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First Report of *Vibrio vulnificus* Outbreak in Farm-Raised Sorubim (*Pseudoplatystoma* sp.) from Brazil

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Abstract: *Vibrio vulnificus* is an opportunistic pathogen in humans and exhibits pathogenic behavior in several aquaculture fish species. To date, in Brazil, there are no reports of *V. vulnificus* outbreaks in farmed fish. However, in 2019, sorubim (*Pseudoplatystoma* sp.) in the grow-out phase with clinical signs and cumulative mortality of 40% was registered. We aimed to identify and characterize the etiological agents of this outbreak. Seven moribund fish were sampled for bacteriological studies, and isolates were obtained from the brain and kidneys. Bacterial identification using matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF/MS) revealed *V. vulnificus*. One isolate, RP4, was used for identification via *dnaJ* and *16S rRNA* gene sequencing and antimicrobial susceptibility evaluation. Sorubim juveniles were experimentally challenged with RP4 isolate via intracelomic injection (IC, 10^7 colony-forming units [CFU] fish^{-1}) and immersion bath (IB, 10^6 CFU mL^{-1} for 30 min). Identities to *V. vulnificus* of *dnaJ* and *16S rRNA* genes by BLAST analysis were higher than 92% and 98%, respectively. Susceptibility to oxytetracycline, florfenicol, and other antimicrobial molecules was also observed. In the IC-challenged group, the mortality rate was 100% with *V. vulnificus* recovered from fish organs (brain, liver, spleen, and kidney). Additionally, splenic endothelium alterations were observed in the IC group. On the contrary, the control and IB groups did not develop any clinical signs, mortality, or bacterial recovery after 7 days of challenge. To the best of our knowledge, this is the first report of pathogenic *V. vulnificus* in farmed *Pseudoplatystoma* sp.

Keywords: vibriosis; neotropical catfish; aquaculture; *Vibrio vulnificus*

Key Contribution: This is the first report of vibriosis in farm-raised sorubim associated with high cumulative mortality. *Vibrio vulnificus* RP4 isolate has pathogenic activity in sorubim following intracoelomic injection and is susceptible to florfenicol and oxytetracycline.

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1. Introduction

Vibrio vulnificus is a flagellated halophilic Gram-negative bacterium that has been associated with opportunistic human illnesses transmitted through contaminated seafood ingestion or via direct wound contact with contaminated water or seafood [1]. *V. vulnificus* has been isolated from freshwater, brackish water, and diseased seawater fish species of aquaculture importance, including *Oreochromis niloticus* [2,3], *Anguilla* sp. [4], *Ctenopharyngodon idellus* [5], and *Trachinotus ovatus* [6] from Asia and Europe since the 1970s [7]. In

Brazil, there have been reports of isolation and molecular identification of *V. vulnificus* from aquatic environments [8,9], human blood (patient who died of septicemia after ingestion of seafood) [10], wild and farmed healthy shellfish [11], and wild captured fish such as *Paralichthys orbignyanus* and *Micropogonias furnieri* [12]. In addition, there are reports of presumptive identification of *V. vulnificus* in sea mammals [13] and sea birds [14] in the country; however, these isolates have not been confirmed at the species level. Accurate *V. vulnificus* identification can be achieved through *dnaJ* gene sequencing [15] or the detection of specific biomarkers, such as the cytotoxin-hemolysin *vvh* gene [16].

Sorubim (*Pseudoplatystoma* sp.) is a neotropical siluriform fish native to South America [17] that has important commercial value in Brazilian aquaculture. Sorubim production reached 11,058 tons in 2022 [18], making it the third most-produced native fish in Brazil. However, with increasingly intensive commercial systems for this fish, propitious conditions for disease occurrence, such as higher stocking density and husbandry stress, are emerging.

To date, there have been reports of Gram-negative bacteria such as *Flavobacterium columnare* [19], *Edwardsiella ictaluri* [20], and *Aeromonas hydrophila* [21], as well as Gram-positive bacteria such as *Streptococcus agalactiae*, *S. iniae*, *S. dysgalactiae* [22], and *Lactococcus garvieae* [23,24] being involved in natural outbreaks in Brazilian sorubim aquaculture. In this study, we describe vibriosis in a Brazilian fish farm of freshwater *Pseudoplatystoma* sp. *V. vulnificus* identification was achieved using matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF/MS) and gene sequencing. Additionally, we assessed the pathogenicity to *Pseudoplatystoma* sp. and evaluated the antimicrobial susceptibility of the RP4 *V. vulnificus* isolate.

2. Materials and Methods

2.1. Outbreak Characterization

The outbreak occurred on a fish farm located at the São Simon reservoir (18°38′31″ S, 50°03′57″ W), Goiás state, in August 2019. Sorubim in the grow-out phase (weighing 400–700 g) showed clinical signs of infection (erratic swimming, melanosis, and hyporexia) and abrupt mortality. Fish mortality of 40% (5400 out of a population of 13,500 individuals) occurred within 1 week of the onset of the disease. The water temperature of the affected net cage was approximately 24 °C.

2.2. Vibrio Vulnificus Identification and Phylogenetic Analysis

2.2.1. Isolation and MALDI-TOF/MS Identification

Seven moribund fish were sampled and subjected to a bacteriological examination. Brain and kidney samples were streaked on Columbia agar (Kasvi, Parana, Brazil) and incubated at 28 °C for 24 h, resulting in the isolation of seven cultures. Bacterial identification was performed using mass spectrometry (MALDI-TOF/MS) with a FlexControl MicroFlex LT mass spectrometer (Bruker Daltonics, Billerica, MA, USA), as previously described by Assis et al. [25]. Subsequently, the isolates were stored at −70 °C, but only RP4 (*V. vulnificus*) was able to reactivate and was used for further analysis.

2.2.2. *dnaJ* and *16S rRNA* Sequencing and Phylogenetic Analysis

The frozen RP4 isolate was thawed and reactivated on marine agar (HiMedia, Mumbai, India) at 28 °C for 24 h. Pure colonies obtained were subjected to DNA extraction using the Maxwell® 16 Tissue DNA Purification Kit in the Maxwell 16 Research Instrument (Promega, Madison, WI, USA). The DNA's quality and quantity were verified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) before being submitted for amplification and sequencing of the *dnaJ* and *16S rRNA* genes. Briefly, *dnaJ* was amplified by PCR using primers DN1-1F (5′-GATYTRCGHTAYAACATGGA-3′) and DN1-2R (5′-TTCACRCCRTYDAAGAARC-3′) as reported by Nhung et al. [15]. Concurrently, *16S rRNA* amplification was conducted using universal primers B37 (5′-TACGGYTACCTTGTTACGA-3′) and C70 (5′-AGAGTTTGATYMTGGC-3′) as reported by Fox et al. [26]. Forward and reverse

PCR amplicons were sequenced using an ABI 3500 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) and subjected to quality control; sequence trimming and contig generation were performed using BioEdit, version 7.2 [27]. Both sequences were evaluated using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 10 November 2023) in the nt/nr database. Sequences from *Vibrio* sp. strains with identity $\geq$ 83% and query coverage $\geq$ 96% were considered for alignment assessment (Table S1) and polishing using the AliView software, version 1.28 [28]. The phylogeny of these sequences was estimated with MrBAYES software, version 3.2 [29] using the GTR nucleotide substitution model (nst = 6) with different substitution rates (rates = invgamma) and two million Markov chain Monte Carlo iterations, as previously described [30]. The resulting phylogram was edited using the graphical viewer FigTree version 3.4.4 [31].

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the disc diffusion method according to the CLSI VET03 guidelines [32]. Briefly, RP4 colonies cultured on marine agar were suspended in a sterile saline solution (NaCl 0.9%) until an absorbance of 0.1 was obtained at 625 nm. This solution was streaked on Muller Hinton agar plates (HiMedia, Mumbai, India) supplemented with NaCl at 1%. Following this, nine antibiotic discs (Oxoid, Basingstoke, UK) were positioned on the plates as follows: lincomycin (10 µg), neomycin (10 µg), erythromycin (15 µg), amoxicillin (10 µg), sulfamethoxazole/trimethoprim (25 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), oxytetracycline (30 µg), and florfenicol (30 µg). The plates were then incubated at 28 °C and the zone of inhibition was measured after 24 h of incubation.

2.4. Experimental Infection Challenge

The pathogenicity of the RP4 isolate was evaluated through experimental infection (Ethics Committee Protocol No. 315/2019). Amazon catfish (*Leiarius marmoratus* × *Pseudoplatystoma corruscans*) juveniles (88 ± 41.23 g) were acquired from a local commercial farm with no disease antecedents. Upon arrival at the laboratory facility, 10% of the total batch of fish were subjected to parasitological (gill and skin biopsy) and microbiological (brain, kidney, and spleen streaking on marine agar plates) analyses. Fish were acclimated in glass aquaria supplied with flow-through freshwater at 28 °C and aerated by a stone diffusor. Fish were fed commercial extruded food for carnivorous species (42% crude protein) two times daily until apparent satiation.

The RP4 isolate stored at −70 °C was reactivated on a marine agar plate at 28 °C. After 24 h incubation, one colony was selected and used for inoculum preparation by bacterial suspension in brain–heart infusion broth (Kasvi, Parana, Brazil). Bacterial growth occurred at 28 °C under 100 rpm agitation until a 0.3 OD_{600} was achieved. Bacterial challenge doses were administered to sorubim juveniles via 0.1 mL of intracoelomic injection (IC, 10^7 colony-forming units [CFU] $fish^{-1}$, n = 6) and 30-min immersion baths (IB, 10^6 CFU mL^{-1}, n = 6). A control group (C, 0.1 mL IC of brain–heart infusion broth, n = 4) was included in this study. All fish in the experimental challenge were kept in 60 L glass aquaria (one aquarium per group) during a 7-d monitoring period under conditions similar to those in the acclimation phase. Fish that died during the experimental challenge and fish that survived at the end of the experiment were subjected to microbiological analysis (brain, kidney, liver, and spleen streaking on sterile marine agar plates).

2.5. Histopathological Evaluation

Organs (brain, spleen, heart, midgut, stomach, swim bladder, kidney, and liver) from all fish (dead and surviving the experimental challenge) were collected and fixed in 10% buffered formalin. Then, paraffin wax sections (4 µm) were obtained after organ immersion in ethanol solutions of increasing concentrations (70–100%), cleared with xylene, embedded in paraffin wax, and sectioned using a semi-automated rotary microtome Leica RM2245 (Leica Biosystems, Wetzlar, Germany). Hematoxylin and eosin staining was routinely

performed for all organ sections, whereas GoodPasture staining was performed on selected tissues for Gram-negative bacterial observation as described by Luna [33].

3. Results and Discussion

3.1. Vibrio Vulnificus Identification and Phylogenetic Analysis

3.1.1. Isolation and MALDI-TOF/MS Identification

During this disease outbreak, bacterial colonies were isolated from the brains (n = 3) and kidneys (n = 4) of the diseased aquaculture species. Bacteriological studies resulted in the isolation of three colonies with different morphologies. The MALDI Biotyper results identified *V. vulnificus* (scores between 1.755 and 2.233) isolates from five fish, and *Edwardsiella tarda* (score 2.348) and *Enterococcus saccharolyticus* (score 2.008) from one fish.

V. vulnificus was identified based on the MALDI-TOF/MS results. The suitability of MALDI-TOF/MS as a tool for *V. vulnificus* identification has been previously reported [34,35]. *Vibrio* species belong to a monophyletic group with high similarity. Thus, phylogenetic analyses provide additional information to support this identification.

3.1.2. *dnaJ* and *16S rRNA* Sequencing and Phylogenetic Analysis

Partial *dnaJ* (727 bp) and *16S rRNA* (1397 bp) sequences obtained from the RP4 isolate were deposited in NCBI (GenBank accession numbers: OP690609 and OP692644) and submitted to BLAST against the NCBI nt/nr database. The RP4 isolate obtained identities between 98.56% and 92.98% with 24 *dnaJ* sequences of strains identified as *V. vulnificus* (Table S1). Comparably, identities between 99.71% and 98.57% with 22 *16S rRNA* sequences identified as *V. vulnificus* were observed (Table S1).

Phylogenetic trees based on *dnaJ* and *16S rRNA* were constructed using these strains. In addition, seven *Vibrio* spp. strains were included in the analysis. Markov chain Monte Carlo simulations with <0.01 average standard deviation of split frequencies allowed the generation of two phylograms (Figures 1 and 2). Mean branches with posterior probability (prob) of 100% for an exclusively *V. vulnificus* cluster were only obtained by *dnaJ* phylogenetic analysis, where the RP4 isolate was located (Figure 1).

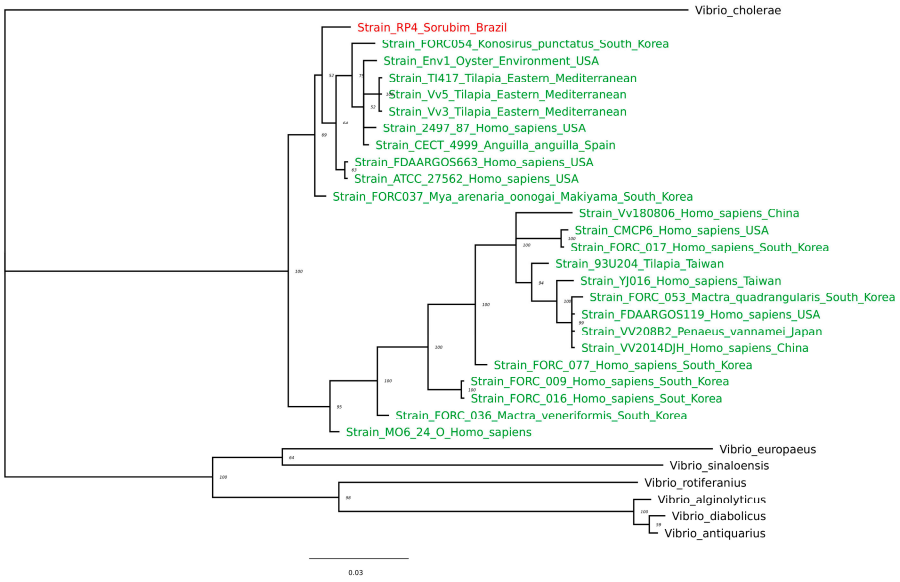

Figure 1. Phylogeny of *V. vulnificus* RP4 isolate from *Pseudoplatystoma* sp. (taxa in red) by *dnaJ* sequence. Phylogenetic tree obtained by Bayesian analysis of 24 *V. vulnificus* (taxa in green), 7 *Vibrio* sp. (taxa in black), and with *Vibrio cholerae* as the root. All *dnaJ* gene sequences were obtained from NCBI. Prob node values are shown as a percent (%).

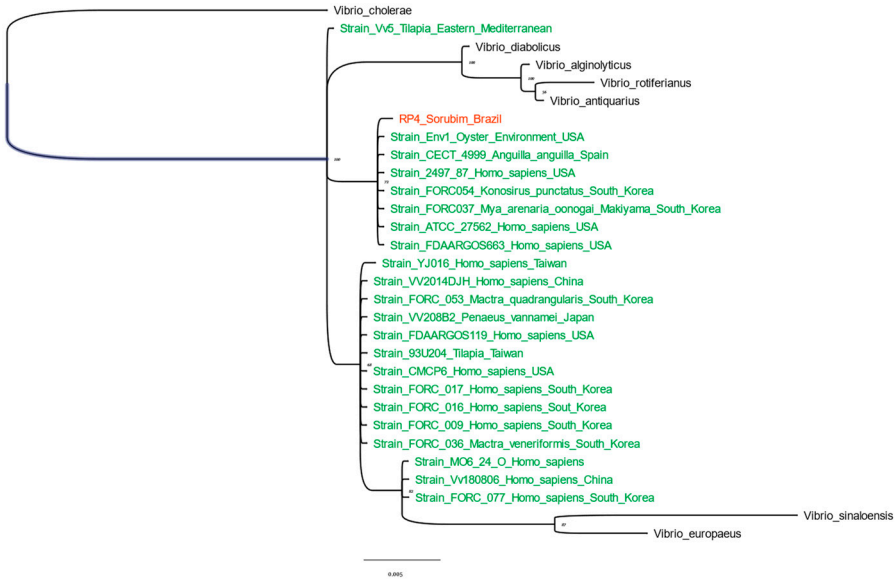

Figure 2. Phylogeny of *V. vulnificus* RP4 isolate from *Pseudoplatystoma* sp. (taxa in red) by *16S rRNA*. Phylogenetic tree obtained by Bayesian analysis of 22 *V. vulnificus* (taxa in green), 7 *Vibrio* sp. (taxa in black), and with *Vibrio cholerae* as the root. All *16S rRNA* gene sequences were obtained from NCBI. Prob node values are shown as a percent (%).

The identity results for *16S rRNA* showed poor species differentiation for *V. vulnificus*, as identities higher than 97% were reached with other *Vibrio* species (e.g., *Vibrio europaeus*, *V. diabolicus*, *V. antiquarius*, *V. alginolyticus*, and *V. rotiferanius*). Even with a clustering threshold of 99% identity for complete *16S rRNA* as proposed by Edgard [36], lower identities were obtained for *16S rRNA* with several *V. vulnificus* strains from NCBI. This explains the failure to cluster all *V. vulnificus* strains into an exclusive species branch, as shown in previous studies [15].

In contrast, the phylogenetic tree based on *dnaJ* identified the RP4 isolate in the exclusive *V. vulnificus* branch. Although *dnaJ* of the RP4 isolate displayed variable identities with other *V. vulnificus* strains, it was sufficient for species discrimination. This can be explained by the lower identity (<86%) of *dnaJ* of the RP4 isolate compared with the other *Vibrio* species evaluated in this study. In addition, a trend of concentrating fish-pathogenic *V. vulnificus* strains (including the RP4 isolate) in a distinct branch was observed. Further evaluations to confirm whether the RP4 isolate belongs to pathovar *piscis* can be conducted using protocols previously reported [37].

Currently, the Vibrionaceae family comprises 190 species distributed in 51 clades where *V. vulnificus* is grouped in the "Vulnificus clade" with two other closely related species: *V. navarrensis* and *V. cidicii* [38]. In the present study, we characterized the RP4 isolate as *V. vulnificus* using the *dnaJ* sequence. In addition, the RP4 isolate showed identity values lower than 85% with closely related species such as *V. navarrensis* and *V. cidicii* (Table S2).

3.2. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility profile of the RP4 isolate (Table 1) was obtained and verified according to the breakpoints described by CLSI and EUCAST [39–41].

The isolate was susceptible to amoxicillin, sulfamethoxazole/trimethoprim, nitrofurantoin, norfloxacin, oxytetracycline, florfenicol, neomycin, and erythromycin. This was contrary to the typical antimicrobial resistance traits observed in other Brazilian isolates of *V. vulnificus* [12,42,43]. On the other hand, despite lincomycin having the lowest inhibition zone (8 mm), it was not possible to find an actual reference for breakpoints for appropriate comparison. However, *V. vulnificus* (strain H 1828/94) isolated from a human clinical case showed the same inhibition zone behavior [44].

Table 1. Antimicrobial susceptibility results of *V. vulnificus* RP4 isolate.

Antibiotic	Inhibition Diameter of RP4 Isolate (mm)	CLSI * (mm)			EUCAST ** (mm)	
		S [a]	I [b]	R [c]	S	R
Neomycin (10 µg)	14 (S)	–	–	–	>12 [1]	<12 [1]
Erythromycin (15 µg)	22 (S)	–	–	–	>12 [2]	<12 [2]
Amoxicillin (10 µg)	23 (S)	>17 [2]	14–16 [2]	<13 [2]	>14 [3]	<14 [3]
Sulfamethoxazole/trimethoprim (25 µg)	26 (S)	>16 [2]	11–15 [2]	<10 [2]	>18 [2]	<18 [2]
Nitrofurantoin (300 µg)	29 (S)	>17 [4]	15–16 [4]	<14 [4]	x	x
Norfloxacin (10 µg)	32 (S)	>17 [4]	13–16 [4]	<12 [4]	>22 [3]	<22 [3]
Oxytetracycline (30 µg)	35 (S)	>15 [5]	12–14 [5]	<11 [5]	>20 [5]	<20 [5]
Florfenicol (30 µg)	34 (S)	>18 [6]	13–17 [6]	<12 [6]	>17 [7]	<17 [7]
Lincomycin (10 µg)	8	–	–	–	–	–

(*): CLSI, Clinical and Laboratory Standards Institute breakpoints (M100-27th Edition and M45-3rd Edition); (**): EUCAST, European Committee for Antimicrobial Susceptibility Testing breakpoints. Version 12.0, 2022; ([a]): Susceptibility; ([b]): Intermediate; ([c]): Resistance; (–): Whit not description on CLSI and/or EUCAST guidelines.; (x): Whit description but with different disc concentrations used in this essay. ([1]): Antimicrobial breakpoint for Enterobacterales submitted against agents of topical use. ([2]): Exclusive antimicrobial breakpoint for *Vibrio* spp. ([3]): Antimicrobial breakpoint for Enterobacterales. ([4]): Antimicrobial breakpoint for Enterobacteriaceae. ([5]): Antimicrobial breakpoint for a closely related chemical molecule, that is, tetracycline, against *Vibrio* spp. ([6]): Antimicrobial breakpoint for a closely related chemical molecule, that is, chloramphenicol, against *Vibrio* spp. ([7]): Antimicrobial breakpoint for a closely related chemical molecule, that is, chloramphenicol, against Enterobacterales.

Overall, the RP4 isolate was susceptible to various antimicrobial molecules, including two antimicrobials licensed for aquaculture purposes in Brazil: florfenicol and oxytetracycline. This information may be useful for the management of fish vibriosis in Brazil.

3.3. Experimental Infection Challenge and Histopathological Evaluation

Mortality rates of 100% and 0% were obtained in the IC (<24 h after inoculation) and IB (7-day observation period) groups, respectively. In addition, recovery of *V. vulnificus* from IC-treated fish organs (brain, liver, spleen, and kidney) was possible after cultivation in Marine agar for 24 h at 28 °C. One fish in the IC group exhibited erratic swimming. Externally, in fish from the IC group, the ventral skin was reddish and the anus was swollen. Internal lesions, including organ congestion and reddish ascitic fluid, were also observed in these fish (Figure 3A,B). Conversely, the Control and IB groups did not develop any clinical signs, internal lesions, or bacterial growth at the end of the assay.

Histopathological examination showed that major lesions were restricted to the spleen of the fish in the IC group, with early necrotic foci and proliferation of Gram-negative bacteria around the necrotized endothelium of the splenic vessels (Figure 3C,D). The other groups (Control and IB) did not show any cellular or tissue alterations.

Complete mortality was observed at a dose of 10^7 CFU fish^{-1} via IC. These results demonstrate the pathogenic behavior of *V. vulnificus* isolate RP4 towards sorubim. Other authors have observed similar mortality and clinical signs 24 h after inoculation with *V. vulnificus* at a lower dose (10^3 CFU fish^{-1}) in tilapia [3] or a higher dose (10^{10} CFU fish^{-1}) in eels [45]. The authors attributed this rapid mortality to peracute septic shock. In the present study, septicemia occurred because it was possible to recover *V. vulnificus* colonies from several organs, including the brain. In addition, necrosis of the endothelial vessels associated with Gram-negative bacillus proliferation observed in the spleen of the IC group supports this peracute septic shock caused by *V. vulnificus* in sorubim. Similarly, histopathological findings associated with vascular disturbances were reported in the renal capillaries of eels [4] and the liver of tilapia [3] challenged with *V. vulnificus*.

Conversely, infection via immersion did not result in mortality or clinical signs after the 7-day observation period. One explanation for the lack of infectivity via immersion could be the relatively short exposure time (30 min) of the fish to the bacterial suspension bath. Previous reports of experimental *V. vulnificus* infection via immersion in fish have shown varying immersion times, ranging from 60 min [46] to over 24 h [3]. However, it is

worth noting that successful infections in fish have been reported with shorter experimental immersion times (less than 60 min) involving other pathogenic bacteria [47], and longer exposure times did not necessarily lead to pathogenic activity in suspected bacteria-infected fish [48].

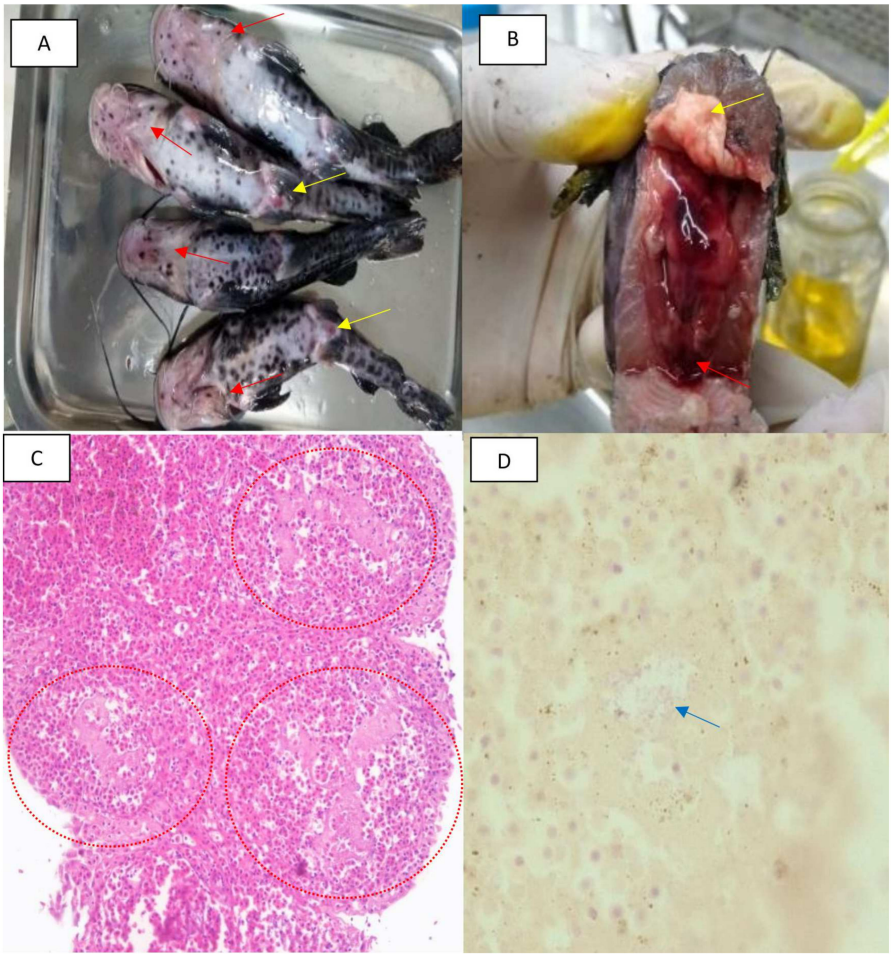

Figure 3. Pathological signs in fish subjected to experimental challenge via intracelomic injection of *V. vulnificus* RP4 isolate (10^7 CFU $fish^{-1}$): (**A**), External lesions as reddish skin (red arrows) and swollen anus (yellow arrows); (**B**), Internal lesions as reddish ascitic fluid (red arrow) and congested swim bladder (yellow arrow); (**C**), foci necrosis areas on spleen (red doted ellipses); (**D**), splenic vessel (blue arrow) with endothelial necrosis and presence of eosinophilic bacilli into the vessel lumen.

On the other hand, failure of the immersion infection could be associated with the inability of *V. vulnificus* to adhere and invade mucosal surfaces. A report [49] suggested that the natural portal of entry for *V. vulnificus* into fish could be the mucosal surfaces of the gills. Additionally, it was observed that the skin mucosa could serve as a reservoir, as *V. vulnificus* can colonize and develop a biofilm structure on fish skin [49]. However, the adhesion and invasion capabilities to fish mucosal surfaces by *V. vulnificus* are not fully understood. There is a report of a lack of pathogenicity in fish after immersion in freshwater (0% salinity) with *V. vulnificus* strains originally isolated from diseased fish inhabiting water with salinities $\geq$ 0.3% [46]. In that report, it was suggested that the inconsistency between water salinity in the infection bath and the strain's salinity isolation may contribute to the failure of immersion infection. Recent studies suggest that exposure to freshwater has a detrimental effect on *V. vulnificus* virulence, specifically with respect to a compound contributing to water salinity. It has been shown that calcium in water (an important salinity contributor in seawater) can trigger biofilm formation while suppressing the motility of *V. vulnificus* [50,51]. In our immersion infection assay, the challenge was

conducted in a freshwater bath with *V. vulnificus* RP4 isolate originating from ill freshwater fish. We speculated that the RP4 isolate was adapted to freshwater conditions and would express virulence in an immersion infection under similar conditions. Unfortunately, this assumption was not validated, and further studies are necessary to verify the effect of salinity on the virulence of RP4 isolate and to elucidate the primary mode of infection of sorubim vibriosis.

4. Conclusions

Taken together, the first disease outbreak caused by pathogenic *V. vulnificus* in farmed *Pseudoplatystoma* sp. in Brazil is described using MALDI-TOF identification, *dnaJ* and *16S rRNA* sequencing, and experimental challenge. The pathogenic behavior was demonstrated to cause mortality and histological damage following intracelomic injection. Under in vitro conditions, this bacterium is susceptible to different antimicrobials, including florfenicol and oxytetracycline, which are licensed for aquaculture purposes by the Brazilian Sanitary Livestock Authority.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9020054/s1, Table S1: *Vibrio* spp. strains used for *dnaJ* and *16S rRNA* genes phylogenetic evaluation.

Author Contributions: Conceptualization, P.C.J.-S. and G.C.T.; Methodology, P.C.J.-S. and G.C.T.; Validation, H.C.P.F. and J.L.R.C.; Formal Analysis, P.C.J.-S., G.C.T. and J.L.R.C.; Investigation, P.C.J.-S., F.Y.T.R., S.B.d.P., S.A.C.M., F.P. and R.C.E.; Resources, G.C.T., F.P. and H.C.P.F.; Data Curation, P.C.J.-S.; Writing—Original Draft Preparation, P.C.J.-S.; Writing—Review and Editing, G.C.T., H.C.P.F., F.Y.T.R. and R.C.E.; Visualization, P.C.J.-S., S.A.C.M. and F.P.; Supervision G.C.T. and H.C.P.F.; Project Administration, G.C.T.; Funding Acquisition, G.C.T. and H.C.P.F. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the CONCEA (Conselho Nacional de Controle da Experimentação Animal) and approved by the Ethics Committee of Federal University of Minas Gerais (Protocol No. 315/2019).

Data Availability Statement: GenBank accession numbers: OP690609 and OP692644.

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Conflicts of Interest: Author Santiago Benites De Pádua was employed by the company Aquivet Saúde Aquática. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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