health crisis experienced previously.

United States. The US, through the U.S. Department of Agriculture (USDA) and Animal and Plant Health Inspection Service (APHIS), maintains TiLV surveillance for suspected cases but does not yet have a sampling plan for epidemiological surveys (USDA; US, 2019) because its aquaculture is more focused on salmon farming. It has had a Surveillance Programme for ISA HPR0 (non-virulent) and HPR-deleted (virulent) since 2002, last revised in 2023 (USDA; US, 2023), consisting of seven components, including laboratory testing (RT-PCR, indirect fluorescent antibody test and viral isolation). Sampling is targeted (n of 5 to 10 moribund or recently dead fish) and carried out by surveillance veterinarians on a routine monthly visit. To carry out the tests, the kidney of a single fish is used per test, with the exception of the viral isolation test where pools of kidney, spleen and heart from up to 5 individuals are permitted. The number of surveillance inspections with sampling varies from month to month, but an average of 10 sites and 100 fish are sampled each month (USDA; US, 2023).

The APHIS/USDA Surveillance Program for ISA establishes a categorization that goes from 1 (when the establishment presents negative results for two or more months) to 6, depending on the number of positive fish and the frequency. Active sites in the bay must undergo outbreak elimination to raise their category to 2, when they will undergo biweekly testing until there have been two months of negative test results. These categories are intended to provide more information to the Program's Technical and Veterinary Council for further evaluations, epidemiological investigations and planning of laboratory surveys.

Canada. In Canada, the Canadian Food Inspection Agency (CFIA) maintains a surveillance program for Atlantic salmon diseases and is supported by another agency, Fisheries and Oceans Canada (DFO). In 2012, the CFIA proposed a surveillance plan for the province of British Columbia to have salmon sampled for three diseases: ISAV, Infectious Hematopoietic Necrosis (IHN) and Infectious Pancreatic Necrosis (IPN). Nearly 5,000 wild fish were tested over more than two years, making this an important surveillance strategy, especially for semi-open systems such as salmon fattening farms (FFA-GovNL; Canada, 2020). On the country's southern coast, in the province of Newfoundland and Labrador, considered one of Canada's largest salmon production centers, a surveillance plan for Atlantic salmon diseases has been implemented that samples fish suspected of any type of mortality. In practice, surveillance is carried out by veterinarians appointed by the facility/company, who must visit the farms at least once a month to inspect them and take samples.

According to the report produced by a committee of fish epidemiologists hired by the local government, the number of samples ranged from 6 to 15 fish (median=10), more than the sampling of 5 fish recommended by the local Aquatic Animal Health Division (AAHD). Assuming that the prevalence of the disease in the 5 fish sampled was conservatively 80% and not 100%, and that the tests were at least 60% sensitive, the protocol adopted, according to these experts, would be able to guarantee 95% confidence in detecting the underlying condition of the diseases (FFA-GovNL; Canada, 2020).

Norway. Outside the American continent, we can refer to Norway's official aquatic animal veterinary service as one of the best structured in the world, which runs surveillance programs against ISA HPR0 and HPR-deleted, Renibacterium salmoninarum (Bacterial Kidney Disease or BKD), Viral Hemorrhagic Septicemia (VHS), IHN, Gyrodactilus salaris and other diseases in Atlantic salmon and rainbow trout species. The Norwegian Food Safety Authority (NFSA) carries out the surveillance programs, while the Norwegian Veterinary Institute is the agency responsible for the epidemiological analyses and risk assessments of the health programs. Since 2019, the Norwegian Veterinary Institute has carried out annual epidemiological surveys for ISA HPR0 and HPR-del with a focus on hatcheries (breeding establishments). There is also systematic sampling in pens and establishments in Infectious Salmon Anemia-free zones (Jansen; Oliveira, 2021; Moldal et al., 2022).

In Norway, all salmonid hatcheries are sampled by the NFSA at most every two years. The sample size is 90 individuals per production unit, randomly selecting 9 fish from 10 different ponds. From this, 30 pools are formed containing 3 fish each which are tested by RT-PCR (Jansen et al., 2022). It is important to note that the ISA surveillance program uses a smaller sample size because the country is not considered a free zone for this virus. The aim of this system is to map the occurrence of ISA HPR0 and HPR-del in the different production types.

As for exotic viruses, the Norwegian official veterinary service establishes a risk-based surveillance routine for VHS and IHN in salmonids, with the aim of documenting the absence and carrying out early detection of these pathogens in order to apply a contingency measures. Norway has been considered an VHS- and IHN-virus-free zone since 1994, although it recorded an outbreak of VHS in 2007 and re-established free status in 2011 (Moldal et al., 2022). The current surveillance model for VHS was developed and implemented in 2016 using a stochastic simulation model (Lyngstad et al., 2016) to replace the very expensive old model that required 10 times as many samples and tests. The current surveillance system is based on routine inspections carried out by the private service (Fish Health Personnel-FHP) in strata with the highest risk of introducing the disease and has a high capacity for detecting VHS in farmed marine salmonids. The FHP carries out six routine inspections a year on salmon farms, when the sites to be sampled are defined based on the risks of infections, stress and increased mortality. Sampling of free-living wild salmonids is also included, due to their high susceptibility. Sampling is targeted at moribund or recently dead fish and the sample size varies greatly between sites (mean sample size of n=5 for rainbow trout and n=9 for Atlantic salmon). The individuals collected are subjected to real-time RT-PCR testing (Gjevre et al., 2016). The surveillance system for VHS based on monitoring with routine inspections at high-risk sites and targeted sampling has been running in Norway since 1980 and offers, according to Lyngstad et al. (2016), a high probability of being SAV-free (95% Probability of Freedom) as it is a highly pathogenic and transmissible disease.

DEFINITION OF SAMPLING

According to the Aquatic Animal Health Code (WOAH, 2019), surveys can be carried out on the entire target population (census) or on a sample. As for the types of surveillance that can be applied, the Aquatic Animals Health Code explains that sampling can be based on probabilistic methods (simple random selection, cluster sampling, stratified sampling, systematic sampling) so that data from the study population can be extrapolated to the target population in a statistically valid way. However, it points out that methods based on non-probabilistic sampling can also be used when it is recognized that sampling some populations of aquatic animals is impractical, in order to optimize the detection of pathogens in a given region. To do this, the sources of information must be fully described and must include a detailed description of the sampling used to select test units.

Another point that should be emphasized is the great difference that exists in relation to epidemiological studies and monitoring of terrestrial animal diseases in terms of the applicability of execution. Prevalence or freedom proof study studies, for example, are usually carried out for various diseases such as Foot and Mouth Disease, Classical and African Swine Fever, Brucellosis bovine, Avian Influenza etc., aimed at assessing the epidemiological situation of states and countries, early detection or recognition of free zones (Cameron, Baldock, 1998; WOAH, 2019; WOAH, 2023). The vast majority of these studies use highly sensitive screening tests such as serological assays, which are much cheaper than the molecular tests used for aquatic animals. In addition, there is no need to sacrifice the animals, and only blood collection is required to obtain serum. For aquatic animals, there is almost always a need to sacrifice the animals, causing losses for the farmer. Laboratory tests using non-lethal samples for TiLV diagnosis (mucus and blood) are still in the testing phase and require further studies to assess the sensitivity and specificity of the tests. (Liamnimitr et al., 2018).

Prevalence studies for aquatic animals have another important limitation, especially when referring to rare diseases or those with low prevalence in the population, which is the need to work with high precision compatible with the expected prevalence of the disease within the target population, resulting in large sample sizes (Epitools, Ausvet; Fejzic; Mardones, 2021). Furthermore, cross-sectional studies are also doomed to unequivocal interpretations if they do not take into account the seasonality and epidemiological characteristics of the target pathogen. In this sense, studies or monitoring carried out at different times of the year can provide more accurate results (Corsin et al, 2009; WOAH, 2019).

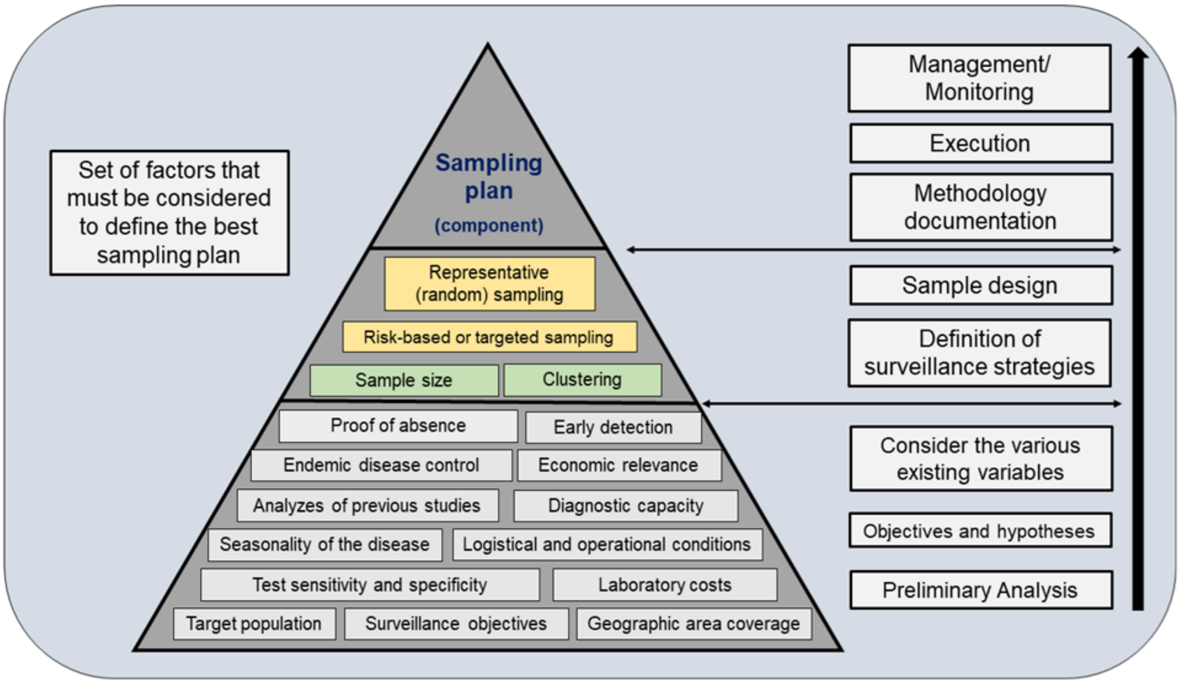

Fig. 3. Overview of the main factors that should be considered when defining the sampling plan for fish disease surveys or monitoring.

Differences between random and targeted sampling

Based on the sampling plans mentioned in this paper, there is a trend towards the use of risk-based surveillance and targeted sampling, given the great logistical and economic difficulties in carrying out epidemiological studies based on random sampling (Lyngstad et al., 2010; Lyngstad et al., 2016).

Currently, a smaller sample is accepted for the investigation of suspected cases of TiLV. The FAO launched a Strategic Manual for the global control of TiLV, admitting that a sample of 5 fish with compatible clinical signs is sufficient for the molecular diagnosis of the disease (FAO, 2021). Another alternative that has been used to reduce laboratory costs is the use of pools of fish and samples for molecular testing using validated methodology (Laurin et al, 2019). To detect subclinically infected fish or for targeted surveillance where many samples are required, tissues with similar weights or volumes from each fish (e.g. 5 fish) can be pooled into a sample (Yamkasem et al., 2021b). In Brazil, the pools used for diagnosing fish diseases in the main laboratories do not usually consist of more than 3 individuals.

Epidemiological studies based on random sampling require a very high n and the authors of the Aquatic Animal Health Code themselves recognize this limitation (WOAH, 2019). For this reason, various veterinary services or groups of researchers have used alternative and economically viable means to carry out epidemiological assessments (US, 2023; Delphino et al., 2019, Barato et al., 2022, Seagri, 2023) or even to declare an area free (Lyngstad et al., 2016) based mainly on targeted sampling.

It is well known that the sensitivity of tests is directly related to the quality of surveillance results (Delphino et al, 2023). When targeted samples (suspect fish) are used, there is a natural increase in the sensitivity of the study (Corsin et al., 2009). Even though there may be cases of positive individuals in asymptomatic fish, there will always be a greater chance of the tests detecting the pathogens when they are symptomatic (Barato et al., 2022). This survey/monitoring model allows for the collection and processing of a much smaller number of samples than those used in randomized design studies, and consequently generates less expenditure on logistics and laboratory analysis. It is therefore possible to gather evidence from all production strata, including the most numerous such as fattening and livelihoods.

At the end of this decade, there was a change in Norway's VHS surveillance program (previously conducted according to Council Directive 91/67/EEC), which was very costly (Hellberg et al., 2009). Expenditure estimates of 652,000 euros (47 euros per individual sample) led the VHS program to seek a risk-based approach and targeted sampling (Lyngstad et al., 2010) associated with other active surveillance components such as clinical inspection (Gjevre et al., 2015).

In the DF, where tilapia production is of low economic importance at a national level, it was decided to carry out two consecutive and complementary epidemiological studies: one based on a targeted sample and the other on higher risk farms (young fish). This monitoring is associated with the execution of other less costly surveillance components such as routine visits to inspect fish farms, investigations into reports of suspected diseases and inspecting fish in fish slaughterhouses. Before defining this model, a prior analysis of logistical and laboratory costs was carried out and in order to carry out a study of the prevalence of six diseases in all production groups (young fish, pay-to-fish and fattening farms) at a minimum cost of USD 8.00 per test, a total of 18,600 molecular tests were estimated based on a DP of 2% for 4 viral diseases and 10% for 2 bacterial diseases, and a minimum cost of approximately USD 140,000.00. Among the farms, many are considered small and work with cycles of a few fish. If, for example, a producer fattened 2000 fish per cycle, if it were sampled, it would be necessary to sacrifice almost 8% of the animals for sampling, many of them completely healthy, generating a considerable economic impact on the farm's income. It is estimated that both studies conducted in the DF, using targeted sampling and RBS, generated an approximate laboratory cost of USD 12,000.00, or about 1/12 of the amount previously estimated.

CONCLUSIONS

Based on the review, it can be concluded that there is a very wide variety of designs and sample sizes among the different health plans and programs for diseases of tilapia and other fish species, with a very strong tendency to use risk-based strategies and targeted sampling with the aim of reducing logistical and laboratory costs. In the Distrito Federal, the only federal unit in Brazil with a surveillance plan for tilapia diseases, the models applied were defined based on strategies aimed at reducing costs and increasing the sensitivity of the research, following a trend widely used in monitoring and studies conducted in other regions and countries.

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CAPÍTULO III

PRODUCTIVE AND SANITARY CHARACTERIZATION OF COMMERCIAL TILAPIA FARMING IN THE DISTRITO FEDERAL, BRAZIL (2021-2022)

Artigo científico para submissão ao periódico ***Aquaculture Reports***

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ABSTRACT

Brasília, Distrito Federal, is among the Brazilian cities with the highest number of tilapia farms, with around 660 farms, of which 112 are commercial. The aim of this study was to characterize the production and health aspects of commercial tilapia farming in the Distrito Federal by applying a semi-structured questionnaire. The farms were categorized according to the degree of vulnerability to the introduction of pathogens and the risk of dissemination using two weighted scorecards tables that evaluated 15 items each. After calculating the mean between the two variables, the farms were classified from A (insignificant risk) to D (high risk). After analyzing the data, it was found that most of the commercial tilapia farms in the Distrito Federal were categorized as B (39; 34.8%) and C (53; 47.3%), representing low and medium risk, respectively. When comparing the different commercial groups, a significant difference ($p<0.05$) was observed between the mean scores of closed system fattening farms and semi-closed fattening and pay-to-fish farms, with closed system fattening such as Biofloc, Aquaponics and Recirculation Aquaculture System being the group of farms with the lowest vulnerability to the entry of pathogens and the lowest risk of spreading diseases. The results obtained can be useful for the official veterinary service during risk-based surveillance strategies for tilapia by categorizing farms in terms of their level of biosecurity, prophylaxis and best practices.

Keywords: tilapia farming; diseases, biosecurity; best aquaculture practices, pathogen introduction; pathogen spread, risk ranking.

1. Introduction

Global tilapia production reached 6.5 million tons in 2022, making it the second most cultivated fish in the world, behind carp (FAO, 2023). In Brazil, the tilapia industry has established itself as an important animal protein commodity, representing the main segment of Brazilian fish farming, with production estimated at 580.000 tons in 2023 and a 65% share of total production. The annual turnover of this industry in Brazil alone exceeds USD 1,2 billion (Peixe-BR, 2024). Brazilian tilapia farming is characterized by diversified production, ranging from small family farms that help supply local markets to large commercial enterprises represented by agro-industrial cooperatives and companies specializing in vertical production. Endowed with great expertise in refrigeration and large-scale production acquired over decades in the production of poultry, pork and beef, these companies have the potential to sell large volumes of tilapia meat on the domestic and foreign markets.

The Distrito Federal (DF) is a Brazilian federative unit made up of a single municipality, Brasília, the capital of Brazil, which is among the Brazilian cities with the largest number of tilapia farms (Brasil, 2022). The DF's aquaculture production data is estimated at 2,000 tons/year, with tilapia accounting for over 88% of this amount (Seagri, 2023). Although this volume is not very representative in Brazil, the segment encompasses approximately 660 tilapia farms, 83% of which are livelihood farms for their own consumption and 17% for commercial purposes (Seagri, 2023). This production chain serves as a source of food and income for hundreds of producers, as well as generating direct and indirect jobs for thousands of people.

Tilapia farming in the DF predominantly consists of small and medium-sized (Raposo, 2024) which play a significant role in supplying fish to the local market, the third largest in Brazil. With an average consumption rate of approximately 14 kg per inhabitant per year, this rate significantly exceeds the national average estimated at 10 kg per inhabitant per year (Borges, 2010; Peixe-BR, 2024). The DF also has 25 fish slaughtering or processing establishments with federal or district inspection, but most of the production processed comes from other states. There is scarce data on the health aspects related to tilapia farms in the Distrito Federal, such as the level of infrastructure and technification, the implementation of best management practices, biosecurity, disease prophylaxis and the frequency of the most impacting diseases, although reports from local health authorities estimate relatively low expected prevalence rates for officially controlled diseases (Seagri, 2023).

The aims of this study were: to characterize the production and health aspects of commercial tilapia farming in the DF, using a semi-structured questionnaire; to categorize and evaluate the biosecurity of farms, as well as the best aquaculture practices of the different production typologies; and to propose an adapted model for categorizing the risk of tilapia farms to help with disease surveillance programs and health risk management.

2. Materials and Methods

2.1 Target population and study site

The study's target population consisted of all commercial tilapia farms (n=112) registered with the animal health service of the Distrito Federal's Secretariat of Agriculture (SEAGRI), identifying three commercial production typologies: 1) hatchery (breeding) or alevin sales establishments; 2) commercial fattening farms subdivided into 2-a) closed system fattening farms and 2-b) semi-closed system fattening farms and 3) recreational fishing establishments ("pay-to-fish"). The classification of production systems was based on Brazilian health legislation (MPA Normative Instruction No. 4/2015; Brasil, 2015), which considers closed systems to be those where tilapia are grown in structures with total water recirculation, aquariums, bioflocs and other similar systems where there is control of water flow and animal movement, and semi-closed systems to be all farms where there is control of fish movement with partial control of water flow, as in the case of earthen ponds (permeable or impermeable), weirs or continuous flow systems (raceways).

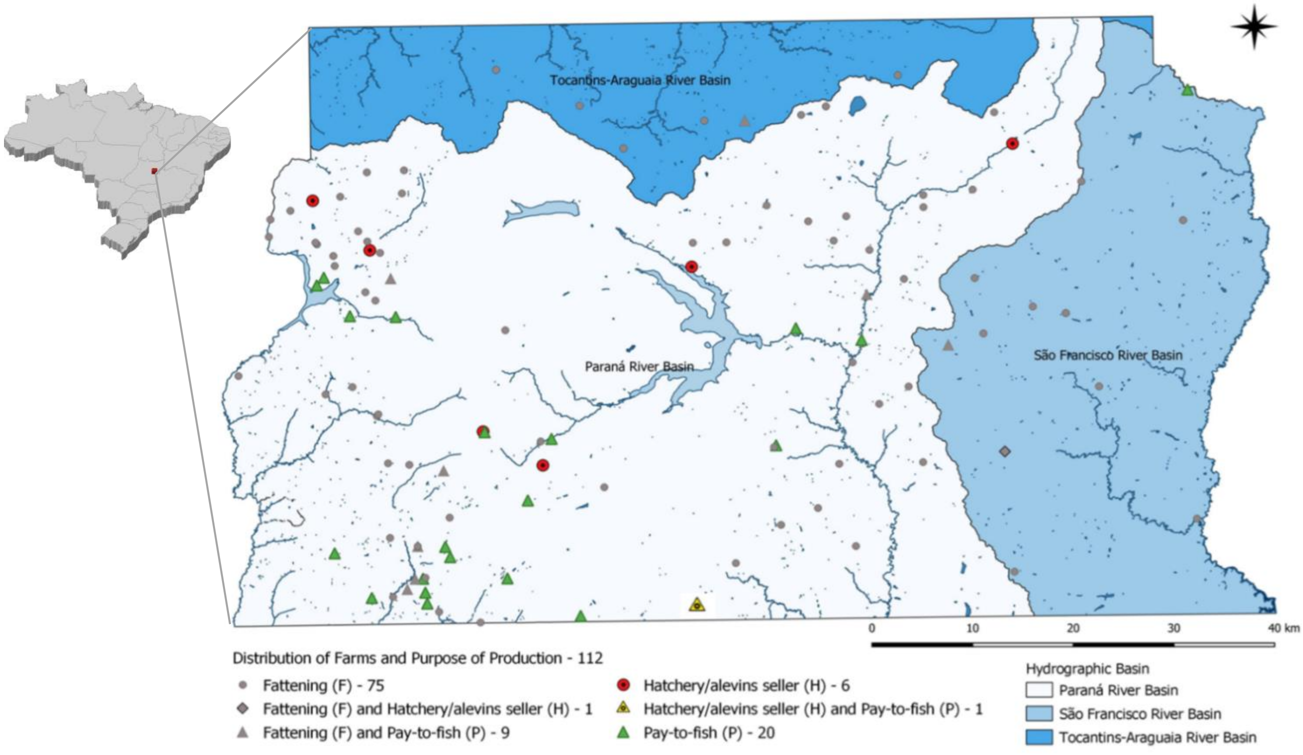

Fig. 1. Spatial distribution of commercial tilapia farms in the Distrito Federal, according to the purpose of production and the national river basins in the territory of the DF.

2.2 Questionnaire and ethical issues

The participating tilapia farmers agreed to take part in the study voluntarily, by signing a term of commitment. The study was approved by the University of Brasilia's Animal Use Ethics Committee under SEI No. 23106.080975/2021-63.

A semi-structured questionnaire covering production and health aspects, including possible risk factors for disease, was drawn up and administered to the participants. The questionnaire contained closed dichotomous, semi-open and multiple-choice questions. In order to define the variables that made up the questionnaire and the respective response structure, three tilapia farmers were randomly selected for a pilot interview, which was also conducted with open-ended questions to better adjust the content and responses. Before the final application, a pre-test was carried out with two other tilapia farmers, also randomly selected, to adjust the accuracy of the answers and data tabulation. The questionnaire was administered by 3 veterinarians and 2 agricultural technicians from the local official veterinary service (OVS) and consisted of 80 questions divided into four sections: producer and farm registration data; socio-economic and production characteristics; characteristics of best practices, prophylaxis and aquaculture biosecurity; and farmers' perception of mortalities.

2.3 Evaluation of atypical health events (AHE)

The farms that answered the questionnaire were monitored between July 2021 and June 2022 (12 months) with constant communication via a smartphone messaging app. The app used was WhatsApp® (Meta Platforms, Inc., California, USA) because it is an easy-to-use social media already used by all farmers. When atypical health events (mortality or observation of clinical signs) were reported, immediate visits were made to assess the health conditions of the production. During these visits, water temperature, dissolved oxygen, pH, toxic ammonia, alkalinity and turbidity were measured in order to rule out mortalities caused by management failures.

2.4 Sanitary categorization of farms

The farms were categorized using a multi-criteria risk analysis that assessed the degree of vulnerability to pathogens entering the production system (vulnerability level; VL), i.e. the establishment's ability to prevent the introduction of a given disease, and the risk of these pathogens spreading to other farms once they have entered the production system (risk of dissemination; RD).

2.4.1 Weighted points table

Weighted scorecards were generated and adapted, based on three previously published models: one for swine breeding farms certified for their degree of vulnerability to external pathogens (Brasil, 2002) and two others for risk classifications/categorizations in commercial fish production (Kleingeld, et al., 2010; Diserens et al., 2013). The scoring was based on criteria and proportions listed in several previous studies (Kleingeld, et al., 2010; Diserens et al., 2013; Oidtmann et al., 2013; Diserens et al., 2017). The adaptation was necessary to adapt the criteria according to the peculiar aspects of tilapia cultures in closed and semi-closed systems, which are the predominant ones in the DF. The data from the questionnaire was used to categorize each individual risk factor using a scale of 0 to 3 where the values 0, 1, 2 and 3 correspond to null, low, medium and high risk, respectively.

The tables were made up of 15 criteria each, totaling 30 verification items. In this way, scores were obtained which allowed the establishments to be classified in terms of their degree of vulnerability to pathogen entry (Table 1) and the risk of dissemination (Table 2). The VL classification ranged from well protected to highly vulnerable to pathogens entry (Table 1), while the RD classification ranged from insignificant risk to high risk (Table 2) for the spread of pathogens. The calculation of the biosecurity level (BL) and the respective categorization were determined from the mean between VL and RD of each farm, according to the formula below, and scores described in Table 3.

$$BL = \frac{VL + RD}{2}$$

The risk scores obtained were converted into risk categories (Class A, B, C and D) in order to determine the BL of the farms.

Table 1. Points table for classifying and assessing the level of vulnerability of tilapia farms with regard to the entry of pathogens. Adapted from models by Diserens et al. (2013), Diserens et al. (2017), Kleingeld, et al., 2010 and NI MAPA-Brasil No. 19/2002.

Variables	Criteria	Points	Score
1. Origin of young forms or matrices, broodstock and adult fish if applicable	Originating from a certified establishment or with regular diagnostic testing of the broodstocks and young fish	0	
	Origin from establishments registered with the OVS, without certification or regular diagnostic tests	2	
	Origin from establishments without origin, without OVS registration or from extractive fisheries	3	
2. Presence of quarantine establishments, ports, airports, national and international aquaculture event parks	There are no quarantine facilities, ports, airports or event parks adjacent to the fish farm or up to 3km from the site	0	
	There are quarantine facilities, ports, airports or event parks adjacent to the fish farm or up to 3km from the site	3	

3. Controlling the entry of people and vehicles into the fish farm	Restricted access and control of the entry of people and vehicles with a disinfection arch, footbath or bath and/or disinfection of fomites	0	
	Restricted access and control of entry (without disinfection) with a low annual flow of people and vehicles and provided that it is not pay-to-fish	1	
	Control of entry with disinfection of equipment (including fishing trays in pay-to-fish) and with a low or moderate flow of people and vehicles	1	
	Control of entry without disinfection (including fishing line in pay-to-fish) and with a large flow of people and vehicles	2	
	Unrestricted access and high flow of people and vehicles without disinfection of equipment, people and vehicles	3	
4. Quarantine and preventive measures before introducing new batches of fish	Does quarantine or only buys from a certified supplier	0	
	Does not quarantine, but uses other measures such as salt baths, stimulants, vaccination, etc.	1	
	Does not quarantine or take any measures with newly acquired animals	2	
5. Water source	Uses water from subterranean sources (artesian well or spring) or spring water or surface sources with 100% effective treatment to eliminate pathogens	0	
	River/stream, reservoir and other surface sources with affluent treatment	1	
	River/stream, reservoir and other surface sources without affluent treatment	2	
	River/stream, reservoir or surface sources with confirmed presence of target disease in the region, without efficient affluent treatment	3	
6. Frequency of fish acquisition (without certified origin)	Up to 2 times a year	0	
	2 to 4 times a year	1	
	5 to 9 times a year	2	
	More than 10 times a year	3	
7. Number of suppliers of young forms (or matrices/broodstock)	Only one supplier per cycle or year	0	
	Switch between more than one supplier in the same cycle or year	2	
8. Presence of domestic land animals	Production system without domestic animal access	0	
	Production system with access for domestic land animals	1	
9. Presence of other aquatic animals	Production system without polyculture	0	
	Production system with polyculture	1	
10. Presence of wild animals	There are protective nets and/or barriers preventing access by birds, reptiles, amphibians and aquatic mammals	0	
	There is poor netting, holes or no protective netting or barriers against wild animals (birds, reptiles, amphibians and aquatic mammals)	2	
	Wild fish are able to access the production system	3	
11. Flooding in the production area	Never occurred	0	
	Has already occurred or may occur	2	
12. Live fish food	No	0	
	Yes	2	
13. Routine feeding	Commercial feed	0	
	Own feed not thermally processed	1	
14. Fertilizing the ponds	Uses only chemical fertilizer or thermally processed products	0	
	Uses organic fertilizer that includes land animal waste	2	
15. Production system	Closed system (e.g. RAS, Bioflocs, etc.) or total control of the water entering the system (through closed pipes)	0	
	Semi-closed system with permeable or impermeable earthen ponds with total control of the water entering the system, always through closed pipes.	0	
	Semi-closed system with water entering through open permeable or impermeable channels	1	
	Semi-open system (e.g. cages in reservoirs) and open system	3	

Classification of farms according to their vulnerability level (VL):

a) Safe Establishment (SE) = score between 0 and 3 points and provided there are no criteria with a score of 2 or 3;
b) Highly Protective Farm (HP) = score between 0 and 3 points and provided there are no criteria with a score of 3;
c) Low Vulnerability Farm (LV) = up to 7 points and as long as there are no criteria with a score of 3 and it does not qualify as a HP;
d) Moderate Vulnerability Farm (MV) = 8 to 10 points;
e) High Vulnerability Farm (HV) = 11 points and above.

Table 2. Points table for classifying and assessing the risk of dissemination of pathogens (RD). Adapted from models by Diserens et al. (2013), Diserens et al. (2017), Kleingeld, et al., 2010 and NI MAPA-Brasil No 19/2002.

Variables	Criteria	Points	Score
1. Trade in young forms	Does not sell young forms;	0	
	Sells young forms with health certification	0	
	Sells young forms without health certification	3	
2. Presence of other tilapia farmer(s) within a radius of 1 km	There are no tilapia farmers within a radius of 1 km	0	
	There are tilapia farmers within 1 km, but few in the region	1	
	There are tilapia farmers and the farm is located in a region or river basin with a high concentration of fish farms	2	
3. Treatment of fish farm effluent	Disposal water is not returned to nature (irrigation, another production unit without aquatic animals, reuse in a closed system)	0	
	Water treatment using methods with 100% efficiency in eliminating pathogens.	0	
	Treatment is carried out, albeit without maximum efficiency for controlling pathogens. E.g. filter system with sand, gravel, activated carbon, biological filter	1	
	Performs some treatment, but not very efficiently to eliminate pathogens. E.g. only a settling tank	2	
	Discharges the water into nature without any treatment	3	
4. Pond cleaning	Empties the water, removes waste from the bottom, applies quicklime or another disinfectant, and makes sanitary void for at least 7 days at the end of each cycle.	0	
	Empty the water, remove waste from the bottom, apply quicklime or another disinfectant and makes sanitary void for at least 7 days only every 2 or more cycles	1	
	Empties the water, removes waste from the bottom, applies quicklime or another disinfectant, but it doesn't make a sanitary void	1	
	Empties the water, removes waste from the bottom, makes sanitary void for at least 7 days, but does not apply quicklime or another disinfectant between cycles	1	
	Only empties and removes waste from the bottom without combining other measures	2	
	Empties the pond after long periods or never empties it for cleaning, disinfection and sanitary void	3	
5. Disinfecting equipment and fomites	Regularly disinfects equipment and objects using a product with a recognized sanitizing action	0	
	Sporadically disinfects equipment and objects	1	
	Rarely or never disinfects equipment and objects	2	
6. Employees and people who visit other fish farms	No employees or people who visit other fish farms	0	
	Has an employee or people who visit other fish farms regularly, but does change clothes and disinfect equipment	0	
	Has an employee or people who regularly visit other fish farms and does not change clothes or disinfect equipment	1	
7. Sharing equipment with other fish farms	Does not share handling equipment	0	
	Shares handling equipment	1	
8. Mixing fish on the same farm	Never mixes fish from different ponds or batches	0	
	Mixes fish from different ponds or batches	1	
9. Moving fish between farms	Never moves. Carries out the entire cycle and only sends for slaughter or consumption.	0	
	Moves, but transport boxes are exclusive and disinfected or use disposable means (e.g. plastic bags)	1	
	Moves using a truck with outsourced transport boxes and takes care to disinfect the boxes	2	
	Moves using a truck with outsourced transport boxes and no care is taken to disinfect the boxes	3	
10. Destination of slaughter-age fish after harvesting	Slaughter in establishments with an official inspection service or rearing for own consumption or only selling young forms	0	
	Slaughter in places without official inspection. E.g. fairs, restaurants,	1	

	fishmongers		
	Unknown destinations, fish caught and taken fresh (in the case of pesque pagues) or fish sold at the farm gate	2	
11. Gutting fish at the edge of streams, rivers, reservoirs, etc.	Not done	0	
	Does	3	
12. Handling moribund fish	Remove alive immediately	0	
	Taken away together with the collection of mortality	1	
	Does not usually remove	2	
13. Collection and disposal of dead fish	Collects dead fish daily and disposes of 100% of the dead for composting, burial, incineration, collection by a cleaning service or other recommended disposal	0	
	Does not regularly collect dead fish, but disposes of 100% for composting, burial, incineration, collection by a cleaning service or other recommended disposal	1	
	Dead fish are disposed of in pits, bushes, grottos, fed to other animals, or left to decompose in the production area	3	
14. Stocking density	Low and moderate density (up to 4 kg per m²)	0	
	High densities (above 4 kg per m²)	1	
15. Type of system and structure	Closed or semi-closed system, impermeable elevated (concret, tarpaulin, plastic and fiberglass ponds) or earthen pond supplied with closed pipes and no external communication before water treatment	0	
	Semi-closed system, earthen pond with permeable or impermeable bottom with communication between the ponds and water outlet from the system by pipe	1	
	Semi-closed system with earthen ponds with permeable or impermeable bottoms and an outlet from the production system through open channels without any protection	2	
	Semi-open (e.g. cage in reservoirs) or open system	3	
Risk of pathogen dissemination (RD): a) Insignificant risk (IR) = score between 0 and 3 points and provided there are no criteria with a score of 3; b) Low risk (LR) = up to 7 points and provided there are no criteria with a score of 3 and it does not fall under IR; c) Moderate risk (MR) = 8 to 10 points; d) High risk (HR) = Above 11 points.			

Table 3. Table of points used to categorize commercial tilapia farms into four different biosecurity classes.

Category	Farm classification	Score and condition for category
Class A	Insignificant risk	BL ≤ 4.0 and as long as there are no criteria with a score of "3" in VL and RD
Class B	Low risk	BL = 4.1 a 7.0
Class C	Moderate risk	BL = 7.1 a 10.9
Class D	High Risk	BL ≥ 11.0

Based on the VL, RD and BL of all the farms, tests were carried out to compare the means between the different types of production and a descriptive analysis of the health profile of commercial tilapia farming in the Distrito Federal.

2.5 Statistical analysis

In this study, we carried out a descriptive analysis of the data from the set of answers to the questionnaire and the categorization of BL based on the mean obtained from two tables of points that assessed the VL and RD, respectively. As it involved all commercial tilapia farms registered with SEAGRI's animal health service, characterizing a census, it was not necessary to calculate confidence intervals for the results.

The VL, RD and BL variables were subjected to the Shapiro-Wilk normality test to check that the data for each group followed a normal distribution. The averages of the categorization scores were compared to determine the strata of greatest vulnerability and risk of spreading diseases using analysis of variance (ANOVA), followed by the Bonferroni post-test to identify the difference between the mean of the typologies assessed ($p<0.05$).

Stata®version 17 (StataCorp 2022, College Station, TX, USA) was used for data organization and statistical analysis.

3. Results and discussion

3.1 Productive and socioeconomic characteristics

Distrito Federal is divided into 33 Administrative Regions and cut by 3 Brazilian river basins which are segmented into 7 micro-basins (Adasa, 2023). Tilapia farms are mainly concentrated in the Brazlândia, Gama, Planaltina and Paranoá regions. Table 4 shows the river basins with the largest number of commercial tilapia farms in the DF.

Table 4. Absolute and proportional number of tilapia farms interviewed in the study by river basin in the Distrito Federal.

Microwatershed	Brazil's watershed	No. Farms	%
Descoberto River	Paraná Basin	36	32,1
São Bartolomeu River	Paraná Basin	34	30,4
Corumbá River	Paraná Basin	17	15,2
Paranoá Lake	Paraná Basin	12	10,7
Rio Preto River	São Francisco Basin	7	6,2
Maranhão River	Tocantins-Araguaia Basin	6	5,4
São Marcos River	Paraná Basin	0	0

Two-thirds (75; 66.0%) of the commercial tilapia farmers in the DF do not have fish farming as their sole source of income, and the activity is a supplement to the family budget. This socioeconomic profile, which combines aquaculture with other sources of income, is similar to other regions of the world, including neighboring countries (Campo-Plata; Manjarrés-Martínez, 2019; FAO, 2022). The figures 2 and 3 illustrate other characteristics of the socio-economic profile of tilapia farm owners in the Distrito Federal.

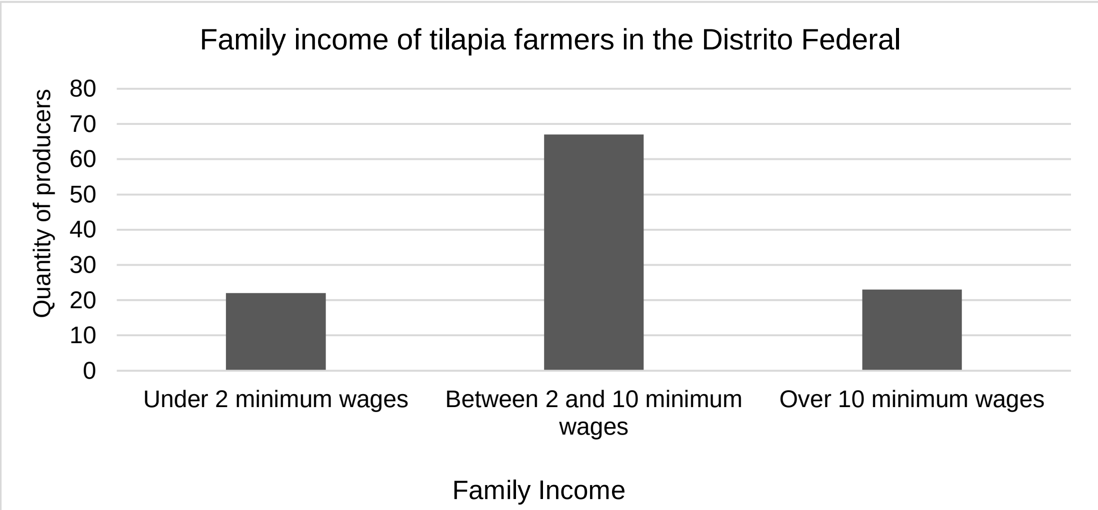

Fig. 2. Graphical representation of the family income of tilapia farmers in the Distrito Federal separated into three socio-economic groups according to monthly income.

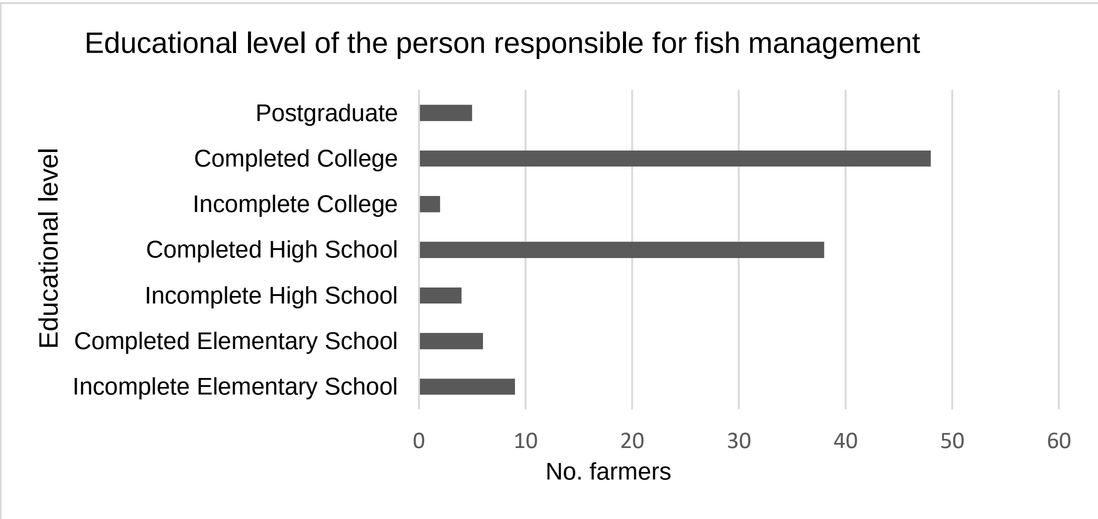

Fig. 3. Graphical representation of the level of education of the person responsible for tilapia production and management.

The highest level of education is found among closed system producers, where 88.8% of farm owners (8/9) have a university degree or post-graduate degree. The second group with the highest level of education is that of alevin producers/sellers, where 87.5% (7/8) of the producers have completed high school, college or postgraduate studies. On the other hand, it is the owners of pay-to-fish farms who have proportionally the lowest level of schooling, as 25.9% (7/27) have no more than primary education. Among the commercial fattening stratum, there was an excellent level of education among the producers, i.e. 81.9% (63/77) had completed high school, college or postgraduate studies.

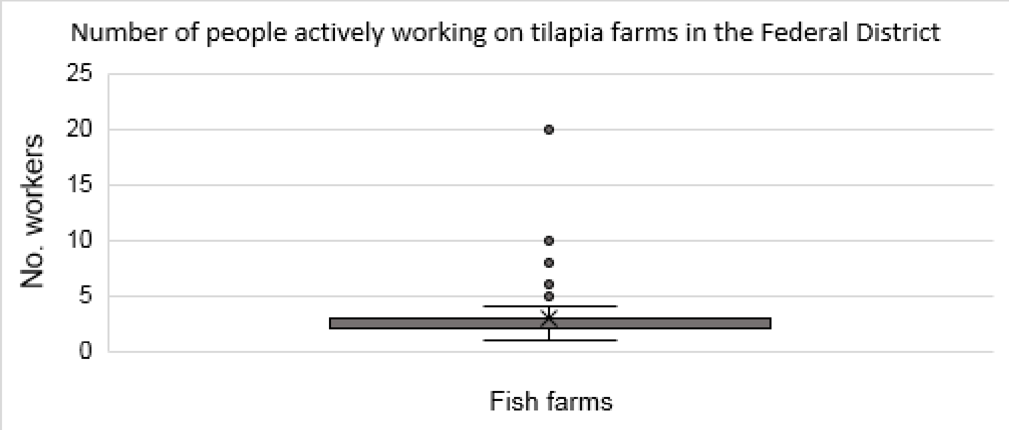

Fig. 4. Box Plot illustrating the distribution of the number of people actively working in tilapia farms in the Distrito Federal. An outlier of 78 employees was hidden from the graph to better visualize the distribution, mean, median and quartiles.

Regarding fish farming labor, 43.8% (49/112) of those interviewed said that they only had their own or family labor, 20.5% (23/112) hired labor and 35.7% (40/112) mixed labor. These results show that the majority of the businesses are small, reflecting the low production and high seasonality of commercialization, with sales peaks during Holy Week. A mean of 3.23 (SD=5.85) workers per farm was observed. Figure 4 illustrates the number of people actively working on fish farms in the DF.

Although the DF is not considered a large tilapia production center, the socio-economic data shows some similarities with other regions of large national production. In the region of the Três Marias reservoir, in the municipality of Morada Nova de Minas, state of Minas Gerais, Brazil, considered the fourth largest tilapia production hub in Brazil and characterized by cage tilapia cultures, 83% of farmers did not depend solely on income from aquaculture and 46.2% (12/26) were classified as family activities (Roriz et al, 2017). In the western region of Paraná, a state in the southern region of Brazil and considered the largest tilapia production center in the country (where the cultivation system in earthen ponds predominates), a recent study identified 4 different networks of fish farmers: a proximity network, represented by around 1,000 families (approximately 43%) who farm tilapia basically for livelihood and trade surplus production during Holy Week and Easter; a recreation and gastronomy network made up of 30 families who use tilapia for tourism, recreational fishing and consumption in their own restaurants; a network of small and medium-sized autonomous farmers who grow and sell their produce to independent slaughterhouses; and a commodities network characterized by a group of 550 families responsible for highly technological fish farms that work under a system of integration with large agro-industrial cooperatives geared towards national and international trade (Brenzan, 2023). In these large centers, the large volume of production is concentrated in a minority group of farms, which does not diminish the importance of small producers in this context, given their social relevance, the supply of high quality animal protein, the generation of family income and the movement of the local economy.

The predominant system in the DF is the semi-closed system, which corresponds to 92.0% (103/112) of all commercial tilapia farms, including three farms that use a mixed system (semi-closed and closed). Among the 12 closed-system farms (9 of which are exclusive), the existing systems are bioflocs (BFT), aquaponics and recirculating aquaculture systems (RAS). There are no farms that produce tilapia in open or semi-open systems (cages in rivers and reservoirs) and this may help explain the low frequency of notifiable and emerging diseases verified in the annual reports of the district veterinary service (Seagri, 2023).

The water sources for the fish farms participating in the study vary widely, with the majority being of subterranean origin (58.0%; 65/112), captured from springs or artesian wells, followed by streams (28.6%; 32/112) and rivers, lakes or reservoirs (13.4%; 15/112). The other production data obtained from the questionnaire is shown in Table 5.

The other production data obtained from the questionnaire is shown in Table 5.

Table 5. General production data from the 112 commercial tilapia farms in the Distrito Federal (2021).

Production parameter	Total	Mean per farm
Annual production (t)	1.248.035[a]	12.001[b]
Tilapia production water area (in m^2)	276.098	3.632
No. of fattening/termination ponds	230	2,9
No. of nursery ponds	100	1,4
Number of tilapia alevins sold (un)	18.000.000	2.250.000[c]
Length of cycle until harvest (in months)	-	8
Stocking density in the fattening phase (Kg/m^2)	-	2,2
Stocking density during the fattening phase ($fish/m^2$)	-	1,8
Mean weight of fish harvested for slaughter/marketing (Kg)	-	0,821

[a]Production volume composed only of the commercial farms participating in the study, without taking into account production for own consumption, livelihood, research and other purposes, whose estimated total is 1.80 tons. In 2021, the production volume was also affected by fish farms temporarily inactive due to the effects of the Covid-19 pandemic.

[b]Mean considering only the fattening and pay-to-fish farms participating in the study.

[c]Mean considering only farms that sell tilapia alevins.

Farmers in the DF usually send tilapia at slaughter weight to more than one destination. Among the participants, 68 (60.7%) tilapia farmers said they sell all or part of their production to third parties, which include neighbors, intermediaries and final consumers who buy and pick up the fish at the farm gate; 57 (50.9%) of them said they harvest for their own use (or for use in their own restaurant, the majority of which is made up of pa-to-fish); 47 (42.0%) said they sell directly to fairs, fishmongers, markets or restaurants; and only 11 (9.9%) mentioned sending their production to slaughterhouses with official inspection. This data, highlighting the low level of aquaculture fish vertical integration, mirrors a widespread scenario across many parts of Brazil (Roriz et al. 2017; Brenzan, 2023) and other high-production countries such as Egypt (Eltholth et al., 2015). In the Três Marias center, 66.7% of fish farms sent their production to slaughterhouses without SAIF/SIE/SIM and 43.% of them sent the fish directly to fairs, restaurants, markets and third parties (Roriz et al., 2017) while in western Paraná, approximately half of the producers sell on the informal market (Brenzan, 2023). In Morada Nova de Minas, in 2016, only 6.7% of farms sold fish to slaughterhouses with official inspection. One hypothesis for the sale of tilapia without official inspection is the lower price offered by slaughterhouses, which is well below the average price obtained in the informal market (Roriz et al, 2017).

3.2 Sanitary characteristics and best aquaculture practices

Among those interviewed, 45.5% (51/112) said they had some knowledge of biosecurity in fish farming. Among the farmers who answered yes, 68.6% (35/51) said they had learned biosecurity measures in specific courses, 56.9% (29/51) cited reading books, manuals and articles on the internet and 43.1% (22/51) mentioned practical learning taught by other people.

Among the farms interviewed, 83.9% (92/112) have some kind of professional assistance for tilapia production, with 79 farms (85.9%) being assisted by companies specializing in technical assistance, rural extension, professional education and management assistance, such as the Technical Assistance and Rural Extension Company (EMATER/DF), the National Rural Apprenticeship Service (SENAR/DF) and the Brazilian Micro and Small Business Support Service (SEBRAE/DF), which offer free or low-cost services to rural producers. Only 6 tilapia farms (6.5%) are assisted exclusively by professionals from the private sector. The type of professional most involved in assisting fish farms was the veterinarian (51/92), followed by zootechnicians (21/92). The wide range of free or low-cost technical assistance may have had a positive effect on the general level of biosecurity in tilapia farming in the Distrito Federal, since the adoption of various best practice and biosecurity measures in production units was carried out after guidance from aquaculture professionals.

When asked about the use of antibiotics in fish farming, only 17 farmers (15.2%) said they used this type of measure for prophylactic and/or therapeutic purposes. The most commonly cited were oxytetracycline (76.5%; 13/17) and florfenicol (23.5%; 4/17), both approved for use in aquaculture in Brazil. In this respect, cage farms in the Três Marias reservoir showed a more pronounced use of antibiotics, with 52% of producers reporting their use in mortality episodes (RORIZ et al., 2017). With regard to the use of vaccines, only one farm in the DF vaccinates tilapia against streptococcosis and ISKNV, in this case juveniles that are sold to cage farms in other states. Studies have shown that tilapia vaccination is economically viable and reduces losses caused by diseases such as SA, FO, Lactococcus sp. and ISKNV in semi-open farms (Delphino et al., 2019b; Campos, 2022). However, there is no more precise information on the economic viability applied to cultivations carried out in closed and semi-closed systems, predominant in the Distrito Federal, where the health challenge is probably lower, even though in most of the simulated scenarios vaccination is justifiable (Delphino et al., 2019).

Analysis of the parameters of the farming water is carried out daily or weekly on 61.6% of the farms (69/112). The most cited measurements were pH (85.5%; 59/69), temperature (63.8%; 44/69), toxic ammonia (52.2%; 36/69), dissolved oxygen (50.7%; 35/69) and nitrate/nitrite (30.4%; 21/69). The fact that almost 40% of commercial producers do not carry out regular water analyses shows that there is still a large proportion of farmers who are unaware of the importance of controlling the physical and chemical conditions of water and their relationship with well-being, immunity and productive performance. Perhaps this is why in the Distrito Federal the vast majority of health events reported to the local health authorities are caused by management failures such as low dissolved oxygen, pH levels that are too acidic and unsuitable for tilapia, sudden drops in water temperature, among others factors (Raposo et al, 2021; Seagri, 2023).

The questionnaire addressed various questions related to biosecurity, disease prophylaxis and best aquaculture practices. All the answers and the respective percentages are shown in Table 6.

Table 6. Responses from tilapia farmers interviewed (n=112) on the adoption of biosecurity measures, disease prophylaxis and best practices in fish farming facilities (%).

Biosecurity measures, prophylaxis and best practices that were asked about	Yes (%)	No (%)
Share equipment with other farms	0	100,0
Share employees with other fish farms	6,2	93,8
Regularly cleaning the ponds	70,5	29,5
Emptying ponds and mechanically removing waste	66,9	33,1
Application of quicklime or other disinfectant	58,9	41,1
Sanitary fallowing for at least 7 days	50,0	50,0
Use of salt in the water for preventive purposes	63,4	36,6
Regular disinfection of fish handling utensils with disinfectant	33,1	66,9
Mix tilapia between ponds and different batches	29,4	70,6
Moving live tilapia between production units	16,0	84,0
Domestic animals with access to the production area	59,8	40,2
External traffic of people or vehicles in production areas	50,0[a]	50,0[a]
Protection net against wild animals	25,0	75,0
Observation of wild animals with access to the production area	80,3	19,7
Observation of flooding in the production area	2,7	97,3
Treatment of water entering the production system (affluent)	9,0[b]	91,0[b]
Treatment of water discharged from the production system (effluent)	38,4[c]	61,6[c]
Fertilizing ponds with animal manure	25,9	74,1
Leftover feed during feedings	14,3	85,7
Gutting fish at the edge of streams, rivers and reservoirs	0,0	99,9
Recording mortality data	34,8	65,2
Daily collection of dead fish	0	100,0
Correct destination of dead fish	82,2	17,8

[a] Overall percentages, including pay-to-fish establishments, that receive visitors for recreation. Without pay-to-fish farms, the percentages change to 68.3 (No) and 31.7 (Yes).

[b] Overall percentages without considering the origin of the affluent. It should be noted that 58% of tilapia farms (65/112) are supplied by groundwater from wells or mines. The percentage of farms that treat their affluent or use groundwater is 63.4%.

[c] Overall percentages without considering the destination of the effluent. Among the farms that do not treat their wastewater, 66.6% (46/69) uses it for irrigation, other animal production units or loss through infiltration, without it flowing into natural bodies of water.

In the case of earthen or elevated ponds, one of the most efficient measures for preventing disease and reducing the load of microorganisms in subsequent batches and cycles is proper pond cleaning (Sadler, Goodwin, 2007; Iwashita, Maciel, 2013). Efficiency in eliminating pathogens is greater when fish farms adopt complete and regular cleaning of the ponds, which combines three measures: emptying the ponds and mechanically removing the waste from the bottom; applying quicklime or a product with a similar disinfectant effect; and fallowing (Iwashita, Maciel, 2013). Table 7 displays the adoption rates of thorough and regular cleaning across the surveyed typologies. Notably, the adoption of thorough cleaning is proportionally lower within the higher-technification stratum, a trend that can be attributed to the specific types of systems utilized by these farms (BFT, RAS or aquaponics). In these farms, the water passes through physical and biological filters or is directly subjected to the action of nitrifying bacteria that degrade nitrogen compounds (Zimmermann et al., 2023) where cleaning at the end of each cycle is not recommended due to the filtration system and additional costs for maturing the water.

Table 7. Stratified comparison of the proportions of commercial tilapia farms in the Distrito Federal that regularly clean their ponds according to the number of measures applied.

Stratum	Total	Farms that adopt at least two measures	%	Farms that use three measures (thorough cleaning)	%
Fattening (closed system)	9	5	55,0	3	33,3
Fattening (semi-closed system)	68	48	70,6	35	51,8
Pay-to-fish	27	11	40,7	9	33,3
Hatchery/ Alevin sellers	8	8	100,0	5	62,5

In practice, the commercial stratum that is least concerned with pond cleaning is the pay-to-fish, and this is most likely a reflection of the characteristics of this type of establishment, destined for leisure and recreational fishing, with the purchase of adult tilapia (in consumption/slaughter weight), in addition to the fact that it is unfeasible for this enterprise to completely empty the ponds and sanitary void, which would certainly lead to a loss of customers and financial turnover.

With regard to disease prophylaxis, 33.0% (37/112) carry out some kind of prophylactic procedure with the fish and fingerlings recently introduced to the farm, with salt baths (25%; 28/112) and quarantine ponds (20.5%; 23/112) being the most commonly used preventive measures. The same percentage of interviewees (37/112) said they regularly disinfect their handling equipment, with 75.7% (28/37) disinfecting it daily or weekly. The most cited disinfectant was sodium hypochlorite (81.1%; 30/37).

Overall, pay-to-fish establishments are the least likely to implement prophylaxis and biosecurity measures. In this stratum, 85.2% (23/27) of the farms allow visitors to enter with their own fishing equipment, and none of them disinfect the utensils at the entrance to the site. In 59.2% (16/27) of the pay-to-fish, visitors are allowed to use their own bait, including live bait.

Another important biosecurity aspect of the DF's pay-to-fish is that 77.8% (21/27) of them have seen customers taking away live tilapia caught at the establishment. Because of these characteristics, pay-to-fish farms are probably more susceptible to the entry of pathogens and have a greater potential for spreading diseases than fattening farms.

Regarding the presence of domestic animals on the production unit, 94.7% (106/112) of the farms interviewed said they had land animals, the majority of which were dogs/cats (85/112) and chickens (81/112) with access to the production area. Although mechanical transmission of pathogens from domestic land animals is biologically possible, there are rare reports of this type of vector in aquaculture. The great advantage of restricting the access of terrestrial domestic animals to the production system is that it preserves good water quality. It is known that the presence of feces from terrestrial animals such as cattle, pigs, birds, dogs and cats can contribute to water contamination by bacteria and other microorganisms capable of causing mortality due to increased oxygen consumption in the system or even contamination of fish by *Salmonella* sp. (Santos, 2015; Fernandes et al., 2018; Ferreira et al, 2021; Galvão; Fabrício, 2023).

Among the aquatic animals mentioned, the species most commonly farmed in tilapia establishments is tambaqui (*Colossoma macropomum*), which is present in 54 farms (48.2%), mostly in pay-to-fish establishments (22/27). It was also observed that in 38.8% of cases (21/54) the tambaqui is in polyculture with tilapia. Although some tilapia diseases are species-specific, there are known risks that other aquatic animal species in polyculture can contribute to the introduction and spread of diseases in the production system, especially those caused by non-specific host pathogens such as *Streptococcus* sp. (Leira et al, 2016) and ISKNV (He et al, 2002).

Wild animals were observed by 80.3% (90/112) of the participating farmers, with fish-eating birds being the most frequently mentioned, followed by amphibians, reptiles and mammals such as otters (*Lutra longicaudis*), giant otters (*Pteronura brasiliensis*) and capybaras (*Hydrochoerus hydrochaeris*). In the case of birds that feed on fish, the risk lies in the possibility of transmitting pathogens through their feces (especially parasitic agents that have birds as their definitive host), regurgitation of fish from neighboring farms or even the mechanical carrying of viruses or bacteria in their moistened plumage (Thatcher, Brites-neto, 1994; Sant'Ana et al., 2012; Scholz, Kuchta, 2016; Galvão; Fabrício, 2023).

Factors related to the risk of spreading diseases between farms were also assessed. The farms' effluent is treated only by 36.6% (41/112), with a settling tank (73.2%; 30/41) and a sand and gravel filter (36.6%; 15/41) being the methods most used by the interviewees. On the other hand, if we add up the production units that use effluent for irrigation (49.1%; 55/112), 100% loss through infiltration/evaporation (17.8%; 20/112) and other animal production units (6.2%; 7/112), there are 62/112 (55.3%) farms that do not dispose of wastewater in natural bodies of water such as streams, rivers and reservoirs. This result is very different from that found by the Paraná State Agricultural Defense Agency in a study carried out on 34 semi-closed and closed system tilapia hatcheries in the north and west of the state, where it was found that 93% of the farms discharged the effluent into nature without any treatment (Adapar, 2014). This data suggests a reduced capacity for pathogen dispersal, corroborating the results of disease investigations carried out by the local official veterinary service over the last 5 years (Seagri, 2023). Table 8 details the proportion of destinations for the water used in fish farms.

Table 8. Destination of effluents from commercial tilapia farming in the Distrito Federal.

Type of effluent destination	n	%
Irrigation	55	49,1
Other natural water body	27	24,1
Same water body where it was abstracted	22	19,6
Loss through infiltration/evaporation	20	17,8
Another animal production unit	7	6,2
Sewage system and other cases	2	1,8
Total destinations without disposal in natural water bodies	62	55,3

* There are production units that have more than one destination for effluent.

Dead fish are major sources of infection and multiplication of pathogens within a production system (Kunttu et al., 2009), which is why it is essential to remove these carcasses from the tilapia cultures immediately. As for the disposal of dead fish, 71.4% (80/112) are buried or composted, data very similar to that found in the Morada Nova de Minas production center (Roriz et al., 2017). The other methods cited include collection by urban cleaning companies (5.3%; 6/112) and incineration (5.3%; 6/112). Among the unsanitary methods, such as throwing the carcasses in the bush or using them to feed other animals was reported by 17.8% (20/112) of the interviewees.

3.3 Farmers' perceptions of clinical signs and mortalities

Health events characterized by atypical mortality or clinical signs are relatively low in the DF. Although 72.3% (81 out of 112) of tilapia farmers have observed them at least once, 69.1%

(56/81) reported events occurring sporadically over the last ten years. With regard to observing clinical signs, 44.6% (50/112) of the farmers surveyed said they remembered some symptoms. The observation of moribund tilapia was more common in pay-to-fish establishments (63.0%; 17/27) and young fish farms (75.0%; 6/8). Fattening farms, which are the majority in the universe of this study, had only 35.1% (27/77) of atypical symptoms and events seen by those responsible for production. The most commonly observed clinical signs are shown in figure 5.

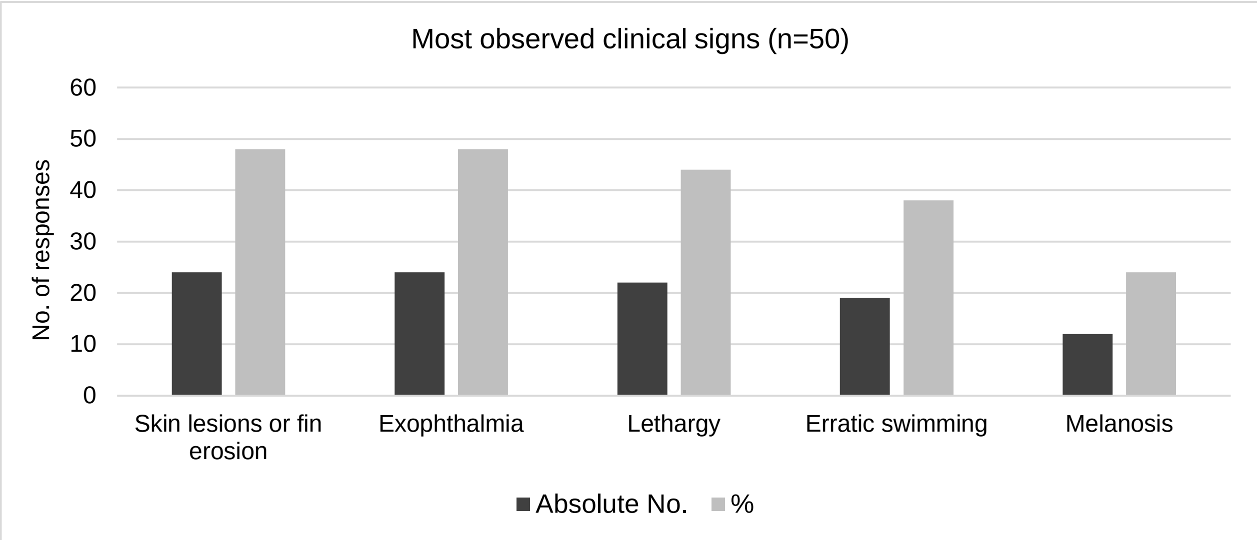

Fig. 5. Absolute number and percentage of clinical signs observed by tilapia farmers in the Distrito Federal

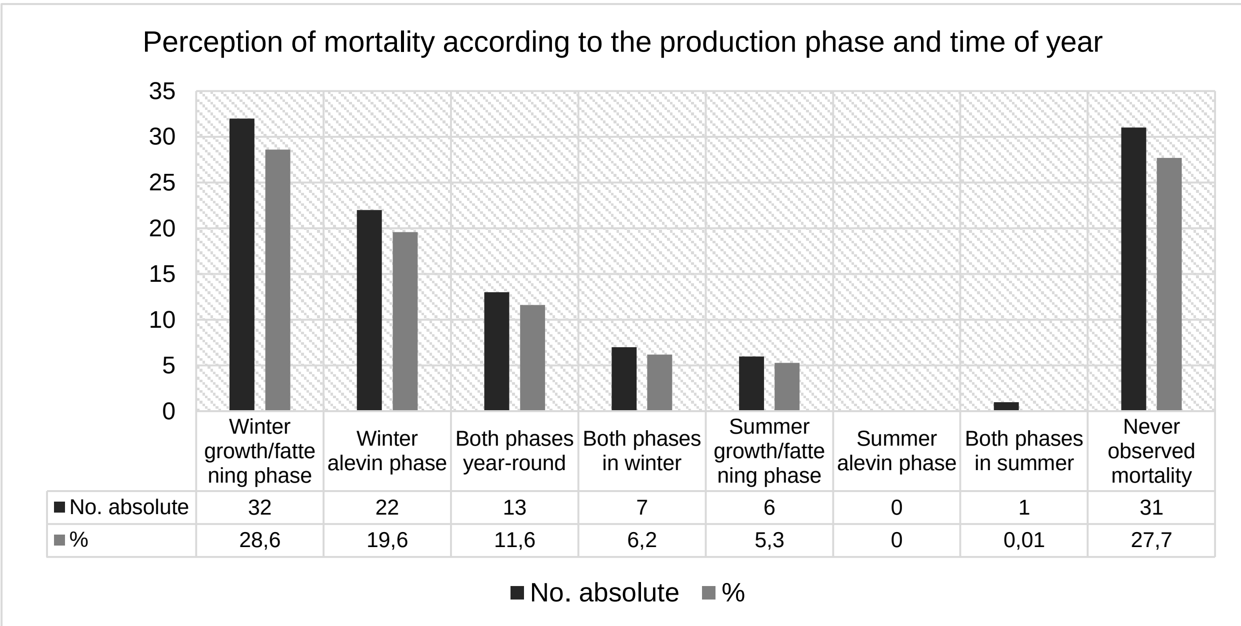

Fig. 6. Perception of mortality of the tilapia farmers participating in the study presented by absolute number and percentage.

Atypical mortalities were seen more intensely in the fall and winter periods, similar to the results seen in the north and west of Paraná where 77% of mortalities were seen more in the winter (Adapar, 2014) and different to the study carried out in the Três Marias reservoir, Morada Nova de Minas, where 69% of fish farmers reported greater losses in the spring and summer periods. This is probably due to the dynamics of the pathogen in relation to climatic conditions and the type of production system, since the semi-open rearing system in cages exposes tilapia much more to environmental health challenges, especially to *Streptococcus* sp. infections (Leal; Figueiredo, 2018; Delphino et al., 2019) and *Lactococcus* sp. (Egger et al., 2023), which are widespread in all large natural bodies of water and become more pathogenic at temperatures above 28ºC (Suhermanto et al., 2019). In the DF, where there is a predominance of semi-closed and closed systems with a large proportion of water abstraction from subterranean sources, the occurrence of these bacteria is low, a fact verified by the perception of the tilapia farmers in the answers to the questionnaire and corroborated by other studies by our group (unpublished data) which observed a very low frequency of these bacteria in all production strata.

The production and sanitary characteristics of fish farms in the Distrito Federal, combined with environmental and climatic factors, prevent streptococcosis and other opportunistic summer diseases from occurring as frequently as they do in other production centers. The DF is located in the Central Plateau region of Brazil, at an average altitude of 1,172m (Brasil, 2019), factors which reflect directly on the water temperature, which remains low during the winter and rarely reaches values above 28ºC in the summer. During visits to investigate atypical health events, water measurements in the fall and winter months, usually taken between 9am and 12pm, had an mean temperature, in degrees Celsius, of 19.60 (SD=2.43), while in the spring and summer months the mean was 25.28 (SD=2.42). In the perception of the participants, management faults (60.5%; 49/81) and water that was too cold (23.4%; 19/81) were the two causes most commonly attributed to mortalities.

3.4 Sanitary categorization of farms

The BL of commercial tilapia establishments was categorized based on the mean of the VL and RD variables. Tables 9 and 10 show the results of the questionnaire responses related to the 30 risk variables identified for continental tilapia production. Only 2 farms were considered class A, while 39, 53 and 18 farms were classified as B, C and D, respectively.

The group of farms made up of pay-to-fish farms had the highest score, representing the stratum with the greatest potential risk of introducing and especially spreading diseases. It was found that the safest farms are the fattening farms with closed cultivation (BL=5.83, SD=1.47), followed by young fish farms (BL=8.00, SD=1.46) and semi-closed fattening farms (BL=8.24, SD=2.20). Among the pay-to-fish establishments, the mean BL was 9.57 (SD=2.63), but if we only consider the 21 establishments with exclusive pay-to-fish activity (disregarding pay-to-fish establishments whose main activity is recreational fishing but which also do fattening), the BL found was 10.21, (SD=3.02), the highest score, with a big difference to the other typologies.

Applying the ANOVA analysis of variance (p_{VL}=0.001, p_{RD}=0.0764 and p_{BL}=0.004) followed by the Bonferroni test, it was found that there was a statistically significant difference ($p<0.05$) between the variables of some groups, as illustrated in Table 11. The typology of fattening farms in a closed system (C-fattening) scored better than the semi-closed fattening (S/C-fattening) and pay-to-fish groups in terms of the VL and BL, although there was no statistical difference between the groups when assessing only the RD. For comparison purposes, the lower the average VL, RD and BL, the better the farm's sanitary infrastructure was considered to be (lower vulnerability to pathogen entry and lower risk of dissemination).

The results of the statistical analysis show that C-fattening farms have the best biosecurity parameters. Although they were few in number (only 9 farms), several common characteristics may help to explain this result, such as not sharing the farming water with fish and wild animals, use of filtering systems, reuse of the same water for long periods (lower frequency of pathogen dispersal), greater technification and specialized technical assistance, acquisition of fish from suppliers registered with veterinary services where sanitary control is carried out, as well as a higher level of education and technical training in aquaculture.

However, the large difference observed between the mean scores of the pay-to-fish farms in relation to the S/C-fattening and alevin producers/resellers typologies could be inferred to be biologically significant, since our observations found a evident difference in the sanitary infrastructure and best practices adopted between the farms.

Considering the categorization of farms, of the 18 fish farms classified as "D" in terms of BL, 33% (6/18) had atypical health events recorded. Among the 53 "C" and 39 "B" establishments, atypical health events were recorded in 15.1% (8/53) and 25.6% (10/39), respectively.

The model used in our study to categorize the risk of tilapia farms is similar to the model used in Switzerland, which classified salmonid farms in terms of the risk of introduction and dissemination for viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN) (Diserens et al., 2013; Diserens et al., 2017), although the score used by these authors for the points table was 0, 1, 2 and 4 points and the number of criteria assessed was less comprehensive than in the present study (6 criteria for the risk of introduction and 7 for the risk of spreading).

In the model applied to Swiss fish farms, the risk factors for the introduction and spread of VHS and IHN were defined and estimated using published data and expert opinions. Among the 357 salmon farms identified in Switzerland, 49.3% were classified as high risk, 49.0% as medium risk and 1.7% as low risk. Even though the Swiss model was stricter, the proportion of tilapia farms with an insignificant risk of disease entry and spread was exactly the same (1.7%), while the proportion of farms classified as high risk was 16.2%, a much lower rate than the Swiss. According to the European Union's Aquaculture Directive 2006/88/EC, the frequency of farm inspections should be derived from their risk levels and this is exactly the surveillance strategy (based on risk) that the Brazilian health authorities have recommended to the state veterinary services (Quali-SV/MAPA audit reports, unpublished data).

Table 9. Distribution of commercial tilapia farms according to the score for vulnerability level, risk of dissemination and biosecurity level with the respective categorization, illustrated by color.

Tilapia farm	Production purpose	Production system	Score			Classification			AHE	Season	
			VL	RD	BL	VL	RD	BL		Cold	Hot
F048	F	S/C	4	3	3.5	LV	IR	A	Yes		S/S
F074	F	C	5	2	3.5	LV	IR	A	No		
P024	P	S/C	5	3	4	LV	IR	B	No		
F034	F	S/C	6	3	4.5	LV	IR	B	No		
F035	F	S/C	6	3	4.5	LV	IR	B	No		
F071	F	C	3	6	4.5	HP	LR	B	Yes	A/W	
F004	F	S/C	6	4	5	LV	LR	B	No		
P016	P	S/C	7	3	5	LV	IR	B	No		
F025	F	S/C	5	5	5	LV	LR	B	No		
F077	F	C	5	5	5	LV	LR	B	No		
F047	F	S/C	4	7	5.5	LV	LR	B	No		
F058	F	C	7	4	5.5	LV	LR	B	No		
F064	F	S/C	6	5	5.5	LV	LR	B	No		
F107	F	C	6	5	5.5	LV	LR	B	No		
F020	F	S/C	5	7	6	LV	LR	B	Yes	A/W	
F021	F	S/C	7	5	6	LV	LR	B	No		
F044	F	S/C	4	8	6	LV	MR	B	No		
F054	F	S/C	5	7	6	LV	LR	B	No		
F061	F	S/C	5	7	6	LV	LR	B	No		
F085	F	S/C	9	3	6	MV	IR	B	No		
P026	P	S/C	8	4	6	MV	LR	B	Yes	A/W	
H001	H	S/C	6	7	6.5	LV	MR	B	Yes	A/W	
F079	F	S/C	10	3	6.5	MV	IR	B	Yes		S/S
F090	F	S/C	6	7	6.5	LV	LR	B	Yes		S/S
F019	F	S/C	6	7	6.5	LV	LR	B	No		
P023	P	S/C	8	5	6.5	MV	LR	B	No		
F032	F	C	6	7	6.5	LV	LR	B	No		
F068	F	S/C	6	7	6.5	LV	LR	B	No		
F072	F	S/C	9	4	6.5	MV	LR	B	Yes	A/W	
F073	F	C	5	8	6.5	LV	MR	B	No		
H003	H	S/C	7	7	7	LV	MR	B	Yes	A/W	
H017	H	S/C	7	7	7	LV	MR	B	Yes	A/W	
H022	H	S/C	8	6	7	MV	MR	B	Yes	A/W	
F002	F	S/C	10	4	7	MV	LR	B	No		
F011	F	S/C	8	6	7	MV	LR	B	No		
F014	F	S/C	9	5	7	MV	LR	B	No		
F065	F	S/C	5	9	7	LV	MR	B	No		
F066	F	S/C	6	8	7	LV	MR	B	No		
F075	F	S/C	6	8	7	LV	MR	B	No		
F089	F	C	7	7	7	LV	LR	B	No		
F095	F	S/C	9	5	7	MV	LR	B	No		
H030	H	S/C	6	9	7.5	LV	MR	C	No		
F009	F	S/C	6	9	7.5	LV	MR	C	Yes		S/S
P028	P	S/C	7	8	7.5	LV	MR	C	No		
F036	F	S/C	7	8	7.5	LV	MR	C	No		
F067	F	S/C	8	7	7.5	MV	LR	C	No		
F070	F	S/C	5	10	7.5	LV	MR	C	No		
F090	F	S/C	7	8	7.5	LV	MR	C	No		
F092	F	S/C	8	7	7.5	MV	LR	C	No		
P098	F	S/C	12	3	7.5	HV	IR	C	No		
F100	F	S/C	8	7	7.5	MV	LR	C	No		
F008	F	S/C	8	8	8	MV	MR	C	No		
F046	F	S/C	7	9	8	LV	MR	C	Yes	A/W	
F053	F	S/C	8	8	8	MV	MR	C	No		
P055	P	S/C	10	6	8	MV	LR	C	No		
F063	F	S/C	11	5	8	HV	LR	C	No		
F083	F	S/C	10	6	8	MV	LR	C	No		
P050	P	S/C	7	9	8	LV	MR	C	No		
P060	P	S/C	7	9	8	LV	MR	C	No		
F010	F	S/C	8	9	8.5	MV	MR	C	No		
F062	F	S/C	7	10	8.5	LV	MR	C	No		
F084	F	S/C	11	6	8.5	HV	LR	C	No		
F088	F	S/C	10	7	8.5	HV	LR	C	No		
F094	F	C	7	10	8.5	LV	MR	C	No		
F097	F	S/C	9	8	8.5	MV	MR	C	No		
F111	F	S/C	10	7	8.5	MV	LR	C	No		
H033	H	S/C	13	5	9	HV	MR	C	Yes	A/W	
F045	F	S/C	6	12	9	LV	HR	C	No		
F059	F	S/C	9	9	9	MV	MR	C	No		
P027	P	S/C	9	9	9	MV	MR	C	No		
P029	P	S/C	7	11	9	LV	HR	C	No		
P042	P	S/C	10	8	9	MV	MR	C	No		
H018	H	S/C	14	5	9.5	HV	MR	C	Yes	A/W	
P031	P	S/C	12	7	9.5	HV	LR	C	No		
F043	F	S/C	7	12	9.5	LV	HR	C	No		
F057	F	S/C	13	6	9.5	HV	MR	C	No		
F069	F	S/C	12	7	9.5	HV	LR	C	No		
F110	F	S/C	10	10	10	MV	MR	C	Yes		S/S
F012	F	S/C	7	13	10	LV	HR	C	No		
F015	F	S/C	11	9	10	HV	MR	C	No		
F052	F	S/C	9	11	10	MV	HR	C	No		
F076	F	S/C	8	12	10	MV	HR	C	No		
F078	F	S/C	12	8	10	HV	MR	C	No		
F082	F	S/C	11	9	10	HV	MR	C	No		
F091	F	S/C	10	10	10	MV	MR	C	No		
P005	P	S/C	7	13	10	LV	HR	C	No		
P038	P	S/C	12	8	10	HV	MR	C	No		
P051	P	S/C	7	13	10	LV	HR	C	No		
P104	P	S/C	11	9	10	HV	MR	C	No		
H037	H	S/C	11	10	10.5	HV	MR	C	Yes	A/W	
F056	F	S/C	9	12	10.5	MV	HR	C	Yes	A/W	
F099	F	S/C	10	11	10.5	HV	HR	C	No		
F109	F	S/C	10	11	10.5	MV	HR	C	Yes	Aut/Win	
P041	P	S/C	11	10	10.5	HV	MR	C	No		
F013	F	S/C	13	9	11	HV	MR	D	No		
F087	F	S/C	13	9	11	HV	MR	D	No		
P007	P	S/C	14	8	11	HV	MR	D	No		
P102	P	S/C	13	9	11	HV	MR	D	No		
P105	P	S/C	12	10	11	HV	MR	D	Yes	A/W	
F081	F	S/C	11	12	11.5	HV	HR	D	Yes	A/W	S/S
F049	F	S/C	13	10	11.5	HV	MR	D	No		
F086	F	S/C	9	15	12	MV	HR	D	No		
F103	F	S/C	12	12	12	HV	HR	D	No		
P040	P	S/C	12	12	12	HV	HR	D	No		
P080	P	S/C	12	12	12	HV	HR	D	Yes	A/W	
F106	F	S/C	12	13	12.5	HV	HR	D	Yes	A/W	
P039	P	S/C	15	10	12.5	HV	MR	D	No		
P108	P	S/C	16	9	12.5	HV	MR	D	Yes	A/W	
F093	F	S/C	10	16	13	MV	HR	D	No		
P112	P	S/C	13	13	13	HV	HR	D	Yes	A/W	
F096	F	S/C	14	13	13.5	HV	HR	D	No		
P006	P	S/C	14	18	16	HV	HR	D	No		

Caption:

- AHE = atypical health event

- Tilapia farms and production purpose

F = fattening
H = hatchery or fingerling seller
P = pay-to-fish

- Production system

C = Closed system
S/C = Semi-closed system

- Score

VL = vulnerability level
RD = risk of dissemination
BL = biosecurity level

SE = safe establishment
HP = high protection
LV = low vulnerability
MV = moderate vulnerability
HV = high vulnerability

IR = insignificant risk
LR = low risk
MR = moderate risk
HR = high risk

- Season

A/W = autumn / winter
S/S = spring / summer

Table 10. Values of the mean, standard deviation and variance of the variables vulnerability level (VL), risk of dissemination (RD) e biosecurity level (BL) of each commercial stratum of tilapia in the DF with the number of farms categorized as A, B, C and D and the p-value of each variable indicating that the groups have an abnormal distribution (p>0.05) according to the Shapiro-Wilk test.

Stratum	**n**	**x̄ (SD)**						**BL Variance**	**No. farms BL categorized**			
		VL	*p*-value	RD	*p*-value	**BL**	*p*-value		**A**	**B**	**C**	**D**
Fattening (closed system)	9	5,66 (1,32)	0,1037	6,00 (2,34)	1,0000	5,83 (1,47)	0,9981	2,1875	1	7	2	0
Fattening (semi-closed system)	68	8,39 (2,55)	0,2322	8,08 (2,98)	0,3930	8,24 (2,20)	0,6457	4,8693	1	27	39	9
Hatchery / Alevin seller	8	9,00 (3,20)	0,6263	7,00 (1,77)	0,4416	8,00 (1,46)	0,1830	2,1428	0	4	4	0
Pay-to-fish	27	10,29 (2,98)	0,3237	8,85 (3,50)	0,4302	9,57 (2,63)	0,9973	6,9558	0	7	14	9
All tilapia farms	**112**	**8,68 (2,87)**	**0,0215**	**8,03 (3,07)**	**0,2005**	**8,35 (2,40)**	**0,3522**	**5,7700**	**2** (1,7%)	**39** (34,8%)	**53** (47,3%)	**18** (16,2%)

x̄ = mean; SD = standard deviation; VL = vulnerability level; RD = risk of dissemination; BL = biosecurity level.

Table 11. Comparison between the strata using the Bonferroni test with the p-value of variance for the variables vulnerability level (VL), risk of dissemination (RD) e biosecurity level (BL), where p<0.05 (*) indicates and statistically significant difference between the groups.

Comparison between strata	VL	RD	BL
F (c) - F (s/c)	0,026*	0,320	0,018*
F (c) - H	0,064	1,000	0,291
F (c) - P	0,000*	0,093	0,000*
F (s/c) - H	1,000	1,000	1,000
F (s/c) - P	0,012*	1,000	0,061
H - P	1,000	0,779	0,498

F (c) = Fattening (closed system); F (s/c) = Fattening (semi-closed system); H = Hatchery / alevin seller; and P = Pay-to-fish.

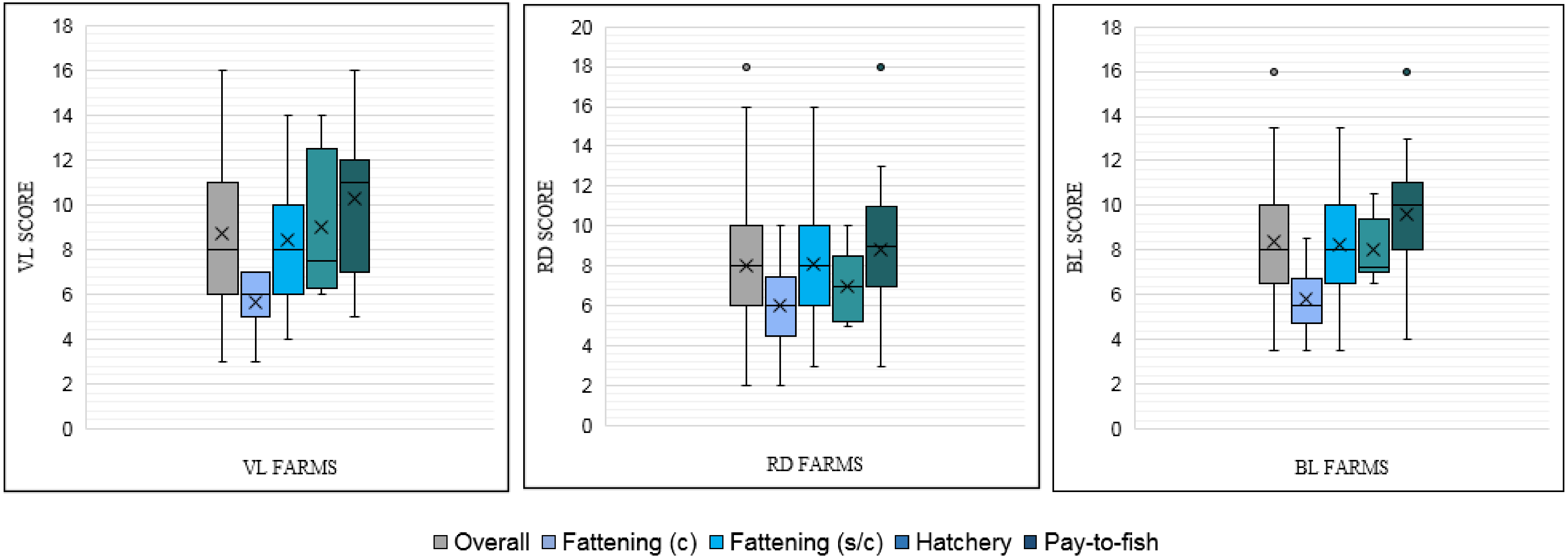

Fig. 7. Graphical representation of the boxplots of the mean VL, RD and BL scores of the farms in the four strata, separated by color according to the image legend.

3.5 Atypical health events

During the in loco evaluations of the atypical health events, the mean values of the water parameters were: dissolved oxygen ($\bar{x}$ = 5.01, SD=1.34 mg/L), pH ($\bar{x}$ = 6.83, SD=0.66 H+) and toxic ammonia ($\bar{x}$ = 0.25, SD=0.07 ppm). None of the AHE found inadequate parameters with lethal levels for tilapia.

A total of 27 health events were investigated, 20 of which occurred during the autumn and winter periods. The main cause of mortality and clinical signs was related to management failures or very low water temperatures that triggered stress and secondary actions by bacterial and parasitic pathogens. The laboratory investigations of cytopathology, histopathology, microbiology and molecular tests carried out on these samples were the subject of another epidemiological study by our research group.

The most common clinical signs observed during the health events were erosive or ulcerative lesions on the skin and fins (40.7%; 11/27), lethargy (33.3%; 9/27), exophthalmos (25.9%; 7/27) and erratic swimming, vertigo or dystaxia (22.2%; 6/27). The four most frequently described clinical signs are exactly the same as those most frequently recalled by farmers during the questionnaire.

Considering the occurrence by strata, 55.5% (15/27) of the events took place in fattening farms, 25.9% (7/27) in young form farms and 18.5% (5/27) in pay-to-fish farms. These results can be explained by the production characteristics of each typology.

Health incidents in fattening establishments follow the proportion of farms with this purpose that took part in the study. In addition to representing the largest stratum in the study, fattening fish are kept on the farm for longer than the other two groups, and are challenged by a series of physical, chemical and biological factors from housing to the finishing phase. The answers to the questionnaire revealed a profile more focused on animal production, including taking care to learn more about fish health, biosecurity and reporting atypical events to the animal health authorities. It is no coincidence that this is the stratum with the highest number of farms classified as B or C in BL.

Hatcheries and farms that sell alevins had proportionally more AHE than the other groups, which can be explained by the dynamic nature of the influx and outflux of fish, the influence of physiological stress due to transportation and the age of the animals, which do not yet have a fully formed immune system against the most common pathogens. With the exception of two establishments that produce young tilapia from matrices and broodstock, the others are resellers who acquire large quantities of alevins from farms in neighboring states and resell batches within the DF, subjecting these fish to a high stress load during their first two months of life.

In our categorization, the 8 young-form establishments were categorized as BL = B or C, even though they had good sanitary and biosecurity infrastructure. The variable responsible for imposing this category limit is the high flow of fish in and out, which is an intrinsic characteristic of the activity of alevin distribution farms and which considerably increases the risks of introducing and spreading pathogens.

As for establishments used for recreational fishing, there was a low proportion of calls in relation to the total. In the previous subheading, results were presented showing that this type of establishment has the worst average rating in terms of biosecurity, prophylaxis and best practices. However, it is believed that the low occurrence of AHE in this type of establishment is related to two main factors. Firstly, due to the short storage time of these fish on site until they are caught and consumed. In addition, we observed in our contact with the owners of these establishments during the questionnaire that fish production is treated as a secondary object. The focus of this type of business is on customer leisure and consumption in their own restaurants. The lesser importance given to animal production was perceived by the lower proportion of reports of AHE during the monitoring system compared to the other strata, since, in the perception responses, the pay-to-fish farms were the segments that most reported observing AHE. There is a hypothesis raised by the pay-to-fish owners themselves that most of the signs observed are related to the fish that are hooked and released in the sport fishing modality, which leads to clinical signs and deaths as a result of injuries caused by hooking and handling.

Based on the categorization of establishments and the history of disease detection, it was assessed that, although the biosecurity level is worse in the pay-to-fish stratum, in practice, it is the S/C-fattening and young fish farms that present the greatest risk, probably due to the high frequency of interstate transit of fish (due to the high possibility of introducing endemic diseases in other regions of the country), higher density of fish per cubic meter and, in the case of fattening, longer exposure time to the environment (Subasinghe, Phillips, 2002; Oidtmann et al. , 2011; Diserens et al., 2011; Oidtmann et al., 2013; Boerlage et al., 2017).

4. Conclusions

It is concluded that tilapia farming in the DF is characterized by a group of small and medium-sized farms, mostly made up of family labour, with low levels of technification and productivity when compared to other Brazilian tilapia production centers. The low frequency of officially controllable diseases observed in the reports from the local veterinary service (Seagri, 2023) is due to a combination of factors, including the type of production system practiced in this Federative Unit (only semi-closed and closed systems), a wide range of specialized technical assistance and a good level of biosecurity in fish farms. Among the measures that may have contributed most to the low vulnerability and low risk of spreading diseases in the DF are the use of subterranean water sources, which are naturally more protected, and the large number of fish farms that dispose of effluent to other production or irrigation units, preventing the spread of pathogens through water discharged into rivers and lakes.

The adapted model used to categorize tilapia farms in terms of vulnerability to the introduction of pathogens and the risk of spreading diseases classified 82% of the farms as having a good or moderate risk (B or C). Fattening farms that raise tilapia using technologies such as RAS, BFT and aquaponics systems have better indicators of biosecurity, best practices and disease prophylaxis than fattening farms in earthen ponds. In the same evaluation, pay-to-fish farms were found to have the worst health indicators overall.

The model for categorizing farms according to their level of biosecurity, prophylaxis and best practices can serve as a basis for the official veterinary service of other tilapia-producing states or countries in risk-based surveillance strategies for tilapia diseases.

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CAPÍTULO IV

EPIDEMIOLOGICAL STUDY OF DISEASES OF ECONOMIC IMPORTANCE IN TILAPIA (*Oreochromis niloticus*) WITH THE FIRST DETECTION OF ISKNV IN THE DISTRITO FEDERAL, BRAZIL (2021-2023)

Artigo científico para submissão ao periódico ***Journal of Fish Diseases***

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ABSTRACT

The Distrito Federal (DF) is a Brazilian federal unit with a large number of fish farmers, mainly tilapia farmers, with around 660 farms. The aim of this study was to determine the frequency of diseases of economic and health importance in tilapia farming in the DF. Two epidemiological surveillance studies were carried out between 2021 and 2023: a study to detect cases of officially controlled diseases based on targeted samples (moribund tilapia) during 12-month monitoring of commercial fattening farms, pay-to-fish and hatcheries/alevin sellers farms with a minimum sample size of five symptomatic fish (n=191), based on 27 visits to farms with atypical health events; and a study that assessed the frequency only in the hatcheries and young fish trade farms using random sampling of 156 fish for exotic diseases and 30 fish for diseases present in Brazil. Only 1 farm was found to be positive for ISKNV and 2 for *Francisella orientalis*, all of which were allochthonous cases, with the pathogens originating in other Brazilian states. The sampling carried out on young fish farms found no officially controlled diseases in the molecular tests. The conclusion is that the Distrito Federal has a low frequency of pathogens considered to be compulsorily notifiable in Brazil and WOAH. The atypical health events were mostly caused by factors related to the inadequate physical-chemical condition of the water for tilapia, triggering ectoparasitosis and bacterial septicaemia secondary to stress. Further studies are needed to assess the dynamics, behaviour and virulence of some pathogens that are not currently on the lists of officially controlled diseases in order to mitigate the risks of new outbreaks and economic losses in the local tilapia production chain. In addition to the valuable health diagnosis, this epidemiological study design that combines two sampling components can serve as a model for other states or countries that need to carry out epidemiological surveys/monitoring of tilapia target diseases.

Keywords: epidemiological study, ISKNV, *Francisela orientallis*, Tilapia lake virus, Tilapia parvovirus, Nervous necrosis virus.

1. INTRODUCTION

Brazil is among the largest producers of animal protein in the world, with the chicken, beef and pork industries standing out, with annual production estimated at 14.52, 10.35 and 4.98 million tons, respectively (ABPA, 2023; Abrafrigo, 2023). However, Brazilian aquaculture has seen strong growth in recent years, with around 1.02 million tons produced in 2023 (Peixe-BR, 2024; ABCC, 2023; Brasil, 2022). This growth has been driven mainly by the tilapia industry, which alone has become the fourth most produced and consumed meat in Brazil, with production of almost 600,000 tons in 2023 and a 57% share of all Brazilian aquaculture production (Peixe-BR).

In the Distrito Federal, one of Brazil's 27 federal units, there are more than 660 tilapia farms registered with the local animal health agency, producing around 2,000 tons of tilapia a year, making Brasília one of the municipalities with the largest number of tilapia farms in Brazil (Brasil, 2022; Seagri, 2023). This production is used to supply the country's capital, which is the third largest fish consumer market in Brazil (Borges, 2010).

Pathogens represent a great sanitary challenge for fish health (Bondad-Reantaso et al., 2005; Figueiredo; Leal, 2008; Stentiford et al., 2012), restricting production and generating socioeconomic impacts for individuals, communities and economies (Adam; Gunn, 2017). Some of them are of greater interest because they impact more strongly on the global tilapia industry, such as the viral agents Tilapia Lake Virus (TiLV) (Eyngor et al., 2014; Aich et al., 2022), Infectious Spleen and Kidney Necrosis (ISKNV) (Fu et al., 2011; Fonseca et al., 2022), Tilapia parvovirus (TiPV) (Du et al., 2019; Liu et al., 2020), Nervous Necrosis Virus (NNV) (Hodneland et al. 2011) and the bacterial pathogens *Francisella orientalis* (*FO*) (Leal et al., 2018; Carreon et al., 2021) *Streptococcus agalactiae* (*SA*) (Kayansamruaj et al., 2014; Leal, Figueiredo, 2018), as well as other emerging diseases (Egger et al. 2023). Among the tilapia diseases considered notifiable in the Federal District are TiLV (WOAH, 2022), *FO* (Brasil, 2015a), SA (Seagri, 2022) and exotic or emerging diseases with major repercussions on the economy such as ISKNV, NNV and TiPV, according to Brazilian health legislation (Brasil, 2015b).

Farming in earthen or elevated ponds, which are predominant in the Distrito Federal (DF), is less vulnerable to the introduction and spread of pathogens than the semi-open system (cages suspended over large water reservoirs) (Delphino et al., 2019; Raposo, 2024). However, cross-sectional studies are needed to assess the epidemiological situation of the region in terms of diseases and the negative impact they cause, which can serve as a parameter for farmers and aquaculture professionals in adapting sanitary management and for health authorities in adopting strategic disease control and eradication measures. Due to the lack of information related to the epidemiology of tilapia diseases in the DF and less costly models for epidemiological studies of fish, the aim of this work was to determine the frequency of the most economically and sanitarily important diseases in tilapia farming in the region using a combination of two complementary surveys based on targeted sampling and production strata at higher risk.

2. MATERIALS AND METHODS

The study was assessed and approved by the Animal Research Ethics Committee (CEUA) of the University of Brasilia (23106.080975/2021-63). The target population selected was made up of all the commercial tilapia farms registered with the Distrito Federal's Secretariat of Agriculture, Supply and Rural Development (SEAGRI), all of which are characterised by farming in semi-closed systems (earthen or elevated ponds) and closed systems (aquaponics, biofloc and recirculation aquaculture system). The study involved two different epidemiological components: a case detection study based on targeted samples and a random sampling study in young tilapia farms (risk stratum). Samples were collected by veterinarians from the DF's official veterinary service.

2.1 Case detection study (monitoring and communication system)

In this method, all 112 commercial tilapia farms registered with the DF's official veterinary service were subjected to a case detection study ("screening") based on monitoring and instant communications to collect targeted samples ("moribund tilapia") and search for the main notifiable tilapia pathogens such as Tilapia Lake Virus (TiLV), Infectious Spleen and Kidney Necrosis Virus (ISKNV), Nervous Necrosis Virus (NNV), Tilapia Parvovirus (TiPV), *Francisella orientalis* (*FO*) and *Streptococcus agalactiae* (*SA*) in all groups of commercial establishments as fattening farms, young fish establishments (hatchery or tilapia alevins trade farm) and recreational fishing farms (pay-to-fish). The RT-qPCR (TiLV and NNV) and qPCR (ISKNV, TiPV, FO and SA) molecular assays were carried out at the Aquavet laboratory at the Federal University of Minas Gerais (UFMG) Veterinary School.

At the same time, a search for severe infestations by ectoparasites was carried out in the same samples used for the diseases mentioned above as they are the largest mortality differentials during investigations of suspected diseases carried out by the official veterinary service since 2018 (SEAGRI, 2023).

This study was carried out between July 2021 and June 2022. All farmers previously responded to a semi-structured questionnaire that served to collect data on the health characterization of local tilapia production and were previously sensitized to report any atypical health event that was seen on their farm (monitoring and communication system). This communication involved both atypical mortality and observation of clinical signs. For 12 months, these farms were monitored by the DF's veterinary service, receiving automatic messages through a smartphone messaging application as a bi-weekly reminder to communicate atypical health events. After each communication, the farmer was immediately visited by a team of veterinarians and technicians from SEAGRI to take samples of moribund animals and analyze water quality in order to rule out gross management failures. The sample size per farm ranged from 5 to 20 fish, using the TiLV Strategic Manual as a reference, which recommends sampling 5 fish with compatible symptoms to diagnose the disease assuming perfect specificity of the tests and an expected prevalence of 0.02 (Yamkasem et al., 2021; FAO, 2021).

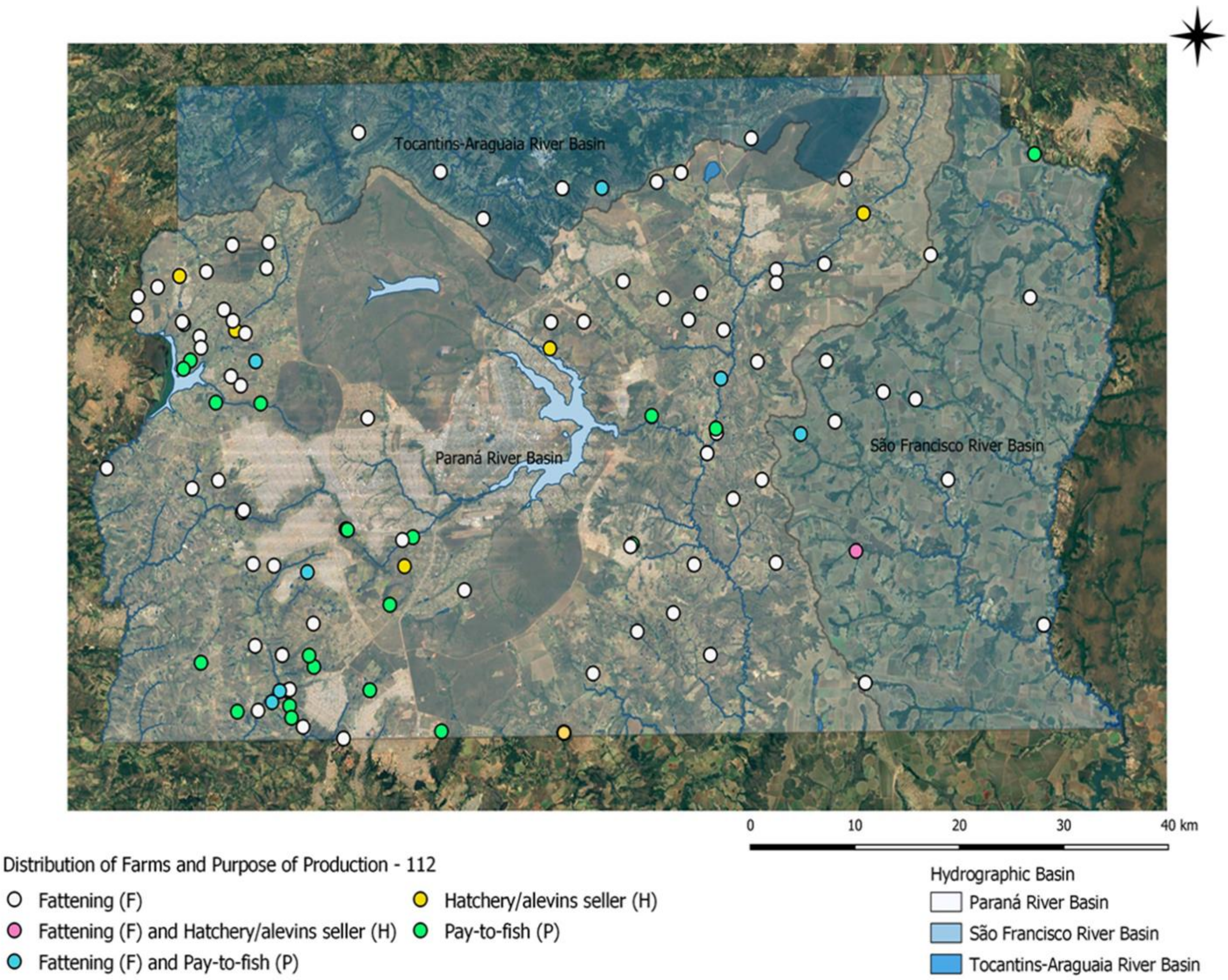

Fig. 1. Spatial distribution of commercial tilapia farms in the Distrito Federal that participated in the study according to production purpose and river basin.

2.2 Random sampling study on young fish farms

After evaluating the groups of farms according to the epidemiological characteristics registered in SEAGRI's computerized system, and based on the conclusions of various authors and studies that mention the main risks of introducing and spreading pathogens (Subasinghe; Phillips, 2002; Kleingeld, et al., 2010; Oidtmann et al., 2011; Diserens et al, 2011; Oidtmann et al., 2013; Oidtmann et al., 2014; Boerlage et al., 2017), it was concluded that the group most at risk is the one formed by farms that manage young fish (hatcheries, trade and resale of tilapia alevins), since they have a volume of inbound and outbound fish movements well above the average for the fattening and pay-to-fish strata. Considering the impossibility of applying random sampling studies to all strata of tilapia farming, justified by the high logistical and laboratory costs, and considering that the number of young fish farms in the DF is much smaller than the other two groups of farms, it was decided to apply this sampling design only to the young fish stratum. At the time of the study, of the 8 establishments registered for the purposes of hatchery or alevin sale, three were inactive (without broodstock and alevin for trade) and the 5 farms that were active during the month of July 2023 were sampled.

The type of design adopted for this monitoring was case detection to demonstrate freedom from disease on the farm. To determine the sample size for each pathogen, the following values were used at herd level: Designe prevalence (Dp): TiLV = 0.02 (exotic in Brazil), ISKNV, *FO*, *Streptococcus* sp. and *Lactococcus* sp. = 0.1 (endemic in several reservoirs in Brazil but with few records in the DF), diagnostic Sensitivity (Se) = 0.95, population Se = 0.95, diagnostic Specificity (Sp) = 1.0 and accuracy of 95%. The pathogen that required the largest sample size was TiLV (n=156 individuals per farm) while for research into the other diseases n=30 fish were used, always including larvae, alevin and broodstock tilapias. The molecular tests were carried out at the Federal Agricultural Defense Laboratory of the Brazilian Ministry of Agriculture and Livestock. (LFDA/MG-MAPA), while the bacterial culture tests for *Streptococcus* sp. and *Lactococcus* sp. were conducted at the Veterinary Medical Microbiology Laboratory/UnB, whose tests and parameters are shown in Table 2. The total sampling was 1,200 tests/fish, with 750 fish being used for molecular diagnosis for TiLV, 150 for ISKNV and *FO* each, and another 150 tests/fish for bacteriological diagnosis of *Streptococcus* sp. and *Lactococcus* sp.

2.3 Sample collection and laboratory tests

In both components, tilapia samples were collected and stored in 95% ethanol (Rawiwan et al., 2021; Brasil, 2022b) keeping fragments of one adult fish per 15 mL tube and 5 whole larvae or alevins (<4 cm) per 50 mL tube. The fish were previously anesthetized using eugenol followed by medullary section, as recommended by CFMV Resolution No. 1000 (CFMV, 2012). DNA extraction kits were used for *FO, SA*, TiPV and ISKNV (Wizard SV Genomic, Promega) and RNA for TiLV and VNN (Cellco Biotech) following the manufacturer's instructions. The extracted nucleic acids were quantified using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and stored at -80◦C until use. Extracted DNA was used for detection of TiPV, ISKNV, *FO* and *SA* using PCR, while extracted RNA was used for detection of TiLV and NNV using reverse transcription quantitative PCR (RT-qPCR). Reactions were performed with a HotStart Taq polymerase kit (Qiagen, Germantown, MD, USA) in a final reaction volume of 25 µl. The reaction mixture consisted of 1 × PCR buffer, 1.0 µM of each PCR primer, 0.2 µM dNTPs, 1.25 U Taq DNA polymerase and 50 ng template DNA. The PCR conditions were as follows: an initial denaturation at 95◦C for 15 min, followed by 30 cycles of 94◦C for 30 s, 58◦C for 1 min and 72◦C for 1 min; final elongation was carried out at 72◦C for 5 min. The primers used were purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). A 96-well Veriti thermal cycler was used and the amplicons were separated on the QIAxcell Advanced using QX DNA Screening Kit (Qiagen). Pathogen detection was carried out using GoTaq® Probe qPCR and RT-qPCR Systems, performed in QuantStudio 7 (Applied Biosystems, UK) according to each manufacturer's instructions. The master mix kits used for the RT-qPCR (AgPath-ID, Life Technologies Corporation) and qPCR (Cellco Biotech) reactions were used in accordance with the manufacturer's instructions and the methods described by Figueiredo et al. (2020) for TiLV, NNV and ISKNV, by Liu et al. (2020) for TiPV and by Assis et al. (2017) for bacterial diseases.

For the 2021/2022 screening, as smaller sample sizes were used, it was decided to process each sample individually. For the 2023 cross-sectional study, in order to reduce laboratory costs, pools of 3 fish were made for each test, in line with the methodology duly validated by the official laboratory. Positive results were retested to reduce the chance of false positives.

Additional samples from moribund fish (brain, kidney, spleen, liver, gills and other organs with macroscopic lesions) were collected for histopathology, fixed in 10% buffered formalin solution and routinely processed. When collecting, fragments of gills and scrapings of skin and gills were also used for direct cytopathological examination in order to estimate the frequency of the most common ectoparasites. For bacterial isolation tests, brain, spleen and kidney samples were collected aseptically for bacterial culture (Oliveira, 2012).

2.4 Statistical analysis

A descriptive analysis of the data was carried out based on the positive results detected by the monitoring and communication system in the three groups of farms participating in the study and the frequency of diseases in the young fish farms. Frequency was determined by dividing the number of positive farms and animals by the total number of participating farms and animals.

In the case detection study based on targeted samples, all commercial farms were monitored, while in the detection study based on random samples all farms in the young fish typology were sampled. Since both studies were census-based, it was not necessary to calculate the confidence interval.

To tabulate and analyze the data, we used the Stata®version 17 program (StataCorp 2022, College Station, TX, USA).

3. RESULTS

In the monitoring and communication system, 27 reports of atypical health events were received from a total of 25 farms during t0’’ he 12 months evaluated, making a total of 191 moribund tilapia sampled and 1,146 tests performed for the six pathogens. The period that concentrated most of the calls was autumn and winter, with 20 (74.1%). A total of 3 farms detected officially controlled diseases in the DF (Table 1). There were 2 fish positives for *FO* from two different farms (a fattening farm and a hatchery), representing 7.4% of the total fish farms sampled and 1.7% of the commercial farms that took part in the monitoring. With regard to viral pathogens, only ISKNV was detected, with 2 positive fish from a single fattening farm, representing 3.7% of the total farms sampled and only 0.8% of the total participating farms. Figure 2 shows the distribution of positive cases by administrative region of the DF.

In the case of ISKNV, the positive fish had ascites, corroded fins, liver congestion and splenic hypoplasia. The most relevant histopathological finding was in the kidney, where a multifocal infiltrate consisting of a moderate amount of lymphocytes and plasma cells was observed in the tubular interstitium. In the two cases of francisellosis detected, only erosive lesions were observed on the skin and fins, with no microscopic findings in the positive fattening farm, while in the positive hatchery farm, signs of exophthalmos, ascites, erratic swimming and the intense presence of granulomas were seen in the histopathological assays of the spleen and cephalic kidney of broodstock and matrices. In the latter case, there was also laboratory detection of other bacteria such as *Edwardsiella tarda* and *Pseudomonas pseudoalcaligenes*, characterizing an atypical multi-causal health event.

The main cause of mortality and clinical signs was related to management failures or very low water temperatures – the mean measured in degrees Celsius was 19.60 (SD=2.43) in the cold months and 25.28 (SD=2.42) in the hot months – which triggered physiological stress and secondary actions by bacterial and parasitic pathogens. In 6 episodes, cytopathology and histopathology findings of gills and skin detected severe infestation by ectoparasites such as *Piscinoodinium pillulare* (2), *Trichodina* sp. (1), *Tripartiella* sp. (1), *Chilodonella hexasticha* (1) and *Dactylogyrus* sp. (1).

With regard to the second epidemiological method applied to assess the frequency of diseases in the stratum of young form establishments, no cases of TiLV, ISKNV, *FO* or Streptococcus sp. were detected. Only one sample from a single farm was positive for the emerging pathogen *Lactococcus petauri*. During the visit to each establishment to carry out this random sampling, no moribund fish or evidence of an atypical health event was found.

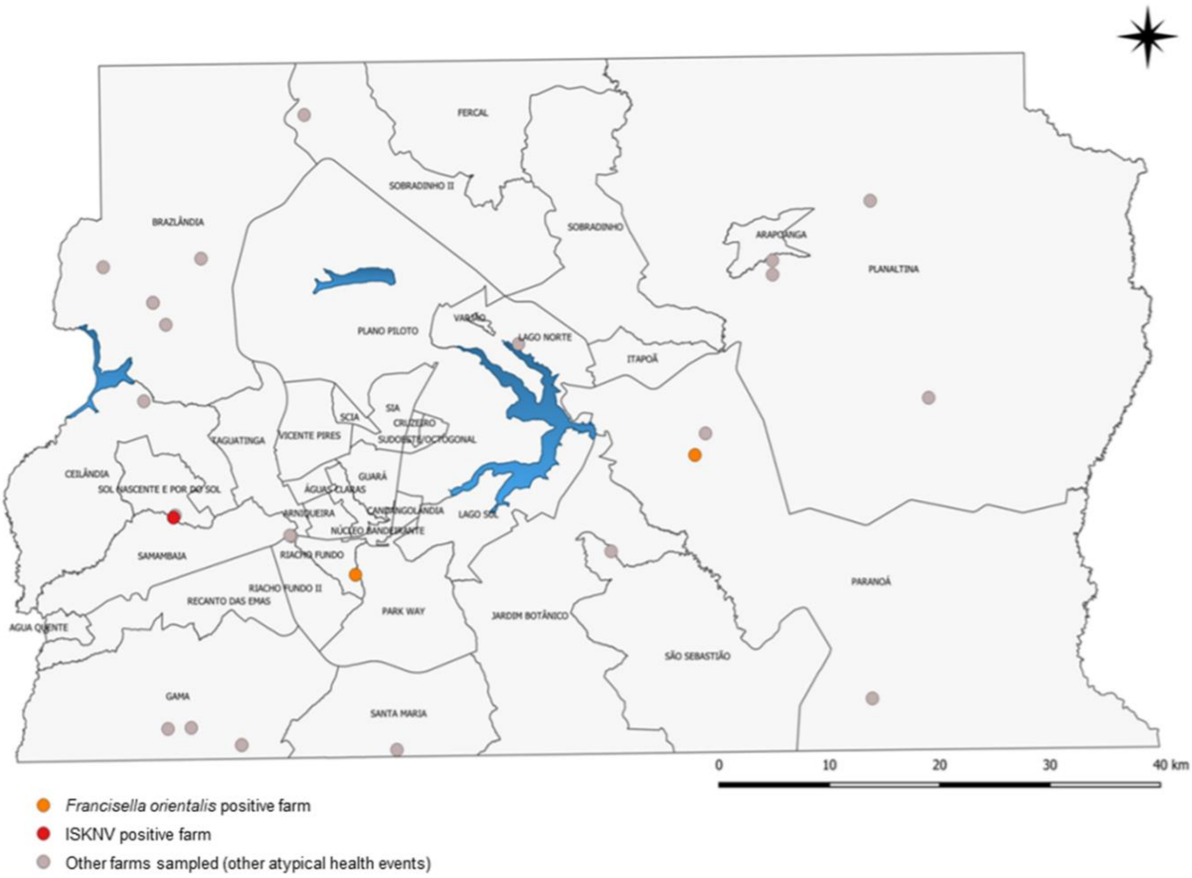

Fig. 2. Spatial distribution of tilapia farms positive for officially controlled diseases in the screening carried out in the Distrito Federal between 2021 and 2022.

Table 1. Molecular evaluation by stratum for TiLV, ISKNV, NNV, TiPV, *FO* and *SA* found by the monitoring and communication system carried out between July 2021 and June 2022 in the DF.

Stratum	n	n positives farms						n positives fish					
		TiLV	ISKNV	NNV	TiPV	FO	SA	TiLV	ISKNV	NNV	TiPV	FO	SA
Fattening (closed system)	1	0	0	0	0	0	0	0	0	0	0	0	0
Fattening (semi-closed system)	14	0	**1**	0	0	**1**	0	0	**2**	0	0	**1**	0
Hatchery / Alevins sale	6	0	0	0	0	**1**	0	0	0	0	0	**1**	0
Pay-to-fish	6	0	0	0	0	0	0	0	0	0	0	0	0
All farms	27	0	**1**	0	0	**2**	0	0	**2**	0	0	**2**	0

n = number of events reported and sampled in 12 months of monitoring

Table 2. Overall result of the laboratory sampling for TiLV, ISKNV, *FO*, *Streptococcus* sp. and *Lactococcus* sp. carried out in young tilapia establishments in the Distrito Federal in July 2023.

Pathogen	Design prevalence	Total No. of hatcheries and alevin sale farms (active)	N of farms sampled	Assay	Se (%)	Sp (%)	n	No. of pools per test	No. of fish per pool	No. of positive pools (5 farms)	No. of positive farms	Relative frequency on farms (%)	Relative frequency in animals (%)
TiLV	0.02	5	5	RT-qPCR	95%	100%	156	52	3	0	0	0	0
ISKNV	0.1	5	5	qPCR	95%	100%	30	10	3	0	0	0	0
F. orientalis	0.1	5	5	qPCR	95%	100%	30	10	3	0	0	0	0
Streptococcus sp.	0.1	5	5	BC + qPCR	95%	100%	30	6	5	0	0	0	0
Lactococcus sp.	0.1	5	5	BC + qPCR	95%	100%	30	6	5	**1**	**1**	20%	3%

Se = teste sensitivity, Sp = teste specificity, n = sample size, CI considered = 95%, BC = bacterial culture.

4. DISCUSSION

For the first time, an epidemiological study of diseases of economic importance to tilapia has been carried out in a federal unit in Brazil. In addition to the unprecedented research into viral and bacterial pathogens, this study also sought to bring local producers closer to the official veterinary service by sensitizing the production chain to the rapid reporting of atypical health events.

Among the diseases detected after applying the two epidemiological components, the first record of ISKNV in the DF stands out. The positive farm had some lethargic tilapia and a mortality rate of approximately 7% of the batch. The clinical signs observed in the positive fish were ascites, splenic hypoplasia, a purplish liver and congested sinusoid capillaries. Some of these findings are similar to the cases of ISKNV detected in semi-open tilapia cultures (cages in reservoirs) in Minas Gerais in 2020, which was one of the first states in Brazil, along with the state of Goiás, to record the presence of this pathogen (Figueiredo et al., 2020; Brasil, 2020). The epidemiological evaluations carried out after this detection showed that it was a fattening producer who purchased 100% of his fingerlings from a distributor located in the neighboring state of Goiás. Even before the molecular diagnosis, the farmer finished the symptomatic batch, emptied all the water, removed waste from the bottom, limed the ponds and carried out a 25-day sanitary void. These procedures helped to eliminate the virus from the system, without any further mortality episodes being observed after the cleaning process.

No samples tested positive for TiLV, NNV or TiPV, corroborating the hypothesis that these viral pathogens are absent in the Distrito Federal and Brazil. Among the diseases considered notifiable in Brazil (MPA-MAPA Ordinance No. 15/2015) and the DF (SEAGRI Ordinance No. 75/2022), only three positive farms were detected, all diagnosed during the screening of moribund tilapia. Although lactococcosis is not listed in any national or international regulations and was not investigated in the first epidemiological method applied, it is the emerging disease that was most involved in outbreaks of mortality in Brazilian tilapia farming between 2019 and 2022 (Egger et al., 2023a; Egger et al., 2023b) and was therefore included in the sample monitoring of young fish farms, where a single sample tested positive.

Among all the results of our study, the absence of detection of *S. agalactiae* in both the targeted sampling and the cross-sectional study of young fish farms was surprising. Although it is not possible to say precisely whether this was related to the very low frequency of this pathogen at field level or to factors related to the sensitivity of the test, it is recommended that, for new frequency studies or prevalence calculations carried out in the DF, the design prevalence value adopted for this pathogen for sample size calculation purposes should be adjusted from 10% to 5% or 2%. The hypothesis that we consider most likely, of low occurrence of streptococcal outbreaks in the DF, may reflect a very common characteristic of bacterial fish diseases in Brazil, with frequent changes in the natural dynamics of diseases, in the epidemiological profile and degree of pathogenicity, with consequent alternation in the etiology of outbreaks and the appearance of emerging and re-emerging pathogens (Mian et al. 2009; Leal et al., 2014; Assis et al., 2017; Delphino et al., 2019; Egger et al., 2023). In addition, it is important to note that the DF's veterinary service has a specific program for the control of streptococcosis (Ordinance No. 75/2022), with sanitation of outbreaks and elimination of positive batches, contributing to the reduction of the presence and dispersion of this agent in the environment and in local fish production.

Given the low number of detections of target pathogens, it was not possible to determine risk factors, correlations or causal relationships with the productive and sanitary characteristics of the farms. However, it was observed that the three farms that tested positive for officially controlled diseases shared some characteristics that were probably determining factors in the introduction of these pathogens: interstate transit, a high flow of incoming fish (with multiple fattening cycles or fish trading) and the absence of quarantine practices for newly acquired fingerlings. These results corroborate other studies which attribute the transit of aquatic animals to the risk factor of greatest epidemiological relevance (Subasinghe; Phillips, 2002; Oidtmann et al., 2011; Diserens et al., 2011; Oidtmann et al., 2013; Boerlage et al., 2017).

Overall, the detection results from targeted samples in all strata of commercial production and random sampling in young fish establishments showed that there is a low frequency of notifiable diseases in commercial tilapia production in the DF. A large number of health events were observed, mainly due to management failures, problems related to the physico-chemical quality of the water (mainly low temperature) leading to the occurrence of parasitosis and bacterial infections secondary to physiological stress, corroborating the trends reported in epidemiological reports of investigations carried out since 2018 by the DF's animal health agency (Raposo et al., 2021; Seagri, 2023). According to the passive surveillance reports of the SEAGRI, severe infestations by *P. pillulare* associated with management failures have been responsible for the majority of mortalities investigated since 2018. Although no tilapia parasite appears on the list of pathogens to be controlled by official bodies, in our study this ectoparasite was present with severe (2/27) or moderate (4/27) infestation in 22.2% of atypical health events (6/27), and was therefore the pathogen most involved in mortalities in the DF. In regions close to the DF, *P. pillulare* is also economically important in commercial fish farms, especially in stressful situations (Sant'Ana et al., 2012). This result differs from that found by Caldas et al. (2020) in a study of the parasite fauna in 10 tilapia farms in the DF, where the genus *Trichodina* sp. was found to be the most prevalent ectoparasite. In our study, trichodinids were the second most common ectoparasitic group in the samples analyzed.

The findings involving ectoparasites, especially the protozoan *P. pillulare*, should attract the attention of local health authorities. The authorities may consider the productive and economic losses caused by these agents in earthen pond systems to be high and assess the need to implement strategies aimed at controlling these pathogens, such as health education on best aquaculture practices, in order to minimize the losses caused by severe infestations in tilapia.

Among the opportunistic bacteria, a survey carried out by SEAGRI based on 27 disease investigations (passive surveillance) carried out between January 2018 and December 2022 described the genus *Aeromonas* sp. as the most isolated in bacterial culture tests (5/27). In the documented investigation forms, the clinical signs most associated with *Aeromonas* sp. septicemic infections were high and acute mortality, erosive or ulcerative and hemorrhagic lesions on the skin, partially or totally corroded fins and tail (Initial and Complementary Investigation Forms; unpublished data). Species such as *A. hydrophila* are widespread in the Brazilian aquatic environment, with their presence in water, organic matter and various fish species (Sebastião et al, 2015). When tilapia homeostasis is lost due to low water temperature or poor management, the fish become highly vulnerable to infection by this opportunistic bacterium (Roberts, 1981), leading to hemorrhagic septicemia and tail rot (Sebastião et al, 2015). It is important to note that there are countries that have specific programs for monitoring and controlling *A. hydrophila*, such as the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA), which monitors the impact of virulent strains of this bacterium, especially in catfish production (USDA, 2021).

There are some characteristics of the fish farming chain in the DF which, in theory, could contribute to the low dispersion of diseases, among which we would highlight the type of system used for tilapia production, which consists entirely of earthen or elevated ponds (Raposo, 2024). Farming in a semi-open system (cages), which is quite common in other states, favors the appearance of outbreaks of mortality caused by various diseases (Kubitza et al., 2013), including streptococcosis, francisellosis, lactococcosis and ISKNV (Delphino et al., 2019; Figueiredo et al., 2020; Egger et al., 2023a). In addition, the Distrito Federal has a wide range of free technical assistance provided by rural extension and apprenticeship organizations. Therefore, absolutely recommended practices such as regular pond cleaning, liming and preventive additives, not sharing management utensils and disposing of effluent for other agricultural activities such as vegetable irrigation certainly help to prevent and control the spread of pathogens.

The strategy adopted to determine the most frequent diseases in the DF used two complementary studies whose sampling designs were aimed at detecting cases. We chose not to use the prevalence method, which is common in veterinary services, due to the high logistical and laboratory costs for aquatic animals caused by the large sample size required when working with exotic diseases that require high precision, as well as not being the best strategy for the proposed objective. The seasonal dynamics presented by fish pathogens (Delphino et al., 2019) and the ability of diseases to emerge from time to time are factors that can interfere with prevalence results, especially if it is executed on a serial way to regularly monitor the effectiveness of the measures of a health program. In this sense, detection studies may be more suitable for the epidemiological assessment of diseases of aquatic animals, especially those that use targeted sampling to optimize logistical and laboratory costs.

In conclusion, it was found that the Distrito Federal has a good sanitary condition, with a low frequency of pathogens considered notifiable under local and international health legislation. Most of the atypical health events were caused by factors related to the inadequate physical and chemical condition of the water for the tilapia species, triggering ectoparasitosis and bacterial septicemia secondary to stress. The few cases of officially controlled diseases detected were related to the interstate transit of fish and the absence of certain practices such as quarantine of newly acquired animals. Studies to evaluate the dynamics, behavior and virulence of some agents such as *Aeromonas* sp. and *P. pillulare* are indicated to support the control and prophylaxis strategies of the production chain and the local health authority in making decisions to implement specific programs to control these pathogens. In addition, this was the first epidemiological study in a Brazilian federative unit aimed at researching viral and bacterial diseases of major economic importance, and could serve as a model for other states or countries that need to conduct epidemiological surveys for better sanitary control of tilapia production.

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CAPÍTULO V

EVALUATION OF A MONITORING AND COMMUNICATION SYSTEM USING AN INSTANT MESSAGING/ SOCIAL MEDIA APPLICATION AS A COMPONENT OF TILAPIA DISEASE SURVEILLANCE

Artigo científico para submissão ao periódico ***Preventive Veterinary Medicine***

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Abstract: In recent years, various approaches and methods have been developed to provide evidence of the presence or absence of diseases. These models have increasingly used risk-based surveillance strategies to improve the cost-effectiveness of surveillance systems for monitoring the dynamics of existing pathogens and early detection of exotic diseases. Considering the use of instant messaging applications as an auxiliary tool in various segments of veterinary medicine, this study aimed to verify the functionality of a monitoring system supported by instant messaging applications (social media) for smartphones as a component of a surveillance program for notifiable diseases of tilapia. The model was applied to 112 commercial tilapia farms registered with the official veterinary service in the Distrito Federal, Brazil, for 12 months between July 2021 and July 2022. There were 27 reports of atypical health events, with 5 to 20 moribund fish being collected and submitted to molecular analysis for *Streptococcus agalactiae*, *Francisella orientalis*, Infectious Spleen and Kidney Necrosis Virus (ISKNV), Tilapia Lake Virus (TiLV), Tilapia Parvovirus (TiPV) and Nervous Necrosis Virus (NNV). The notification rate per farm was 0.241 compared to 0.045 in the traditional passive surveillance system, showing that the smartphone app monitoring and communication system had 5 times more reports of atypical health events than the traditional system. The component proved capable of contributing to the early detection of pathogens in population strata where it is not logistically or financially feasible to carry out random sampling studies. Further studies are recommended to assess the effectiveness, economic viability and quantification of the sensitivity of this model as a surveillance component. In addition, the incorporation of artificial intelligence tools for better management of responses and targeting of suspicions may be indicated.

Keywords: tilapia, diseases surveillance, monitoring, smartphones, messaging applications.

1. Introduction

Tilapia is one of the most important species for global aquaculture and is recognized by the Food and Agriculture Organization of the United Nations as one of the most important production chains for global food security (FAO, 2018). To ensure the sustainable production of this animal protein, FAO, WOAH and international health authorities have established guidelines for member countries such as the implementation of surveillance programs for the most impactful pathogens, such as Tilapia Lake Virus (TiLV) (FAO, 2021) and other emerging diseases (WOAH, 2019). These programs should focus on the early detection of exotic and emerging diseases, the control and eradication of pathogens, as well as the certification or compartmentalization of disease-free areas in order to guarantee access to national and international trade in this protein (Thurmond, 2003; WOAH, 2019; Cameron et al., 2020; Bondad-Reantaso, et al., 2021).

In this context, the official veterinary service (OVS) plays an important role in monitoring the dynamics of pathogens, defining and implementing risk mitigation strategies and controlling diseases that negatively impact the tilapia industry. Over the last few decades, various approaches and methods have been continuously developed to provide evidence of the presence or absence of diseases (Cameron, Baldock, 1998; Josseran, Fouillet, 2013; Bondad-Reantaso, et al., 2005; Lyngstad et al., 2016; Bondad-Reantaso, et al., 2021). One of these methods is risk-based surveillance, which is a method that aims to improve the cost-effectiveness of surveillance systems for detecting diseases or proving the absence of diseases, and to provide support for strategic and operational decision-making (Stärk et al., 2006).

At the same time, digital technologies are transforming veterinary practice, offering new possibilities for improving diagnoses and communication between professionals and their respective audiences. The use of smartphones and social media during visits to farms has shown good potential as a tool for improving the animal health services provided by veterinarians (Karimuribo et al., 2016). In the field of animal health, we highlight, for example, the use of digital tools developed exclusively for sending information and reporting suspected diseases such as the iSIKHNAS system, launched in 2013 in Indonesia in partnership with the Australian official veterinary service, which has become a successful national information system on animal production and health in that country (Ausvet, 2022) and the e-Sisbravet system used in Brazil so that anyone can send communications about suspected syndromic diseases of terrestrial animals (Brasil, 2020).

When it comes to social media, we have generally observed that these networks have been used to quickly and effectively share clinical cases, promote discussions and disseminate knowledge (Bernardo et al., 2013; Mekaru, Brownstein, 2014; Kedrowicz et al., 2016; Englar, 2017; Trittmacher et al., 2021; Woodard et al., 2021; Veiga et al, 2022; Lemos et al., 2023).

The OVS of the Distrito Federal, Brazil, represented by the local Secretariat of Agriculture (SEAGRI), has maintained a remote service channel since the Covid-19 pandemic through a multiplatform instant messaging application where farmers can request services and communicate with the animal health defense service. Among the various services offered is the possibility of reporting suspected notifiable diseases such as nervous, vesicular and hemorrhagic syndromes and atypical mortalities of the most diverse production species. From 2020 to 2023, the period in which this service remained in force, more than half of the notifications made by producers and veterinarians to the OVS/DF regarding passive surveillance (PS) took place via the instant messaging channel (SEAGRI, unpublished data), proving to be an important communication tool between the official service and rural producers. In addition, notifications received via the messaging app have major advantages over traditional PS methods (notifications made in person or by telephone call) as there is the possibility of sending photos or videos of suspect animals, clinical signs and lesions presented, allowing for a more effective prior clinical-epidemiological assessment.

However, the potential of smartphone messaging apps as a tool to help aquatic animal disease surveillance programs is still little explored. In addition to passive surveillance, there are other possibilities for using this tool in disease surveillance systems, such as epidemiological monitoring. The objectives of this study were to present a tilapia disease surveillance model based on epidemiological monitoring carried out using a social media/instant messaging application for smartphones and to evaluate the functionality and effectiveness of this monitoring and communication system (MCS) as a component of a surveillance program for notifiable tilapia diseases, comparing the results obtained with traditional PS methods.

2. Materials and Methods

All commercial tilapia farms registered with the OVS/DF (n=112) took part in this study, divided into three different commercial groups: i) breeding, larviculture or alevin sales farms (n=8); ii) closed and semi-closed fattening farms (n=77); and iii) recreational fishing farms (pay-to-fish) (n=27). The participating producers received an invitation letter explaining the purpose of the study and signed a voluntary participation agreement, undertaking to authorize messages to be sent to their private telephones, to report atypical health events and to facilitate the veterinary service's access to the farm during sampling. This study was approved by the Ethics Committee for Animal Use of the University of Brasilia under number 23106.080975/2021-63.

2.1 Monitoring and communication system

This system combines active and passive surveillance characteristics to obtain targeted samples, as part of the disease surveillance plan carried out by the OVS/DF's fish health program. The pathogens investigated were *Streptococcus agalactiae* (*SA*), *Francisella orientalis* (*FO*), Infectious Spleen and Kidney Necrosis Virus (ISKNV), Tilapia Lake Virus (TiLV), Tilapia Parvovirus (TiPV) e Nervous Necrosis Vírus (NNV). The component used differs from traditional PS models based on spontaneous notification from an individual who has an established relationship with a particular production unit. In the MCS, participants were previously sensitized to instantly report any atypical health events involving tilapia, whether serious, such as high mortality, or mild, such as just the observation of moribund tilapias. The aim was to create a rapid communication channel, using a popular app/social media easily accessible to all producers, in order to investigate the causes of mild health events that would naturally not be reported in the PS system, but which could be mild and sub-clinical outbreaks of target diseases listed in surveillance programs.

The messaging and social media application WhatsApp® (Meta Platforms, Inc., California, USA) was used, since all the participating tilapia farmers were users of this social media. For 112 fish farmers, automatic messages were sent every two weeks reminding them to participate in the study. Among the options requested by the message, the farmer could type "1" in the event of an atypical health event that day or week and "2" in the absence of health events. The message also stated that tilapia farmers could report the occurrence of moribund tilapia or atypical mortalities at any time, regardless of the fortnightly automatic transmission line.

2.2 Sampling

Each time a positive report was made, the farm was visited immediately or within a maximum of 12 hours from the time the health event was reported. The system was maintained for a period of 12 months, from July/2021 to June/2022, in order to include the different seasons and increase the sensitivity of the survey for both typical winter pathogens such as ISKNV and *Francisella orientalis* (Assis et al., 2017; Figueiredo et al., 2020) and summer pathogens such as TiLV and *Streptococcus* sp. (Eyngor et al., 2014; Suhermanto et al., 2019).

Each tilapia farm was considered an epidemiological unit. Five to 20 moribund fish were collected per farm and, although there are important differences in the patterns of diagnostic specificity and sensitivity that can lead to false negatives/positives, the number of fish per epidemiological unit was considered adequate to allow the detection of at least one positive individual. The minimum sample size established was based on the n used by the Strategic Manual for TiLV (Yamkasem et al., 2021; FAO, 2021) which recommends collecting at least 5 moribund fish with clinical signs compatible with the disease and other fish disease surveillance plans based on targeted sampling (Lyngstad et al., 2016; FFA-GovNL, 2020; Barato et al., 2022). In cases where the number of moribund animals did not reach the minimum number, the sample was supplemented with tilapia caught in the ponds with the greatest history of mortality or observation of symptomatic fish.

Before sampling, the clinical signs and macroscopic findings were described and catalogued in spreadsheets. To euthanize the live fish, the active ingredient eugenol was used (1 mL of clove oil and 10 mL of ethanol for 1 L of water) followed by a physical method (National Council for the Control of Animal Experimentation - CONCEA, 2013; Resolution No. 1000 of the Federal Council of Veterinary Medicine - CFMV, 2012). After necropsy for macroscopic observation of internal structures, 1 cm^2 of brain, spleen, kidney, liver and gill were stored in tubes with 95% ethanol for molecular tests and 10% formalin for histopathological tests, with a minimum ratio of liquid to tissue volume of 1:5 (Rawiwan et al., 2021; Brasil, 2022), keeping tissue fragments from one adult fish per 15 mL tube or 5 whole alevins (< 4 cm) per 50 mL tube, duly identified with the establishment's code, for individual testing of each fish sampled. Skin and gill scrapings were also taken for direct examination and the search for ectoparasites in all the fish.

During the visits, a general assessment of production and sanitary conditions was carried out, measuring water temperature, dissolved oxygen, pH, toxic ammonia, alkalinity and turbidity in order to rule out mortalities caused by serious management failures.

2.3 Molecular diagnosis

The samples were sent to and processed at the Aquatic Animal Diseases Laboratory (Aquavet) of the Veterinary School of the Federal University of Minas Gerais – UFMG. DNA extraction kits were used for *FO*, *SA*, TiPV and ISKNV (Wizard SV Genomic, Promega) and RNA for TiLV and VNN (Cellco Biotech) following the manufacturer's instructions. The extracted nucleic acids were quantified using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and stored at -80◦C until use. Extracted DNA was used for detection of TiPV, ISKNV, FO and SA using PCR, while extracted RNA was used for detection of TiLV and NNV using reverse transcription quantitative PCR (RT-qPCR). Reactions were performed with a HotStart Taq polymerase kit (Qiagen, Germantown, MD, USA) in a final reaction volume of 25 µl. The reaction mixture consisted of 1 × PCR buffer, 1.0 µM of each PCR primer, 0.2 µM dNTPs, 1.25 U Taq DNA polymerase and 50 ng template DNA. The PCR conditions were as follows: an initial denaturation at 95◦C for 15 min, followed by 30 cycles of 94◦C for 30 s, 58◦C for 1 min and 72◦C for 1 min; final elongation was carried out at 72◦C for 5 min. The primers used were purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). A 96-well Veriti thermal cycler was used and the amplicons were separated on the QIAxcell Advanced using QX DNA Screening Kit (Qiagen). Pathogen detection was carried out using GoTaq® Probe qPCR and RT-qPCR Systems, performed in QuantStudio 7 (Applied Biosystems, UK) according to each manufacturer's instructions. The master mix kits used for the RT-qPCR (AgPath-ID, Life Technologies Corporation) and qPCR (Cellco Biotech) reactions were used in accordance with the manufacturer's instructions and the methods described by Figueiredo et al. (2020) for TiLV, NNV and ISKNV, by Liu et al. (2020) for TiPV and by Assis et al. (2017) for bacterial diseases.

For the 2021/2022 screening, as smaller sample sizes were used, it was decided to process each sample individually. For the 2023 cross-sectional study, in order to reduce laboratory costs, pools of 3 fish were made for each test, in line with the methodology duly validated by the official laboratory. Positive results were retested to achieve maximum specificity.

2.4 *Statistical analysis*

Excel (Microsoft Office 365®) and Stata®version 17 (StataCorp 2022, College Station, TX, USA) statistical programs were used to record data, categorize responses and perform descriptive statistical analysis. In order to compare the results of the MCS with the traditional passive surveillance system (PS), we used the ratio of the mean monthly notifications (MMN), expressed in the formula below:

$$R_{MMN} = \bar{X}_{MCS} : \bar{X}_{PS}$$

The data on the traditional passive surveillance system for the last five years (2018-2022) was taken from the SEAGRI report (2023), which recorded the receipt of 27 notifications of suspected diseases in aquatic animals in the DF, 23 of which involved the tilapia species. To compare the results between the components in terms of notification rate, we used ratio and proportion tests, according to the following formula, where NRF = notification rate per farm, n = number of notifications received and p = universe of farm.

$$NRF = n / p$$

$$Ratio = (n_{MCS} / p_{MCS}) / (n_{PS} / p_{overall})$$

For the purposes of calculating the proportion, we considered p=112 for MCS and p=600 for PS, as this was the approximate number of aquaculture farms registered in the Distrito Federal between 2018 and 2022 (SEAGRI, 2023).

3. Results

During the established period, 27 communications/samples were taken from a total of 25 different production units (22.32%; 25/112) that presented some atypical event in tilapia production. Table 1 summarizes the results of the MCS and compares the ratios and proportions with data from the traditional passive surveillance system extracted from reports from the Distrito Federal's animal health service.

Table 1. Comparison between the MCS carried out between Jul/2021 and Jun/2022 with historical data from the traditional passive surveillance system for the period 2018-2022 in the Distrito Federal.

Surveillance system	Notifications	Period evaluated (in months)	Monthly Mean	% notification per farm	R_{NRF}	R_{MMN}
PS	27	60	0,45	0,045	1,0	1
MCS	27	12	2,25	0,241	5,35	5

PS = passive surveillance, MCS = monitoring and communication system, R_{TNP} – ratio of notification rate per farm, R_{MMN} – ratio of the mean monthly notifications.

Considering the number of reports by production group, it was observed that the proportion of reports of atypical health events was higher at hatcheries and alevin sales farms (75.0%; 6/8), followed by pay-to-fish farms (22.2%; 6/27) and commercial fattening farms (19.5%; 15/77). With regard to communications/collections, 20 (74.1%; 20/27) were carried out in the autumn and winter months. The number of communications in each month during the period evaluated and the polynomial trend expressing the results are shown in the time graph in Fig. 1.

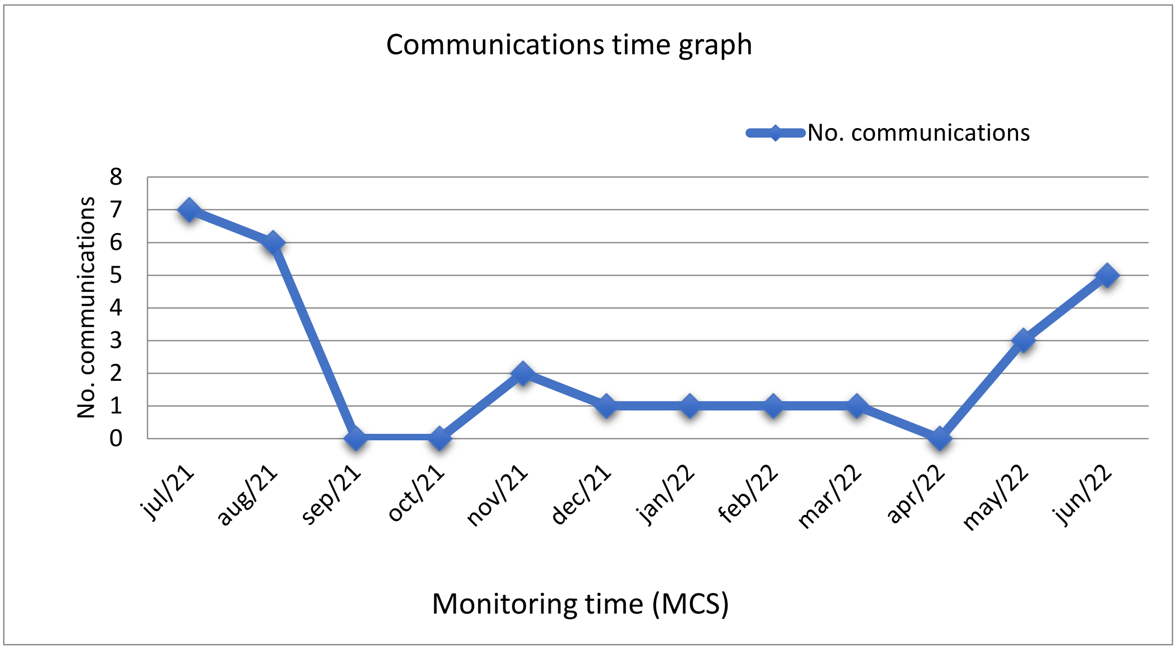

Fig. 1. Time distribution of communications made by tilapia farmers during the MCS held between Jul/2021 and Jun/2022.

The most common clinical signs observed during health events were erosive or ulcerative lesions on the skin and fins (40.7%; 11/27), lethargy (33.3%; 9/27), exophthalmos (25.9%; 7/27) and erratic swimming, vertigo or dystaxia (22.2%; 6/27).

The cause of mortality was confirmed in most cases (22/27; 81.48%), based on clinical, anatomopathological, parasitological, molecular, environmental and physico-chemical analyses of the pond water. The information and photos sent by the producers via the app in each case helped to direct clinical suspicions, as well as facilitating quick technical visits to the production systems.

As for the number of positive farms detected by MCS, the results were as follows: ISKNV=1 and FO=2. No animals or farms tested molecularly positive for TiLV, TiPV and NNV viruses. The results of the detection of infectious diseases and investigations into other causes of health events such as severe infestations by parasitic agents and adverse water conditions for tilapia farming (low temperature, acid pH, dissolved oxygen deficit, inadequate levels of toxic ammonia and other physico-chemical factors) normally associated with opportunistic bacterial infections are shown in Table 2.

Table 2. Distribution of MCS communications carried out between Jul/2021 and Jun/2022 according to the seasons in Brazil and the results of the investigations into each health event reported.

Season	Collection date	Collection No.	Production purpose	RT-qPCR/ qPCR positives	Severe ectoparasitism	Management failures and inadequate temperature
Autumn and winter	07/02/2021	1	Only fattening			Low temperature; High toxic ammonia levels
	07/05/2021	2	Only fee-fishing pond (pay-to-fish)			Low temperature; Low dissolved oxygen; High toxic ammonia levels
	07/05/2021	3	Only fee-fishing pond (pay-to-fish)			Low temperature
	07/06/2021	4	Hatchery or alevin seller			Low temperature
	07/13/2021	5	Hatchery or alevin seller			Low temperature
	07/16/2021	6	Only fattening			Low temperature
	07/26/2021	7	Only fattening		*Piscinoodinium pillulare*	Low temperature
	08/05/2021	8	Hatchery or alevin seller			Low temperature; Low dissolved oxygen
	08/12/2021	9	Only fattening			Low temperature
	08/13/2021	10	Only fee-fishing pond (pay-to-fish)		*Chilodonella hexasticha*	Low temperature
	08/19/2021	11	Only hatchery or alevin seller	*Francisella orientalis*	*Dactylogyrus* sp.	Low temperature
	08/20/2021	12	Only fattening		*Trichodina* sp. *Tripartiella* sp.	No abnormalities observed in water parameters
	08/25/2021	13	Only fee-fishing pond (pay-to-fish)			Low temperature; Low dissolved oxygen
Spring and summer	09/10/2021	14	Only fattening		*Piscinoodinium pillulare*	No abnormalities observed in water parameters
	11/12/2021	15	Only fattening	ISKNV		No abnormalities observed in water parameters
	11/16/2020	16	Only fattening			No abnormalities observed in water parameters
	12/30/2021	17	Only fattening			No abnormalities observed in water parameters
	01/18/2022	18	Only fattening			No abnormalities observed in water parameters
	03/18/2022	19	Only fattening			No abnormalities observed in water parameters
	04/13/2022	20	Only fattening			No abnormalities observed in water parameters
Autumn and winter	05/18/2022	21	Only hatchery or alevin seller			Low temperature
	05/28/2022	22	Only fattening			Low temperature
	06/06/2022	23	Only fattening	*Francisella orientalis*		Low temperature; Low dissolved oxygen
	06/23/2022	24	Only hatchery or alevin seller			Low temperature
	06/24/2022	25	Only fattening			Low temperature
	06/26/2022	26	Only hatchery or alevin seller			Low temperature
	06/29/2022	27	Only fee-fishing pond (pay-to-fish)		*Piscinoodinium pillulare*	Very acidic pH

RT-qPCR and qPCR tested for TiLV, NNV, ISKNV, TiPV, *S. agalactiae* e *F. orientalis*.

4. Discussion

For the first time, a study has analyzed the applicability of a relevant tilapia disease monitoring system using a social media app, compared to the conventional PV method. The results of the study revealed that the MCS method, although simple, was easy to execute, extremely practical and allowed for rapid decision-making by the professionals involved in diagnosing diseases, as well as strengthening ties between the production system and the animal health agency.

All MCS participants had access to the previous messages sent by transmission line, and no difficulties were reported in operationalizing the responses. In almost all the cases reported, producers only informed the number corresponding to the observation of an atypical health event. The veterinarian operating the system, when he saw any positive response, contacted the notifying farmer directly via private message or telephone call to request more information, including videos or photos, to ensure that it really was a mortality event or clinical sign.

In terms of effectiveness, the MCS proved to be superior to the traditional passive surveillance system. This superiority was evident in both the increased number of reports received by the animal health service and the broader distribution of atypical events across the different production groups. MCS received five times more communications (27 in 12 months) compared to PS over five years (27 in 60 months). The MCS was also superior in the notification rate per farm (NRF), which was proportionally much higher (R_{NRF}= 5.35). Even though the universe of farms shows large differences (MSC = 112 and PS = 600), it is believed that the results are closely linked to the ease and speed of use of the application, which is used intensively by farmers. However, it should be considered that the surveillance components were not carried out or implemented simultaneously and that this timeless implementation may have influenced the direct comparison due to changes in external factors, technological advances and the awareness of producers to report suspected diseases over time.

The breadth of communications between all strata of commercial purpose was also observed. According to data from SEAGRI (2023), most of the notifications from 2018 onwards were from fattening (11) and subsistence (8) farms, followed by just 5 from young-form farms (3 different notifications/investigations on the same establishment) and 3 originating from pay-to-fish farms. In the analysis by MCS strata, the higher proportion of reports of atypical health events observed in the group of hatcheries and alevin sellers may be related to the age and stage of the fish, which are immunologically more susceptible to disease when compared to the growth and fattening stages. In addition, young forms are subjected to various stressful procedures such as handling with nets, biometrics and transportation (in the case of alevin sellers, who buy very small fry and fingerlings to resell to fattening farmers), which can trigger clinical signs and secondary mortalities. This result corroborates the understanding of several authors who consider this group to be the one with the greatest health risk (Kleingeld, 2010; Diserens et al., 2011; Oidtmann et al., 2011; Oidtmann et al., 2013; Oidtmann et al., 2014).

The group with the lowest notification rate (lowest frequency of health events) was commercial fattening, curiously the largest stratum, made up of 77 fattening farms. The most likely hypothesis is that the fattening farms in the DF have better sanitary conditions than the pay-to-fish farms, and that biosecurity measures, prophylaxis and best practices are observed with greater intensity.

As shown in Fig. 1, the MCS received a considerably higher share of communications in the autumn and winter months in Brazil (74.1% of communications). In some periods, a concentration of mortality reports was observed in short time intervals, usually after an atypical weather event (e.g. intense cold wave or very heavy rain). Reports from the DF's official service had already pointed to winter as the period of greatest occurrence of atypical health events since 2018 (SEAGRI, 2023). The stress caused by low water temperatures can affect tilapia and tropical fish, causing production losses and secondary mortality due to different pathogens (Sun et al., 1992; Sant'Ana et al., 2012; Hassan et al., 2013; Pereira et al., 2019).

Among the diseases surveyed during the MCS, there was a low frequency of pathogens considered endemic in Brazil, such as *FO* and ISKNV, and no detection of exotic pathogens such as TiLV, TiPV and NNV. The estimated prevalence at farm level for the pathogens considered endemic in Brazil was previously estimated in the DF at values lower than 5%. Although the MCS cannot determine the actual prevalence of the diseases, the results of this component not only confirmed the expected trend of low frequency of officially controlled diseases in tilapia production in the Distrito Federal, but also allowed the first detection of ISKNV, something that no other surveillance component had done.

As of 2018, the DF had recorde d only 3 cases of diseases that could be controlled by the local veterinary service, 1 case of *FO* and 2 cases of *Streptococcus* sp. (SEAGRI, 2023). In a case detection study carried out in 2023 based on random sampling carried out only at tilapia hatchery and alevin sale farms, no cases of TiLV, ISKNV, *SA* or *FO* were detected (Federal Agricultural Defense Laboratory - Ministry of Agriculture, Livestock and Food Supply; 2024 analytical report SEAGRI, unpublished data).

It should be noted that the surveillance components used in the Distrito Federal have different coverage. For example, the laboratory sampling component in young fish establishments is applied only to hatcheries and the alevin sale farms; the inspection component in fish agro-industries carries out surveillance on loads of fish from fattening; the MCS component carried out surveillance in all commercial strata identified in Brazil's capital and the passive surveillance component is the broadest of all, involving any type of establishment, including farms without commercial purpose, such as subsistence breeding. The results of all the surveillance components applied in the DF show that the MCS is highly sensitive in relation to the others, and that it has the highest capacity to detect notifiable diseases compared to the other components. However, it is important to note that the monitoring system based on social media or any other more economical surveillance methodology based on the screening of moribund fish is not capable of providing quantitative estimates of epidemiological data, nor of guaranteeing, within a certain confidence interval, the extrapolation of the results to the respective target population of the study, as well as being less reliable than random sampling studies for assessing the impact of interventions and disease control measures.

In the case of the only ISKNV-positive farm found in the MCS, we found that this farmer buys tilapia alevins from an alevin seller located in the state of Goiás, neighboring the Distrito Federal. For cases of allochthonous infections such as this, the laboratory sampling component in young-form establishments would be unable to detect them, which demonstrates the importance of not restricting surveillance to just one component or group of farms, especially when seeking to carry out surveillance of diseases that are not very prevalent or are exotic in an zone, state or country (Hadorn, Stärk, 2008).

An important limitation that should be highlighted for the use of this component is the degree of veracity of the tilapia farmers' responses during the interaction on the messaging app. In order to reduce the bias caused by untruthful responses, all tilapia producers participated voluntarily, receiving a letter beforehand informing them that the study would not trigger any fiscal activities by the official veterinary service. Even so, we can't rule out the possibility that some participants may have omitted to report the atypical health event out of fear of any restrictive measures that could be imposed by the OVS/DF, or even neglected to respond positively because they were busy at the time of our message. However, we presume that this rate of untruthfulness was very low, given that during the period of application of the MCS, the veterinary service carried out visits as part of the active surveillance component (without prior call) to 30 tilapia farms for survey and clinical inspection, and in none of them was there any evidence of mortality or clinical signs. Even though this percentage of on-site checks is small in the universe of participating farms (26.8%; 30/112), the fact that many visits were carried out randomly in different groups presupposes that the responses had a high proportion of veracity.

With regard to the use of social instant messaging apps on smartphones, we believe that they can be a notable source for recording reports of suspected diseases due to their great accessibility and the fact that they are widely used in all social classes. The possibility of immediately sending photos or videos of sick animals can also help to direct suspicions, because if the veterinarian makes the visit and finds only dead fish, with the rapid deterioration of the carcasses, he may have difficulty defining the suspicions at the time of the clinical and epidemiological investigation. In domestic mammals, a similar study showed great advantages in using this system to receive information and images/videos from the field, facilitating the work of the veterinary diagnostic laboratory in most calls (Lemos et al., 2023).

In this surveillance component model, producers' responses to automatic messages and the direction of the investigation in cases of atypical health events were controlled manually by an operator with a degree in veterinary medicine. However, for a larger number of monitored farms, additional tools available through social media applications (some of which are not free) would probably be recommended to help with data/response management. For example, for monitoring diseases in a population made up of a large number of fish farms, it would be highly recommended to incorporate artificial intelligence based on pre-established responses so that the producer could provide additional information about the atypical health event, helping to better target suspicions.

In view of the above, we assume that the monitoring and communication system using messaging apps can be effective as a component of disease surveillance, as well as having advantages over conventional systems, although sensitivity quantification and economic viability studies are needed to prove this hypothesis.

In conclusion, the MCS based on messaging applications for reporting atypical health events has fulfilled its purpose of being an effective component for disease surveillance, with advantages over traditional passive and active surveillance components. The component proved capable of contributing to the early detection of pathogens in populations or strata where it was not logistically or financially feasible to carry out random sampling studies, such as the numerous groups of fattening and pay-to-fish farms. The results of the MCS, if added to other surveillance components, make it possible to determine the frequency of the main pathogens for official control in tilapia farming. However, it is suggested that further studies be carried out to assess effectiveness and quantify sensitivity, evaluate artificial intelligence tools for managing responses and prove the economic viability of this component compared to traditional methods based on random sampling and/or routine visits.

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ANEXO I

Universidade de Brasília
Comissão de Ética no Uso Animal

Brasília, 12 de abril de 2022.

CERTIFICADO

Certificamos que o projeto de ensino intitulado " **CARACTERIZAÇÃO SANITÁRIA E EPIDEMIOLÓGICA DOS PRINCIPAIS PATÓGENOS DE TILÁPIAS NO DISTRITO FEDERAL**", SEI n.º 23106.080975/2021-63, sob responsabilidade do Professor Fabiano José Ferreira de Sant'Ana, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA, e foi avaliado e aprovado pela Comissão de Ética no Uso Animal (CEUA) da Universidade de Brasília, na 171ª reunião ordinária, realizada em 11/4/2022. Este projeto foi aprovado para utilização de *Oreochromis niloticus* (100 machos e 100 fêmeas), provenientes de Propriedades de piscicultura do DF.

O presente certificado é válido pelo período de 30/5/2022 à 31/7/2022.

Universidade de Brasília UnB

Carina Krewer

Dra. Carina da Costa Krewer

Coordenadora da CEUA – UnB

Comissão de Ética no Uso de Animais CEUA

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

ANEXO II

GOVERNO DO DISTRITO FEDERAL
Secretaria de Estado da Agricultura, Abastecimento e Desenvolvimento Rural – SEAGRI/DF
Subsecretaria de Defesa Agropecuária – SDA
Diretoria de Sanidade Agropecuária e Fiscalização – DISAF

TERMO DE COMPROMISSO

Eu, ..., portador(a) do CPF/CNPJ nº ...., proprietário(a) ou responsável legal pelos animais criados na propriedade, denominada ...., localizada no Núcleo Rural/Localidade de ...., Distrito Federal, cadastrada sob o nº (SIDAGRO) ...., declaro, para todos os fins, que participarei voluntariamente do **"Estudo de caracterização produtiva, sanitária e epidemiológica da estreptococose, franciselose e outras doenças de tilápias no Distrito Federal"**. Para tanto, aceito participar do questionário científico do estudo e autorizo que peixes de minha propriedade sejam amostrados e testados caso seja verificada a possibilidade de presença desses patógenos em minha criação.

Comprometo-me também a auxiliar o(s) médico(s) veterinário(s) e técnicos em agropecuária da SEAGRI/DF durante as coletas de amostras, caso minha propriedade seja selecionada, a fim de facilitar os trabalhos realizados pelos profissionais.

Por ser verdade, firmo o presente Termo de Compromisso.

Brasília/ DF, ... de .... de 2020.

....

Assinatura do proprietário ou responsável

ANEXO III

GOVERNO DO DISTRITO FEDERAL
Secretaria de Estado da Agricultura, Abastecimento e Desenvolvimento Rural – SEAGRI/DF
Subsecretaria de Defesa Agropecuária – SDA
Diretoria de Sanidade Agropecuária e Fiscalização – DISAF
Gerência de Saúde Animal – GESAN
Coordenação de Sanidade de Animais Aquáticos de Cultivo – CAAQ/DF

ESTUDO DE CARACTERIZAÇÃO PRODUTIVA, SANITÁRIA E EPIDEMIOLÓGICA DA TILAPICULTURA – SEAGRI/DF

1 IDENTIFICAÇÃO DO ESTABELECIMENTO

Código do Produtor (SIDAGRO):	Código da fazenda no estudo:
Entrevistado:	Proprietário:
Nome do estabelecimento:	Cód. do estabelecimento:
Localidade:	Região Administrativa:
E-mail:	Telefone:

2 **Coordenadas geográficas da piscicultura:**

S: Latitude:* ______º _________´ _______" W: Longitude:* ________º ________' ________"

CARACTERÍSTICAS SOCIOESCONÔMICAS

3 **Realiza outras atividades remuneradas:** [ ] Não [ ] Sim

4 **Qual a renda familiar do proprietário da exploração?** [ ] Até 2 salários mínimos [ ] Entre 2 e 10 salários mínimos [ ] Acima de 10 salários mínimos

5 **Quem toma as decisões de manejo na criação das tilápias? (principal responsável pelas atividades)**

[ ] Proprietário da fazenda ou seu familiar [ ] Funcionário/caseiro sem formação técnica [ ] Profissional da área contratado e que presta serviços contínuos

6 **Grau de instrução do responsável pela criação:** [ ] Fundamental incompleto [ ] Fundamental completo [ ] Médio incompleto [ ] Médio completo

[ ] Superior incompleto [ ] Superior completo [ ] Pós-graduado [ ] Sem escolaridade

7 **Mão-de-obra:** [ ]Própria/Familiar [ ]Contratada [ ]Ambos [ ] Terceirizada (associações, cooperativas, etc.) | 8 **Número de funcionários:**

9 **O proprietário, familiar ou funcionário responsável pelo manejo já participou de curso(s) ou treinamento(s) na área de piscicultura?** [ ] Não [ ] Sim

10 **Há assistência técnica?** [ ] Não [ ] Sim | 11 **Se SIM, quem presta?** [ ] Emater [ ] SEAGRI [ ] Universidades [ ] Embrapa [ ] Profissional Particular [ ] Outro

12 **Qual a formação do profissional que presta assistência? (pode marcar mais de uma opção)**

[ ] Não há assistência [ ] Zootecnista [ ] Eng. de Pesca [ ] Veterinário [ ] Eng. Agrônomo [ ] Biólogo [ ] Eng. de Aquicultura [ ] Técnico [ ] Outro:

CARACTERÍSTICAS DA PRODUÇÃO

13 **Finalidade da produção:** [ ] Engorda (E) [ ] Fornecedor de alevinos (F) [ ] Recreação/ Pesque Pague (PP) | 13.1 **Se mais de uma, qual a principal?** [ ] E [ ] F [ ] PP

14 **Existem animais terrestres na fazenda?** [ ] Não [ ] Sim | 14.1 **Se SIM, quais espécies?**

15 **Existem outras espécies aquáticas?** [ ] Não [ ] Sim | 15.1 **Se SIM, as tilápias estão em policultivo? (mesmo tanque com outras espécies)** [ ] Não [ ] Sim

15.2 **Se SIM, quais espécies?** [ ] Tambaqui [ ] Outros peixes redondos [ ] Pintado/ surubins [ ] Crustáceos [ ] Moluscos (ex. ostras) [ ] Outros:

16 **Sistema de produção:** [ ] semi-fechado (tanque/viveiro) [ ] fechado (recirculação com filtro, biofloco etc.) [ ] Ambos

17 **Qual o tipo de tanque/viveiro que utiliza?** [ ] Superfície [ ] Escavado permeável [ ] Escavado impermeável [ ] Outro:

18 **Fonte da água de produção:** [ ] reservatório [ ] lago/lagoa [ ] córrego [ ] rio [ ] subterrânea (mina ou poço)

19 **Bacia Hidrográfica (consultar anexo se houver dúvida):** [ ] Descoberto [ ] Maranhão [ ] Corumbá [ ] S. Bartolomeu [ ] Lago Paranoá [ ] São Marcos [ ] R. Preto

20 **Quantidade de tilápias:** 20.1[ ] adultas em fase de engorda 20.2[ ] alevinos e juvenis 20.3[ ] matrizes

21 **A quantidade acima é a mesma da última estocagem?** [ ] Não [ ] Sim | 22 **Se NÃO, quantos peixes estocou por último?** [ ] ________________

23 **Nº tanques de engorda:** | 24 **Nº tanques com alevinos:** | 25 **Lâmina d'água total (m²):**

26 **Realiza biometria da massa viva de tilápias?** [ ] Não [ ] Sim

26.1 **Densidade de estocagem nos viveiros de engorda em kg/m²** (dividir o peso médio da biomassa de engorda pela lâmina d'água total):

27 **Número de ciclos por ano:** [ ] Menos de um ciclo [ ] 1 ciclo [ ] 2 ciclos [ ] 3 ou mais ciclos

28 F **Número de alevinos de tilápia distribuídos por ano:**

29 **Duração do ciclo de produção (estocagem até despesca em meses):** | 30 **Peso médio de despesca (g):** | 31 **Produção anual (em kg):** / 31.1 **Produção mensal (em kg):**

32 **Origem das larvas/alevinos/juvenis/matrizes:** [ ] produzidos no local [ ] fornecedor do DF [ ] fornecedor do entorno [ ] fornecedor de fora do DF/entorno

32.1 **Fornecimento:** [ ] sempre o mesmo fornecedor [ ] mais de um fornecedor no mesmo ciclo [ ] alterna fornecedor entre ciclos

33 **Se o fornecedor for de fora do DF, qual a UF?**

34 Nome do distribuidor:	35 Frequência de aquisição dos alevinos: [ ] mensal [ ] semestral [ ] mín. anual
36 Primeiro destino das tilápias despescadas (pode marcar mais de uma opção): [ ] Estabelecimentos de abate/ beneficiamento com inspeção SIF/SID/SIE/SIM [ ] Estabelecimentos de abate/beneficiamento sem inspeção [ ] Direto para mercados/ feiras/ restaurantes [ ] Terceiros (destino desconhecido) [ ] Consumo próprio	
37 Possui vizinho produtor de tilápia ou em um raio de 1 Km? [ ] Não [ ] Sim	38 Usa alimento vivo p/ os peixes? (artêmia, minhoca, larvas etc) [ ] Não [ ] Sim
39 Alimentação dos animais: [ ] Ração comercial [ ] Ração feita em associações/cooperativas [ ] Ração feita na própria propriedade [ ] Outra:	
40 Acrescenta alguma substância na ração? [ ] Não [ ] Prebiótico/ probiótico [ ] Vitamina C [ ] Antibiótico [ ] Imunoestimulante [ ] Outro:	
40 Se usa antibiótico, qual? [ ] Florfenicol [ ] oxitetraciclina [ ] off-label com outro, qual?	
CARACTERÍSTICAS DE BIOSSEGURIDADE E BOAS PRÁTICAS	
41 Está aderido ao programa de BPA da SEAGRI? [ ] Não [ ] Sim	42 Possui conhecimento sobre biosseguridade na piscicultura? [ ] Não [ ] Sim
42.1 Se SIM, onde se informou sobre? [ ] Leitura de livros, manuais técnicos, pesquisa internet etc. [ ] Fiz cursos da área [ ] Alguém me falou e me ensinou medidas.	
43 Alevinos (ou matrizes) são adquiridos com GTA (Guia de Trânsito Animal)? [ ] Não [ ] Sim [ ] Às vezes	
44 Peixes adultos terminados (ou alevinos distribuídos) são comercializados com GTA? [ ] Não [ ] Sim [ ] Às vezes	
45 Sabe se o seu fornecedor de alevinos tem cadastro em serviço de defesa? [ ] Não possui cadastro [ ] Possui cadastro [ ] não sei informar	
46 Sabe se a larvicultura realiza algum dos procedimentos descritos abaixo nos alevinos? (pode marcar mais de uma) [ ] Tratamento com antibiótico [ ] Vacinação [ ] Teste diagnóstico (1x/ano) [ ] Não realiza [ ] Não sabe informar	
47 Realiza algum procedimento para os animais que são introduzidos? [ ] Não [ ] Sim 47 Se SIM, qual(is)? [ ] tanque de quarentena [ ] Banho de sal [ ] Vacinação [ ] Teste diagnóstico [ ] Vit. C [ ] Trat. com antibiótico [ ] Outros:	
48 Compartilha equipamentos de manejo da exploração com outras propriedades? [ ] Não [ ] Sim	
48 Se compartilha, realiza procedimentos de desinfecção? [ ] Não [ ] Sim.	
49 O(s) funcionário(s) da fazenda tem contato ou presta(m) serviços para outras pisciculturas? [ ] Não [ ] Sim	
49 Se SIM, costuma trocar ou desinfetar roupas, botas e utensílios próprios quando sai de uma fazenda para a outra? [ ] Não [ ] Sim	
50 Realiza limpeza dos tanques/viveiros? [ ] Não [ ] Sim. 50.1 Se SIM, com que frequência? [ ] Mais de uma por ciclo [ ] 1 após cada ciclo [ ] Após 2 ou mais ciclos	
50 Se SIM, marque os métodos usados: Esvaziamento da água e remoção mecânica dos dejetos de fundo: [ ] Não [ ] Sim Aplicação de cal virgem ou outro desinfetante no fundo: [ ] Não [ ] Sim Vazio sanitário de pelo menos 7 dias: [ ] Não [ ] Sim	
51 Utiliza sal comum na água? [ ] Não [ ] Sim 51.1 Se SIM, qual o motivo: [ ] preventivo [ ] sinais clínicos [ ] mortalidade [ ] outro	
52 Realiza desinfecção dos utensílios (redes, puçá e outros)? [ ] Não [ ] Sim 52.1 Se SIM, qual desinfetante é usado? ______________________________________	
52.2 Com que frequência? [ ] sempre que usa [ ] Diário [ ] Semanal [ ] Mensal [ ] Trimestral [ ] Por ciclo [ ] Após 2 ou mais ciclos	
53 Mistura peixes entre tanques? [ ] Não [ ] Sim	54 Movimenta peixes vivos entre propriedades? [ ] Não [ ] Sim
54.1 Se SIM, utiliza caminhão e caixas de transporte terceirizados? [ ] Não [ ] Sim	54.2 As caixas são desinfetadas pelo transportador? [ ] Não [ ] Sim [ ] Não sei
55 Pessoas ou veículos alheios acessam as áreas de produção (tanques, água, equipamentos da fazenda etc.) [ ] Não [ ] Sim	55.1 Se SIM, qual a frequência? [ ] Diário [ ] Semanal [ ] Mensal [ ] Raramente
56 PP As pessoas externas utilizam equipamentos de pesca próprios? [ ] Não [ ] Sim	56.1 Se SIM, os equipamentos são desinfetados na entrada? [ ] Não [ ] Sim
57 PP As pessoas externas utilizam iscas próprias? [ ] Não [ ] Sim	58 PP Pessoas que pescam no local já levaram peixes vivos embora?[ ] Não [ ] Sim
59 Outros animais domésticos tem acesso aos tanques? (ex: cães, gatos, bovinos, aves) [ ] Não [ ] Sim	
60 Existe rede de proteção contra animais silvestres/aves? [ ] Não [ ] Sim	
61 Animais silvestres têm acesso aos tanques? (ex: garças e aves pescadoras, lontras, ariranhas, anfíbios, répteis etc.) [ ] Não [ ] Sim	
62 A água utilizada para tilapicultura passa por algum tipo de tratamento? [ ] Não [ ] Sim, qual(is)?:	
63 Já observou inundações na área de produção? [ ] Não [ ] Sim	
64 Realiza tratamento da água antes de descartá-la? [ ] Não [ ] Sim, qual(is)?_____________	

GOVERNO DO DISTRITO FEDERAL
Secretaria de Estado da Agricultura, Abastecimento e Desenvolvimento Rural – SEAGRI/DF
Subsecretaria de Defesa Agropecuária – SDA
Diretoria de Sanidade Agropecuária e Fiscalização – DISAF
Gerência de Saúde Animal – GESAN
Coordenação de Sanidade de Animais Aquáticos de Cultivo – CAAQ/DF

65 **A água usada na produção tem qual(is) destino(s)?** [ ] Mesmo corpo d'água de captação [ ] Outro corpo d'água natural [ ] Rede de Esgoto
[ ] Outra unidade de produção animal [ ] Irrigação [ ] Perda por infiltração [ ] Água 100% reutilizada para os peixes após filtração mecânica e biológica

66 **Utiliza adubação dos tanques com esterco ou dejetos de suínos, bovinos e outros animais?** [ ] Não [ ] Sim

67 **Durante o arraçoamento dos peixes, costuma sobrar ração na água ou fundo do tanque?** [ ] Não [ ] Sim

68 **Quais manejos (manipulações estressantes) realiza durante o ciclo?** [ ] Transferências entre tanques de fases diferentes [ ] Classificação e/ou cálculo de biomassa
[ ] Vacina [ ] Coleta de mortalidade [ ] Despesca [ ] Outras:

68.1 **Com que frequência ocorrem esses manejos estressantes?**
[ ] Diário [ ] Semanal [ ] Mensal [ ] Trimestral [ ] Semestral ou apenas em final de ciclo

69 **Realiza evisceração na borda do córrego, rio ou reservatório?** [ ] Não [ ] Sim

70 **Já observou sinais de doença ou anormalidades nas tilápias dentro do tanque?** [ ] Não [ ] Sim

70.1 Se SIM, qual(is)? [ ] Natação errática [ ] Escurecimento da pele [ ] Olho saltado [ ] Lesões de pele/nadadeiras [ ] Outras:

70.2 **Quando observa peixes moribundos:** [ ] Retira vivo imediatamente [ ] Retira vivo junto com a coleta de mortalidade [ ] Espera morrer para retirar junto à coleta de mortalidade

71 **Qual a atitude realizada em casos de sintomas ou mortalidade? (pode marcar mais de uma opção)**
[ ] Não faz nada [ ] Chama a Assistência técnica ou universidade [ ] Notifica ao serviço de defesa sanitária da SEAGRI [ ] Banho de sal [ ] Antibiótico ou desinfetante [ ] Registra os índices da mortalidade [ ] Faz análises de água (oxigênio, pH, Amônia, Nitrato etc.) [ ] Interrompe fornecimento de ração

72 **Registra mortalidade estimada?** [ ] Não [ ] Sim 72.1 **Se SIM, com que frequência?** [ ] diária [ ] semanal [ ] mensal [ ] por ciclo

73 **Coleta os peixes mortos da produção?** [ ] Não [ ] Sim 73.1 **Se SIM, com que frequência?** [ ] 2 ou mais vezes por dia [ ] diária [ ] semanal [ ] intervalo > 1 semana

74 **Problema de mortalidade ocorre mais frequentemente na:** [ ] Fase de alevinos/juvenis no verão [ ] Fase de alevinos/juvenis no inverno
[ ] Fase de engorda no verão [ ] Fase de engorda no inverno [ ] ambas as fases no verão [ ] ambas as fases no inverno [ ] ambas as fases no ano inteiro

75 **Já se deparou com mortalidade alta de alevinos?** [ ] Não [ ] Sim 75.1 **Se SIM, mudou de fornecedor?** [ ] Não [ ] Sim 75.2 **Se SIM, qual foi o resultado?**
[ ] Mudei de fornecedor e a mortalidade alta diminuiu ou cessou [ ] Mudei de fornecedor mas a mortalidade nunca baixou ou cessou

76 **Quando ocorreu um quadro de mortalidade anormal mais recente?** [ ] em até 1 mês [ ] até 6 meses [ ] até um ano [ ] mais de uma ano

77 **Destino dado aos animais mortos:**
[ ] Compostagem /Enterrio [ ] Incineração [ ] Digestão ácida ou alcalina [ ] Recolhimento por empresa especializada ou SLU
[ ] Destinado a unidade processadora [ ] Jogado de volta para a o córrego, rio ou represa [ ] Outra:

78 **Avaliação periódica da qualidade da água?** [ ] Não [ ] Sim	78.1 **Se SIM, quais parâmetros são avaliados?** [ ] Temperatura [ ] pH [ ] Oxigênio dissolvido [ ] Amônia [ ]Nitrito/Nitrato [ ] Outros:	78.2 **Se SIM, com que frequência é realizado?** [ ] Diário [ ] Semanal [ ] Mensal [ ] Semestral [ ] Anual [ ] Por demanda

78.3 **Se SIM, notou se existe muita variação de temperatura, pH e demais parâmetros entre cada mensuração?** [ ] Não [ ] Sim

PERCEPÇÃO DOS PRODUTORES

79 **Como você caracteriza a última mortalidade ocorrida na fazenda durante um mesmo ciclo?**
[ ] Nunca observei mortalidade [ ] Mortalidades baixas e esporádicas [ ] Mortalidades baixas e frequentes
[ ] Acumulado de mortalidade alta durante todo o ciclo [ ] Mortalidade alta e aguda (morte rápida da maioria dos peixes em curto período)

80 **A que você atribui como causa da última mortalidade na fazenda?**
[ ] Nunca observei grande mortalidade [ ] Falhas de manejo (problema com aerador, baixo nível de oxigênio na água, qualidade ruim da água, excesso de ração, densidade de peixes acima da capacidade, contaminantes químicos na água, etc.) [ ] Temperatura muito fria [ ] Doenças infecciosas, fúngicas ou parasitárias
Outro:

Nome do servidor que realizou a entrevista:____

Formação: [ ] Médico Veterinário [ ] Técnico em Agropecuária

Assinatura:____