Scholars Research Library
European Journal of Zoological Research, 2021, 9 (5): 16
(http://scholarsresearchlibrary.com)

ISSN: 2278–7356

**Article type:** Research Article

**ISSN:** 2278-7356

**Year:** 2021

**Volume:** 9

**Issue:** 5

**Article numbers:** ejzr-21-41747



# Design of a protocol for the detection of parvovirus interspecies through Polymerase Chain Reaction

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**ABSTRACT**

*Parvoviridae is a family of small, non-enveloped viruses possessing a single-stranded DNA genome of approximately 5,000 bases. Virions in this family infect a wide variety of hosts, from insects to primates. Human, canine, feline, porcine, and rat hosts stand out. Therefore, it is important to develop a diagnostic method that can demonstrate the presence of the viruses belonging to this family in any host species. For this work, canine Parvovirus type 2 (CPV-2) and Carnivore protoparvovirus 1 (FPLV) were used as interspecies models, as they are prevalent diseases in the clinic of small animals.*

*One of the leading causes of hemorrhagic enteritis in dogs around the world is caused by canine Parvovirus type 2 infection, Canine Parvovirus. Feline panleukopenia produces a picture similar to canine parvovirus but also generates a significant*

*decrease in leukocytes. Therefore, Veterinarians need to have a highly sensitive diagnostic technique for both clinical pictures. In the present work, the foundations were laid for the implementation of a protocol that uses the conventional Polymerase Chain Reaction (PCR) to detect a DNA fragment of CPV-2 and FPLV from feces of dogs or cats with signology and symptoms corresponding to parvovirus.*

*For the generation of the primers capable of detecting the newly named viruses, the GenBank database was first searched for nucleotide sequences of the complete CPV-2 and FPLV genome. With this information, 15 nucleotide sequences of the complete genome of both viruses were chosen and aligned using the Clustal Omega Software to obtain the consensus sequence of the complete genome of both viruses. Subsequently, the percentage of conservation of the genes coding for the canine and feline Parvovirus proteins was obtained. It was found that the genes that code for the NS1 protein are the most conserved, so they were used to generate the primers to be used in the Polymerase Chain Reaction (PCR) for diagnosis.*

**Keywords:** Polymerase Chain Reaction, *Parvoviridae*, leukocytes



## INTRODUCTION

### *Canine parvovirus*

In 1967 the first parvovirus infecting canines was detected [1]. Canine Parvovirus type 2 was later detected in 1978, after emerging as a pandemic due to the lack of previous immunity in dogs, which allowed the rapid spread of the virus around the world [2]. The origin of the virus is unclear, but it is postulated that it arose as a variant of the Feline Panleukopenia Virus (FPLV). Among other hypotheses about the emergence of this virus, it is postulated that it would be a mutation of the FPLV present in the vaccine or an adaptation of parvoviruses that affect wild carnivores such as foxes and minks [3]. The detected CPV-2 continued to evolve and in 1980 its first variant was identified, called CPV-2a, a variant that presented substitutions in some amino acids of the sequence that forms the VP2 protein of the viral capsid. In 1984 a new variant was detected from CPV-2a and was named CPV-2b. Both spread rapidly around the world and in a short time completely displaced the original virus (CPV-2). In 2000, a new variant from CPV-2b was described in Italy, which was called CPV-2c, and which was also rapidly detected in other parts of the world. CPV-2, known mainly to be the causative agent of acute hemorrhagic enteritis, is one of the most important viruses of veterinary interest worldwide. Since its first characterization in the 70s, it has been established as a pathogen that mainly affects canines, both domestic and wild, globally, with high morbidity (100%) and an estimated mortality rate of 10% in adult dogs. and over 90% in puppies [4].

### *Taxonomy, structure, and viral genome*

CPV-2 is classified within the Parvoviridae family, Parvovirinae subfamily [5], and was recently included in the Protoparvovirus genus, as a member of the carnivorous Protoparvovirus 1 species that also groups, among others, FPLV [6]. The etiological agent was characterized for the first time in 1978 from fecal samples and tissues of affected individuals [7]. The virus is small, 18 to 26 nm in diameter, without an envelope, with an icosahedral capsid composed of three structural proteins (VP1, VP2, and VP3), and a single-stranded DNA genome of approximately 5,000 nucleotides. This strand encodes

two structural proteins (VP1 and VP2) and two non-structural proteins (NS1 and NS2). The viral capsid is composed of 60 protein subunits (capsomeres), which are made up of 90% of the VP2 protein and 10% of the VP1 protein. VP2 is the most numerous [5] and is highly conserved [8], participates in the recognition of the host, and the nuclear translocation of the viral particle [9].

VP1 plays a role in the infectivity of the virus [8]. A third structural protein can be found, VP3, which is not detected in all viruses, and which originates from the action of a host protease on VP2 which undergoes a cleavage process and becomes VP3, which allows the interaction of the viral particle with cell membranes [9]. The NS1 and NS2 proteins have essential roles for virus invasion and replication. NS1 is the majority non-structural protein and performs functions as a promoter of viral DNA replication, a regulator of its transcription, and as a cytotoxic component as it is capable of inducing apoptosis through caspases and has helicase and ATPase properties [10]. The functions and properties of NS2 have been less studied, but it is believed that its main role is to regulate the transport of the viral particle from the cytoplasm into the nucleus [11]. Because the genome of parvoviruses is small and only encodes a few proteins, these viruses are highly dependent on the host cell for their replication. The proteins necessary for viral replication are found only in the S phase of the cell cycle [12], so replication occurs in the nucleus of constantly dividing cells, such as fetal, newborn, or newborn cells. of intestinal tissue from young or adult animals [5]. By the action of DNA polymerase, the single strand of virus DNA is converted into a double strand. Using cellular RNA polymerase II, two classes of messenger RNA (mRNA) are generated, one of greater length that codes for non-structural proteins and another of shorter length that codes for capsid proteins [12].

*Pathogeny*

The most common route of entry is through the oropharyngeal region [13], through contact with feces of infected animals or contaminated surfaces [4]. The virus has an incubation period of 3 to 10 days [14], initially replicates in the lymphoid tissue of the region [15], in the mesenteric lymph nodes and the thymus, then it spreads through the blood to the crypt epithelium in the mucosa of the small intestine [1]. CPV-2 directly affects the cells of the intestinal crypts, leading to the destruction and shortening of the intestinal villi, which prevents the absorption of nutrients, resulting in diarrhea. The deterioration of the intestinal mucosa allows blood to escape into the intestinal lumen and the passage of bacteria from the intestine to the blood [4]. Lymphoid tissue is also affected and the destruction of lymphocytes will produce immunosuppression, predisposing to secondary infections [16]. Rarely does the virus affect the myocardium and this occurs when the mother does not have antibodies [15] and the puppy acquires the infection within the first week of life when myocardial cells are rapidly dividing [13].

*Clinical features*

Enteric infection presents with anorexia, depression, vomiting, abdominal pain, and eventually fever. Diarrhea can be severe and hemorrhagic, especially in puppies. Due to diarrhea and vomiting, dehydration occurs quickly. Symptoms appear three to five days after the virus enters the body and the death of the puppy can occur three days after the appearance of clinical signs [16]. When CVP-2 infects the myocardium, clinical signs are usually evident throughout the litter [17] and death occurs from

congestive heart failure. 70% of puppies will die within the first eight weeks and 30% will undergo pathological changes in the organ, which will cause death weeks or months later [16].

*Immunization*

Puppies acquire antibodies through colostrum, which gives them protection against the virus in the first weeks of life. The highest rates of infection are seen in puppies older than six weeks of age. These maternal antibodies interfere with the immunization of the puppies through vaccination. Maternal antibody titers equal to or greater than 1:80 confer immunity to puppies. When the titer is 1:40 it does not confer immunity, but it is capable of interfering with the active immunization of puppies [18]. Active immunization is done through the administration of vaccines, 90% of puppies respond to it at twelve weeks of age, when maternal immunity has declined. Generally, polyvalent vaccines are used [4], in Chile the Sextuple vaccine is used, which is the antigenic preparation that includes the canine Distemper virus, canine Parvovirus, Adenovirus type I and II, and canine parainfluenza virus as live virus modified; and Leptospira interrogans serovares canicola and icterohaemorragiae as a bacterin [19]. The vaccines marketed in Chile are registered with the Agricultural and Livestock Service [20].

*Diagnosis*

The clinical diagnosis of parvovirus tends to be complicated on some occasions due to the similarity of the clinical signs to other pathologies such as that caused by a coronavirus and also coccidiosis, in the case of the enteric manifestation [5]. Therefore, the diagnosis through clinical signs is only presumptive and must be confirmed by a diagnostic test. The ELISA test and IC immunochromatography are the routine methods used in the veterinary clinic because they are simple, fast, cheap, and usually have an acceptable sensitivity [4]. The ELISA test is quick and simple, but a great variability in its sensitivity has been found in various studies, 81.8% [21], 56.2% [22] and 18.4% [23], although it can detect the three circulating CPV-2 variants [24]. The IC immunochromatography technique delivers the result in a short time, but its sensitivity does not exceed 50% [5]. Another of the tests used to detect the virus or antigen viral includes hemagglutination that is quick and simple to detect parvovirus in feces using porcine, feline, or Rhesus monkey erythrocytes [4] but is less sensitive than other tests such as viral isolation or chain reaction of the polymerase (PCR) (Desario et al., 2005), in addition to the fact that some variants of CPV-2 lack hemagglutinating activity [25]. A variation of this test is hemagglutination inhibition, which is more specific since specific antibodies against the viral antigen are used [22]. It is also possible to perform electron microscopy for diagnosis, but CPV-1 and CPV-2 are morphologically identical, which does not allow their identification [16]. PCR has proven to be the most sensitive test for the detection of canine parvovirus and in its conventional form, it has a sensitivity of 93.15% [22]. This test has become the technique of choice in cases of dogs with clinical signs that are negative in other diagnostic tests [15].

*Feline parvovirus*

Feline panleukopenia is the syndrome of clinical disease caused by infection with Carnivore protoparvovirus 1 (FPLV). Both feline parvovirus (FPLV) and canine parvovirus (CPV) can cause feline panleukopenia, although canine parvovirus infections in cats are rare. Feline parvovirus causes 95% of cases, while 5% is caused by variants of canine parvovirus, specifically CPV-2a, CPV-2b, and CPV-2c [26].

*Taxonomy, structure, and viral genome*

FPLV belongs to the Parvoviridae virus family, Parvovirinae subfamily, Protoparvovirus genus [27]. Feline Panleukopenia was first identified as a viral cause in 1928 [28], cats were successfully vaccinated against FPLV in 1934 using formalin-inactivated tissue extracts from infected cats [29]. In 1964 FPLV was isolated from infected cat tissue culture, allowing the development of inactivated tissue culture vaccines and modified live virus vaccines [30]. With the increase with the progressive adoption of cat vaccines by pet owners, FPLV became a rare disease diagnosis in companion animal veterinary practice in several countries [31].

Carnivore protoparvovirus 1 is a small, non-enveloped, single-stranded DNA virus with a 5.1 kb genome that encodes 2 main genes, non-structural (NS) and structural protein. The NS gene encodes the NS1 and NS2 proteins involved in DNA replication, capsid assembly, and intracellular transport, while the structural gene encodes the VP1 and VP2 virus capsid proteins. The viral capsid is composed of 60 protein subunit molecules (approximately 10% VP1 and 90% VP2) arranged in icosahedral symmetry [26].

*Pathogeny*

Carnivore protoparvovirus 1 is a highly contagious and resistant virus, capable of persisting in infected facilities for 1 year. In infected cats, the virus is shed in large amounts in all excretions, including saliva, urine, feces, and vomit. The main portals of infection are the gastrointestinal (GI) tract through orofecal transmission and, less frequently, the respiratory tract through the inhalation of aerosolized viruses. In the field, transmission is predominantly indirect by fomites [26]. After infection, Carnivore protoparvovirus 1 bind to its cellular receptor, TfR, a transmembrane protein that is expressed in many tissues [32]. Virions enter cells through clathrin-mediated endocytosis and colocalize with transferrin in endosomes before entering the cytoplasm to allow viral DNA to access the nucleus [33]. Viral DNA is released from the capsid and replicates through double-stranded RNA intermediaries in the nucleus of the cell. The virus does not have its DNA polymerase and must "sequester" that of the host for replication to occur. Since the virus can only replicate in S-phase cells, it has a tropism for lymphatic tissue, bone, bone marrow, intestinal crypt epithelium, and newborn tissues that are still actively replicating, FPLV can replicate in Purkinje cells of the cerebellum in neonates less than 10 days old [26]. Viral replication in lymphoid tissue oropharyngeal occurs 18 to 24 hours after infection, and viremia can be detected within 2 to 7 days after infection. Clinical disease occurs in cats after 2 to 10 days of incubation. Excretion of the virus in feces can occur in the absence of clinical signs (subclinical infections), or before clinical signs of the disease are detected. Low-level virus shedding can persist for more than 6 weeks. Transplacental infection can also occur, resulting in miscarriage, mummified fetuses, stillborn kittens (early gestation), or kittens born with central nervous system deficit (late gestation) [26].

*Clinical signs*

FPLV or CPV-2 infection can be clinical or subclinical. High seroprevalence rates in some unvaccinated adult cat populations suggest that subclinical infections are common in young adult cats. The determinants of the clinical disease include age, immune status, and co-infections with intestinal parasites, viruses, and bacteria [34]. The disease can be acute, resulting in sudden death from septic shock without prior signs, especially in kittens less than 2 months of age. The most common presentation is characterized by an acute course of the disease over several days with a high fever of 40ºC, lethargy, anorexia, vomiting, diarrhea, and severe dehydration. Only a few of these signs may be present, vomiting generally precedes diarrhea, and unlike dogs with CPV-2 enteritis, hemorrhagic diarrhea is much less common in cats [35]. There may be hypersalivation due to nausea. Abdominal palpation can be painful and reveal thickened intestinal segments and/or enlarged mesenteric lymph nodes [36]. Myocarditis is a recognized complication of CPV-2 infection in puppies, but convincing evidence is lacking to support the role of parvovirus infection in cats with myocarditis. Depending on the stage of pregnancy in which infection occurs, infected females may abort or give birth to kittens with the central nervous system and eye defects, including cerebellar hypoplasia, hydrocephalus, hydranencephaly, retinal dysplasia, and optic nerve hypoplasia. Common complications that generally result in the death of the animal include circulatory shock, septicemia, and disseminated intravascular coagulation. Cats with FPLV are also susceptible to coinfections as a result of severe immunosuppression [26].

*Immunization*

The World Small Animal Veterinary Association (WSAVA) vaccination guidelines and the feline vaccination advisory panel report of the American Association of Feline Practitioners (AAFP) recommend a vaccination program for those beginning 6 to 8 weeks after age [26]. In Chile, this virus is part of a vaccine that is applied in a protocol way in cats from puppies and must be revaccinated annually. This vaccine is Triple feline, an antigenic preparation that contains feline Parvovirus, feline Herpes virus, and Calicivirus as modified live viruses [19]. Vaccine registered in the Agricultural and Livestock Service [20].

*Diagnosis*

As diagnostic confirmation tests, fecal antigen tests, polymerase chain reaction (PCR), or viral isolation are used. Fecal antigen enzyme-linked immunosorbent test kits designed to detect CPV in dogs can be used for the diagnosis of FPLV in cats, as they detect both CPV-2a-c and FPLV antigen in feline feces [37]. A diagnosis of FPLV based on a negative result of the enzyme-linked immunosorbent test with fecal antigen should never be ruled out [38]. Polymerase chain reaction assays can be used to confirm the diagnosis of FPLV in cases that have a negative fecal antigen test result in patients whose clinical presentation suggests the disease. Commercial PCR assays are typically quantitative PCR assays that will amplify and detect DNA of carnivorous Protoparvovirus1, but may not distinguish between feline (FPLV) and canine (CPV-2) strains. False positives can occur in recently vaccinated cats [39].

## MATERIALS AND METHODS

Using the GenBank database, the nucleotide sequence of 15 complete genomes of Canine Parvovirus and Feline Parvovirus was obtained. The search for canine Parvovirus in Genbank yielded 4514 sequences, most of them being the VP2 gene sequences, 306 VP1, and VP2 sequences, and 145 sequences the complete genome of the virus that includes the NS1, NS2, VP1, and VP2 genes.

Regarding Feline Parvovirus, when searching GenBank for feline parvovirus, only 93 sequences were found, but when including feline panleukopenia, 702 sequences were found, of which the vast majority were VP2, similar to what happened when searching for canine Parvovirus genome sequences. Both search names were used for the Feline Parvovirus case. 25 sequences include VP1 and VP2, and 22 sequences of the entire virus genome.

Fifteen sequences from the whole genome of canine Parvovirus and 15 sequences from the whole genome of feline Parvovirus were used. Annex 1 contains the GenBank access code for the sequences used. The 30 nucleotide sequences of the entire genome were aligned using Clustal Omega software to obtain the consensus sequence and locate all the common areas. The consensus sequence that was obtained is showing in Table 1.

**Table 1**: Consensus sequence (AMC1) for complete genome

**AAGCATTTTCGTTTGTTTTTAAATGTGACAACGTCCAACTAAATGGAAAGGATGTTCGCTGGAACAACTATACCA**
**AACCAATTCAAAATGAAGAGCTAACATCTTTAATTAGAGGAGCACAAACAGCAATGGATCAAACCGAAGAAGAA**
**GAAATGGACTGGGAATCGGAAGTTGATAGTCTCGCCAAAAAGCAAGTACAAACTTTTGATGCATTAATTAAAAA**
**ATGTCTTTTTGAAGTCTTTGTTTCTAAAAATATAGAACCAAATGAATGTGTTTGGTTTATTCAACATGAATGGGG**
**AAAAGATCAAGGCTGGCATTGTCATGTTTTACTTCATAGTAAGAACTTACAACAAGCAACTGGTAAATGGCTACG**
**CAGACAAATGAATATGTATTGGAGTAGATGGTTGGTGACTCTTTGTTCGGTAAACTTAACACCAACTGAAAAGAT**
**TAAGCTCAGAGAAATTGCAGAAGATAGTGAATGGGTGACTATATTAACATACAGACATAAGCAAACAAAAAAAG**
**ACTATGTTAAAATGGTTCATTTTGGAAATATGATAGCATATTACTTTTTAACAAAGAAAAAAATTGTCCACATGAC**
**AAAAGAAAGTGGCTATTTTTTAAGTACTGATTCTGGTTGGAAATTTAACTTTATGAAGTATCAAGACAGACAAAC**
**TGTCAGCACACTTTACACTGAACAAATGAAACCAGAAACCGTTGAAACCACAGTGACGACAGCACAGGAAACAA**
**AGCGCGGGAGAATTCAAACTAAAAAGGAAGTGTCAATCAAATGTACTTTGCGGGACTTGGTTAGTAAAAGAGTA**
**ACATCACCTGAAGACTGGATGATGTTACAACCAGATAGTTATATTGAAATGATGGCACAACCAGGAGGTGAAAA**
**TCTTTTAAAAAATACACTTGAAATTTGTACTTTGACTTTAGCAAGAACAAAAACAGCATTTGAATTAATACTTGAA**
**AAAGCAGATAATACTAAACTAACTAACTTTGATCTTGCAAATTCTAGAACATGTCAAATTTTTAGAATGCACGGA**
**TGGAATTGGATTAAAGTTTGTCACGCTATAGCATGTGTTTTAAATAGACAAGGTGGTAAAAGAAATACAGTTCTT**
**TTTCATGGACCAGCAAGTACAGGAAAATCTATTATTGCTCAAGCCATAGCACAAGCTGTGGGTAATGTTGGTTGT**
**TATAATGCAGCAAATGTAAATTTCCATTTAATGACTGTACCAATAAAAATTTAATTTGGATTGAAGAAGCTGGTA**
**ACTTTGGTCAACAAGTTAATCAATTTAAAGCAATTTGTTCTGGACAAACAATTAGAATTGATCAAAAAGGTAAAG**
**GAAGTAAGCAAATTGAACCAACTCCAGTAATTATGACAACTAATGAAAATATAACAATTGTAAGAATTGGATGTG**
**AAGAAAGACCTGAACATACACAACCAATAAGAGACAGAATGTTGAACATTAAGTTAGTATGTAAGCTTCCAGGA**
**GACTTTGGTTTGGTTGATAAAGAAGAATGGCCTTTAATATGTGCATGGTTAGTTAAACATGGTTATGAACCAACC**
**ATGGCTAACTATACACATCATTGGGGAAAAGTACCAGAATGGGATGAAAACTGGGCGGAGCCTAAAATACAAGA**
**AGGTATAAATTCACCAGGTTGCAAAGACTTAGAGACACAAGCGGCAAGCAACCTCAGAGTCAAGACCAAGTTCT**
**AACTCCTCTGACTCCGGACGTAGTGGACCTTGCACTGGAACCGTGGAGTACTCCAGATACGCCTATTGCAGAAA**
**CTGCAAATCAACAATCAAACCAACTTGGCGTTACTCACAAAGACGTGCAAGCGAGTCCGACGTGGTCCGAAATA**
**GAGGCAGACCTGAGAGCCATCTTTACTTCTGAACAATTGGAGGAAGATTTTCGAGACGACTTGGATTAAGGTAC**
**GATGGCACCTCCGGCAAAGAGAGCCAGGAGAGGTAAGGGTGTGTTAGTAAAGTGGGGGGAGGGGAAAGATTTA**
**ATAACTTAACTAAGTATGTGTTTTCTTATAGGACTTGTGCCTCCAGGTTATAAATATCTTGGGCCTGGGAACAGT**
**CTTGACCAAGGAGAACCAACTAACCCTTCTGACGCCGCTGCAAAAGAACACGACGAAGCTTACGCTGCTTATCT**
**TCGCTCTGGTAAAAACCCATACTTATATTTCTCGCCAGCAGATCAACGCTTTATAGATCAAACTAAGGACGCTAA**
**AGATTGGGGGGGGAAAATAGGACATTATTTTTTTAGAGCTAAAAAGGCAATTGCTCCAGTATTAACTGATACACC**
**AGATCATCCATCAACATCAAGACCAACAAAACCAACTAAAAGAAGTAAACCACCACCTCATATTTTCATCAATCT**
**TGCAAAAAAAAAAAAAGCCGGTGCAGGACAAGTAAAAAGAGACAATCTTGCACCAATGAGTGATGGAGCAGTTC**
**AACCAGACGGTGGTCAACCTGCTGTCAGAAATGAAAGAGCTACAGGATCTGGGAACGGGTCTGGAGGCGGGGG**

**TGGTGGTGGTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGACGGAATTTAAATTTTTGGAAA**
**ACGGATGGGTGGAAATCACAGCAAACTCAAGCAGACTTGTACATTTAAATATGCCAGAAAGTGAAAATTATAAA**
**AGAGTAGTTGTAATTAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTTTAGATGATACTCATGTACAAATT**
**GTAACACCTTGGTCATTGGTTGATGCAAATGCTTGGGGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATTGTT**
**AATACTATGAGTGAGTTGCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTTGTTTTAAAGACTGTTTCAGAAT**
**CTGCTACTCAACCACCAACTAAAGTTTATAATAATGATTTAACTGCATCATTGATGGTTGCATTAGATAGTAATA**
**ATACTATGCCATTTACTCCAGCAGCTATGAGATCTGAGACATTGGGTTTTTATCCATGGAAACCAACCATACCAA**
**CTCCATGGAGATATTATTTTCAATGGGATAGAACATTAATACCATCTCATACTGGAACTAGTGGCACACCAACAA**
**ATATATATCATGGTACAGATCCAGATGATGTTCAATTTTATACTATTGAAAATTCTGTGCCAGTACACTTACTAAG**
**AACAGGTGATGAATTTGCTACAGGAACATTTTTTTTTGATTGTAAACCATGTAGACTAACACATACATGGCAAAC**
**AAATAGAGCATTGGGCTTACCACCATTTCTAAATTCTTTGCCTCAATCTGAAGGAGCTACTAACTTTGGTGATAT**
**AGGAGTTCAACAAGATAAAAGACGTGGTGTAACTCAAATGGGAAATACAGACTATATTACTGAAGCTACTATTAT**
**GAGACCAGCTGAGGTTGGTTATAGTGCACCATATTATTCTTTTGAAGCATCTACACAAGGGCCATTTAAAACACC**
**TATTGCAGCAGGACGGGGGGGAGCGCAAACAGATGAAAATCAAGCAGCAGATGGTGATCCAAGATATGCATTT**
**GGTAGACAACATGGTCAAAAAACTACTACAACAGGAGAAACACCTGAGAGATTTACATATATAGCACATCAAGA**
**TACAGGAAGATATCCAGAAGGAGATTGGATTCAAAATATTAACTTTAACCTTCCTGTAACAAATGATAATGTATT**
**GCTACCAACAGATCCAATTGGAGGTAAAACAGGAATTAACTATACTAATATATTTAATACTTATGGTCCTTTAAC**
**TGCATTAAATAATGTACCACCAGTTTATCCAAATGGTCAAATTTGGGATAAAGAATTTGATACTGACTTAAAACC**
**AAGACTTCATGTAAATGCACCATTTGTTTGTCAAAATAATTGTCCTGGTCAATTATTTGTAAAAGTTGCGCCTAAT**
**TTAACAAATGAATATGATCCTGATGCATCTGCTAATATGTCAAGAATTGTGACTTACTCAGATTTTTGGTGGAAA**
**GGTAAATTAGTATTTAAAGCTAAACTAAGAGCATCTCATACTTGGAATCCAATTCAACAAATGAGTATTAATGTA**
**GATAACCAATTTAACTATGTACCAAATAATATTGGAGCTATGAAAATTGTATATGAAA**

The AMC sequence is the result of the alignment of the 30 nucleotide sequences in the Clustal Omega software. Subsequently, to determine what is the percentage of conservation of each of these genes, the 30 nucleotide sequences of the genes that code for NS1, NS2, VP1, and VP2 were aligned separately (Table 2).

**Table 2**. Percentage of conservation of canine and feline Parvovirus genes

| | NS1 | NS2 | VP1 | VP2 |
|---|---|---|---|---|
| Total bases | 1937 | 1900 | 2155 | 1653 |
| Communes bases | 1832 | 1796 | 1976 | 1546 |
| Percentage | 94.57 | 94.53 | 91.69 | 93.52 |

The gene with the highest percentage of conservation corresponds to NS1 (94.57%), which makes it an optimum candidate to implement the diagnosis of parvovirus. Thus, the consensus sequence of the 30 alignments of the genes encoding NS1 is showing in Table 3.

**Table 3.** Consensus sequence of NS1

**AAGCATTTTCGTTTGTTTTTAAATGTGACAACGTTCAACTAAATGGAAAGGATGTTCGCTGGAACAACT**
**ATACCAAACCAATTCAAAATGAAGAGCTAACATCTTTAATTAGAGGAGCACAAACAGCAATGGATCAA**
**ACCGAAGAAGAAGAAATGGACTGGGAATCGGAAGTTGATAGTCTCGCCAAAAAGCAAGTACAAACTTT**
**TGATGCATTAATTAAAAAATGTCTTTTTGAAGTCTTTGTTTCTAAAAATATAGAACCAAATGAATGTGTT**
**TGGTTTATTCAACATGAATGGGGAAAAGATCAAGGCTGGCATTGTCATGTTTTACTTCATAGTAAGAAC**
**TTACAACAAGCAACTGGTAAATGGCTACGCAGACAAATGAATATGTATTGGAGTAGATGGTTGGTGAC**
**TCTTTGTTCGGTAAACTTAACACCAACTGAAAAGATTAAGCTCAGAGAAATTGCAGAAGATAGTGAAT**
**GGGTGACTATATTAACATACAGACATAAGCAAACAAAAAAAGACTATGTTAAAATGGTTCATTTTGGAA**
**ATATGATAGCATATTACTTTTTAACAAAGAAAAAAATTGTCCACATGACAAAAGAAAGTGGCTATTTTT**
**TAAGTACTGATTCTGGTTGGAAATTTAACTTTATGAAGTATCAAGACAGACAAATTGTCAGCACACTTT**
**ACACTGAACAAATGAAACCAGAAACCGTTGAAACCACAGTGACGACAGCACAGGAAACAAAGCGCGG**
**GAGAATTCAAACTAAAAAGGAAGTGTCAATCAAATGTACTTTGCGGGACTTGGTTAGTAAAAGAGTAA**
**CATCACCTGAAGACTGGATGATGTTACAACCAGATAGTTATATTGAAATGATGGCACAACCAGGAGGT**
**GAAAATCTTTTAAAAAATACACTTGAAATTTGTACTTTGACTTTAGCAAGAACAAAAACAGCATTTGAA**
**TTAATACTTGAAAAAGCAGATAATACTAAACTAACTAACTTTGATCTTGCAAATTCTAGAACATGTCAA**
**ATTTTTAGAATGCACGGATGGAATTGGATTAAAGTTTGTCACGCTATAGCATGTGTTTTAAATAGACAA**
**GGTGGTAAAAGAAATACAGTTCTTTTTCATGGACCAGCAAGTACAGGAAAATCTATCATTGCTCAAGC**
**CATAGCACAAGCTGTGGGTAATGTTGGTTGTTATAATGCAGCAAATGTAAATTTTCCATTTAATGACTG**
**TACCAATAAAAATTTAATTTGGATTGAAGAAGCTGGTAACTTTGGTCAACAAGTTAATCAATTTAAAGC**
**AATTTGTTCTGGACAAACAATTAGAATTGATCAAAAAGGTAAAGGAAGTAAGCAAATTGAACCAACTC**
**CAGTAATTATGACAACTAATGAAAATATAACAATTGTGAGAATTGGATGTGAAGAAAGACCTGAACAT**
**ACACAACCAATAAGAGACAGAATGTTGAACATTAAGTTAGTATGTAAGCTTCCAGGAGACTTTGGTTT**
**GGTTGATAAAGAAGAATGGCCTTTAATATGTGCATGGTTAGTTAAACATGGTTATGAATCAACCATGG**
**CTAACTATACACATCATTGGGGAAAAGTACCAGAATGGGATGAAAACTGGGCGGAGCCTAAAATACAA**
**GAAGGTATAAATTCACCAGGTTGCAAAGACTTAGAGACACAAGCGGCAAGCAATCCTCAGAGTCAAGA**
**CCAAGTTCTAACTCCTCTGACTCCGGACGTAGTGGACCTTGCACTGGAACCGTGGAGTACTCCAGATA**
**CGCCTATTGCAGAAACTGCAAATCAGCAATCAAACCAACTTGGCGTTACTCACAAAGACGTGCAAGCG**
**AGTCCGACGTGGTCCGAAATAGAGGCAGACCTGAGAGCCATCTTTACTTCTGAACAATTGGAAGAAGA**
**TTTTCGAGACGACTTGGATTAA**



Subsequently, the Oligo Prefect software was used to generate the primers, the NS1 consensus sequence containing 1938 nucleotides was copied, the regions where there was no base correspondence in the 30 sequences were excluded and the range to be used by the primers was asked. let it be 450-550 nucleotide bases. The program generated 5 primers which are shown in table 4.

**Table 4.** Primers generated from the NS1 consensus sequence.

| Size (bp) | Sequence | % GC | Tm (ºC) | ΔTm (ºC) |
|---|---|---|---|---|
| 460 | AAACCACAGTGACGACAGCA | 50.00 | 60.11 | 0.21 |
| | GCTTGTGCTATGGCTTGAGC | 55.00 | 59.90 | |
| **451** | **TGACGACAGCACAGGAAACA** | **50.00** | **59.82** | **0.08** |
| | **GCTTGTGCTATGGCTTGAGC** | **55.00** | **59.90** | |
| 454 | CAGTGACGACAGCACAGGAA | 55.00 | 60.25 | 0.35 |

| | | | | |
|---|---|---|---|---|
| | GCTTGTGCTATGGCTTGAGC | 55.00 | 59.90 | |
| 466 | CCGTTGAAACCACAGTGACG | 55.00 | 59.70 | 0.2 |
| | GCTTGTGCTATGGCTTGAGC | 55.00 | 59.90 | |
| 453 | AGTGACGACAGCACAGGAAA | 50.00 | 59.54 | 0.36 |
| | GCTTGTGCTATGGCTTGAGC | 55.00 | 59.90 | |

For the choice of the optimal pair of primers, two parameters were considered,% GC obtained from the amount of Guanine and Cytosine present in the designed starter and the Melting Temperature (Tm (ºC)) from which the difference of temperature (ΔT ° C) to decide the best pair of splitters to use.

In this way, the second pair of starters are chosen for the PCR protocol (Table 4), since it has a good% GC and the temperature delta is the lowest of the generated starters. These primers must be synthesized and tested for canine or feline parvovirus. Upon obtaining the results, the fragment obtained must be sent to a commercial company to validate its identity. The sequence delivered by this company must be entered into the BLAST software.

## DISCUSSION

From the Parvoviridae family, the virus that has been most studied is Canine Parvovirus type 2 (CPV-2) and of which there is a greater number of sequences of its genome in the GenBank database concerning Carnivore protoparvovirus 1. Of the 4514 sequences of canine Parvovirus type 2, most are of the genes that code for VP2, 306 sequenced VP1 and VP2 and there are only 145 sequences of the complete genome of the virus including NS1, NS2, VP1, and VP2. About Carnivore protoparvovirus 1, of the 702 sequences that GenBank has, the vast majority are of the genes that encode VP2, similar to what happened with canine Parvovirus type 2, 25 sequences include VP1 and VP2, and only 22 of the complete genome of the virus. The fact that the vast majority of the sequencing is for the genes that code for VP2 occurs because the literature describes that the viral capsid is made up of 90% of the VP2 protein and 10% of the VP1 protein. says that VP2 is the most numerous [5] and is highly conserved [8]. But when carrying out this project it was shown that the genes that code for the NS1 protein are more conserved than those that code for VP2. The percentage of conservation of genes that code for NS1 is 94.57%, for VP2 93.52%. Although the difference in the percentage of conservation of NS1 for VP2 is only 1.05%, it makes it possible to postulate a new target gene for the diagnosis of canine or feline Parvovirus.

## CONCLUSION

Interspecies diagnosis is possible from a PCR protocol since the differences between nucleotide sequences of canine Parvovirus type 2 and Carnivore protoparvovirus 1 are minimal. The common areas can be used for the design of specific partitions. It is important to consider the genes that code for NS1 for the diagnosis of interspecies Parvovirosis since their conservation percentage is high.

Carrying out this tutorial represents the possibility of removing previously acquired knowledge and updating it, considering the advancement of information and technology, since the existence of these bioinformatics tools does not allow having any pretext to face the molecular detection of any pathogen of interest. veterinarian or how to achieve its differentiation, although its genome is highly conserved between different animal species.

## ANEXO 1

GenBank accession number of the complete canine Parvovirus and feline Parvovirus genome sequence.

| | **Canine Parvovirus** | **Feline Parvovirus** |
|---|---|---|
| 1 | MF510158.1 | MH559110.1 |
| 2 | MF510157.1 | MG924893.1 |
| 3 | MF134808.1 | KX685354.1 |
| 4 | MF805798.1 | KX900570.1 |
| 5 | MF805797.1 | KP280068.1 |
| 6 | MF805796.1 | EU659115.1 |
| 7 | MH476585.1 | EU659114.1 |
| Carter and Saunders, 2007 | Carter and Saunders, 2007 | Carter and Saunders, 2007 |
| 9 | MH476583.1 | EU659112.1 |
| 10 | EU659121.1 | EU659111.1 |
| 11 | EU659120.1 | KP019621.2 |
| 12 | EU659119.1 | KX434462.1 |
| 13 | KM457130.1 | KX434461.1 |
| 14 | KM457129.1 | MG764511.1 |
| 15 | KM457128.1 | MG764510.1 |



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