Journal of Food Safety

WILEY

Journal of Food Safety

ORIGINAL ARTICLE OPEN ACCESS

Monitoring of *Salmonella* spp. in Native Fish Farms in the Pantanal and Cerrado Biomes, Brazil: Serotype Diversity, Antimicrobial Resistance Profiles, and Multivatiate Patterns

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Received: 1 August 2025 | **Revised:** 22 October 2025 | **Accepted:** 27 October 2025

Keywords: antimicrobial sensitivity | aquaculture | food safety | multiple correspondence analysis | *Salmonella* Newport | *Salmonella* Saintpaul

ABSTRACT

Mato Grosso State, Brazil, is the main producing region of native farmed round fish. This study aimed to investigate the occurrence of *Salmonella* spp. in fish farms located in the Pantanal and Cerrado biomes, across eight municipalities, focusing on the hybrid species tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*); to analyze possible multicausal associations contributing to contamination during the fish farming phase; to characterize the circulating serotypes; and to assess the antimicrobial resistance profiles. A total of 184 samples were tested for *Salmonella* spp. following protocols from the International Organization for Standardization (ISO) and confirmed by polymerase chain reaction (PCR). *Salmonella* spp. was detected in 88% (22/25) of the fish farms and in 31.5% (58/184) of the samples. Contamination was confirmed in fish farms from all eight municipalities investigated, reinforcing the widespread circulation of the pathogen. Among the positive samples, 60.3% were from fish, with viscera showing the highest detection rate, while sediment/soil, pond water, and animal feces were the most frequent environmental sources. Serotyping revealed 10 distinct serotypes, with *S*. Saintpaul and *S*. Newport predominant, alongside *S*. Reading, *S*. Abaetetuba, and other less common serotypes of epidemiological relevance. The associations between environmental and management factors contributed to 57% of the explained variance in *Salmonella* spp. occurrence, and contamination was significantly higher during the dry season. Resistance was most frequent against azithromycin (44%) and sulfonamides (38%), although no multidrug-resistant strains were identified. The high occurrence of *Salmonella* spp. during the fish farming phase demonstrates that associated factors contribute to contamination and the persistence of strains with resistance profiles from the early production stages. These findings highlight weaknesses in on-farm biosecurity that must be addressed, while joint actions with good manufacturing practices in processing plants are also needed to mitigate risks. The circulation of diverse and epidemiologically relevant serotypes emphasizes the need for integrated surveillance under a One Health perspective in Brazilian aquaculture.

1 | Introduction

Salmonella spp. is recognized as one of the main bacterial pathogens transmitted through water and food contaminated by fecal contact, causing public health damages and productive and socioeconomic sector loss; therefore it is kept under surveillance throughout the world. (Porto et al. 2023; Wang et al. 2022; Ferrari et al. 2019). Cases of human infection by *Salmonella enterica* serotypes are generally associated with failures in hygienic-sanitary control of the water used, as well as other microbiological safety failures during the handling or treatment of food throughout the production chain. (Surya et al. 2022; Fernandes et al. 2021; Pastro et al. 2019).

Salmonella spp. is widely distributed throughout the environment, and its natural habitat is the intestinal tract of several animal species (birds, reptiles, amphibians, and mammals) (La Tela et al. 2021; Dróżdż et al. 2021). In aquatic biomes, *Salmonella* spp. is often associated with contamination by domestic, agricultural, and industrial effluents, promoting contamination risk in the fish production chain, extractive fishing, aquaculture, or both (Chahouri et al. 2022; Saingam et al. 2020; Klase et al. 2019; Fernandes et al. 2018).

Salmonella spp. occurrence in fish and fish products has been reported in several countries, with farmed freshwater fish one of the most investigated (Porto et al. 2023). Minimally processed samples (Padovani et al. 2022; Surya et al. 2022; Gawish et al. 2021), frozen samples (Don et al. 2020; Cunha-Neto et al. 2019), processing samples in slaughterhouses (Surya et al. 2022; Fernandes et al. 2021), or samples from fish farming (Custodio et al. 2023; Mota and Majolo 2021; dos Santos et al. 2019), show that fish products are subject to *Salmonella* spp. contamination risk at different sites throughout the production chain.

Mato Grosso State is currently one of the largest producing regions of native farmed fish in Brazil, with 40,500 tons. Brazilian production of native fish includes the species tambaqui (*Colossoma macropomum*), pirapitinga (*Piaractus brachypomus*) and its hybrid tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) (PEIXE BR 2024; Silva and e Barros 2020). Remarkable zootechnical attributes such as the ease of obtaining juveniles, good growth potential, high rusticity and great acceptance by the market consumer are among the main advantages for native fish farming with tambatinga, which has been gaining strength with the reduction of wild tambaqui and superior quality when farmed in captivity (Silva et al. 2022; da Costa et al. 2021; de Moraes et al. 2017).

The semi-intensive system in ponds and excavated dams is the main model of fish farming production in Brazil, representing around 80% of the activity (Valenti et al. 2021). Therefore, research on the potential for captive native fish to act as accidental carriers of *Salmonella* spp. strains, as well as the identification of contamination sites associated with the possible source of the detected serotypes are relevant data to be obtained to inform knowledge and decision measures to mitigate the problem (dos Santos et al. 2019). At the same time, there is little or no conclusive information in the scientific literature, as existing studies are limited and very specific. Therefore, the generation of scientific data in this regard will contribute to the characterization of *Salmonella* spp. contamination concerns and will allow the restructuring of the native fish production chain, through strategic interventions and technical and technological production improvements.

The presence of *Salmonella* spp. in native fish in Brazil has been reported in commercialized samples. As they are widely sold in minimally processed form, contamination of carcasses may result from multiple sources and handling steps from capture to the final product (Padovani et al. 2022; de Almeida and Morales 2021; Moura et al. 2021; Pastro et al. 2019; Cunha-Neto et al. 2019; Viana et al. 2016). In contrast, fewer studies have addressed the microbiological conditions of native fish during the farming phase and within production sites (Mota and Majolo 2021; dos Santos et al. 2019; de Mello et al. 2010), which justifies the present research approach. In this context, this study investigated the occurrence of *Salmonella* spp. in native fish farms located in the Pantanal and Cerrado regions of Mato Grosso, Brazil; characterized the diversity of serotypes and antimicrobial resistance profiles of the isolates; and evaluated the multifactorial factors associated with contamination during the farming phase.

2 | Material and Methods

2.1 | Sampling

2.1.1 | Study Area and Fish Farms

Sampling was conducted in 25 fish farms located across eight municipalities in the Cerrado and Pantanal regions of Mato Grosso State, Central-West Brazil (Figure 1), during both the rainy season (November–April) and the dry season (May–October) between 2021 and 2022 (Alvares et al. 2013). Farmers were invited to voluntarily participate in the study. All farms shared the common characteristic of employing a semi-intensive farming system in excavated ponds.

The study area encompassed two distinct biomes with contrasting climatic conditions. The Cerrado biome, represented by the municipalities of Chapada dos Guimarães, Cuiabá, Jangada, Rosário Oeste, and Várzea Grande, is characterized by a tropical savanna climate with marked seasonality, where rainfall is concentrated between November and April. The Pantanal biome (similar to a wetland biome), represented by Nossa Senhora do Livramento, Poconé, and Santo Antônio do Leverger, consists of lowland floodplain areas with seasonal inundation and high water retention in ponds during the wet season. For analytical purposes, fish farms were classified into these two groups (Cerrado and Pantanal) according to the predominant biome of each municipality, following the same grouping adopted in the Statistical Analysis section.

To characterize rainfall during the study period, pluviometric data from the National Institute of Meteorology (INMET 2021; Instituto Nacional de Meteorologia (INMET) 2021) were used. The Cuiabá station (code A901) was selected as representative of the municipalities of Cuiabá, Várzea Grande, Nossa Senhora do Livramento, Poconé, and Santo Antônio do Leverger, while the

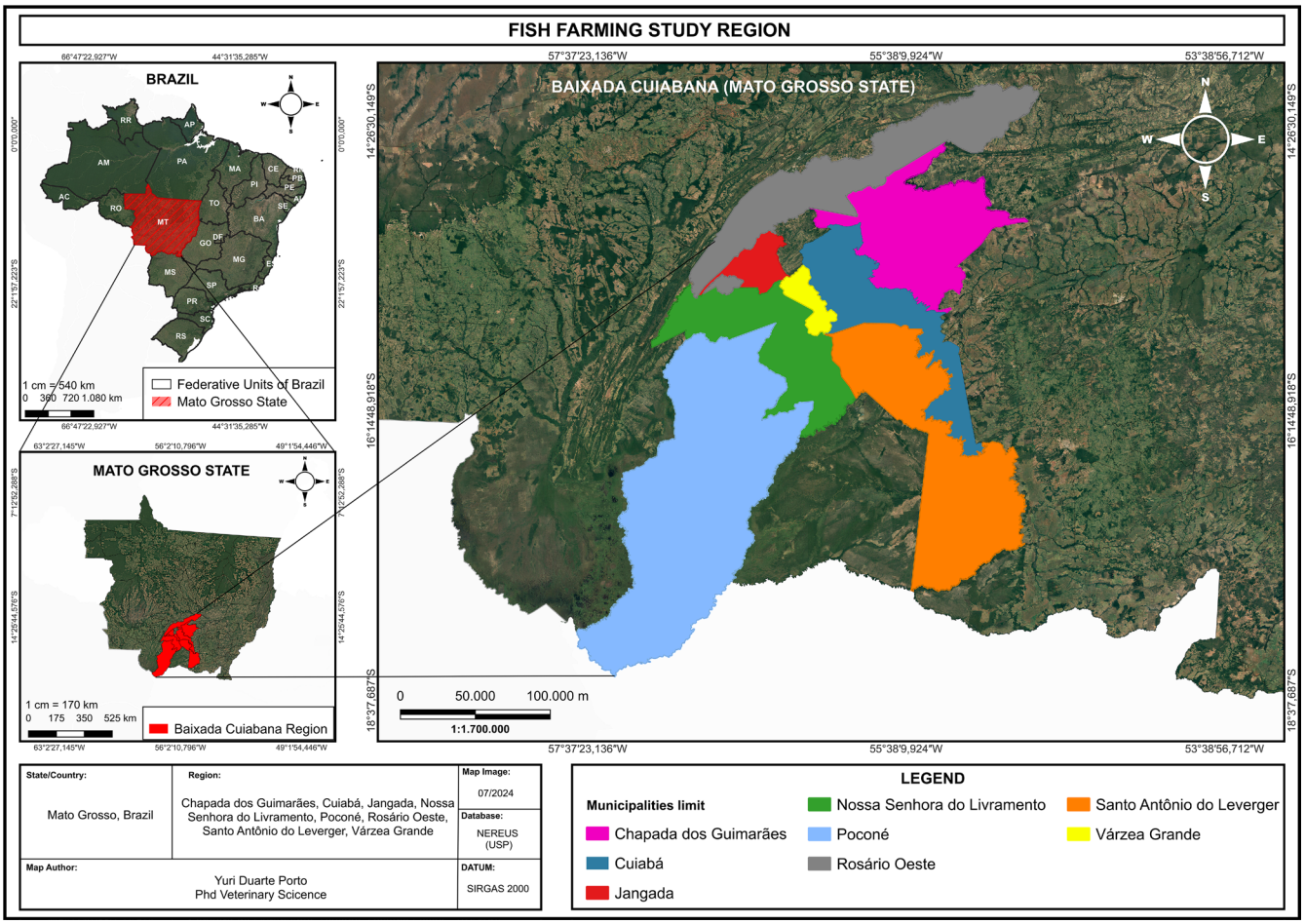

FIGURE 1 | Geographic map of the municipalities in Mato Grosso State, Brazil, where 25 fish farms were sampled from November 2021 to June 2022. The study encompassed municipalities located in two biomes: Cerrado (Chapada dos Guimarães, f21–f23; Cuiabá, f8, f25; Jangada, f9; Rosário Oeste, f19; Várzea Grande, f2, f3, f12, f15, f16) and Pantanal (Nossa Senhora do Livramento, f4–f6, f10, f11, f13, f14, f17; Poconé, f18; Santo Antônio do Leverger, f1, f7, f20, f24). The figure illustrates the spatial distribution of the sampled fish farms across both biomes. Source adapted from Instituto Brasileiro de Geografia e Estatística (IBGE) 2017.

Campo Verde station (code A912) was adopted as representative of Chapada dos Guimarães, due to its proximity and similar elevation within the highland Cerrado region. The Rosário Oeste station (code A944) was included to represent the municipalities of Rosário Oeste and Jangada. Rainfall totals, calculated as the mean of the 2021–2022 records from these representative INMET stations, ranged from 642 to 869 mm during the rainy season (November–April) and from 71 to 188 mm during the dry season (May–Oct), encompassing the variability observed across the eight municipalities sampled and reflecting the climatic conditions that influence fish farming in these regions.

2.1.2 | Experimental Design

In fish farming, one pond was randomly selected for collection. Specimens of tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) were collected per fish farming. A total of 72 fish samples with an average weight of 1.3 (±0.7) kg were sampled and analyzed. Also, samples of fish pond water (25) and fish pond sediments/soil from the bottom and slopes of ponds (25) were collected; as well as samples of fish feed (25) used on each fish farm (Porto et al. 2023).

In the laboratory, the fish were prepared by analytical units of anatomical portion (Porto et al. 2023), represented by 25 sample units from external washing of the fish (scales), 25 gill units, 25 units of costal muscle (without scales and without skin) also 25 units of viscera (esophagus, stomach, liver, intestine, and feces), Then, a set of individual analytical units per fish farm was analyzed by microbiology method.

Fecal pooled samples (pets, production animals—chicken, goat, sheep, cattle and horse, and free-living wild animals—birds and mammals) were collected from nine fish farms (f17, f18, f19, f20, f21, f22, f23, f24, and f25). Those were found near the breeding ponds, which included feces of capybara (*Hydrochoerus hydrochaeris*) and Brazilian tapir (*Tapirus terrestris spegazzinii*), endemic to the region of Mato Grosso, Brazil.

2.1.3 | Sample Collection

The fish were captured with bait and hook and placed individually in a sterile transparent polyethylene bag (5000 mL) and placed in an isothermal box (50 L) with ice blocks. All collected samples were packaged in the same way. A sterile glass bottle

was used to collect 1000 mL of water from the breeding pond at a random point, while individually in other sterile transparent polyethylene bags (720 mL) samples of sediment/soil from the bottom and slopes of the breeding ponds were placed (collected from five random points), fish feed samples, and fecal samples from pools of animals outside fish production.

2.1.4 | Epidemiological Inquiry

When collecting fish farm samples, a checklist was applied that gathered information from the breeding environment and production management to identify possible sources of contamination by *Salmonella* spp., with the aim of assisting in the diagnosis and supporting the analysis of possible multicausal associations of contamination according to the Data Analysis section. The checklist used was adapted from the Aquaculture Establishment Registration Form (Annex I—National Program for the Health of Farmed Aquatic Animals—"Aquaculture with Health"—Ministry of Fisheries and Aquaculture—IN, 2015) (Ministério da Agricultura e Pecuária 2015) and covered four main topics: (I) water source; (II) production system—pond sites, renewal water management and treatment (affluent/effluent); (III) fish farming—aquatic species, breeding cycles/production phases, practice of other animal production; (IV) biosecurity—observe the presence of terrestrial animals (domestic and wild) in the breeding areas or if there were protective barriers in the nurseries, traffic control (people and vehicles), handling equipment (standardized), handlers (uniforms/equipment personal protection—PPE), feed (commercial or artisan manufacturing, inspected, storage, and rodent control).

2.2 | Analysis Units

Each bagged fish was weighed and peptone saline (0.1%, w/v, Oxoid, Basingstoke, UK) in a ratio of 1:4 (mL/g; diluent/g sample) was added, followed by manual homogenization for 5 min (International Organization for Standardization (ISO) 2015). The resulting rinse composed the analytical unit of the external surface of the fish (scales) to be carried out according to the Microbiological Analysis section. Then, from each fish, the other analytical units were sequentially extracted: gills, unilateral costal muscles, and viscera (esophagus, stomach, liver, intestine, and feces), with the aid of scalpel blades, sterile tweezers, and a stainless-steel tray.

2.3 | Microbiological Analysis of *Salmonella* spp.

The analysis followed the protocol based on ISO 6579-1 (International Organization for Standardization (ISO) 2017). Then, 25 g (25 mL) of each sample unit was inoculated in Buffered Peptone Water (BPW) and incubated for 24 h at 37°C in a BOD oven. An aliquot (0.1 mL) was transferred to 10 mL tubes with selective Rappaport-Vassiliadis broth (RVS) and 1 mL to tubes with 9 mL Tetrathionate broth (TT) and incubated for 24 h at 42°C ± 5°C and 37°C. In the next step, they were sown on Xylose Lysine Deoxycholate (XLD) agar, incubated at 37°C for 24 h, and on Brilliant Green agar (BGA), incubated for 24 h at 41.5°C for inoculum extracted from RVS broth, and incubated at 37°C for inoculum extracted from TT broth. After growth, up to five typical colonies on XLD (translucent with a red halo with a black center or not) and on BGA (translucent with a red halo) were selected and isolated on Nutrient agar, incubated at 37°C for 24 h. Subsequently, they were subjected to biochemical tests on Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA). Colonies with presumptive positive results for *Salmonella* spp. were conducted for subsequent analysis by conventional PCR to confirm the microbiological culture result.

2.4 | DNA Extraction

The extraction of chromosomal DNA from the isolates was carried out by thermal lysis. Briefly, 1 mL of overnight culture was centrifuged (15,000 rpm, 5 min), the pellet was resuspended in ultrapure water, heated at 100°C for 10 min, cooled at −20°C for 10 min, and the supernatant containing DNA was stored at −20°C. The procedure followed the thermal lysis protocol described by De Medici et al. (2003), with minor adaptations.

2.5 | Polymerase Chain Reaction (PCR)

The *hilA* gene was used, which amplifies the 497 bp product, specific for *Salmonella* spp. (Guo et al. 2000). The reaction was carried out in a final volume of 25 μL, containing ultrapure water (15.05 μL), 1× reaction buffer (2.5 μL), 3.5 mM $MgCl_2$ (1.75 μL), 0.2 mM dNTPs (Fermentas) (0.5 μL), 1 μM of forward primer (CTGCCGCAGTGTTAAGGATA) and reverse primer (CTGTCGCCTTAATCGCATGT) (Fermentas) (1 μL each), 1 U of Taq DNA polymerase Platinum (Fermentas) (0.2 μL) and approximately 40 ng/μL of bacterial DNA (3 μL). The reaction conditions were: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min in the Veriti thermocycler (Applied Biosystems). PCR products were analyzed on 1.5% agarose gel electrophoresis.

2.6 | Antimicrobial Assay

Fifty-two *Salmonella* spp. strains isolated from fish and pond site samples were randomly selected for analysis by disk diffusion technique, for in vitro antimicrobial susceptibility analysis according to the Clinical and Laboratory Standards Institute (CLSI 2021). Previously, each strain was inoculated into Müller-Hinton broth and incubated at 37°C for 16 to 18 h. Turbidity was adjusted to the McFarland scale of 0.5 (approximately 108 CFU/mL) by swarming on a Müller-Hinton agar plate. Then, the discs containing antimicrobials were distributed equidistantly and then incubated at 35°C for 16–20 h (Bauer et al. 1966). The 11 disk-diffusion antibiotics used were: Penicillin class—ampicillin (10 μg), Macrolides class—azithromycin (15 μg), Cephem class—cefepime (30 μg), cefoxitin (30 μg), and ceftiofur (30 μg), Fluoroquinolone class—ciprofloxacin (5 μg), Phenicol class—chloramphenicol (30 μg), Folate Pathway Inhibitor class—trimethoprim and sulfamethoxazole (25 μg), and sulfonamides (300 μg), Carbapenems class—imipenem (10 μg), and Nitrofuran class—nitrofurantoin (300 μg). Inhibition zones were measured and classified as sensitive (S), intermediate (I) or

resistant (R) strains according to the Enterobacteriaceae section (CLSI 2021). Strains with resistance to antibiotics from three or more chemical classes were classified as Multidrug Resistant (MDR) (Magiorakos et al. 2012).

2.7 | Serotyping of *Salmonella* spp.

A subset of isolates was submitted for serotyping to a reference laboratory (Imunova Análises Biológicas, Curitiba, Brazil). The procedure followed method MB004 V003, in accordance with ISO 6579-3:2014 "Microbiology of the food chain—Part 3: Guidelines for serotyping of *Salmonella* spp." and the Kauffmann–White–Le Minor scheme (ISO 2014, Grimont and Weill 2007, WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur). Isolates received in stock agar were streaked onto Brilliant Green Agar (selective) to verify purity and onto Nutrient Agar (non-selective) for serotyping. Confirmation was first performed using polyvalent O antisera, followed by somatic (O) antigen testing (groups O:2, O:4, O:7, O:8, O:9, O:9; 46, O:3; 10, O:1; 3; 19, O:11, O:15, O:19, O:21, O:23, O:46). When agglutination was observed, phase inversion and flagellar (H) antigen identification were performed to complete antigenic characterization. Antigenic identification was carried out according to the Kauffmann–White–Le Minor scheme. Some isolates could not be recovered or remained pending due to non-agglutination or cross-reactions, and these were classified as non-typeable (N/T) or undetermined for reporting purposes.

2.8 | Data Analysis

The data generated were tabulated and evaluated through exploratory analyzes aimed at describing the presence or absence of *Salmonella* spp. Prevalence was estimated as the number of positive samples out of the total samples analyzed. The variables used in the study to investigate possible associations with the *Salmonella* spp. occurrence were categorized (see Table S1). Non-parametric tests Chi-Square, Mann-Whitney test, and Spearman Correlation test were applied (Conover 1999). For all statistical tests, a significance level of $\alpha = 0.05$ was adopted.

Multivariate exploratory analysis was performed using Multiple Correspondence Analysis (MCA) to investigate associations (i.e., co-occurrence patterns relative to independence) between *Salmonella* spp. occurrence ("salmonella"), and categorical variables including season of sampling ("season"), regional biome in which the fish farm is located ("biome"), production system and water renewal management ("system"), and sample matrix collected ("sample") (see Table S1) (Alves et al. 2020; Kluger 2018; Greenacre et al. 2014; Greenacre 1988). MCA was conducted on the complete disjunctive (indicator) matrix with χ^2 distances; category proximity in the map indicates association (higher-than-expected co-occurrence), whereas separation indicates weaker association relative to independence.

All data analysis was performed using the statistical package R version 4.1.3 (R Core Team 2022). The libraries used were "tableone" (Yoshida and Bartel 2022), to perform non-parametric tests; "ggpubr" (Kassambara 2023), to generate graphics; "FactoMiner" (Le et al. 2008) and "factoextra" (Kassambara and Mundt 2020) for multivariate analysis.

3 | Results

3.1 | Bacterial Isolation and Distribution

It was possible to confirm the identification of 110 *Salmonella* spp. strains isolated. Table 1 shows that the *Salmonella* spp. distribution per fish farm was heterogeneous, ranging from a single strain (fish farms f3, f5 and f6) to 11 strains (fish farm f10).

Salmonella spp. strains were isolated from fish in 62.7% (69/110), predominantly from viscera (esophagus, stomach, liver, and intestine, with feces or not) representing 26.4% (29/69) of the strains. *Salmonella* spp. strains were isolated from pond sites in 17.3% (19/41) and sediment/soil samples had the highest number of isolates (Table 1).

3.2 | *Salmonella* spp. Occurrence

Salmonella spp. occurrence was 88% (22/25) of fish farms, being detected in 31.5% (58/184) samples. The contaminated samples with *Salmonella* spp. presented heterogeneous distribution, varying from one to six samples contaminated per fish farm (Table 1). Just three fish farms (12%; 3/25) did not present *Salmonella* spp. strains in their samples.

The diagnosis of *Salmonella* spp. indicated its presence in the fish farms sampled across the eight towns of this investigation. *Salmonella* spp. was detected in 10 fish farms in towns within the Pantanal region and in 12 fish farms situated in the Cerrado region (Figure 1).

3.2.1 | Sample Sites

Salmonella spp. was detected in 60.3% (35/58) of fish samples and 39.7% (23/58) of farm environment samples (Table 1). The highest *Salmonella* spp. occurrence frequency was in fish samples following viscera 31.4% (11/35), gills 28.6% (10/35), muscle 25.7% (9/35), and in the external washing of the fish 14.3% (5/35). In samples from the fish farming pond sites (39.7%; 23/58) there was a higher contamination frequency following: fish pond sediments/soil (10/23), fish pond water (9/23) and fecal samples from other animals external to fish farming (4/23).

Salmonella spp. was detected in both fish and the farming environment samples in 13 fish farms located in seven municipalities (except Cuiabá). A fish farm located in Cuiabá municipality had *Salmonella* spp. detected just in environmental samples (feces). In fish farms f21 and f23 (Cerrado) and f10 (Pantanal) *Salmonella* spp. were isolated in all fish analysis units (surface, gills, muscles, and viscera). In fish farm f20 (Cerrado) *Salmonella* spp. was isolated in all environmental samples (fish pond water and fish pond sediment/soil and other animals' feces), and in fish farm f24 (Pantanal) it was isolated from fish pond water and fish sediment/soil, and no detection in animal fecal samples.

TABLE 1 | Frequency and distribution of *Salmonella* spp. isolates identified by the *hilA* gene in the fish farms sampled.

		Municipalities (n = fish farm)							
		Chapada dos guimarães	**Cuiabá**	**Jangada**	**Nossa Sra. do livramento**	**Poconé**	**Rosário oeste**	**Sto. Antônio do leverger**	**Várzea grande**
		(n = 3)	**(n = 2)**	**(n = 1)**	**(n = 8)**	**(n = 1)**	**(n = 1)**	**(n = 4)**	**(n = 5)**
Sample site	**Total *Salmonella* spp. isolated (%)**	***Salmonella* spp. isolated (fish farm)**							
Fish samples	69 (62.7)								
Scale	13 (11.8)	3 (f21); 1 (f23)	0	0	3 (f10)	0	0	2 (f20); 4 (f24)	0
Gills	15 (13.6)	2 (f21); 2 (f23)	0	1 (f9)	1 (f10)	3 (f18)	1 (f19)	2 (f1); 1 (f24)	1 (f2); 1 (f16)
Muscle	12 (10.9)	3 (f21); 1 (f22); 1 (f23)	0	0	2 (f10); 1 (f11); 1 (f17)	0	0	1 (f20)	1 (f3); 1 (f12)
Viscera	29 (26.4)	2 (f21); 2 (f22); 2 (f23)	0	0	3 (f10); 2 (f11); 1 (f13); 2 (f14); 2 (f17)	4 (f18)	6 (f19)	0	3 (f2)
Environmental samples	41 (37.3)								
Water	16 (14.5)	1 (f22); 3 (f23)	0	2 (f9)	1 (f5); 2 (f11)	2 (f18)	0	2 (f20); 1 (f24)	2 (f12)
Sediment/soil	19 (17.3)	2 (f22); 1 (f23)	0	0	2 (f6); 2 (f10); 1 (f14)	0	3 (f19)	1 (f20); 2 (f24)	3 (f15); 2 (f16)
Feces*	6 (5.5)	1 (f22)	2 (f25)	—	2 (f17)	1 (f18)	0	0	—
Another sample									
Fish feed	0	0	0	0	0	0	0	0	0
None**	—	—	f8	—	f4	—	—	f7	—
(n) Total (%)	110 (100.0)	27 (24.6)	2 (1.8)	3 (2.7)	28 (25.5)	10 (9.1)	10 (9.1)	16 (14.5)	14 (12.7)

Note: *Feces: nine pooled feces' samples were collected in nine fish farms: f17, f18, f19, f20, f21, f22, f23, f24, and f25. **None: no samples had *Salmonella* spp. detected.

The feed samples analyzed showed the absence of *Salmonella* spp. in all fish farms.

3.3 | Epidemiological Survey

Information on fish farming sites and management was collected. The data were tabulated in a checklist with four main themes: (I) water source; (II) production system; (III) fish farming; and (IV) biosecurity (Table 2).

The fish farms are far from each other and have their own peculiarities. However, it was possible to identify similar general characteristics of the environment and fish farming management, with no notable distinction between fish farms located in the Pantanal region and those located in the Cerrado region. (Figure 1 and Table 2). In general, there was no established standard for the dimensions of any fish pond (height × width × depth) within the same fish farm, with local topography and rainwater reservoirs possibly being the determining factors. Therefore, it was possible to observe complete heterogeneity in the depth of the water used in the ponds of all the fish farms visited.

All the fish ponds on the farms were of the type dug into the ground and without covers. Cultivation water was obtained almost exclusively through rainwater impoundment, with a few exceptions (f8, f15, f21, f22, f23), when the water source was also obtained in part from a nearby river spring (Table 2). No specific management of fish farming was observed to renew water in the ponds, except for maintaining the volume of the pond when there was water support from the river. There was also no prior sanitary treatment of the water from the ponds or production effluents.

As for fish farming, there was a predominance of juveniles acquired from other producers for fattening and finishing for sale, in polyculture with other native fish species of commercial importance or with species accidentally introduced into breeding grounds, without production purposes. Two fish farms (f2 and f19) produced from farming to the finishing phase. It was observed that all fish farms carried out some other animal production activity (chicken, pigs, goats, sheep, and cattle) or vegetable production (vegetables, corn, and tubers) concomitantly with fish farming (Table 2).

All the fish ponds have essentially rustic characteristics, exposed to environmental and climatic factors, and it was possible to notice weaknesses in biosecurity management. Therefore, it was observed that pets, production animals and free-ranging animals (birds and other terrestrial wild animals) had free access to the fish pond. No sanitary controls were observed when moving vehicles or people during production. It was observed that there was no standardization of utensils for exclusive use in the management of the fish pond and a lack of standardized uniforms/equipment for personal protection (PPE) for handlers. The lack of such requirements became more evident during fish feeding management (manual or mechanized support), and mainly during the fishing phase, carried out completely manually in all fish farms visited.

All fish farms used commercial feed (inspected and within the expiration date) purchased in bags (50 kg). No storage feed was observed in silo structures. Some fish farms had warehouse-like structures for storing stacked feed bags, often in direct contact with the warehouse floor, and the presence of pets was also observed. Other fish farms have adapted storage in PVC drums housed in covered structures to protect them from daily climate action. No temperature and humidity control were observed in the storage of feed. No practices for integrated pest control in fish farms were observed (Table 2).

3.4 | Statistical Analysis

A significant difference ($p < 0.01$) was found in *Salmonella* spp. detection between samples collected in the rainy season (November–April) and the dry season (May–October) (Table 3). The prevalence of *Salmonella* spp. contamination observed during the dry season was double that observed in the rainy season. There were no significant differences in *Salmonell*a spp. detection between fish farming regions (Pantanal and Cerrado), water management in fish ponds (water resource only from rain with renewal only during the rainy season or management with minimum water renewal ponds with rainwater and support from a nearby river), sample types (fish or fish farm environment), unit of analysis (scales, gills, muscles, viscera, fish pond water and fish pond sediment/soil), fish weight sampled and number of fish collected for pool analysis (Table 3).

A significant statistical difference ($p < 0.01$) was found in the number of *Salmonella* strains isolated from samples collected in different seasons, with a higher number of isolates obtained during the dry season (Table 4). No significant differences were observed for the other variables analyzed, indicating that the observed variations were not statistically meaningful.

There was no correlation between the quantity of *Salmonella* strains isolated and the weight of the fish sampled ($p = 0.711$), or with the quantity of fish collected in ponds ($p = 0.803$) for microbiological analysis.

Using Multiple Correspondence Analysis (MCA), a two-dimensional graph was generated with the axes obtained from the data matrix (Figure 2). The first dimension (axis 1) explained 36.3% of the variance, and dimension two (axis 2) explained 20.7% of the variance, totaling 57% of the explained variance. Therefore, the total percentage obtained represents how much the variability of the studied data set can be understood. The five variables were considered active, observing their contributions to the formation of the axes, presented in Table S2. To interpret the axes, it was possible to observe associations for all variables. In this general framework, it was possible to define dimension 1 (axis 1) as the dimension of intrinsic and extrinsic factors of fish farming management and environment, as the variables "season," "system," and "biome" demonstrated the contribution of associations with *Salmonella* spp. contamination. In dimension 2, called the result relationship dimension, the "environment" and "fish" modalities contribute with associations to maintain the *Salmonella* spp. contamination cycle in fish pond sites (see Table S2, and additional graphical outputs are available in Figure S1).

TABLE 2 | Main productive characteristics of the 25 fish farms (checklist) by municipalities and identification of *Salmonella* spp. positive samples.

Fish farming productive characteristics (Main checklist topics)	Municipalities/(*n*) fish farm							
	Chapada dos guimarães	Cuiabá	Jangada	Nossa Sra. do livramento	Poconé	Rosário Oeste	Sto. Antônio do leverger	Várzea grande
	(*n* = 3)	(*n* = 2)	(*n* = 1)	(*n* = 8)	(*n* = 1)	(*n* = 1)	(*n* = 4)	(*n* = 5)
Water source								
Only by water rain	—	f25	f9	f4; f5; f6; f10; f11; f13; f14; f17	f18	f19	f1; f7; f20; f24	f2; f3; f12; f16
Rain/river	f21; f22; f23	f8	—	—	—	—	—	f15
Fish farming								
Rearing/fattening	f21; f22; f23	f8; f25	f9	f4; f5; f6; f10; f11; f13; f14; f17	f18	f19	f1; f7; f20; f24	f3; f12; f15; f16
Polyculture	f21	f8	f9	f6; f10; f11; f13	f18	f19	f20; f24	f2; f3
Another animal or vegetal production	f21; f22; f23	f8; f25	f9	f4; f5; f6; f10; f11; f13; f14; f17	f18	f19	f1; f7; f20; f24	f2; f3; f12; f15; f16
Production system								
Water renewal (only rainy season)	—	f25	f9	f4; f5; f6; f10; f11; f13; f14; f17	f18	f19	f1; f7; f20; f24	f2; f3; f12; f16
Minimal water renewal	f21; f22; f23	f8	—	—	—	—	—	f15
Treatment (affluent/effluent)	—	—	—	—	—	—	—	—
Biosecurity								
Barriers (sampled fish pond)	none	none	none	none	none	none	none	none
Traffic control	none	none	none	none	none	none	none	none
Handlers (uniform/PPE)	none	none	none	none	none	none	none	none
Standardized equipment	none	none	none	none	none	none	none	none
Rodent catching trap	none	none	none	none	none	none	none	none
Salmonella spp.								
Fish samples	f21; f22; f23	—	f9	f10; f11; f13; f14; f17	f18	f19	f1; f20; f24	f2; f3; f12; f16
Fish pond samples	f22; f23	f25	f9	f5; f6; f10; f11; f14; f17	f18	f19	f20; f24	f12; f15; f16
NSD*	—	f8	—	f4	—	—	f7	—

*NSD-no *Salmonella* spp. detected.

TABLE 3 | Chi-square test results of the variables studied in relation to the presence or absence of *Salmonella* spp.

Variable		Categories	Outcome					
			Salmonella negative $n = 96$		*Salmonella* positive $n = 54$		*Salmonella* prevalence (%)	*p*
Season	(%)	Dry	33	(34.4)	33	(61.1)	50.0	< 0.01*
		Rainy	63	(65.6)	21	(38.9)	25.0	
Biome	(%)	Pantanal	43	(44.8)	23	(42.6)	34.8	0.929
		Cerrado	53	(55.2)	31	(57.4)	36.9	
System	(%)	Rainwater	81	(84.4)	39	(72.2)	32.5	0.116
		Rainwater/river	15	(15.6)	15	(27.8)	50.0	
Sample	(%)	Fish	65	(67.7)	35	(64.8)	35.0	0.857
		Environment	31	(32.3)	19	(35.2)	38.0	
Unit of analysis	(%)	Scale	20	(20.8)	5	(9.3)	20.0	0.576
		Gills	15	(15.6)	10	(18.5)	40.0	
		Muscle	16	(16.7)	9	(16.7)	36.0	
		Viscera	14	(14.6)	11	(20.4)	44.0	
		Fish pond water	16	(16.7)	9	(17.7)	36.0	
		Fish pond sediment/soil	15	(15.6)	10	(18.5)	40.0	
(*n*) Fish	(%)	1 specimen	13	(20.0)	3	(8.6)	18.7	0.230
		≥ 2 specimens	52	(80.0)	32	(91.4)	38.1	
(Weight) fish	(%)	< 1.3 Kg	29	(44.6)	15	(42.9)	34.1	1.000
		≥ 1.3 Kg	36	(55.4)	20	(57.1)	35.7	

*Significant at level $\alpha = 0.05$.

Figure 2 presents the graph of the variables in the plane delimited by axes 1 and 2 of the MCA, with the modalities (black triangle) and the negative result points (red point) and positive result points (blue point), distributed in the four quadrants of the two dimensions presented in Figure S1. The proximity between the categories and their location within the same quadrant indicates possible associations between the parameters of the studied topic.

In general, through the ellipses representing the outcome in the cloud chart (Figure 2), some level of overlap between the categories was observed; therefore, it did not indicate an exclusively heterogeneous grouping of predominant factors that led to the detection of *Salmonella* spp., However, it was possible to observe cases of positive and negative detection with similar characteristics. Therefore, a highlight can be observed in quadrants 1 and 4, where the categories "dry," "environment," "pantanal," and "rainwater" were closely contained in the same quadrants with the predominant occurrence of blue points in the cloud (Figure 2). This result demonstrated that the joint detection of *Salmonella* spp. occurred more frequently in environmental samples collected during the dry season (between May and October). Then, the graph showed an association between these categories and the detection of *Salmonella* spp., with this prevalence being a statistically significant difference (Table 3). Therefore, in the dry period, the lower rainfall associated with management with low water renewal possibly increases the risk of *Salmonella* spp. occurring in fish farming environments (fish pond water and fish pond sediment/soil), and favors fish contamination, mainly via the oral-fecal route, in addition to the adaptive capacity on the surfaces and gills of the fish that were observed. Furthermore, during this same period of the year, wild animals look for other sources of water to drink, accessing fish ponds, as there are no effective barriers to prevent the movement of these animals into fish ponds, favoring an increase in the risk of contamination through their feces.

In quadrant 2, it was possible to observe that there was co-occurrence in the sampling of fish farms in the Pantanal region ("pantanal") and ponds without water renewal ("rainwater") in both positive and negative detection for *Salmonella* spp. (Figure 2). This co-occurrence translates, therefore, into sampling fish farms located in the Pantanal biome that have ponds predominantly supplied by accumulated rainwater and without renewal management, and in the same way it was observed in quadrant 4 for sampling fish farms in the region of Cerrado and nurseries supplied with some water renewal ("rainwater/river") (Figure 2). Although the graph demonstrates proximity between these categories with positive cases of *Salmonella* spp. detection (co-occurrence), no statistically significant difference was observed (Table 3).

TABLE 4 | Mann-Whitney test results of the variables studied in relation to the number of *Salmonella* spp. strains isolated.

Variable	Category	Positive categories (*n*, %)	*Salmonella* strains (*n*, %)	*p*
Season	Dry	33 (61.1)	68 (65.4)	< 0.01*
	Rainy	21 (38.9)	36 (34.6)	
Biome	Pantanal	23 (42.6)	43 (41.3)	0.806
	Cerrado	31 (57.4)	61 (58.7)	
System	Rainwater	39 (72.2)	75 (72.1)	0.064
	Rainwater/river	15 (27.8)	29 (27.9)	
Sample	Fish	35 (64.8)	69 (66.3)	0.713
	Environment	19 (35.2)	35 (33.7)	
Unit of analysis	Scale	5 (9.3)	13 (12.5)	0.540
	Gills	10 (18.5)	15 (14.4)	
	Muscle	9 (16.7)	12 (11.5)	
	Viscera	11 (20.4)	29 (27.9)	
	Fish pond water	9 (16.7)	16 (15.4)	
	Fish pond sediment/soil	10 (18.5)	19 (18.3)	
(*n*) fish	1 specimen	3 (8.6)	5 (7.2)	0.126
	≥ 2 specimens	32 (91.4)	64 (92.8)	
(weight) fish	< 1.3 kg	15 (42.9)	29 (42.0)	0.964
	≥ 1.3 kg	20 (57.1)	40 (58.0)	

*Significant at level $\alpha = 0.05$.

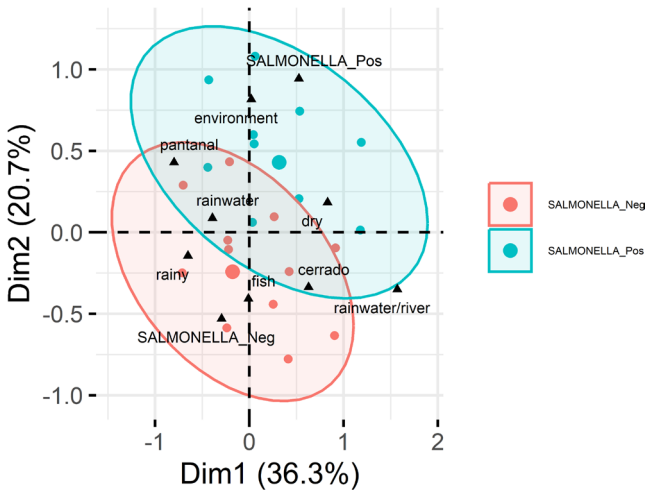

FIGURE 2 | Multiple Correspondence Analysis (MCA) two-dimensional graph. The Dimension 1 (intrinsic and extrinsic factors of fish farming management and environment) explained 36.3% of the variance, and the Dimension 2 (result relationship dimension) explained 20.7% of the variance. Modalities cloud of the variables "season," "biome," "system," "sample," and "salmonella", for detection of *Salmonella* spp. in 25 fish farms in the Brazilian Pantanal and Cerrado region, Mato Grosso, Brazil, November 2021–June 2022—Axes 1 and 2 with the addition of ellipses for the active variable molecular microbiological diagnosis.

In quadrant 3, the categories "fish" and "rainy" were observed to be most associated with the occurrence of a negative diagnosis of *Salmonella* spp. (Figure 2). The fish and the fish farming environment (fish pond water, and fish pond sediment/soil) are closely linked. In some fish farms (59%; 13/22), the detection of *Salmonella* spp. occurred simultaneously in samples from fish farming environments and fish, but no statistically significant difference was observed in the detection of *Salmonella* spp. between the samples analyzed (Table 3).

3.5 | Antimicrobial Profile

Salmonella spp. strains (52) were evaluated for susceptibility to 11 antimicrobials. There were no *Salmonella* spp. strains classified as multidrug resistant (MDR). The most frequently resistant antimicrobials (R) were Azithromycin (44.2%, 23/52), Sulfonamides (38.5%, 20/52), Nitrofurantoin (5.8%, 3/52), Trimethoprim and Sulfamethoxazole (3.8%, 2/52), and Ampicillin (1.9%, 1/52).

3.6 | Serotyping of *Salmonella* spp.

Serotyping of isolates recovered from fish and aquaculture environments revealed a diversity of circulating serotypes in the Pantanal and Cerrado biomes. Seven distinct serotypes were

confirmed, with *Salmonella* Saintpaul ($n = 10$), *S*. Newport ($n = 7$), and *S*. Abaetetuba ($n = 4$) being the most frequent, followed by *S*. Reading ($n = 3$). Less common serotypes included *S*. Eastbourne, *S*. Kaapstad, and *S*. Atakpame (one isolate each). In addition, some isolates could not be typed (non-typeable, N/T) or showed cross-reactions that did not allow unequivocal distinction between closely related serotypes (e.g., *S*. Fillmore/Tshiongwe and *S*. Chester/Sandiego). The distribution of serotypes encompassed different isolation matrices (muscle, viscera, gills, scales, pond water, sediment/soil, and animal feces), indicating multiple sources of contamination within fishponds. The predominant serotypes were detected in both biomes, reflecting the multifactorial and persistent nature of *Salmonella* spp. contamination in native fish farming (Table 5).

4 | Discussion

The results of this research point to the presence of *Salmonella* spp. in a natural biome (Brazilian Pantanal and Cerrado) in which native farmed fish sites are in production systems in excavated ponds. It was observed that throughout the fish farming phase, the fish are exposed to a cycle of *Salmonella* spp. contamination within the ponds by dispersion through the pond water, contact with the soil/sediment accumulated at the bottom and slopes of the excavated fish ponds, and by the direct and indirect influence of other animals (pets, birds, wild and farm animals). These animals freely come into contact with fish ponds, and through their feces, they contribute as a source of contamination and increase the risks of introducing and maintaining the circulation of this pathogen in the fish farming environment in general. Furthermore, animal feces commonly found around fish ponds may be leached into the ponds by climatic action (rain) or mechanical action (handlers/utensils/machinery). When fish ponds are contaminated, therefore, the main form of infection of fish by *Salmonella* is fecal-oral transmission, forming a cycle between positive and negative individuals, with different sources of contamination identified in several studies, including water, animals, pets, wild animals and farm animals (Dróżdż et al. 2021; La Tela et al. 2021; dos Santos et al. 2019; Li et al. 2017; Gauthier 2015). In general, we found that fish can be contaminated with *Salmonella* spp. both at the beginning of the farming period and in the most terminal phase for slaughter, as both juveniles and adults sampled of different weights were positive in detection, without a statistically significant result.

The check list provided information on the locations of fish farming and management and challenges in correcting and mitigating *Salmonella* spp. contamination of native fish farms. It was observed that production with limited water availability (rainwater accumulation, essentially) is an adaptation of the production system installed through large dams and dams with a high volume of water; therefore, it does not allow constant water exchange, in both sampled regions. However, it could not be confirmed that management without water renewal is an isolated "sanitary failure", as several pathogens (bacterial, parasitic, yeast) can be introduced into ponds through previously contaminated fish or through the water supply, for example. In this system of extensive fish farms, there was no treatment of the water that supplies the ponds or the effluents, which would facilitate the introduction of other pathogens. However, low water renewal management could be considered an aggravating factor for *Salmonella* contamination, due to the fact that there is no way to dry the fish pond and carry out asepsis between farming cycles, associated with the lack of control over the other animal access to the fish pond.

Multiple associated factors may explain the high percentage of occurrence of contaminated fish farms observed in this study (88%), with a frequency of 31.5% of the total samples collected (environmental and fish), with 59% (13/22) of farms having both positive samples. This characterizes poor sanitary control in native fish farming, showing weaknesses in farming management that need to be corrected, as it is a pathogen of public health importance capable of remaining circulating during the fish farming cycle. The management of smaller ponds would possibly facilitate drying between farming cycles, applying animal control in fish farming sites, standardizing uniforms and PPE (Personal Protective Equipment) and equipment for exclusive use of fish farming by handlers during management and farming fish; these are measures to improve biosafety and mitigate *Salmonella* spp. occurrence in low water renewal farming fish systems.

TABLE 5 | Serotypes of *Salmonella* identified in native fish farms from the Pantanal and Cerrado biomes, Brazil.

Serotype	Isolates (*n*)	Fish farms (ID)	Matrices	Biome (s)
S. Saintpaul	10	f12, f15, f16, f22, f23	G, M, V, W, S, F	Cerrado
S. Newport	7	f10, f17, f21, f23	Sc, G, M, V, F	Pantanal, Cerrado
S. Abaetetuba	4	f22, f24, f25	W, S, F	Pantanal, Cerrado
S. Reading	3	f9, f10, f24	Sc, G, S	Pantanal, Cerrado
S. Eastbourne	1	f3	M	Cerrado
S. Kaapstad	1	f2	V	Cerrado
S. Atakpame	1	f18	V	Pantanal
Total	27	—	—	—

Note: Serotyping was performed at a reference laboratory (Imunova Análises Biológicas, Curitiba, Brazil) following ISO 6579-3:2014 and the Kauffmann–White–Le Minor scheme (ISO 2014; Grimont and Weill 2007). IDs refer to fish farms included in the study. Additional isolates ($n = 6$) were classified as non-typeable (N/T) due to the absence of agglutination with the available O antisera. Two isolates presented cross-reactions that did not allow unequivocal distinction between closely related serotypes (*S*. Fillmore/Tshiongwe and *S*. Chester/Sandiego).
Abbreviations: F, animal feces; G, gills; M, muscle; S, sediment/soil; Sc, scales; V, viscera; W, pond water.

Other research has reported positive *Salmonella* spp. diagnosis in native fish sampled from fish farms, with different approaches such as fish farm number sampled, the sampling carried out on each farming site, the collection method (by surface swabs) and microbiological isolation (direct in selective broth for *Salmonella* spp.). Mota and Majolo (2021) reported 6.67% (3/45) *Salmonella* spp. presence in *Colossoma macropomum* (tambaqui) feces in a single excavated fish pond of the three fish farms that were monitored. dos Santos et al. (2019) identified *Salmonella* spp. presence in 6.9% (12/173) of *Colossoma macropomum* (tambaqui) fecal samples in three fish farms out of four visited from excavated ponds, with a low percentage of water renewal in management. de Mello et al. (2010) identified a positive sample (8.33%) in a pool of liver samples from *Brycon microlepis* (piraputanga) in a fish farm sampled monthly during rainy and dry periods.

Salmonella spp. has been widely reported with a weak association with the fish intestinal tract as a natural habitat, and there is barely any information about salmonellosis affecting fish. However, in a production scenario where the natural farming sites allow contamination of fish ponds, it can be considered that farmed fish end up becoming accidental hosts. In this sense, the findings obtained in the present investigation confirmed that viable cells of *Salmonella* spp. can be harbored mainly in the gastrointestinal tract of farmed tambatinga. During the removal of samples from the viscera (esophagus, stomach, liver, and intestine), no macroscopic anatomical changes were observed that would characterize lesions suspected of disease. dos Santos et al. (2019) detected *S. Heidelberg*, *S.* Hadar and *S. panama* from *Colossoma macropomum* (tambaqui) feces and other farmed native fish. The same authors identified *S.* Hadar serotype as capable of transiently colonizing the tambaqui intestine and being eliminated in feces for at least 40 days. Furthermore, they carried out histopathological examinations on various organs of the fish and found no lesions caused by *Salmonella* (dos Santos et al. 2019).

In the present study, the most frequent serotypes identified were *S.* Saintpaul and *S.* Newport, both recovered from fish (muscle, viscera, gills, scales) and environmental matrices (pond water, sediment, feces) in farms from the Pantanal and Cerrado. The simultaneous detection of these serotypes in fish tissues and in the farming environment reinforces the fecal–oral cycle of contamination already discussed in this study and highlights the persistence of epidemiologically relevant serotypes throughout the production system. Considering that both *S.* Saintpaul and *S.* Newport have been implicated in major foodborne outbreaks worldwide (Panzenhagen et al. 2025), their circulation in native fish farming sites represents a concrete overlap between environmental contamination and potential public health risks.

Other serotypes, such as *S.* Reading, *S.* Abaetetuba, *S.* Eastbourne, *S.* Kaapstad, and *S.* Atakpame, although less frequent, illustrate the epidemiological diversity of *Salmonella* strains in native fish farms. Of note, *S.* Abaetetuba was detected in pond water and animal feces, reinforcing the role of environmental reservoirs and wildlife access as key drivers of contamination in fishponds. Similarly, *S.* Reading was recovered from gills and sediment, indicating that this site may serve as a temporary niche for colonization and survival. These observations are consistent with reports that aquatic environments can act as reservoirs for multiple *Salmonella* serotypes, sustaining their persistence and recirculation in fish farming ecosystems (Kim et al. 2025).

Taken together, the identification of multiple serotypes in the Pantanal and Cerrado biomes expands the understanding of the sanitary risks associated with *Salmonella* spp. dissemination in native aquaculture. These findings align with earlier reports of diverse serotypes in Brazilian fish farming, such as *S. Heidelberg*, *S.* Hadar, *S. panama*, and *S. typhimurium* (dos Santos et al. 2019; Fernandes et al. 2021; Cunha-Neto et al. 2019). More broadly, reviews on *Salmonella* in aquaculture highlight that the diversity of circulating serotypes reflects multiple contamination routes and underscores the importance of integrated biosecurity approaches (Arthur et al. 2009). The confirmation of both epidemiologically relevant and less common serotypes in this study reinforces the need for interventions in fish farm management, addressing not only water renewal practices but also the control of animal access and the prevention of cross-contamination from the environment to fish and vice versa.

The results also indicate that the skin, scales, gills, and muscles are sites of colonization and installation of *Salmonella* spp. in tambatinga. Gills have already been reported as a source of *S. panama* in *Brycon orbignyanus* (piracanjuba) and tambaqui (*Colossoma macropomum*) (dos Santos et al. 2019).

Salmonella spp. isolation sites are most frequently reported in the gastrointestinal tract (Porto et al. 2023), therefore, in native fish processing plants, the evisceration step is at greater risk, capable of generating cross-contamination during handling (Fernandes et al. 2021). Corroborating this fact, *S.* Abony, and *S.* Schwarzengrund have already been detected in 5.76% of samples of frozen native fish products in Mato Grosso (Cunha-Neto et al. 2019), as well as *S.* Ndolo, *S.* Mbandaka, *S. typhimurium*, *S.* Rough and *S.* O:16 in the processing of tambatinga in slaughterhouses (Fernandes et al. 2021). Both studies confirm failures in the efficiency of the sanitary control system in the slaughterhouse to eliminate a dangerous pathogen. However, there was no information on the primary *Salmonella* source detected in the samples. In the present study, the results support this information, pointing out that fish coming from fish farms may be contaminated not only in the viscera, but also on the surface, gills, and muscle of the carcasses, increasing the risk of cross-contamination during handling.

The findings of the present study point to a primary source of *Salmonella* spp. contamination on farms during fish management, as *Salmonella* spp. have been identified in environmental sites (fish pond sediment/soil, water, and animal feces). The weaknesses in sanitary control in the farming system are commonly observed on the farms visited, where the main source of *Salmonella* infection is fecal-oral transmission between positive and negative individuals (dos Santos et al. 2019). However, other sources such as water, wild animals and insects may be associated with transmission (Chahouri et al. 2022; Meletiadis et al. 2022). Wild birds observed in fish farms can introduce and spread pathogenic bacteria around fish farms, through water or other direct contamination of the environment (soil leaching). Sanitary controls were also not observed in traffic (vehicles and

people), being considered another crucial risk of *Salmonella* contamination in several farm animals (Bakhshandeh et al. 2022).

It was possible to identify *Salmonella* spp. in pooled animal feces samples in 44% (4/9) of the farms analyzed for this sample. This highlights a potential source of cross-contamination in breeding ponds, since pets, wild animals and farm animals may carry *Salmonella* spp. Among these, the free access of wild birds and capybaras (*Hydrochoerus hydrochaeris*) stood out, considered common reservoirs of *Salmonella* spp. (dos Santos et al. 2019; Farikoski et al. 2019). It was not possible to collect feces from other species of wild animals such as alligators, snakes and frogs accidentally caught during collections. These wild species are endemic to the Pantanal region and other regions in Brazil; therefore, they can act as potential sources of *Salmonella* spp. due to free access to fish ponds, in the same way as wild animals in other regions in other countries (La Tela et al. 2021).

Salmonella spp. was not detected in feed samples collected directly from the storage sheds. Most fish feed is extruded, a process that combines high vapor pressure with high temperature (130°C), thus eliminating most contaminants (Sarker 2023). However, Parker et al. (2022) show evidence that the prevalence of *Salmonella* detection in finished foods and in milling equipment and the environment continues to be a challenge for feed processing. The same authors argue that the risks of introducing *Salmonella* into a farm through animal feed should, therefore, be based on the individual characteristics of the factory regarding HACCP management practices, including microbial monitoring.

The absence of *Salmonella* spp. in fish feed did not confirm this route as a source of contamination in fish farming. This fact corroborates the results of dos Santos et al. (2019) who also did not detect *Salmonella* in fish farming feed, but the risks of contamination due to failures in quality control during manufacturing cannot be ruled out (Parker et al. 2022). The possibility of contamination during food handling cannot be ruled out, as the handlers did not use PPE (Personal Protective Equipment), nor did they standardize the use of fish farming equipment. Furthermore, no traps were observed to control rodents inside the feed storage sheds (Table 4), these animals being a risk factor for *Salmonella* spp. contamination.

The use of MCA to observe environmental site characteristics and fish farming management added important information for the study investigating the occurrence of *Salmonella* spp. in fish farms during the farming phase. In this sense, the MCA revealed that the management factors associated with the fish farming environment are relevant in the characterization of positive cases of *Salmonella* detection, as in the multidimensional analysis there was good discrimination of these cases. In addition to the association with positive cases, it was observed that during the dry season there was a statistically significant difference in the occurrence of *Salmonella* spp. in relation to the rainy season.

The MCA also showed other important aspects for the study by highlighting the dimension of intrinsic and extrinsic factors of fish farming management (dimension with the greatest explained variance, Figure 2) as a potential way of understanding and identifying the fish farm *Salmonella* detection, such as aspects associated with the management factor of water renewal in fish pond ("system") and the biome in which the fish farm is located ("biome"). The presence of the dimension of intrinsic and extrinsic factors of breeding management that relate to environmental characteristics may suggest that these components can be potentially improved. In this sense, the best path would be interventions in fish farming management, as it would help in resolving the general sanitary condition.

In general, the results obtained in this study support the need for an approach to solving the sanitary problem of *Salmonella* contamination in the farming phase in fish farms, considering the specific relevance of the different categories studied. Observation of the dimensions helped to identify multiple factors associated with contamination by *Salmonella* spp. related to fish farm management or environmental characteristics of sites, which can result in more assertive interventions through good agricultural practices, considering that aspects related to management are potentially more modifiable than environmental ones.

The discovery of native fish contaminated with *Salmonella* spp. in farming sites revealed in this study confirms that cross-contamination occurs in slaughterhouses during the processing of native fish; therefore the potential risks of damage to public health and economic damage are a reality in this fish production chain. *Salmonella* spp. strains with a phenotypic profile of antimicrobial resistance were identified. This is an alert for the occurrence of resistant strains in fish production systems that are unusual hosts of *Salmonella* spp.

5 | Conclusions

The high *Salmonella* spp. occurrence found in fish farms highlights the sanitary challenges of the native fish production chain. The pathogen was detected in environmental matrices and in multiple fish tissues (scales, gills, muscles and intestines), evidencing the high risk of multiple cross-contamination in processing plants and detection by health authorities. In addition to prevalence, serotyping revealed a diversity of circulating strains, with *S*. Saintpaul and *S*. Newport being predominant and of recognized public health relevance, alongside less frequent serotypes such as *S*. Reading and *S*. Abaetetuba.

Environmental factors and fish farming management practices, especially during the dry period, were associated with contamination, reinforcing the need for the implementation of good agricultural practices, water management improvements, and stricter biosecurity measures in fish farms. Finally, the identification of strains with antimicrobial resistance phenotypes raises concern about the circulation of resistant *Salmonella* in aquaculture systems that are not traditional hosts of this pathogen, emphasizing the importance of continuous monitoring and preventive interventions.

This study provides novel data on the diversity of *Salmonella* serotypes in native fish farms in the Pantanal and Cerrado biomes, offering new insights into contamination routes in Brazilian aquaculture. These results also reinforce the need for integrated

surveillance under a One Health perspective, given the close interface between aquaculture, the environment, and public health.

Author Contributions

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Acknowledgments

The authors thank the participating fish farmers, the Mato Grosso Aquaculture Association (AQUAMAT), and the institutions involved in the project for their support and collaboration throughout the study, including the Agricultural Defense Institute of Mato Grosso (INDEA-MT), the Federal Rural University of Rio de Janeiro (UFRRJ), and the Embrapa Food Technology Unit (Embrapa Agroindústria de Alimentos, RJ). The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Funding

This work was supported by the Brazilian Agricultural Research Corporation (EMBRAPA) [code SEG: 20.19.03.049.00.00]; the Secretaria de Estado de Desenvolvimento Econômico de Mato Grosso (SEDEC-MT), Cuiabá, Mato Grosso, Brazil [SEDEC-PRO-2022/02316, Project number 3.008.004]; and the D.Sc. (Y.D.P.) fellowships provided by CNPq (National Council for Scientific and Technological Development), Brazil [141486/2019-8].

Ethics Statement

All procedures involving animals were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Mato Grosso (UFMT), under protocol number 23108.051127/2022-16, in accordance with the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** jfs70041-sup-0001-Supinfo.docx.