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Article

Validation of a Health Characterization Model for Tilapia Farming in a Brazilian Federative Unit

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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 validate a health characterization model for commercial tilapia production using the production chain in the Distrito Federal (DF), one of Brazil's 27 federative units, by applying a semi-structured questionnaire. A total of 112 farms were categorized according to the degree of vulnerability to the introduction of pathogens and the risk of dissemination using two weighted scorecard 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). 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 in the mean scores between closed-system fattening farms and both semi-closed fattening farms and pay-to-fish farms. Closed-system fattening farms, such as those using biofloc, aquaponics, and recirculation aquaculture systems, showed the lowest vulnerability to pathogen entry and the lowest risk of disease spread. The study's findings provide valuable health information for the official veterinary service of the DF, enabling the categorization of farms, identification of production units, and determination of the most vulnerable strata. Furthermore, the model can be easily applied by private companies and by official veterinary services in other states or countries with significant tilapia production that need to implement risk-based surveillance programs for tilapia farms.

Keywords: tilapia; biosecurity; best aquaculture practices; pathogen introduction; pathogen spread; risk ranking

Key Contribution: The validation of a specific model for tilapia farming in earthen or elevated tanks for different production purposes has provided valuable information for determining the farms and production types with the highest health risks. These data can be used by the global tilapia industry to better manage health risks.

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1. Introduction

Global tilapia production reached 6.5 million tons in 2022, making it one of the most cultivated fish in the world [1]. In Brazil, the tilapia industry has become an important animal protein commodity, representing the leading segment in Brazilian fish farming. In 2023, the production reached 580,000 tons, accounting for 65% of the total fish farming output. It is a diversified production, ranging from small family farms that help supply local markets to large commercial enterprises represented by companies specializing in vertical fish production and agroindustrial cooperatives with great expertise in refrigeration and large-scale production acquired over decades in the production of poultry, pork, and beef [2]. The annual turnover of the tilapia industry in Brazil alone exceeds USD 1.2 billion [2].

The aquaculture sector can be significantly impacted by disease, which is why there are a variety of risk assessment methods that have been published to classify or categorize fish farms, mostly focused on salmonids, to enable the use of risk-based surveillance and health risk management strategies [3–7]. However, there is not much information on risk classifications for tilapia farming in regions where closed and semi-closed systems predominate. Likewise, the health aspects related to tilapia farms in many regions of Brazil, such as the Distrito Federal, are unknown. It is only known that the occurrence of pathogens that require official control by the official veterinary service is low, according to information available in local surveillance reports [8].

Therefore, the aim of this study was to validate a model for categorizing tilapia farms based on the risks of introducing and spreading viral and bacterial pathogens, based on a general assessment of the structure of the farms and a semi-structured questionnaire applied to farmers. The location chosen in which to apply the model was the Distrito Federal (DF), which is one of Brazil's 27 federative units and has tilapia farming as a major segment of its aquaculture production [8]. In addition, the aim was to carry out a health characterization of commercial tilapia farming in the DF in order to identify the production strata with the greatest health risk to help the local veterinary service's surveillance and risk management programs.

2. Materials and Methods

2.1. Study Area Characterization

Brazil has 27 federative units, including 26 states and the Distrito Federal. As the smallest federative unit, the Distrito Federal was chosen to validate the model created to categorize tilapia farms in terms of the risk of introducing and spreading notifiable pathogens. The DF is made up of a single municipality, Brasília, which is among the Brazilian cities with the largest number of tilapia farms [9]. The DF's aquaculture production data are estimated at 2000 tons/year, with tilapia accounting for over 88% (1760 tons/year) of this amount [8]. Although this volume is not very representative within the Brazilian universe, the segment includes approximately 660 tilapia farms, 83% of which are livelihood farms for individual consumption and 17% for commercial purposes [8]. This production chain serves as a source of food and income for hundreds of fish farmers, as well as generating direct and indirect employment for thousands of people [10].

According to published data, tilapia farming in the DF is mainly made up of small and medium-sized fish farms that contribute to supplying the local market, the third largest in Brazil, with an average consumption of approximately 14 kg/inhabitant/year, well above the Brazilian average, which is estimated at 10 kg/inhabitant/year [2,8,10]. 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 [8].

2.2. Target Population

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). These farms were categorized into 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 spatial distribution of farms of each typology is shown in Figure 1. The classification of production systems follows the Brazilian health legislation, MPA Normative Instruction No. 4/2015 [11], which defines closed systems as those where tilapia are raised in structures with total water recirculation, aquariums, bioflocs, and other similar systems that control water flow and animal movement. Semi-closed systems include farms that control fish movement with partial control of water flow, such as earthen ponds (permeable or impermeable), weirs, or continuous flow systems (raceways).

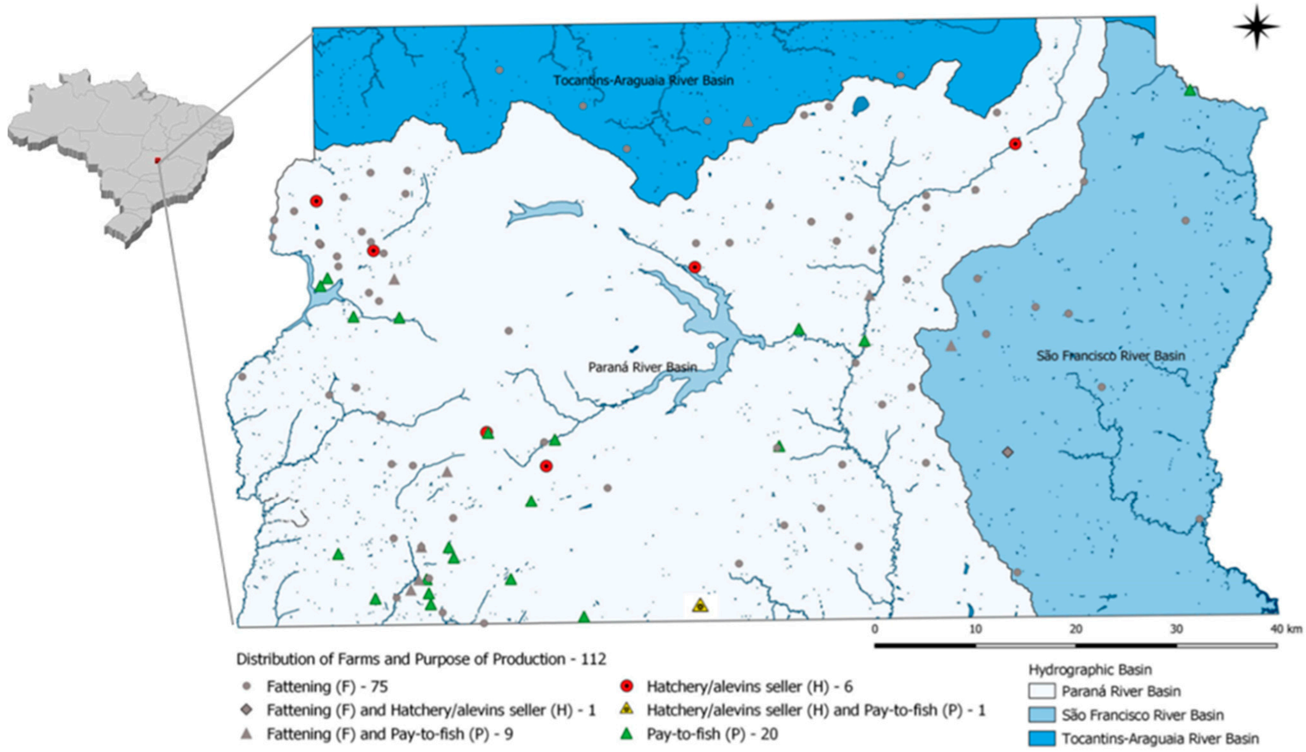


Figure 1. Spatial distribution of commercial tilapia farms in the Distrito Federal, according to the purpose of production and the national river basins in the territory of the DF.

2.3. *Questionnaire*

All 112 tilapia farmers voluntarily agreed to participate in the study by signing digital terms of commitment. Informed consent was obtained from all subjects involved in the study. The study was approved by the University of Brasilia's Animal Use Ethics Committee under number 23106.080975/2021-63.

A semi-structured questionnaire covering production and health aspects was developed and administered to the participating farmers. The questionnaire included closed dichotomous, semi-open, and multiple-choice questions. In order to define the variables and the response structure, three tilapia farmers were randomly selected for a pilot interview, which was conducted with open-ended questions to refine the content and responses. Before the final application, a pre-test was carried out with two randomly selected tilapia farmers to adjust the accuracy of the answers and data tabulation. The questionnaire was administered by three veterinarians and two 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. Complementary and individual information for each farm was taken from the registry/computerized system of the animal health service of the DF Secretariat of Agriculture.

2.4. *Evaluation of Atypical Health Events (AHE)*

To validate the risk classification of fish farms, our research group conducted a parallel epidemiological study to investigate notifiable pathogens, focusing especially on viral diseases that had not been detected in the DF until now. The farms that answered the questionnaire were monitored from July 2021 to June 2022 (12 months) with constant communication via a smartphone messaging app. WhatsApp® (Meta Platforms, Inc., Menlo Park, CA, USA) was used due to its easy use and familiarity among all breeders. When atypical health events (mortality or observation of clinical signs) were reported, immediate visits were made by veterinarians from the official service to assess the health conditions of the production. In addition to water temperature, water samples were measured for dissolved oxygen, pH, toxic ammonia, alkalinity, and turbidity, aiming to rule out mortalities caused by management failures.

2.5. Health 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).

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 [12] and two others for risk classifications/categorizations in commercial fish production [3,4]. The scoring was based on criteria and proportions listed in several previous studies [3–6,13]. 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. Data from the questionnaire and SEAGRI's computerized system were 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.

Each table consisted of 15 criteria, totaling 30 verification items. This structure allowed for the scoring and subsequent classification of the establishments based on their degree of vulnerability to pathogen entry (Table 1) and their risk of disease dissemination (Table 2). The VL classification ranged from well-protected to highly vulnerable to pathogen entry according to the legend in Table 1, while the RD classification ranged from insignificant risk to high risk (Table 2) for the spread of pathogens according to the legend in Table 2. 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.

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

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 of different works [3,4,6,12].

Variables	Criteria	Points	Score
1. Origin of young fish or 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 3 km from the site	0	
	There are quarantine facilities, ports, airports, or event parks adjacent to the fish farm or up to 3 km from the site	3	

Table 1. *Cont.*

Variables	Criteria	Points	Score
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	Quarantines 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 effluent treatment	2	
	River/stream, reservoir, or surface sources with confirmed presence of target disease in the region, without efficient effluent 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	

Table 1. *Cont.*

Variables	Criteria	Points	Score
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 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 of different works [3,4,6,12].

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	

Table 2. *Cont.*

Variables	Criteria	Points	Score
4. Pond cleaning	Empties the water, removes waste from the bottom, applies quicklime or another disinfectant, and makes sanitary fallowing for at least 7 days at the end of each cycle	0	
	Empties the water, removes waste from the bottom, applies quicklime or another disinfectant, and makes sanitary fallowing 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 does not make sanitary fallowing	1	
	Empties the water, removes waste from the bottom, makes sanitary fallowing 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 fallowing	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	Slaughters in establishments with an official inspection service or rearing for own consumption or only selling young forms	0	
	Slaughters in places without official inspection, e.g., fairs, restaurants, fishmongers	1	
	Unknown destinations, fish caught and taken fresh (in the case of pay-to-fish), or fish sold at the farm gate	2	
11. Gutting fish at the edge of streams, rivers, reservoirs, etc.	Not performed	0	
	Performed	3	

Table 2. *Cont.*

Variables	Criteria	Points	Score
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 m^2)	0	
	High densities (above 4 kg per m^2)	1	
15. Type of system and structure	Closed or semi-closed system, impermeable elevated (concrete, 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 $\leq$ 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 $\geq$ 11.0

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

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.

2.6. Statistical Analysis

In this study, we carried out a descriptive analysis of the data from the questionnaire responses and the categorization of BL based on the mean scores from two point tables that assessed VL and RD, respectively. The VL, RD, and BL variables were subjected to the Shapiro–Wilk normality test to check if the data for each group followed a normal distribution. The averages of the categorization scores were compared to identify 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.

The plan was to validate the risk classification model by evaluating the applicability of the questionnaire and the characterization of tilapia farm facilities. After identifying the farms and strata with the highest health risk, the idea was to correlate the farms categorized as highest risk with the results of an epidemiological study conducted at the same time. Due to the low detection of viral pathogens, which was the main target of the study, the validation of the risk classification was carried out solely on the basis of descriptive and observational analyses.

3. Results and Discussion

3.1. Productive and Socioeconomic Characteristics of the Distrito Federal

According to the results of the questionnaire, two-thirds (75; 66.0%) of the commercial tilapia farmers in the DF do not have fish farming as their sole source of income, but as a supplement to the family budget. This socioeconomic profile, which combines aquaculture with other sources of income, is similar across the world, including neighboring countries [14,15].

In 43.8% (49/112) of the farms, the fish farming labor force is family-owned, while 20.5% (23/112) rely on hired labor, and 35.7% (40/112) use a combination of both (mixed labor). These results show that the majority of the businesses are small-scale, characterized by low production and high seasonality of commercialization, with sales peaks during Holy Week. Although the DF is not considered a large tilapia production center, the socio-economic data show some similarities with other regions of large national production. For instance, in the Três Marias reservoir located in the municipality of Morada Nova de Minas, in the state of Minas Gerais, southeastern Brazil, which is considered the fourth-largest tilapia production hub in the country 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-run operations [16]. In the western region of Paraná, the largest tilapia production center in Brazil, a recent study identified various networks of fish farmers, including family livelihood farms, leisure and gastronomy networks, and highly technified farms integrated with large agroindustrial cooperatives. Although most production is concentrated in a few large farms, small producers play a crucial role by providing high-quality animal protein, generating family income, and supporting the local economy [17]. Similar to DF, many farmers in Paraná do not depend exclusively on aquaculture for their income (more than half of the farmers), highlighting common socio-economic patterns in different regions.

As for the production system, 92.0% (103/112) of the commercial tilapia farms cultivate in a semi-closed system, including three farms that use a mixed system (semi-closed and closed). Among the 12 closed-system farms (9 of them exclusive), the existing systems are bioflocs (BFT), aquaponics, and water recirculation systems (RAS). The average annual production of tilapia per farm was 12,001 kg, with a stocking density during the fattening phase of 2.2 Kg/m^2 (standard deviation, SD = 0.14) in semi-closed ponds and 5.54 Kg/m^2 (SD = 6.06) in closed system ponds and an average slaughter weight of 0.821 kg (SD = 0.046).

The water sources vary widely, with the majority being of subterranean origin (58.0%; 65/112), such as springs or artesian wells, followed by streams (28.6%; 32/112), and rivers, lakes, or reservoirs (13.4%; 15/112).

There was minimal vertical integration in the aquaculture sector. Only 11 (9.9%) reported sending their production to slaughterhouses with official inspection, while the majority of producers send their production directly to fairs, restaurants, and the final consumer. This scenario is common in much of Brazil [16,17] and other high-production countries such as Egypt [18]. In the Três Marias reservoir, 66.7% of fish farms sent their production to slaughterhouses without official inspection [16]. In western Paraná, approximately half of the producers sell on the informal market [17].

3.2. Health Characteristics and Best Aquaculture Practices

Almost half of the interviewees (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 six tilapia farms (6.5%) are assisted exclusively by professionals from the private sector. 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 practices 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. With regard to vaccination, only one farm in the DF vaccinates tilapia against streptococcosis and ISKNV—in this case, juveniles that are sold to farms in other states. Studies have shown that tilapia vaccination is economically viable and reduces losses caused by diseases such as Streptococcus agalactiae and ISKNV in semi-open farms [19,20]. In the DF, where farming is mostly carried out in semi-closed/closed systems, and where the health challenge is probably lower, fish vaccination was also recommended by these authors, according to economic viability simulations also carried out for this scenario [19].

Around 40% of commercial farmers do not carry out regular water analyses, demonstrating a lack of knowledge about the importance of controlling the physical and chemical conditions of water and their relationship with well-being, immunity, and production performance. Perhaps this is why in the DF the vast majority of health events reported to the local health authorities are caused by management failures such as dissolved oxygen deficits, pH levels that are too acidic and unsuitable for tilapia, and sudden drops in water temperature, among others [8,21].

The questionnaire encompassed a range of topics related to biosecurity, disease prophylaxis, and best aquaculture practices. The responses, along with their respective percentages, are detailed in Table 4.

For earthen or elevated ponds, one of the most efficient measures for preventing disease and reducing microbial load in subsequent batches and cycles is proper pond cleaning [22,23]. 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 [23]. 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 [24] where cleaning at the end of each cycle is not recommended due to the filtration system and additional costs for maturing the water. 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 fallowing, which would certainly lead to a loss of customers and financial turnover.

Table 4. 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 (%)
Sharing equipment with other farms	0	100.0
Sharing 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 of establishments, including pay-to-fish, 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) use it for irrigation, other animal production units, or loss through infiltration, without it flowing into natural bodies of water.

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.

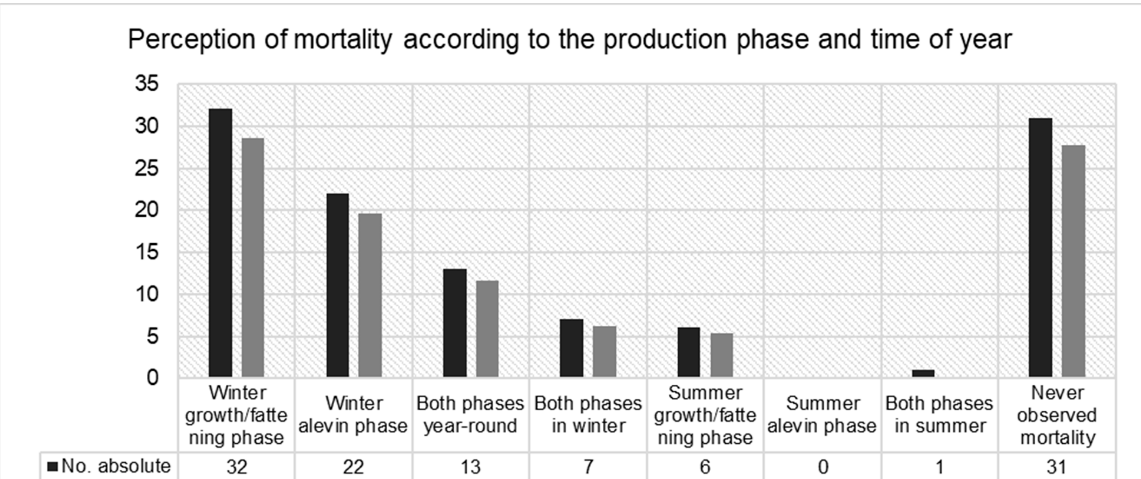
Factors related to the risk of disease spreading between farms were also assessed. Only 36.6% (41/112) of the farms treated their effluent, with the most used methods being a settling tank (73.2%; 30/41) and a sand and gravel filter (36.6%; 15/41). On the other hand, if we add up the production units 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 [25]. These data suggest a reduced capacity for pathogen dispersal, corroborating the results of disease investigations carried out by the local official veterinary service over the past 5 years [8,26].

Dead fish are major sources of infection and multiplication of pathogens within a production system [26], 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 those found in the Morada Nova de Minas production center [16]. The other methods cited include collection by urban cleaning companies (5.3%; 6/112) and incineration (5.3%; 6/112). Among the unsanitary methods, 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 past 10 years.

Atypical mortality events were seen more intensely in the fall and winter periods (Figure 2), similar to the results seen in semi-enclosed systems in southern Brazil [25]. In tilapia production in pond cages, losses were higher in the spring and summer [16]. 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 [25] 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 [16]. 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 [27,28] and *Lactococcus* sp. [29], which are widespread in all large natural bodies of water and become more pathogenic at temperatures above 28 °C [30]. The DF is located in the Central Plateau region of Brazil, at an average altitude of 1172 m [31], factors which reflect directly on the water temperature, which remains low during the winter and rarely reaches values above 28 °C in the summer. During visits to investigate atypical health events, water measurements in the fall and winter months, usually taken between 9 a.m. and 12 p.m., had a 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.



	Winter growth/fattening phase	Winter alevin phase	Both phases year-round	Both phases in winter	Summer growth/fattening phase	Summer alevin phase	Both phases in summer	Never observed mortality
No. absolute	32	22	13	7	6	0	1	31
%	28.6	19.6	11.6	6.2	5.3	0	0.01	27.7

Figure 2. Perception of mortality of the tilapia farmers participating in the study presented by absolute number and percentage.

3.4. Health Categorization of Farms

The BL of commercial tilapia establishments was categorized based on the mean of the VL and RD variables. Tables 5–7 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. Figure 3 compares the score data for the production strata in Boxplot form.

Table 5. 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 Farms	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		

Table 5. *Cont.*

Tilapia Farms	Production Purpose	Production System	Score			Classification			AHE	Season	
			VL	RD	BL	VL	RD	BL		Cold	Hot
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	A/W	
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	

Table 5. *Cont.*

Tilapia Farms	Production Purpose	Production System	Score			Classification			AHE	Season	
			VL	RD	BL	VL	RD	BL		Cold	Hot
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**		

Table 6. Values of the mean, standard deviation, and variance of the variables vulnerability level (VL), risk of dissemination (RD), and 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	$\bar{x}$ (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%)

$\bar{x}$ = mean; SD = standard deviation; VL = vulnerability level; RD = risk of dissemination; BL = biosecurity level.

Table 7. Comparison between the strata using the Bonferroni test with the *p*-value of variance for the variables vulnerability level (VL), risk of dissemination (RD), and biosecurity level (BL), where $p < 0.05$ (*) indicates a 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; P = pay-to-fish.

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 perform fattening), the BL found was 10.21 (SD = 3.02), the highest score, with a big difference compared to the other typologies.

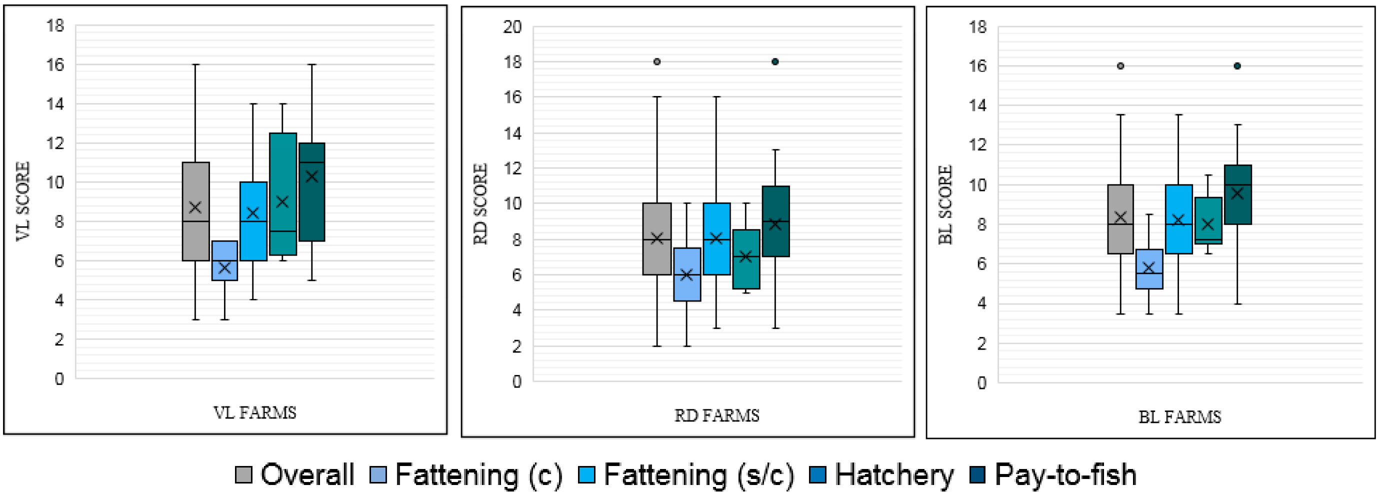


Figure 3. Graphical representation of the boxplots of the mean VL, RD, and BL scores of the farms in the 4 strata, separated by color according to the image legend.

Applying the ANOVA analysis of variance (pVL = 0.001, pRD = 0.0764, and pBL = 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 7. 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 nine 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 producer/reseller typologies could be inferred to be biologically significant, since our observations found an evident difference in the sanitary infrastructure and best practices adopted between the farms.

Caption:

- AHE = atypical health event
- Tilapia farms and production purpose

  F = fattening
  H = hatchery or alevin 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

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 laboratory investigations of cytopathology, histopathology, microbiology, and molecular tests carried out on these harvests were the subject of an epidemiological study conducted by our research group and will be published shortly. All the AHE were investigated for the main tilapia viral pathogens, such as Tilapia Lake Virus (TiLV), Infectious Spleen and Kidney Necrosis Virus (ISKNV), Tilapia parvovirus (TiPV), and Nervous Necrosis Virus (NNV), but only one farm tested positive for ISKNV. This was the first detection of a viral pathogen in the Distrito Federal.

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, parasitic, and fungal pathogens. The laboratory investigations of cytopathology, histopathology, microbiology, and molecular tests carried out on these samples were the subject of an epidemiological study conducted by our research group, which will be published later. 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 s (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 the first few months of their lives.

In our categorization, the eight young fish 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. First is 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 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.

3.6. Overall Assessment of the Farm Categorization Model

In our assessment, the model proved to be applicable and was able to characterize commercial tilapia farming in the DF in terms of health aspects. Based on the categorization of establishments and the history of disease detection, it was assessed that, although the sanitary infrastructure 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; this is probably due to the high frequency of fish transit with different regions of Brazil (interstate transit), increasing the possibility of introducing endemic diseases in other regions of the country, and, in the case of fattening, longer exposure to the environment [5,13,32–34]. Knowledge of the health profile of the production strata and tilapia farms most vulnerable to the introduction or spread of diseases of economic importance has enabled the local veterinary service to create a strategic risk-based surveillance plan for notifiable diseases in the tilapia industry [8].

The risk assessment criteria used in our model were designed to categorize tilapia farms and were defined based on the epidemiological characteristics of tilapia viral diseases (TiLV, ISKNV, TiPV, and NNV). The model has some similarities to the one adopted by authors who classified salmonid farms in Switzerland in terms of the risks of introduction and dissemination for viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN) [4,6], 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 these authors' model was more rigorous, 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 [4], 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 audit reports/Department of Animal Health of the Ministry of Agriculture, Livestock and Food Supply of Brazil, unpublished data).

Although the initial intention was to complete the validation of the model using field data, it was not possible to establish correlations between the categorized farms and the results of research into notifiable pathogens such as TiLV, ISKNV, TiPV, and NNV, since the parallel epidemiological study conducted by the same group of researchers did not find enough positive farms to carry out reliable calculations of the correlation between the risk classification of the farms and the occurrence of outbreaks of notifiable diseases. Of the 27 AHE observed and investigated with molecular assays, a single case of viral disease was detected (ISKNV). Diserens et al. (2017) presented the same limitations in their validation study carried out in Switzerland. The authors mentioned the occurrence of only five cases of farms positive for VHS or IHN in the ten years prior to the study period, impeding the progress of statistical validation at the field level.

Despite this important limitation, we evaluated the relationship between AHE and the risk classification established by the model to observe trends. The highest occurrence of AHE occurred on farms classified as D in terms of BL (33.3%; 6/18). Among the 53 establishments classified as C, 15.1% (8/53) had AHE in the months evaluated, and of these 8, 37.5% were classified as high vulnerability in terms of VL. And among the 39 establishments classified as B, atypical health events were present in 25.6% (10/39), and of these 10, 40% had a moderate VL score. These results show that the data are consistent, with a higher number of AHE being observed on farms with a lower BL or VL score.

Finally, it is possible to establish a relationship between the classification of the only farm positive for ISKNV and the entry and occurrence of the disease. This farm was BL = C, with a moderate vulnerability (MV) and a low risk (LR) of dissemination. It is a fattening fish farmer who buys tilapia fingerlings from an uncertified young fish farm, a supplier in another state, and who systematically cleans the ponds, limes them, and sanitary fallows them before new stocking of fish. In other words, the categorization identified that the producer had a considerable risk of introducing a pathogen (and in this case, we highlight interstate movement as a probable risk factor) and at the same time a good capacity to prevent the spread of the virus to other farms or its maintenance. It is important to note that, even before learning of the virus diagnosis, the farm finished the cycle of the batch of tilapia that showed mortality, and carried out the cleaning, disinfection, and fallowing procedures.

For all the above reasons, we presume that the proposed model is sufficiently valid to adequately characterize a federative unit, state, municipality, region, or country. Even using a large number of variables, no difficulties were observed in managing data from tilapia farms. The model, which was specifically designed to list the health risks of tilapia production in closed and semi-closed systems, made it possible to determine the farms and production strata at greatest risk. The model has been replicated at the DF's animal health agency in its risk-based surveillance program for TiLV and other notifiable tilapia diseases.

4. Conclusions

The model for categorizing tilapia farms in terms of vulnerability to the introduction of pathogens and the risk of dissemination of diseases proved to be a useful, applicable, and sufficiently valid tool for characterizing the health of a given target population. The application of this model classified 82% of tilapia farms in the Distrito Federal as "good" or "moderate" risk (B or C), results that are consistent and in line with the history of the low frequency of notifiable diseases in the DF. 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 assessment, it was found that pay-to-fish farms have the worst health indicators, although there is a need to relativize the risks due to the type of activity (S/C-fattening farms keep susceptible fish for much longer and young fish farms have much more intense ingress/egress of fish from different regions).

The method adopted also provided valuable information for the DF's veterinary service, which can subsequently draw up a surveillance plan focused on farms and production strata with the highest health risk. In addition, we believe that this model can be used for risk management both by private companies that integrate different tilapia farms and by veterinary services in other states and countries with more representative tilapia production.

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Informed Consent Statement: All 112 tilapia farmers voluntarily agreed to participate in the study by signing a digital term of commitment. Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data will be made available upon a suitable request from the corresponding author.

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