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Review Article
Livestock Diseases

Tilapia disease surveillance and sampling plans for epidemiological surveys and monitoring: a review[1]

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ABSTRACT.- Raposo RS, Delphino MKVC, Rezende LPO, Sant'Ana FJF. **Tilapia disease surveillance and sampling plans for epidemiological surveys and monitoring: a review**. *Pesquisa Veterinária Brasileira 45:e07593, 2025.* Laboratório de Diagnóstico Patológico Veterinário, Universidade de Brasília, SRB, Área Especial, Galpão 04, Granja do Torto, Brasília, DF 70636-020, Brazil. E-mail: santanafjf@yahoo.com

The tilapia industry has been expanding increasingly 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 in the environment and between farms. 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 from 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.- [**Vigilância de doenças de tilápias e planos amostrais para inquéritos epidemiológicos e monitoramento: uma revisão.**] 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 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.

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INTRODUCTION

The tilapia industry has been expanding increasingly 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 – a rich source of protein of high biological value, and the commercial aspects – 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). 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).

The World Organisation for Animal Health (WOAH) maintains a list of diseases and pathogens of major importance for international trade that includes two pathogens capable of causing serious mass mortality events (MMEs) in tilapia: tilapia lake virus (TiLV) (Eyngor et al. 2014, Aich et al. 2022) and infection with *Megalocytivirus pagrus1* (which represents a group of iridoviruses that includes infectious spleen and kidney necrosis – ISKNV) (Fu et al. 2011), the latter of which was only included in May 2024 (WOAH 2024). WOAH member countries also have their pathogen lists subject to official notification and control, which include various other viruses and bacterioses, which are generally emerging and exotic pathogens in the respective country or region. In Brazil, for example, francisellosis (*Francisella orientalis*) has been a listed pathogen since 2015, although the disease is already widely established in the main tilapia production centers (Brasil 2015).

Therefore, this study aimed 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 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 (2017) 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) to compare over time, space or other factors; or aimed at supporting case detection to respond to individual cases, for example as part of a disease control or eradication program. For diseases currently absent in the population, surveillance can aim to demonstrate the absence of the disease or infection to facilitate safe trade or to confirm successful eradication; or to carry out early detection to enable the 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, Tan et al. 2023, WOAH 2023). The main components of fish disease surveillance systems are shown in Figure 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. These methods can be consulted in the World Organisation 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).

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. In contrast, the passive surveillance method is

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

based on clinical, epidemiological and laboratory investigations of suspect animals, and is the most sensitive component of surveillance systems (WOAH 2019).

On the other hand, syndromic surveillance is characterized by using 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, 2014, Diserens et al. 2017, Brasil 2020, Seagri 2023). The fundamental principle of RBS is targeting 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, 2013). For European Union countries, legislation requires fish farms to be individually classified regarding 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 of 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). However, it 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; (12) surveillance in bigger picture (Bondad-Reantaso et al. 2021).

The surveillance system can make great strides if it were supported by a National or Regional Aquatic Organism Health Strategy (NAOHS or RAOHS) using a Progressive Management Pathway for Aquaculture Biosecurity (PMP/AB) with a focus on risk-based surveillance to optimize resources and review of epidemiological monitoring strategies to underpin decision-making (FAO 2023).

During the implementation of the system, the surveillance methodology for a given group of diseases may involve more than one activity or component to generate 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 mass mortality event (MME). 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, some diseases 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, DU et al. 2019, Machimbirike et al. 2019, Kembou-Ringert et al. 2023). In addition to these, there are also emerging diseases, which are those that appear suddenly, causing MME 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).

The socioeconomic impact caused by TiLV on the global tilapia trade was estimated at USD 7.5 billion per year in 2016 (Bacharach et al. 2016). In the last eight years, the disease has expanded globally, and the economic impact caused by the virus could be much greater. Bacterial diseases are also responsible for very large losses that can exceed USD 6 billion (Maldonado-Miranda et al. 2022).

In this context, we highlight TiLV as the main disease of economic interest, 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 – Bondad-Reantaso 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 (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.

SURVEILLANCE AND SAMPLING PLANS

Tilapia disease surveillance and sampling plans used in Brazil

In Brazil, the "Ministério de Agricultura, Pecuária e Abastecimento" (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 – "Ministério da Pesca e Aquicultura" (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 public health repercussions as an official control disease. MAPA currently monitors three tilapia diseases based on the investigation of suspected cases: TiLV, ISKNV and *Francisella orientalis*. The state agencies are responsible for investigating suspected cases and taking samples 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 programs (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 carried out have been limited to farms in certain regions or reservoirs. Among the state agencies, there is notable difficulty in carrying out surveys to monitor fish and aquatic animal diseases, probably due to limited financial, logistical, laboratory and human resources (professionals specialized in aquatic animals). Main factors that should be considered when defining the sampling plan for fish disease surveys or monitoring are showed in Figure 3.

Epidemiological survey by the Paraná Agricultural Defense 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 (five to seven 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 confidence interval (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 hatchery 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 centers (Adapar 2014).

Epidemiological study at the Morada Nova de Minas production center. Between 2015 and 2016, a group of researchers conducted a longitudinal study on tilapia farms 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 to characterize

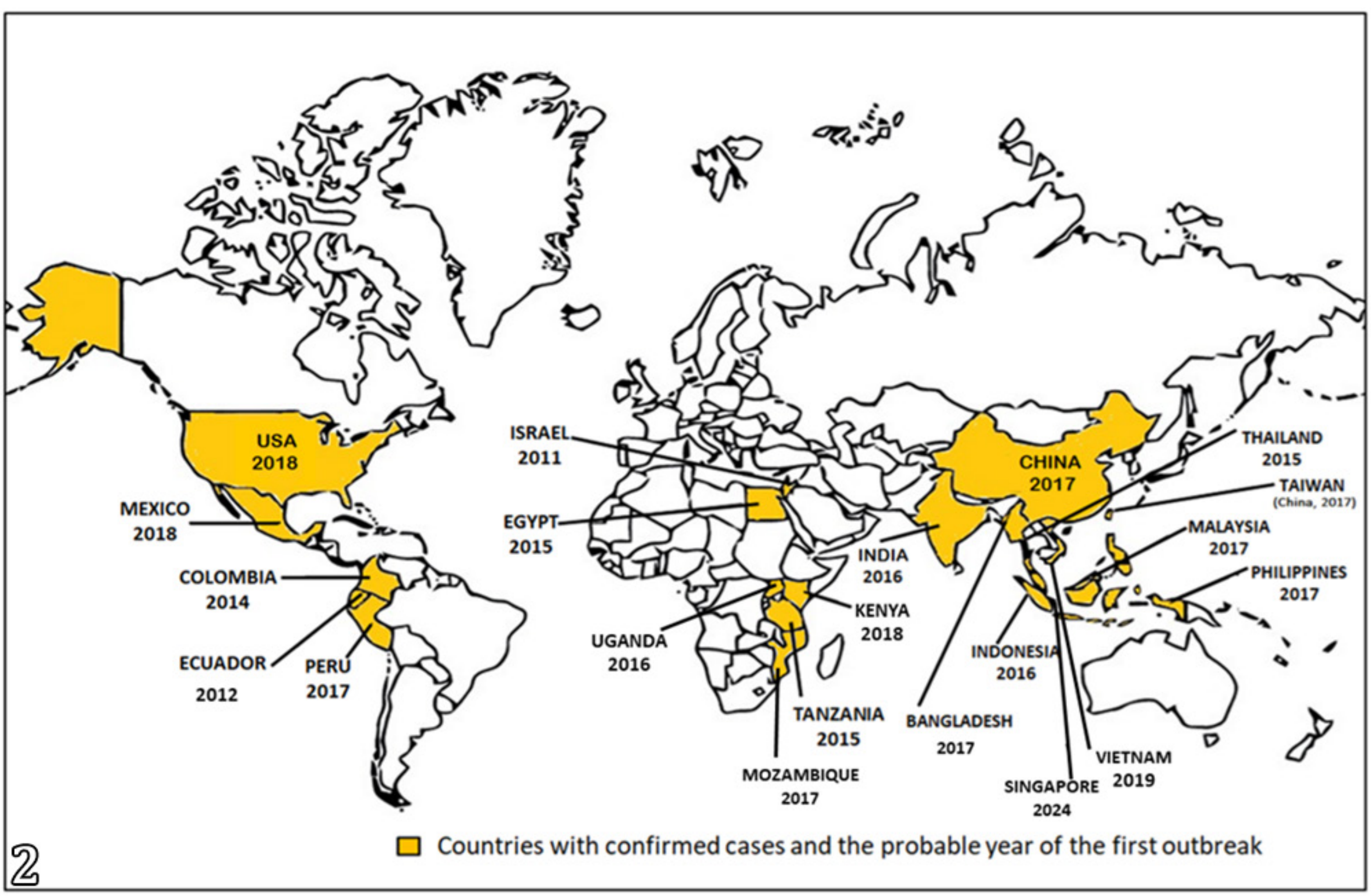

Fig. 2. Global distribution of countries with TiLV outbreaks and the probable year of introduction. Source: Eyngor et al. (2014), Dong et al. (2017), Tsofack et al. (2017), He et al. (2023).

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 pagrus 1*)	Africa, Asia, North and South America	No	High mortality of young forms, immunosuppression, splenic hypoplasia, nonspecific signs (secondary bacterial diseases)	High	Yes	Yes	Yes	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) Rajendran et al. (2023)
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 *Streptococcus 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)
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 *Lactococcus garvieae* and *Lactococcus 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 World Organisation for Animal Health (WOAH), Food and Agriculture Organization (FAO) and health authorities in member countries, [c] The list of notifiable diseases in aquatic animals ("Ministério da Pesca e Aquicultura" – 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.

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 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 were 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. Still, all of them have had a regional scope, such as the epidemiological monitoring of francisellosis conducted in the state of São Paulo on six 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). The epidemiological study carried out on eight 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 officially controlled diseases list (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 "Secretaria de Estado da Agricultura, Abastecimento e Desenvolvimento Rural" (Secretariat of Agriculture, Supply and Rural Development – Seagri), which is the DF's animal health authority, has a voluntary health certification program for biosecure establishments free of TiLV and monitored for ISKNV, *F. orientalis* 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 "Plano Distrital de Vigilância de Doenças e Boas Práticas em Aquicultura" (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 (MME, clinical signs or low performance/grow-out) reported by farms of fattening, pay-to-fish and hatchery tilapia who had previously been sensitized by the local veterinary service

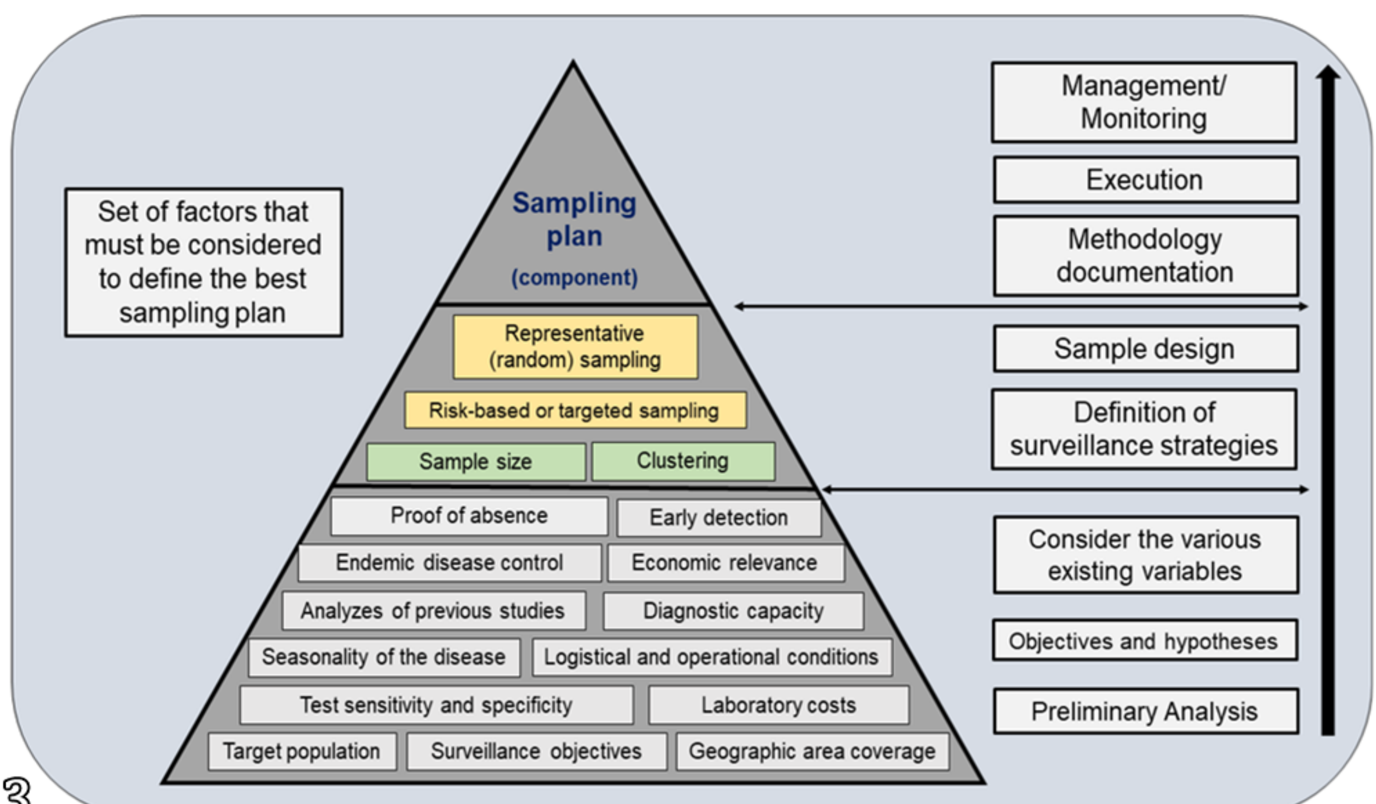

Fig. 3. Overview of the main factors that should be considered when defining the sampling plan for fish disease surveys or monitoring.

(Raposo 2024, Raposo et al. 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, *F. 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 the "Faculdade de Agronomia e Medicina Veterinária" of the "Universidade de Brasília" (FAV/UnB).

The current plan aims to monitor the prevalence of pathogens in Brazil 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 randomly, plus moribund tilapia seen at the time of the visit. To sample the other diseases with a registered presence in Brazil, 30 random individuals were 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 three 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 4 cm 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 "Instituto Colombiano Agropecuario" (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 (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 five alevins over 15 g or 12 pools of 10 alevins under 15 g for Reverse transcription polymerase chain reaction (RT-PCR) testing (Bondad-Reantaso 2021). On one hand, the study served to assess the prevalence of TiLV in the most epidemiologically important stratum, the suppliers of genetic material. On the other hand, 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 conducted an epidemiological study using samples of dying tilapia (5 to 10 fish per farm/lot; sampling completed with asymptomatic fish) in 13 country departments 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 "Autoridad Nacional de Sanidad e Inocuidad en Pesca y Acuicultura" (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 three for broodstock, five for alevins over 15 g, 10 for alevins under 15 g and 100 for try or larvae under 1 g live weight. In 2020, Sanipes launched an official surveillance plan for the period 2020 and 2021 that included surveillance for TiLV, *F. orientalis* 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 five individuals and collections at two periods (May/June and October/November) to evaluate different fish cycles and seasons (Sanipes – Perú 2020). Due to the lack of published results, it is not possible to analyze the effectiveness of the plan. However, 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 "Programa Sanitario Específico de Vigilancia y Control de la Anemia Infecciosa del Salmón" (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 established control of this virus after the health crisis experienced previously.

United States. The US, through the US 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 2019) because its aquaculture is more focused on salmon farming. It has had a Surveillance Program for ISA HPR0 (non-virulent) and HPR-deleted (virulent) since 2002, last revised in 2023 (USDA 2023), consisting of seven components, including laboratory testing (RT-PCR, indirect fluorescent antibody test and viral isolation). Sampling is targeted (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, except for the viral isolation test, where pools of kidney, spleen and heart from up to five individuals are permitted. The number of surveillance inspections with sampling varies monthly, but an average of 10 sites and 100 fish are sampled each month (USDA 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, then 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, which 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: Infectious Salmon Anemia Virus (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 – Canadá 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 six to 15 fish (median = 10); more than the sampling of five fish recommended by the local Aquatic Animal Health Division (AAHD). Assuming that the prevalence of the disease in the five 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 – Canadá 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) conducts surveillance programs. In contrast, 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, focusing on hatcheries (breeding establishments). There is also systematic sampling in pens and establishments in Infectious Salmon Anemia-free zones (Jansen & Oliveira 2022, Moldal & Garseth 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, and nine fish were randomly selected from 10 different ponds. From this, 30 pools are formed containing three fish each, which are tested by RT-PCR (Jansen & Moldal 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, aiming to document the absence and early detection of these pathogens to apply contingency measures. Norway has been considered a 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 & Garseth 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 by the private service (Fish Health Personnel – FHP) in strata with the highest risk of introducing the disease. It 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 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 to optimize the detection of pathogens in a given region. To do this, the sources of information must be fully described and include a detailed description of the sampling used to select test units.

Another point that should be emphasized is the great difference between epidemiological studies and the 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, 2023). Most 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. There is almost always a need to sacrifice aquatic 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 consider 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).

Differences between random and targeted sampling

Based on the sampling plans mentioned in this study, there is a trend towards using 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, 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 five fish with compatible clinical signs is sufficient for the molecular diagnosis of the disease (Tang et al. 2021). Another alternative that has been used to reduce laboratory costs is using 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., five 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 three individuals.

Epidemiological studies based on random sampling require a very high number, and the Aquatic Animal Health Code authors 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 (Delphino et al. 2019, Barato et al. 2022, Seagri 2023, USDA 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. 2014).

In 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. 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 four viral diseases and 10% for two 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 2,000 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 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.

CONCLUSION

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 to reduce 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.

Ethical statement.- No approval of research ethics committees was required to accomplish the goals of this study since no animal experiments were performed.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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Data availability statement.- The authors confirm that the data supporting the findings of the study are available within the article. Derived data supporting the findings of this study are available from the corresponding author upon request.

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