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[*Comunicação*]

First description of porcine parvovirus in aquatic matrice - Rio dos Sinos Basin, Southern Brazil

[*Primeira descrição de parvovírus suíno em matriz aquática – Rio dos Sinos, Sul do Brasil*]

G. *Mohr*[1], M. *Demoliner*[2], N.M.S. *Röhnelt*[1], K.G. *Oliveira*[3], A. *Henzel*[4*].

[1] Graduate, Universidade Feevale, Novo Hamburgo, RS, Brasil
[2]Instituto de Ciências da Saúde (ICS), Universidade Feevale, Novo Hamburgo, RS, Brasil
[3] Undergraduate, Instituto de Ciências da Saúde, ICS, Universidade Feevale, Novo Hamburgo, RS, Brasil
[4] Practitioner, Novo Hamburgo, RS, Brasil

Urban and rural areas tend to experience a disorderly population growth, especially due to inappropriate or absent planning, as well as the expansion of agriculture, deforestation, and the lack of effluent treatment, have contributed to environmental impacts (Borges and Athayde 2017). Surface and groundwater matrices are predisposed to changes in the physical, chemical and biological characteristics owing to the substances derived from industrial, agricultural and anthropogenic activities, (Gomes *et al.*, 2018).

The Sinos River Basin (SRB) is an important river in the metropolitan region of Rio Grande do Sul (RS) State. The basin supplies approximately 1.5 million inhabitants and is distributed heterogeneously across 32 municipalities. Its extension comprises different land and occupation characteristics, including preserved areas with a high population density (FEPAM, 2019). The SRB was divided into three stretches (upper, middle, and lower). The upper stretch (from Caraá to Rolante) is 25km long and has a high slope, rapid water flow, and a low population density. It is surrounded by small farms with diverse agriculture and dairy cattle, swine, and poultry (subsistence properties), in which dogs and cats currently transit among the farm animals. The middle stretch (Taquara and Sapiranga) runs 125km and has a higher population density. In the lower stretch (from Campo Bom to the mouth of the Jacuí river), with a length of 50km, it appears flat, with a slow water flow. The area has a high population density and industrial concentration, and important streams supply large urban centers (FEPAM, 2019).

SRB has been the target of several studies mainly due to the low rates of effluent treatment that contribute to detection and identification of contaminants as pathogens viral origin (Dalla Vechia *et al.,* 2012; Demoliner *et al.,* 2021; Girardi *et al.,* 2019). These studies have shown the presence and persistence of viruses in different environmental matrices such as water and sediment (Girardi *et al.,* 2019). It is important to note that enteric viruses can be carried by water matrices over long distances and, when present in the environment, represent an imminent threat to water resources, humans, and animals (Rigotto *et al.,* 2010).

Among the viral groups involved in fecal-oral transmission, the most prevalent in environmental matrices are Adenovirus (AdV), Rotavirus (RV), Hepatitis A virus (HAV), and Norovirus [NoV] (Dalla Vecchia *et al.,* 2012; Girardi *et al.,* 2019; Rigotto *et al.,* 2010). Nevertheless, parvovirus has the same route of dissemination (oral-fecal), are ubiquitous in nature, resistant to environmental pressure and able to infect a wide variety of hosts (Cotmore and Tattersall 2014); but only a few studies have investigated the presence of them in environmental matrices. In humans, Human

*Corresponding author: henzelvet@gmail.com
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parvovirus (HBoV) genomes have been detected in a river in Germany (Hamza *et al.,* 2009), in a sewage in Finland (Räsänen *et al.,* 2010) and Blinkova *et al.* (2009) detected in effluent samples from the United States; in Brazil, it was detected in the surface waters of the metropolitan region of RS (Kluge *et al.,* 2013). While for animal parvoviruses, Chicken parvovirus (ChPV) and turkey parvovirus (TuPV) were described in Spain after the mapping of fecal contamination of avian origin in the environment (Carratalà *et al.,* 2012) and a raccoon canine parvovirus (CPV-like) was detected in the surface water at the Education Environmental Center, Novo Hamburgo City [RS] (Gartner *et al.,* 2022), carried out by some authors of this present article.

Parvoviruses are small, non-enveloped viruses with a single-stranded genome that belong to the *Parvoviridae* family. Parvoviruses of veterinary importance in domestic and production animals belong to the *Parvovirinae* subfamily and genus *Protoparvovirus*, which includes CPV, feline parvovirus (FPV), and porcine parvovirus [PPV] (Cotmore and Tattersall, 2014).

CPV infections in domestic and wildlife carnivores are generally associated with a high morbidity and lethality, particularly in young puppies (Buonavoglia *et al.,* 2001). FPV infects domestic cats and other *Felidae* members. Infected puppies more than six weeks old can present with anorexia, fever, vomiting, hemorrhagic diarrhea, and leukopenia (Stuetzer and Hartmann, 2014). PPV infection in swine results in reproductive disorders, commonly known as stillbirth, mummification, embryonic death, and infertility (SMEDI) syndrome (Streck and Truyen, 2020). Nowadays, in addition to the care in the circulation of animals infected with viruses, such as porcine circovirus, fever classical swine, senecavirus, and influenza within and between properties, attention to fomites and people carrying viruses such as employees and deliverers has gained strength. This is due to the importance of biosecurity strategies and their impact on both animal health and the economy.

Following this scope, the present study aimed to analyze the presence of genome from PPV, CPV, and FPV in surface and groundwater from farms and recreational areas, which are both located in the rural areas of the SRB.

Samples from recreational points were obtained from the Cascata do Chuvisqueiro, Parque das Laranjeiras, and Balneário João Martins Nunes, which are distributed along the SRB. Five samples were collected weekly during the following periods: a) November to December 2015, b) January to February 2016, c) November to December 2016, and d) January to February 2017. The number of collections and their temporal distribution were established according to the National Environment Council (Conama) Resolution no. 274/00 (Conselho, 2000), which recommends several collections within five weeks. The selection criteria for the sampling points included different rivers belonging to different municipalities, places with few nearby residents, far from urban centers, and those that usually receive high numbers of visitors during the summer (Rohnelt *et al.,* 2020).

Farm collections were conducted between November and December 2015 on 34 rural properties in 11 municipalities. Table 1 shows information on the municipalities.

Both recreational and rural areas were obtained from two previous studies (Rohnelt *et al.,* 2020; Demoliner *et al.,* 2021), in which the viral concentration of AdVs (human and animal species) and coliforms were analyzed. And the period in which the collections were carried out is due to the stretch in which both authors completed their course completion work, named TCC in Brazil – trabalho de conclusão de curso (Rohnelt *et al.,* 2020; Demoliner *et al.,* 2021).

Once collected, the samples (500mL) were rested in sterile bottles and transported to the Feevale University Molecular Microbiology Laboratory (LMM) and refrigerated at 4°C until processing. Samples were concentrated by ultracentrifugation assays (Dalla Vecchia *et al.,* 2012), following this procedure 36mL aliquots were centrifuged using the Sigma® 3-30KS equipment (Germany rotor 12150-H apparatus) for three hours at 41,000×g at 8°C, and the precipitates were resuspended and vigorously homogenized through the use of a vortex for 1 min with 1 ml of Tris-EDTA buffer (pH 8.0).

Table 1. Distribution of rural properties by municipality and stretch of Sinos River Basin (SRB) where surface and groundwater samples were collected.

Stretch SRB	Cities	Number of proprieties
Upper	Caraá	2
	Santo Antônio da Patrulha	4
Midle	Rolante	2
	Riozinho	3
	Taquara	3
	Igrejinha	3
	Araricá	4
	Sapiranga	5
	Campo Bom	4
Lower	São Leopoldo	1
	Nova Santa Rita	3
Total	11	34

Legend: Sinos River Basin (SRB). Source: adapted from Demoliner *et al.* (2021).

The genetic material was extracted after concentration. DNA extraction was performed from an initial volume of 200ul of a concentrated sample using the BioPur® kit according to the manufacturer instructions. The final eluted volume was stored in DNAse/RNAse free microtubes and kept at -80ºC freezer until PCR processing.

The first standard required PCR to detect CPV and FPV genomic isolates. Marketed vaccine Vanguard HTLP 5/CV were used. VP2 (viral protein) capsid protein was used as the target gene. The amplicons produced 583 base pairs (bp). Differentiation between FPV and CPV is performed using a sequencing assay. Reaction steps included the following: 95°C for 5', followed by 35 cycles of 95°C for 30" and gradient
CPV-555For (5'-CAGGAAGATATCCAGAAGGA-3') and CPV-555Rev (5'-GGTGCTAGTTGATATGTAATAAACA-3'), primer annealing (ranging from 50°C to 60°C for 30"), and final extension of 72°C for 1', as described by Buonavoglia *et al.* (2001).

PCR for PPV was previously standardized using the characterized strain, PPV type 1 (named 27A). This study was kindly supported by André Streck, a researcher at the University of Caxias do Sul, RS, Brazil. Amplification cycles were performed according to the protocol described by Hao *et al.* (2011). Reaction steps included the following: 95°C for 5', followed by 40 cycles at 94°C for 30", and annealing gradient of PPV-For (5'-AATTAGGCCAGCTCAGGTAGGATA-3') and PPV-Rev (5'-TGTTGTTGTGTGTTGTTGAATAGG- 3') primers (ranging from 52.3°C at 61°C for 1'), followed by a final extension at 72°C for 1'. VP2 is also a target gene for PPV detection, with an amplicon at 661pb.

Amplicons were subjected to sequencing and subsequent molecular analyses. The alignments used in this research were conducted in the NCBI BLAST (BLASTn) program. The phylogenetic relationship between the genomic fragments obtained by sequencing and the nucleotide fragments available from GenBank was reconstructed using the Molecular Evolutionary Genetics Analysis version 7.0 (MEGA 7.0) program (Kumar *et al.,* 2016) and the neighbor-joining method (Saitou and Nei, 1987).

Positive samples detected by PCR were subjected to viral isolation according to the cell culture specificity. Samples positive for PPV were inoculated into the porcine kidney (PK-15), and if positive samples were detected for CPV/FPV, they were inoculated into Madin Darby Canine Kidney (MDCK) cells to evaluate the cytopathic effect (ECP). Fifty microliters of the positive sample were inoculated into the plaque culture of each cell. Cell cultures were kept in a CO_2 atmosphere (5%) at 37°C for 5 days (each passage) to observe the ECP.

A total of 124 samples from the rural area across 11 municipalities were analyzed (see Table 1). Eighty-six samples were from the groundwater (spring and artesian well) and 38 samples were from the surface water (streams, weir, and river); and in recreational points, 59 samples were possible to be analyzed.

Only one of the samples tested was positive for PPV (LMM-2924). CPV and FPV were not detected in the present study. LMM-2924, collected in 2015, from groundwater (spring water) of a small rural property in Nova Santa Rita city. Likeness, sequencing of the PPV-type 1 (PPV-1) showed nucleotide similarities with the isolates from Germany AY684871 and AY684864 isolates in 2001 and 2002 (Zimmermann *et al.,* 2006), with values of 99.7% and 99.3%, respectively (data not shown). In this study Zimmermann *et al.* (2006) analyzed VP1/VP2 (2187 nt) region into sequencing, being VP2 was only target of our PCR with 661pb of amplicon.

LMM-2924 cells were subjected to a viral infectivity assay using cultured PK-15 cells. It was subjected to three passages of five days each. No ECP was detected in all passages; and it was submitted again for nucleic acid extraction, PCR, and sequencing, which confirmed to be PPV-1.

In this study, we detected the presence of the PPV genome in water matrix samples, and to our knowledge this has been the first report of detection in environmental matrices in Brazil. Furthermore, parvovirus as well as other waterborne viruses are a threat to public health, it exhibits long-term permanence in environmental matrices, resistance to abiotic factors (precipitation and temperature), and the ability to infect a wide range of hosts; but still few studies seek to detect it in environmental matrices.

CPV, FPV, and the PPV have been described worldwide, including in Brazil. Most of what have been detected are from "fresh" biological samples and describes issues of pathologies sources, seroprevalence, evolution, and molecular characterization (Dezengrini *et al.,* 2007; Streck and Truyen, 2020).

The PPV detected in this present study showed similarity with German isolates in 2021 and 2022 - as shown in the results item. The German isolates were originated from clinical samples of tissues (lung, liver and lymph nodes) from aborted fetuses and rectal swabs from sows with reproductive failure (Zimmermann *et al.,* 2006). However, this study did not consider historic infections, disease pre-existence, number and species of animals on the property, vaccination status of animals, distribution of precipitation rates, or seasonal variation of groundwater when sampling was performed. Demoliner *et al.* (2021) and Rohnelt *et al.* (2020) objective was just to analyze the presence of AdV (regardless of the species) as an indicator of contamination and hosts origin. Although we have no way of knowing whether the PPV detected in this study came from sick animals and which syndrome the pigs were affected by as well as subclinical infection. What is less likely is that the PPV detected is of strain vaccine origin, since the studied region is more subsistence agricultural than commercial. But even so, we cannot confirm any epidemiological relationship.

As mentioned, the samples used in this study were previously tested for AdVs. Demoliner *et al.* (2021) detected the following AdVs with the respective rate: human adenovirus (HAdV) in 48.8% of samples, canine mastadenovirus (CAV) in 19.7%, bovine mastadenovirus (BAdV) in 17.4%, avian denovirus (AvAdV) in 15.1%, and porcine mastadenovirus (PAdV) in 3.5% of the samples from the groundwater. In surface waters, the HAdV genome was detected in 44.7% of the samples, CAV in 42.1%, BAdV in 28.9%, and PAdV and AvAdV in 13.1%. Rohnelt *et al.* (2020) detected HAdV, CAV, BAdV, and PAdV in 86.4%, 42.4%, 37.3%, and 28.8% of samples, respectively. However, PPV detected in the present study originated from the rural propriety located in Nova Santa Rita from groundwater kind from spring (Table 1). Specifically, the LMM-2429 were negative for any AdVs (Demoliner *et al.,* 2021).

The detection of animal and human viruses in such scenario points to their potential for zoonotic transmission. It is known that this does not happen to AdV and parvovirus because the infection of both viruses is specie specific. Nonetheless, this could also be the case for other infections, such as RV; since RVs are a major cause of severe gastroenteritis in humans and animals and can cause zoonotic infections. RVA, RVB, and RVC are found in mammals including humans, swine, and cattle (Trovão *et al*., 2019).

However, if it were possible to carry out broader research, such as being able to access the deeper water points that surround these water sources, it would be possible to understand the source of contamination. Despite these factors, this study could not specify whether the virus had been transmitted through the soil (by infiltration) or had reached the top of the well via runoff from water contaminated with excrement or animal products, or directly by excreta arranged near the well.

To clarify these issues and discover the origin of this contaminant in groundwater, it is necessary to conduct a more detailed investigation to analyze the climate, considering the rainfall index, epidemiological profile of the animal, and the occurrence of parvovirus in swine populations in this region. It would also be interesting to assess the percolation of animal viruses in local soil, as they can be traced in the soil profile of the aquifer recharge areas where the studies were performed.

Nevertheless, animal parvovirus (raccoon CPV-like) has already been detected in the surface water sample collected in 2018 (Gartner *et al.,* 2022). It was also detected in the lower stretch of the SRB; however, it was in a region different from the area analyzed in the current study. Raccoon CPV-like was detected around Lomba Grande, a suburb of the city of Novo Hamburgo, and PPV-1 was detected in a rural area of the city of Nova Santa Rita; the distance between these points was approximately 40km.

These findings indicate the importance of continued research of parvovirus presence in the environment, mainly in rural areas which are located the spring. They also highlighted the need for protective measures to maintain the quality and sustainability of natural water. Thus, to minimize such negative impacts, it would be relevant to implement actions such as a) improving the basic sanitation system; b) waste collection in households mainly in rural areas; c) construction of isolated septic tanks to avoid contamination of water wells; d) advice on abstraction, water use, and measures for disinfection of wells and/or water for human and animal consumption; e) guidance on recycling nutrients and organic matter (e.g., swine manure) through anaerobic digestion so it does not contaminate water sources; and f) implementation of biosecurity strategies.

Keywords: PPV, CPV, FPV, PCR, sanitary barrier

RESUMO

Presença do parvovírus suíno (PPV), parvovírus canino (CPV) e parvovírus felino (FPV) em amostras de água em áreas rurais da Bacia do Rio dos Sinos, sul do Brasil, entre 2015 e 2017, foi investigada. A metodologia empregada foi a coleta de água superficial e subterrânea, utilizando-se a técnica de PCR para detecção do genoma viral, sequenciamento para caracterização molecular e isolamento em cultivo celular para análise de infectividade viral nas amostras positivas na PCR. Foi analisado um total de 183 amostras de água, e o genoma do PPV foi detectado em uma única amostra. Até onde se sabe, esta é a primeira descrição da presença do genoma do PPV em matrizes ambientais no Brasil. Os resultados deste estudo destacam os desafios na biosseguridade, no monitoramento e na gestão hídrica em áreas rurais produtivas.

Palavras-chave: PPV, CPV, FPV, PCR, barreira sanitária

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