**Research Article** 



# Distribution and epidemiology of the infectious pancreatic necrosis virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*) in Peru

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**ABSTRACT.** The infectious pancreatic necrosis virus (IPNV) is a highly pathogenic virus that affects the aquaculture of rainbow trout (*Oncorhynchus mykiss*) and can cause mortality rates that exceed 90% in the juvenile stage. This study aims to know the distribution and frequency of IPNV presentation in trout production farms in the seven main producing states of the species in Peru. Prevalence values of IPNV have been identified in the states of Cusco (4.05%), Puno (3.81%), and Huancavelica (0.23%). These results showed the distribution and epidemiology of the IPNV that, until 2019, was absent in the country. Likewise, it represents the need to implement prevention and control measures against the virus to reduce the risk of dissemination to free states of IPNV. Finally, improving sanitary management against IPNV reduces its economic and productive impact on rainbow trout aquaculture in Peru.

**Keywords:** *Oncorhynchus mykiss*; rainbow trout; infectious pancreatic necrosis virus; IPNV; epidemiology; Peru

## **INTRODUCTION**

The contribution of world aquaculture to fish production efficiency has grown significantly during the last decades (FAO 2020). In Peru, the aquaculture industry is emerging sustainably, has, on average, increased by 6.31% over the last decade, and the total production of 141,090 t of aquatic animals (RNIA 2021) is becoming an important economic sector (Saldarriaga & Regalado 2017). The national aquaculture production is mainly represented by the rainbow trout (Oncorhynchus mykiss), white shrimp (Penaeus vannamei), Peruvian scallop (Argopecten purpuratus) and tilapia (Oreochromis niloticus), among which the rainbow trout stand out for being the oldest fish to be introduced to the Peruvian fluvial ecosystem and for its high level of national production (Ramírez-Gastón et al. 2018).

Trout farming in Peru is increased at a growing rate of 10.6% since 2009. The activity is mainly concentrated

in the Andean highlands and, based on the 2020 production, is distributed in the states of Puno (65.43%), Pasco (14.89%), Huancavelica (8.39%), and Junín (5.46%); the other 15 states with trout production represents less than 2% of the annual total of 51,910 t (RNIA 2021).

Rainbow trout is a main economic and animal protein source of high biological value for the highland communities (Montesinos 2018). According to National Aquaculture Cadaster (PRODUCE 2021), 3280 rainbow trout farmers are registered across the country. The typical farms are managed without technical support, with different levels of production and technology. Peruvian trout farming has been exposed to the persistent risk of certain pathogens, especially due to the intensification of aquaculture production in recent years, which is aggravated by stress factors, the impact of the aquaculture food systems industry (Naylor et al. 2021).

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The infectious pancreatic necrosis virus (IPNV) is the causative agent of infectious pancreatic necrosis (IPN) disease. This double-stranded RNA (dsRNA) virus belongs to the *Aquabirnavirus* genus within the Birnaviridae family (Wolf et al. 1960, Hill & Way 1995). IPN is a highly contagious disease with global distribution (Ariel & Olesen 2002, Munro & Midtlyng 2011).

Worldwide, salmonids production has been mainly affected by the IPN, causing significant losses, which leads to risks in environmental, social, and economic sustainability in aquaculture activity (Ruane et al. 2007, Lakshmi et al. 2019) because of the high mortality rates in the early stages of the species, and the ability of the pathogen to spread through carrier animals for many years (Stoskopf 2015). Furthermore, the aquatic environment contaminated by IPNV could spread the disease, and the water supply represents dissemination sources of the pathogen (Murray 2006a, Escobar-Dodero et al. 2019).

Studies have shown that the spread of IPNV is unpredictable because infected fish may not present clinical signs. Therefore, it is necessary to evaluate the presence and distribution at the national level through specific surveillance, considering that the transmission can occur horizontally through excreta and fluids, as well as the vertical route (Ruane et al. 2009). It should be noted that 95% of the production of rainbow trout in Peru comes from eggs imported from the USA, Spain, Denmark, South Africa, Chile, Ireland, and the Isle of Man (SANIPES 2020).

In Peru, the presence of the virus was reported in the states of Cusco and Puno by the National Fisheries Agency (SANIPES 2019). Nevertheless, the distribution and the possible impact that IPNV can cause in Peruvian trout farming is unclear. The present study aims to determine the distribution of the disease and its occurrence in aquaculture trout farming in Peru.

# MATERIALS AND METHODS

### Study area and sampling

Rainbow trout (*Oncorhynchus mykiss*) of different life stages were collected from freshwater farms in Ancash, Cusco, Huancavelica, Junín, Lima, Pasco, and Puno states during the period November 2019 until October 2020. The study included farms with three production stages: hatcheries, on-growing, and those carrying out both production types (complete cycle). The sampled life stages were fry, juveniles, and adults.

For this study, we considered an epidemiological unit as a fish farm or a group of fish farms that share a water body (springs, river, lake, or lagoon) with another farm. The sampling was carried out in epidemiological units, following the recommendations of the Aquatic Animal Health Code (OIE 2021), defined as a group of animals that share approximately the same risk of exposure to a pathogenic agent with a defined location. For the calculation of the sample size was used the Epitools software (Sergeant 2018) was based on an expected prevalence of 5%, which means a total of 60 animals grouped by five animals per pool in each epidemiological unit. Hence, pools with less or more than five animals were rejected. The total number of farms sampled was 85, distributed in the states of Ancash (4), Cusco (14), Huancavelica (10), Junín (19), Lima (2), Pasco (3), and Puno (33), and whose water sources were springs, rivers, lagoons, and lake.

The fieldwork included the water temperature measures, photographic registry, and fish necropsies, where clinical signs were recorded. Prior to sampling, the fishes were euthanized with benzocaine. The kidney, liver, and spleen were collected and placed in cryovials with 96% ethanol, transported in isothermal boxes at temperatures of 4°C, and sent to the SANIPES diagnostic laboratories in the constitutional Province of Callao.

#### **RNA** extraction and purification

Kidney, liver, and spleen tissue samples were obtained for the extraction and purification of RNA using columns, through the commercial RNeasy Mini Kit (Qiagen, Germany), according to the manufacturer's instructions. The extracted RNA was eluted with molecular biology grade water and stored at -80°C until use.

## qRT-PCR assays

Subsequently, the IPNV genetic material was amplified using the real-time PCR technique using specific sequences of primers and probes for IPN amplification and an ELF1 internal positive control following Tapia et al. (2015) (Table 1).

## **Fluorescence analysis**

The rqRT-PCR assays were analyzed from the exponential plots with amplification fluorescence following the manufacturer recommendations of the real-time thermal cycler (QuantStudio5/Applied Biosystems). The samples that were positive for the qRT-

**Table 1.** Sequences of primers and probes for the amplification of infectious pancreatic necrosis virus. Adapted from Tapia et al. (2015).

Code	Sequencing	Size of the amplified fragment
VP1 F	5'- GTTGATMMASTACACCGGAG -3'	
VP1 R	5'- AGGTCHCKTATGAAGGAGTC -3'	159 pb
Probe VP1	56FAM-TACATAGGC-ZEN-AAAACCAAAGGAGACAC -3'	
ELF1 F	5'- CCCCTCCAGGACGTTTACAAA -3'	
ELF1 R	5'- CACACGGCCCACAGGTACA -3'	57 pb
ELF1 probe	5'- FAM- ATCGGCGGTATTGGAAC -3'	

PCR test were considered positive for IPNV. A cycle threshold (Ct) below 40 was considered positive. Subsequently, all positive results were subjected to agarose gels by conventional electrophoresis to verify the presence of the genetic material.

#### Statistical analysis

The data was analyzed using R tidyverse and stats packages (v4.0.2). The variables' frequency, prevalence, and associations were evaluated using the chisquare test ( $\chi^2$ ). The logistic regression models elaborated on the presence of IPNV (positive qRT-PCR) as the dependent variable, and the independent variables were considered the production stages of farms, life stages of rainbow trout, the water source of the fish farms, IPN clinical signs, and anatomicpathological alterations, using a binomial method allowed to calculate the odds ratio (OR). Finally, maps were created to visualize the distribution of IPNV cases in the states of Peru using QGIS 3.6 software (QGIS 2018).

## RESULTS

A total of 1581 samples from seven states of Peru were evaluated. The results of IPN allowed us to calculate the disease prevalence obtained in the positive states, as shown in Table 2, where the prevalence was reported in Cusco (4.05%), Puno (3.81%), and Huancavelica (0.23%). Moreover, the results showed no presence of the IPNV in the states of Ancash, Lima, Junín, and Pasco.

The results indicated a higher proportion of positive cases in on-growing rainbow trout (*Oncorhynchus mykiss*) farms and a lower proportion in the hatcheries and those that carry out the complete cycle on their farms (Table 2). The statistical analysis showed a significant value, which could mean a degree of association between the occurrence of IPN and the

production stages. The logistic regression analysis showed OR values of 6.898 in on-growing rainbow trout farms, 3.195 in hatcheries, and 0.027 in those contemplating both activities concerning the presentation of the disease.

The sample distribution highlights the IPN cases in the country (Fig. 1), showing positive states and a higher number of cases in Cusco and Puno. The relation between the different life stages and the occurrence of IPN showed significant results and OR values of 0.452 in alevins, 0.473 in juveniles, and 0.207 in adults (Table 3). Furthermore, the relation between the fish farm's main water source and the occurrence of IPN also showed significant results, and OR values of 0.491 in the lake, 0.238 in the lagoon, and 0.242 in springs.

Finally, the clinical signs and pathological alterations of 260 batches of rainbow trout were evaluated, and the OR was calculated in those variables that demonstrated a significance level (Table 4). The results showed that melanosis 11.9% (31/260) and splenomegaly 13.5% (35/260) were the most frequently found clinical signs and pathological alterations.

#### DISCUSSION

The present study is the first to describe the distribution and epidemiology of IPNV in aquaculture populations of rainbow trout (*Oncorhynchus mykiss*) in Peru, which has been described worldwide in several species of wild and farmed fish (Rodriguez-Saint-Jean et al. 2003).

The IPNV showed prevalence in fish farms in the central and southern highlands, in the states of Cusco (4.05%), Huancavelica (0.23%), and Puno (3.81%). Previous studies have described prevalence variation of the IPNV and an increasing trend through time as it happened between 1990 to 2002 in Scotland, which ranged from 0.6% to 12.5% (Bruno 2004). The study showed the absence of IPNV in farms in the states of

	Total samples <sup>1</sup>	Prevalence (%)	Types of production stages in fish farms						
Department			Hatcheries		On-growing		Complete cycle farms <sup>2</sup>		
			Negative	Positive	Negative	Positive	Negative	Positive	
Ancash	84	0	0	0	24	0	60	0	
Cusco	182	4.05	0	0	128	18	20	16	
Huancavelica	172	0.23	12	0	10	2	148	0	
Junín	430	0	0	0	106	0	324	0	
Lima	24	0	0	0	0	0	24	0	
Pasco	60	0	24	0	36	0	0	0	
Puno	629	3.81	240	24	278	87	0	0	

**Table 2.** Infectious pancreatic necrosis prevalence, by department (2019-2020). <sup>1</sup>Pool size of 5 animals, <sup>2</sup>Production centers where both operations are carried out (hatchery and on-growing).

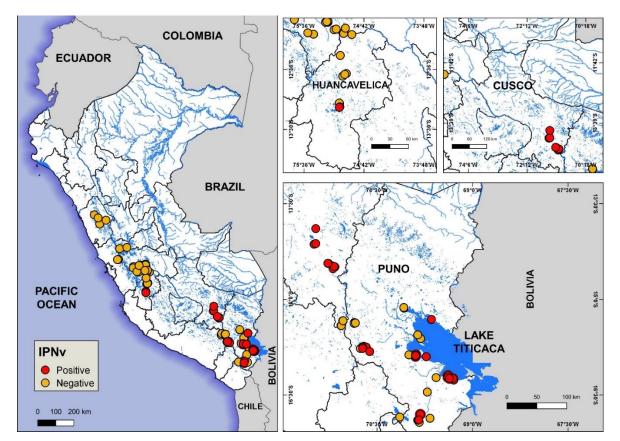


Figure 1. Distribution of the infectious pancreatic necrosis virus (IPNV) on rainbow trout (*Oncorhynchus mykiss*), Peru (2019-2020).

Ancash, Lima, Junín, and Pasco. However, it is necessary to implement sanitary measures due to the spreading potential of the disease (Murray et al. 2003, Peeler et al. 2007). The findings of the present study are similar to those of Ortega et al. (2016), which found the presence of IPNV in the main states of Mexico that produce rainbow trout. The highest proportion of samples collected belonged to the department of Puno (39.78%), which is the main producer of rainbow trout (65.43%) of total national production and concentrated 20.9% of formal aquaculture farms of the species (PRODUCE 2021). Lake Titicaca is the more important aquatic resource in the Puno state, where the presence of the IPNV has been identified in most sampling areas that were part of

Stage	A	dult	Ju	venile	А	levin			
IPN	Total	Positive	Total	Positive	Total	Positive	Stage	P-value	$OR^1$
Ancash	0	0	36	0	48	0	Alevin	0.00849	0.452
Cusco	45	4	34	10	103	20	Alevin	0.00849	0.432
Huancavelica	18	2	66	0	88	0	T	0.00503	0.473
Junín	0	0	227	0	203	0	Juvenile		
Lima	0	0	12	0	12	0	A .114	3.86e <sup>-10</sup>	0.207
Pasco	0	0	12	0	48	0	Adult		
Puno	48	13	375	55	206	43			

**Table 3.** Frequency of presentation of infectious pancreatic necrosis (IPN) according to the life stage of rainbow trout (*Oncorhynchus mykiss*), by department (2019-2020). <sup>1</sup>OR: odds ratio.

**Table 4.** Chi-square test results ( $\chi^2$ ) and odds ratio of pathological alterations findings and clinical signs in rainbow trout (*Oncorhynchus mykiss*) concerning the presentation of infectious pancreatic necrosis, Peru (2019-2020).

Clinical signs	Chi-square test $(\chi^2)$	Odds ratio			
Clinical signs	P-value (2-tails)	(confidence interval)			
Melanosis	0.01127	2.317 (1.197-4.487)			
Eye hemorrhage	0.01897	2.305 (1.133-4.689)			
Anal congestion	0.00006272	4.076 (1.979-8.395)			
Anal prolapse	0.02783	9.422 (0.8364-106.1)			
Pathological alterations					
Transparent fluid	0.002086	3.967 (1.563-10.07)			
Bloody fluid	0.007469	4.952 (1.373-17.87)			
Yellow liver	0.01716	4.86 (1.17-20.19)			
Splenomegaly	0.01187	2.439 (1.2-4.955)			
Reduced pancreatic fat	0.01716	4.86 (1.17-20.19)			

the study, which means there is a higher probability of IPN outbreaks. Similar results were reported by Ball et al. (1971) in the UK, showing that the virus's presence in a lake leads to the spread of the pathogen throughout the region (Ruane et al. 2007).

Accordingly, it has been identified that there is a 6.9 times probability of occurring IPNV in on-growing fish farms, which may be due to the higher number of fish farms with deficient biosecurity measures that increase the risk of spread and recurrence of the pathogen (Murray 2006a,b, 2013). Moreover, the fish that become infected and survive IPN outbreaks become carriers for at least six years (Yamamoto 1975, Holopainen et al. 2017) and, consequently, are sources of infection to new batch (Roberts & Pearson, 2005, Ruane et al. 2009, Bruno et al. 2013, Escobar-Dodero et al. 2019).

IPNV has survived for long periods in the aquatic environment and in different organisms such as wild fish, piscivorous birds, crustaceans, and mollusks (Munro & Midtlyng 2011). In addition, it has been reported that the IPNV can travel more than 19 km from farm outbreaks (McAllister & Bebak 1997). As reported in other countries, the IPNV prevalence reported in this study will likely increase during the following years if prevention and control measures are not established at the national level (Bruno 2004).

Previous studies have reported that IPN could lead to up to 90% mortality rates in alevins (Vestergård-Jørgensen & Bregnballe 1969), growth retardation (Roberts & Pearson 2005) by causing irreversible pancreatic damage, affecting the performance of the surviving fish, and substantial economic losses to the industry (Munro & Midtlyng 2011). Furthermore, IPN affects mainly fish in the first stages of life (Roberts & Pearson 2005, Evensen & Santi 2008), similar to the present study reported where the highest probability of occurrence was in alevins and juveniles. In Peru, as the IPNV increases its prevalence and incidence, it could cause low production rates and predisposition to other infectious diseases, affecting the sustainability of rainbow trout aquaculture.

Mutoloki & Evensen (2011) highlighted the importance of vertical transmission of IPNV through genetic material. However, it is unlikely that the spread of the disease in Peru is due to the trade of infected embryonic eggs since the transport of one batch can be distributed to different fish farms in different states, which is related to the prevalence of the disease that has been only identified in three states.

More than 95% of embryonic eggs are imported from several countries with free IPNV certification (SANIPES 2020), and most hatcheries use first-use water sources, reducing the probability of horizontal transmission of the virus into the hatcheries (Peeler et al. 2007). However, these farms have deficient biosecurity measures, which could not allow them to prevent and detect disease outbreaks, increasing the risk of spreading IPNV (Peeler et al. 2007, Mulei et al. 2018).

Likewise, the transportation of live fish is considered an activity that generates the greatest risk of introducing and spreading IPNV (Murray et al. 2006a). Therefore, it is necessary to strengthen traceability systems which are the responsibility of the public and private sectors. Likewise, understanding the possible spread routes are important to prevent outbreaks and control diseases in aquatic animals (Peeler & Taylor 2011, Murray 2013).

Concerning the clinical findings reported in the present study, melanosis, anal congestion, anal prolapse, and ocular hemorrhage are similar to those reported in previous studies (Evensen & Santi 2008, Smail & Munro 2012, Eriksson-Kallio et al. 2020).

The main anatomic-pathological alterations reported in the study were splenomegaly, yellow liver, and transparent and bloody abdominal fluid, which are also described in the scientific literature as compatible with the occurrence of IPN (Munro & Midtlyng 2011, Smail & Munro 2012, Dopazo 2020).

For decision-making on aquaculture health at the national level, it is necessary to use tools such as zoning and disease management areas (Murray 2013) that are based on Geographic Information System, similar to the National Aquaculture Cadaster that is currently available in Peru (Aguilar-Manjarrez et al. 2010, Calle-Yunis et al. 2020). The implementation of the disease control plan must consider the country's geography, access, and the availability of human resources specialized in aquatic health.

As stated before, IPNV is more resistant than other aquatic pathogens, more persistent in the environment, and to different physical and chemical disinfection procedures (Murray 2006b, Munro & Midtlyng 2011). As a consequence, it is necessary to apply measures to prevent its spread, such as vaccination (Munro & Midtlyng 2011), restriction of movement of live animals (Dopazo 2020), improvement of biosecurity measures (Rodriguez-Saint-Jean et al. 2003), and the elimination of infected batch (Holopainen et al. 2017).

During the study, some modifications were done in the sampling points due to external factors, such as the alteration of some farms sampled in certain states, so in those situations, they were altered by other farms with similar productive characteristics to try to reduce for any methodological bias in the results obtained. In Lima and other states, some problems were recorded during sampling. Especially at the beginning, when the animals' size did not fit the present study's required standards. So, they were discarded and not considered in the statistical analysis. In the same way, there were problems due to sanitary restrictions on people's movement during the COVID-19 pandemic.

The study provides information on the epidemiology of IPNV in Peru. It highlights the need to apply sanitary measures to control the disease and prevent the introduction to regions with a free status, which would affect the sustainability of the aquaculture activity.

Likewise, it is necessary to develop studies that allow the tracing of the origin and source of IPNV by using genotyping in order to implement control strategies such as elimination of the contamination source (Bain et al. 2008, Barrera-Mejía et al. 2011, Manríquez et al. 2017, Büyükekiz et al. 2018, Mulei et al. 2018, Ulrich et al. 2018, Tapia et al. 2019). In addition, it is necessary to distinguish and quantify the activities that increase the risk of introducing and spreading IPNV in rainbow trout farming in Peru.

In conclusion, the study provides the first epidemiology information on IPNV in Peru, with prevalence data in the states of Cusco (4.05%), Puno (3.81%), and Huancavelica (0.23%), located in the central and southern Andean regions of the country, and highlighted the need to apply sanitary measures for control the disease and prevent the spreading to IPNV free states, which contribute to the sustainability of the aquaculture activity.

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