



Molecular Epidemiology of Aquaculture Pathogens in Rainbow Trout (*Oncorhynchus mykiss*) using Real-time PCR in Peruvian Andean Environments

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ABSTRACT

Background: Rainbow trout (*Oncorhynchus mykiss*) is one of the main aquaculture species cultivated in the high Andean regions of Peru, providing food security and rural socioeconomic development. However, the area is frequently affected by infectious agents that compromise fish health and generate significant economic losses. The objective of this study was to determine the prevalence and regional distribution of the main infectious pathogens in rainbow trout using molecular techniques.

Methods: Biological samples were collected from five producing regions (Junín, Puno, Pasco, Lima and Ayacucho) and analyzed using real-time quantitative polymerase chain reaction (qPCR) for the detection of relevant bacterial and viral pathogens in trout farming.

Result: The results showed the presence of various etiological agents, with *Flavobacterium* sp. being the most prevalent pathogen, representing 46.2% of positive samples. Other pathogens such as infectious pancreatic necrosis virus (IPNV), *Weissella* sp., *Aeromonas* sp. and *Yersinia* sp. were also identified. A higher frequency of infectious pathogens were found in the Junín and Puno regions, coinciding with areas of greatest production activity. These findings highlight the need to strengthen health surveillance, biosecurity and timely diagnosis programs in intensive high-Andean systems, to reduce the health and economic impact of infectious diseases on trout production in Peru.

Key words: Andean aquaculture, Fish pathogens, *Flavobacterium* sp., Infectious pancreatic necrosis virus, Molecular detection, qPCR, Rainbow trout.

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) farming in the high Andean regions of Peru represents an activity of considerable economic, social and nutritional importance for rural communities. This cold-water species, introduced decades ago, has adapted well to environments characterized by clear waters, high Andean basins and altitudes ranging from approximately 2,300 to 4,000 meters above sea level. These conditions have favored the development of trout aquaculture in regions such as Puno, Junín, Pasco and Ayacucho (MINAM, 2021). In addition to contributing to regional food security, trout farming promotes diversification of production systems and generates employment opportunities in areas with limited economic alternatives (Sierra Exportadora, 2019). Similar roles of aquaculture in supporting rural livelihoods and nutritional security have been reported in other developing regions, highlighting its relevance as a sustainable production strategy (Jaies *et al.*, 2020; Özcan, 2023).

From a technical perspective, environmental conditions in the highlands-including moderate temperatures, high dissolved oxygen levels and spring water sources-are advantageous for trout growth and production performance (Mullisaca, 2020). Consequently, trout aquaculture has become an important component of sustainable rural

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development, linking local production systems with regional and national markets. However, the expansion of aquaculture also involves significant sanitary risks, particularly infectious diseases that may affect productivity and the economic sustainability of farming units. Previous studies have emphasized that disease outbreaks represent one of the major constraints to aquaculture intensification, particularly in systems with increasing stocking densities and limited biosecurity measures (Jaies *et al.*, 2020).

Among the main pathogens affecting salmonid aquaculture are bacterial agents such as *Flavobacterium columnare*, the causative agent of columnaris disease and viral pathogens such as Infectious Pancreatic Necrosis Virus (IPNV). *F. columnare* is a globally distributed pathogen responsible for skin and gill lesions, fin erosion and high mortality rates in cultured fish (Declercq *et al.*, 2013; Thunes *et al.*, 2023). IPNV is widely recognized as one of the most important viral pathogens in salmonid aquaculture, with reported mortality rates that can reach up to 90% in susceptible populations (Ulloa-Stanojlovic *et al.*, 2022). In addition, emerging and opportunistic pathogens have been increasingly reported in aquaculture systems, highlighting the complexity of disease dynamics in intensively managed fish populations (Kumar *et al.*, 2017).

Advanced molecular techniques such as real-time PCR (qPCR) have significantly improved disease diagnosis in aquaculture systems. These methods enable highly sensitive and specific pathogen detection, quantification of infectious load and monitoring of pathogen dynamics within production systems (Abdelsalam *et al.*, 2023). For high-Andean aquaculture, molecular surveillance represents a strategic tool for early outbreak detection, improved biosecurity decision-making and the implementation of targeted sanitary management strategies. Molecular approaches have also been recognized as essential tools for strengthening epidemiological surveillance and improving disease control in aquaculture production systems (Jaies *et al.*, 2020).

Despite these methodological advances, information on the spatial distribution and epidemiological patterns of infectious pathogens in Andean trout farming remains limited. In Peru, few studies have evaluated the prevalence and regional distribution of major pathogens in cultured trout using molecular diagnostic approaches (Ulloa-Stanojlovic *et al.*, 2022). This knowledge gap limits the capacity to prioritize intervention areas, design effective surveillance programs and implement evidence-based disease management strategies.

Therefore, this study aims to determine the prevalence and regional distribution of major infectious agents in rainbow trout from five regions of Peru using real-time PCR. Specifically, the study addresses the following research questions: What is the molecular prevalence of key bacterial and viral pathogens in farmed rainbow trout in different Peruvian regions? and are there differences in pathogen distribution among trout-producing regions in the high Andean system? We hypothesize that: (i) infectious pathogens are present in multiple trout-producing regions

of Peru and (ii) prevalence varies according to regional production and environmental conditions.

MATERIALS AND METHODS

Location

Samples were taken in Junín, Puno, Pasco, Lima and Ayacucho. These areas are located between 2,800 and 4,200 meters above sea level and are characterized by cold, clean water bodies, conditions favorable for intensive and semi-intensive trout farming. Samples were obtained from certified production units and authorized fish farms during the study period. The geographic coordinates and approximate altitudes of each facility were recorded for spatial analysis and epidemiological reference.

Animals and sampling

A total of 473 rainbow trout (*Oncorhynchus mykiss*) were sampled from aquaculture establishments located in major trout-producing regions of Peru between October 2024 and March 2025. The sample size was defined to ensure broad geographic representation of production systems and to increase the probability of detecting infectious agents circulating in farms reporting health problems. Although the sampling was not probabilistic, the number of samples analyzed is comparable to or greater than that reported in epidemiological surveillance studies of pathogens in salmonid aquaculture. Biological samples were collected from trout of different ages and production stages directly in the field. Fish handling followed animal welfare and aquaculture biosecurity protocols. Samples were subsequently transported under refrigerated conditions to the Ichthyopathology Laboratory of the Faculty of Biological Sciences, Universidad Nacional del Altiplano, Puno, Peru, for processing. All procedures involving animals were conducted in accordance with institutional guidelines for animal care and use in research and were approved by the Ethics Committee of the Faculty of Veterinary Medicine and Zootechnics, Universidad Nacional del Altiplano, Puno, Peru (Ethical Approval Certificate No. 05-2024-FMVZ-UNA-PUNO). From each specimen, tissues with diagnostic relevance were sampled according to clinical suspicion, including gills, liver, spleen, kidney and integumentary lesions when present. Samples were preserved in sterile cryovials and transported under refrigerated conditions to the laboratory. Sampling was conducted using a purposive sampling design, focusing on farms that had reported clinical signs compatible with infectious disease or recent mortality events. Therefore, the results should be interpreted as evidence of pathogen presence and distribution in affected production systems rather than as an estimate of population-level prevalence.

Nucleic acid extraction

Bacterial DNA was extracted from fish tissues (kidney, spleen and external lesions) using the GeneJET commercial kit based on silica columns, strictly following

the manufacturer's protocol. For the detection of Infectious Pancreatic Necrosis Virus (IPNV), viral RNA was extracted using a GeneJET commercial RNA-specific kit, ensuring RNase-free conditions. The concentration and purity of the nucleic acids were assessed by fluorometric analysis and the samples were stored at -20°C (DNA-RNA) until analysis.

qPCR assays for bacteria and virus

All qPCR reactions were performed in a final volume of 20 µL, containing 10 µL of Master Mix, 1 µL of each primer (forward and reverse), template nucleic acid and nuclease-free water. Amplifications were carried out in a real-time thermocycler (Quantabio), including positive controls, negative controls (no-template control) and an internal amplification control to verify reaction performance and detect potential inhibition. A cycle threshold (Ct) value ≤ 35 was considered positive for pathogen detection, while samples with Ct values between 35 and 38 were reanalyzed to confirm amplification. Only reactions showing the expected amplification profile and, when applicable, a characteristic melting curve were considered valid.

The identification of *Yersinia ruckeri* was performed using primers YER8 (Forward: GCGAGGAGGAAGGG TTAAGTG) and YER10 (Reverse: GAAGGCACCAAGGCA TCTCTG). The PCR thermal profile consisted of an initial denaturation at 94°C for 1 min, followed by 40 cycles of denaturation at 94°C for 30 s and annealing/extension at 60°C for 1 min (Altinok *et al.*, 2001). Detection of *Flavobacterium psychrophilum* was used primers Fp_16S1 (Forward: GAGTTGGCATCAACACAC) and Fp_16Sint1 (Reverse: TCCGTGTCTCAGTACCAG), targeting the 16S rRNA gene. Amplification conditions included an initial denaturation at 96°C for 10 min, followed by 40 cycles of 96°C for 30 s, 56°C for 30 s and 72°C for 30 s (Madsen *et al.*, 2005). The detection of *Aeromonas salmonicida* used primers Asal-aopO-F (Forward: AGCTCATCCAATGTTTCG GTATT) and Asal-aopO-R (Reverse: AAGTTCATCGTGCTGTTCA), which target the aopO gene. The PCR program consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s and annealing at 62°C for 30 s (Gustafson *et al.*, 1992). Molecular identification of *Weissella tructae* used universal primers 27F (Forward: AGAGTTTGATCMTGGCTCAG) and 1492R (Reverse: TACGGTTACCTTGTTACGACTT),

targeting the 16S rRNA gene. The amplification protocol included an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 52°C for 30 s and 72°C for 90 s, with a final extension at 72°C for 7 min (Fusco *et al.*, 2015). Infectious Pancreatic Necrosis Virus (IPNV) was detected by one-step RT-qPCR using primers WB1 (Forward: CCGCAACTTACTTGAGATCCATTATGC) and WB2 (Reverse: CGTCTGGTTCAGATTCCACCTGTAGTG). The reaction mixture was prepared in a final volume of 20 µL containing 10 µL of qPCR Master Mix, 0.4 µL of reverse transcriptase, 1 µL of each primer, 1 µL of template RNA and nuclease-free water to complete the final volume. Thermal cycling conditions included reverse transcription at 50°C for 2 min, initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 10 s and 60°C for 60 s (Bowers *et al.*, 2008).

Statistical analysis

The prevalence of each infectious agent was determined using simple proportions relative to the total number of samples analyzed. Absolute and relative frequencies were estimated by region and by etiological agent. Fisher's exact test was used to assess differences in pathogen distribution between regions. Additionally, heat maps and a correspondence chart were created to visualize epidemiological patterns between agents and production areas. The level of statistical significance was set at $p < 0.05$. The analysis was performed using R statistical software.

RESULTS AND DISCUSSION

Oncorhynchus mykiss samples from five high Andean regions of Peru were analyzed. Molecular diagnosis using real-time PCR allowed the identification of several bacterial and viral pathogens associated with trout production systems. Among the detected agents, *Flavobacterium* sp. showed the highest frequency, representing 46.2% (95% CI: 37.12-55.19) of positive detections, confirming predominance in the evaluated aquaculture systems. There were a considerable proportion of cases with Infectious Pancreatic Necrosis Virus (IPNV) 31.5% (95% CI: 20.85-42.16), followed by *Weissella* sp. 14.2% (95% CI: 7.52-20.79). Whereas *Aeromonas* spp. and *Yersinia* spp. were detected at lower frequencies (Table 1). The confidence intervals indicate the variability and precision of the estimated prevalence for each pathogen. In particular, the

Table 1: Prevalence of bacterial and viral pathogens detected by qPCR in *Oncorhynchus mykiss* from five high-Andean regions of Peru.

Etiological agent	Total	Positives	Prevalence	CI (95%)
<i>Aeromonas</i> sp.	91	4	4.4	0.18-8.61
<i>Flavobacterium</i> sp.	117	54	46.2	37.12-55.19
IPNV	73	23	31.5	20.85-42.16
<i>Weissella</i> sp.	106	15	14.2	7.52-20.79
<i>Yersinia</i> sp.	86	5	5.8	0.87-10.76
Total	473	62	13.1	10.07-16.15

CI: Confidence interval; IPNV: Infectious pancreatic necrosis virus.

relatively wider interval observed for some agents reflects differences in detection frequency among sampled farms and regions. When the distribution was evaluated by region, *Flavobacterium* sp. was detected in all evaluated areas, with a higher number of positive cases in Junín and Puno, suggesting a widespread distribution consistent with endemic occurrence in freshwater aquaculture systems. In contrast, the presence of IPNV showed greater regional variability, with notable detection in Puno and Junín. *Weissella* sp. was mainly detected in production units where coinfection with other pathogens was observed, supporting its role as an opportunistic microorganism (Table 2). Multivariate analysis using simple correspondence analysis (Fig 1 and 2) showed the association patterns between pathogens and regions. The analysis indicated a closer relationship between *Flavobacterium* sp. and the main trout-producing regions, particularly Puno and Junín, while other pathogens showed weaker or more dispersed associations across regions.

The results of this study indicate that trout farming in the Peruvian highlands is exposed to a complex sanitary

scenario characterized by the circulation of multiple bacterial and viral pathogens. Of the 473 samples analyzed, 62 were positive for at least one etiological agent, corresponding to an overall prevalence of 13.1%. These findings confirm that infectious diseases remain an important constraint for rainbow trout (*Oncorhynchus mykiss*) production, particularly in intensive and semi-intensive systems at high altitude.

Flavobacterium sp. was the most frequently detected pathogen (46.2%), supporting previous reports that identify this genus as one of the most important bacterial agents affecting salmonid aquaculture worldwide. Species such as *Flavobacterium psychrophilum*, the causative agent of Bacterial Cold Water Disease, have been associated with high mortality in early life stages and significant economic losses in trout farming (Ramsrud *et al.*, 2007; Starliper, 2011). The higher number of detections in Junín and Puno suggests that environmental and production conditions in these regions may facilitate pathogen persistence and transmission, particularly in systems with high stocking densities and shared water sources.

Table 2: Regional comparison of positive and negative detections of major infectious agents in rainbow trout (*Oncorhynchus mykiss*).

Region	Etiological agent	Negative	Positive	Total	p-value
Ayacucho	<i>Aeromonas</i> sp.	3	0	3	0.0764
	<i>Flavobacterium</i> sp.	1	2	3	
	<i>Weissella</i> sp.	3	0	3	
	Total	7	2	9	
Junín	<i>Aeromonas</i> sp.	21	0	21	0.001
	<i>Flavobacterium</i> sp.	12	16	28	
	IPNV	14	6	20	
	<i>Weissella</i> sp.	17	7	24	
	<i>Yersinia</i> sp.	21	0	21	
	Total	85	29	114	
Lima	<i>Aeromonas</i> sp.	5	0	5	0.113
	<i>Flavobacterium</i> sp.	5	5	10	
	IPNV	2	0	2	
	<i>Weissella</i> sp.	7	3	10	
	<i>Yersinia</i> sp.	5	0	5	
	Total	24	8	32	
Pasco	<i>Aeromonas</i> sp.	5	0	5	0.549
	<i>Flavobacterium</i> sp.	6	1	7	
	IPNV	5	0	5	
	<i>Weissella</i> sp.	4	1	5	
	<i>Yersinia</i> sp.	5	2	7	
	Total	25	4	29	
Puno	<i>Aeromonas</i> sp.	43	4	47	0.001
	<i>Flavobacterium</i> sp.	34	25	59	
	IPNV	21	15	36	
	<i>Weissella</i> sp.	50	4	54	
	<i>Yersinia</i> sp.	42	1	43	
	Total	190	49	239	

IPNV: Infectious pancreatic necrosis virus.

Infectious Pancreatic Necrosis Virus (IPNV) was the second most prevalent agent (31.5%). This virus is widely distributed in salmonid aquaculture and is known for its ability to persist in asymptomatic carriers and spread through vertical and horizontal transmission (Hill and Way, 1995; Ulloa-Stanojlovic *et al.*, 2022). The greater number of positive cases observed in Puno and Junín may be related to the higher intensity of production and the frequent movement of fry between farms, highlighting the importance of strengthening sanitary certification programs for eggs and juveniles.

The detection of *Weissella* spp. (14.2%) is also noteworthy, as this genus has been associated with emerging disease outbreaks in trout aquaculture. Previous

studies have reported pathogenic species such as *Weissella ceti* causing septicemia in cultured trout (Figueiredo *et al.*, 2015). In the present study, the detection of *Weissella* sp. may indicate subclinical circulation of the pathogen, which could become clinically relevant under stress conditions, coinfections, or environmental deterioration.

In contrast, *Aeromonas* sp. and *Yersinia* sp. showed relatively low prevalences. These bacteria are commonly considered opportunistic pathogens within aquatic microbiota and tend to cause disease when fish are exposed to stress or immunosuppression (Charette and Boychuk, 2021). Although the frequency was low, the presence of these bacteria suggests a potential role in mixed infections under unfavorable production conditions.

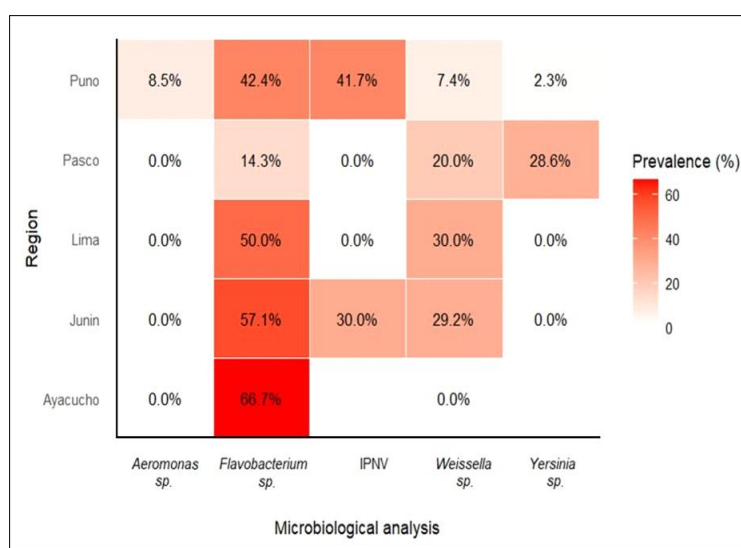


Fig 1: Simple correspondence analysis showing the relationship between detected infectious agents (*Flavobacterium* sp., IPNV, *Weissella* sp., *Aeromonas* sp. and *Yersinia* sp.) and the evaluated regions (Ayacucho, Junín, Lima, Pasco and Puno) in rainbow trout aquaculture systems in Peru.

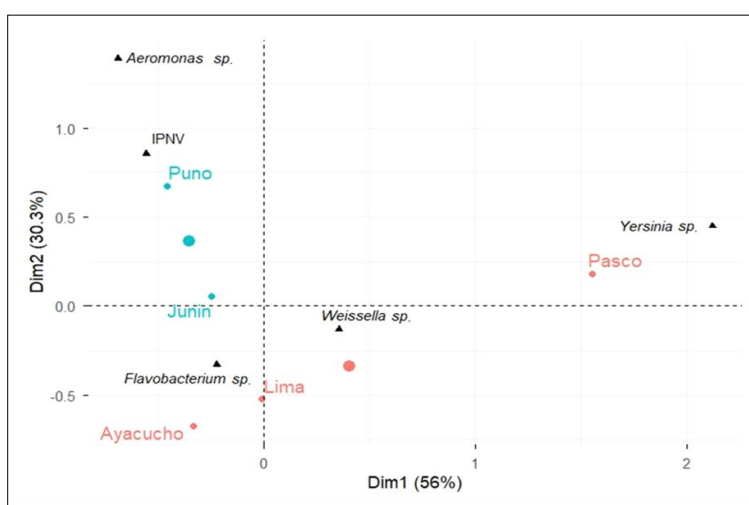


Fig 2: Spatial distribution pattern of infectious agents identified in rainbow trout farms from five Peruvian regions based on correspondence analysis. The proximity between variables in the plot indicates stronger epidemiological association between specific pathogens and regions with higher detection frequency.

Regional differences observed in pathogen distribution indicate that epidemiological dynamics are influenced by production intensity, hydrological connectivity between farms and local management practices. Regions with higher aquaculture activity, particularly Puno and Junín, showed greater pathogen diversity and number of positive cases, which is consistent with patterns reported in other salmonid-producing regions.

From a management perspective, these findings highlight the need to strengthen biosecurity measures, improve sanitary monitoring programs and promote the use of molecular diagnostics such as qPCR for early detection of pathogens in aquaculture systems. In addition, coordinated surveillance strategies between farms and regional authorities could help reduce pathogen dissemination within shared watersheds.

This study has some limitations that should be considered when interpreting the results. First, the use of purposive sampling focused on farms with clinical suspicion of disease may overestimate pathogen prevalence. Second, the cross-sectional design does not allow evaluation of seasonal variation or temporal dynamics of infections. Finally, although qPCR provides high sensitivity for pathogen detection, it does not necessarily differentiate between active infection and the presence of pathogen genetic material.

Future research should incorporate probabilistic sampling designs, longitudinal monitoring and genomic characterization of circulating strains to better understand pathogen epidemiology in high-altitude aquaculture systems.

CONCLUSION

This study demonstrates that trout farming in the Peruvian highlands faces a complex health scenario, characterized by the simultaneous presence of bacterial and viral agents of high epidemiological importance. *Flavobacterium* sp. was identified as the predominant pathogen, followed by IPNV and *Weissella* sp., confirming a central role in the dynamics of diseases affecting rainbow trout in high-altitude production systems. The regional differences observed reflect the influence of production and environmental factors on the distribution of pathogens, highlighting the need to strengthen management practices, biosecurity and health monitoring. The use of sensitive molecular techniques allowed for the accurate detection of subclinical infections; demonstrating value as a fundamental tool for epidemiological surveillance. Taken together, these findings underscore the urgent need to implement comprehensive prevention and control programs that contribute to reducing the impact of diseases and improving the sustainability of national aquaculture production.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All procedures involving animals were conducted in accordance with established guidelines for the care and use of experimental animals. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine and Zootechnics, Universidad Nacional del Altiplano, Puno, Peru (Ethical Approval Certificate No. 02-2020-FMVZ-UNA-PUNO).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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