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Case Report
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Detection of feline panleukopenia virus (*Carnivore protoparvovirus 1*) in free-ranging *Panthera onca* in Brazil[1]

Thalita P.P.S. Cruz[2]*, Thais O. Morgado[3], Karen R. Ribeiro[3], Luciano Nakazato[2] and Valéria Dutra[2]

ABSTRACT.- Cruz T.P.P.S., Morgado T.O., Ribeiro K.R., Nakazato L. & Dutra V. 2023. **Detection of feline panleukopenia virus (*Carnivore protoparvovirus 1*) in free-ranging *Panthera onca* in Brazil.** *Pesquisa Veterinária Brasileira 43:e07331, 2023*. Laboratório de Microbiologia Veterinária e Biologia Molecular, Hospital Veterinário, Universidade Federal de Mato Grosso, Av. Fernando Corrêa da Costa 2367, Bairro Boa Esperança, Cuiabá, MT 78060-900, Brazil. E-mail: thaly.prii@hotmail.com

The decline in the jaguar population confirms how much the species is vulnerable to extinction in Brazil. It also indicates the degradation of its natural habitat's environmental integrity and quality. Studies claim that large felids are susceptible to feline panleukopenia virus (FPV) and are presumptively diagnosed clinically in Brazil. A free-living jaguar (*Panthera onca*) cub was found unconscious and rescued due to a possible hit-and-run in the savannah of Mato Grosso. During recovery, it exhibited clinical and hematological signs consistent with FPV infection. The PCR was positive for FPV, with 99.61% identity between the FPV sequences available in the GenBank database through the BLAST tool. Due to habitat restrictions, certain diseases threaten wild cats and habitat encroachment by domestic animals can alter the pattern of spread of pathogens. We highlight the importance of the molecular diagnosis and phylogenetic analysis of FPV to elucidate how it has reached wild felids.

INDEX TERMS: Panleukopenia, jaguar, molecular diagnosis, phylogenetic analysis.

RESUMO.- [Detecção do vírus da panleucopenia felina (*Carnivore protoparvovirus 1*) em *Panthera onca* de vida livre no Brasil.] O declínio da população de onça-pintada confirma o quanto a espécie está vulnerável à extinção no Brasil, indicando também a degradação da integridade ambiental e da qualidade de seu *habitat* natural. Estudos afirmam que felinos de grande porte são suscetíveis ao vírus da panleucopenia felina (FPV) e são diagnosticados clinicamente de forma presuntiva no Brasil. Um filhote de onça-pintada (*Panthera onca*) de vida livre foi encontrado inconsciente e resgatado devido a um possível atropelamento no cerrado do Mato Grosso. Durante a recuperação, apresentou sinais clínicos e hematológicos compatíveis com infecção por FPV. A PCR foi positiva para FPV, com 99,61% de identidade entre as sequências de FPV disponíveis no banco de dados GenBank por meio da ferramenta BLAST. Devido a restrições de *habitat*, certas doenças ameaçam felinos selvagens e a invasão de *habitat* por animais domésticos pode alterar o padrão de propagação de patógenos. Destacamos a importância do diagnóstico molecular e da análise filogenética do FPV para elucidar como ele atinge os felídeos silvestres.

TERMOS DE INDEXAÇÃO: Panleucopenia, onça-pintada, diagnóstico molecular, análise filogenética.

INTRODUCTION

The jaguar is currently considered a species vulnerable to extinction in Brazil, according to the Red Book of Brazilian Fauna (Porfirio 2019). This species is considered an indicator of environmental integrity or quality, as its conservation depends directly on the supply of natural prey and habitat quality (Porfirio 2019). Diseases are a growing threat to wild cats due to habitat restriction and encroachment by domestic animals, which can directly or indirectly spread pathogens and alter disease patterns (Furtado & Filoni 2008). Due to the decline in the jaguar population, epidemiological and health studies involving wild animals have been conducted (Silveira et al. 2018).

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[2] Laboratório de Microbiologia Veterinária e Biologia Molecular, Hospital Veterinário, Universidade Federal de Mato Grosso (UFMT), Av. Fernando Corrêa da Costa 2367, Bairro Boa Esperança, Cuiabá, MT 78060-900, Brazil. *Corresponding author: thaly.prii@hotmail.com

[3] Departamento de Clínica Médica de Animais Silvestres, Hospital Veterinário, Universidade Federal de Mato Grosso (UFMT), Av. Fernando Corrêa da Costa 2367, Bairro Boa Esperança, Cuiabá, MT 78060-900, Brazil

Committee on Taxonomy of Viruses (ICTV) considers both canine parvovirus type 2 (CPV-2) and feline panleukopenia virus (FPV) belonging to the same species, along with mink enteritis virus (MEV) and raccoon parvovirus (RaPV), that have been recently included in the species *Carnivore protoparvovirus 1*, member of the *Protoparvovirus* genus (family Parvoviridae, subfamily Parvovirinae) (Cotmore et al. 2019, Mira et al. 2019).

Carnivore protoparvovirus 1 causes severe enteric disease with a high fatality rate in pets (especially in puppies and young kittens non-immunized), as well as in a wide range of susceptible and endangered wild hosts of extinction (Decaro et al. 2020, Tegegne et al. 2022). Both CPV-2 and FPV have been detected in wild carnivores of different genera across the world, with cross-species transmission at the domestic-wildlife interface still evident in some countries, such as South America, making virus dynamics and evolution rather complex (Santana et al. 2022). Feline panleukopenia is a highly contagious disease caused by the feline panleukopenia virus (FPV), a small, linear, non-segmented, non-enveloped species of feline parvovirus (Rice 2017). The viral genome is a single strand of DNA with tropism in mitotically active tissues (Martins Del Barrio 2016).

The affected young cats present with the classic manifestation of the disease, including intestinal crypts and bone marrow cell depletion, leading to diarrhea that can be hemorrhagic, anorexia, vomiting, and white cell depletion (neutropenia and lymphopenia) (Stuetzer & Hartmann 2014). Direct contact between carnivores is not necessary for efficient transmission (Demeter et al. 2010).

This report addresses the detection of FPV DNA in a jaguar cub, the first molecular diagnosis of FPV in a naturally infected jaguar in Brazil.

CASE REPORT

A jaguar (*Panthera onca*), approximately three months old, was found on the side of the road in the Cerrado of Mato Grosso, suspected to have been run over. The animal was rescued, referred for emergency evaluation, and received symptomatic treatment based on suspicion of cranioencephalic and abdominal trauma. Following an improved state of consciousness, the animal presented with a seizure controlled by diazepam administration and treated with phenobarbital.

The animal was then referred to a tertiary veterinary hospital, where CSF and blood samples were collected for hematological, cytological, and molecular tests for canine distemper virus, *Neospora caninum*, *Toxoplasma gondii*, *Cryptococcus* spp., *Ehrlichia* spp., *Babesia* spp., *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Coccidioides* spp. as well as serological tests for distemper, leishmaniasis, feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV). All tests were negative.

Approximately two months after the rescue, the animal was apparently healthy with no laboratory abnormalities and was thus transferred to a larger enclosure for its well-being. In this area, the presence of stray domestic animals is observed in the neighborhood, and three weeks after transfer to the enclosure, the animal presented with anorexia, emesis, diarrhea, and stereotyped pacing behavior. Blood count revealed severe leukopenia (300 leukocytes/mm^3), preventing the white cells' differential count. The symptoms were consistent with feline panleukopenia; therefore, whole blood and rectal swab samples were collected for the molecular diagnosis of FPV. The following day, the animal died and was referred for necropsy.

Necropsy revealed alterations suggestive of parvovirus infection, such as dehydration, anemia, distended segments of intestinal loops, with evident vascularization on the serous surface, thickened mucosa with a slightly yellowish granular appearance (Fig.1). It was also observed enteritis, multifocal crypt necrosis, moderate infiltration of mononuclear cells, shortening of intestinal villi, and lymphoid depletion. In the CNS, the encephalic meninges were opaque with an edematous appearance characterized by flattened gyri and thinned sulci. The frontal, parietal, and occipital cortices exhibited polioencephalomalacia (cerebrocortical necrosis) (Fig.2). Microscopically, severe depletion of mucosa-associated lymphoid tissue (MALT), diffuse and marked necrotic enteritis

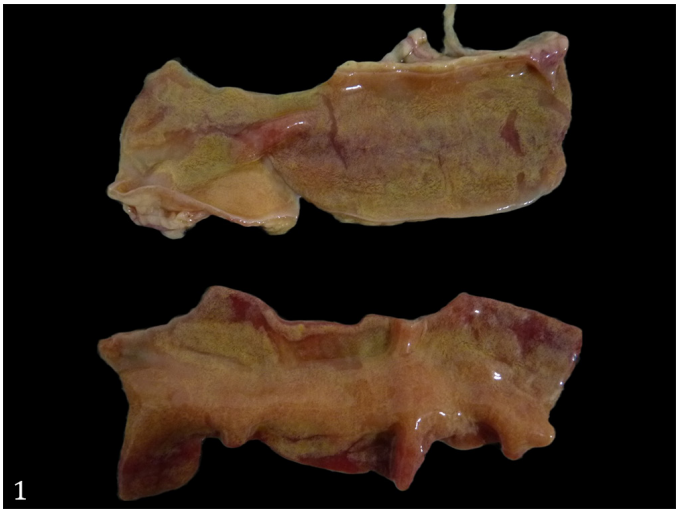

Fig.1. Macroscopic alterations observed in the jaguar's gastrointestinal system (intestine) affected by feline panleukopenia. Distended segments of intestinal loops, with evident vascularization on the serous surface, thickened mucosa, with a slightly yellowish granular appearance.

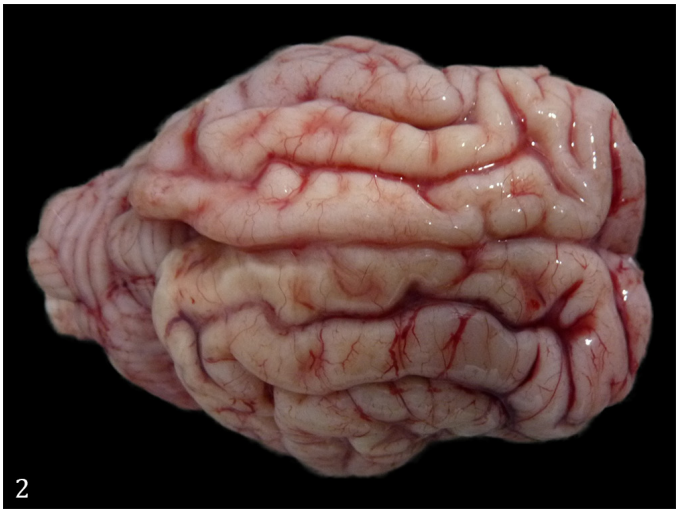

Fig.2. Brain. Macroscopic alterations observed in the central nervous system of the jaguar affected by feline panleukopenia. Meninges were opaque with an edematous appearance characterized by flattened gyri and thinned sulci.

with necrosis of crypt cells, fusion and flattening of villi and submucosal edema was observed in the small intestine (Fig.3).

According to the manufacturer's instructions, DNA extraction from the rectal swab was performed using the MagMAX Sample Extraction Kit (Thermo Fisher®). PCR was performed using oligonucleotides 5′-ATGAGTGATGGAGCAGTTCAACC-3′ and 5′-GGATCACCATCTGCTGCTTG-3′, as described by Yoon et al. (2009), which amplifies an 1127bp fragment (a gene that encodes a fragment of the VP2 viral capsid protein) and ultrapure water used as a negative control.

The reactions were performed in a thermocycler (SimpliAmp Thermo Cycler, Thermo Fischer Scientific™) as described by Yoon et al. (2009). The PCR products were subjected to agarose gel electrophoresis, stained with GelRedTM (Nucleic Acid Gel stain, Biotium®) at 10V/cm, and visualized in a ChemiDoc™ XRS photodocumenter using Image Lab™ software to verify the results. The molecular mass marker used was ladder 100bp (Ludwig®). Obtaining the specific PCR product for the gene that encodes a fragment of the VP2 viral capsid protein at the height of 1,127bp, according to the positive control used in the reaction, confirmed the detection of feline parvovirus in the tested sample, indicating, thus, feline panleukopenia.

The product obtained by PCR was purified using the GE Healthcare Illustra GFX™ Kit and used in the sequencing reaction, together with BigDye Terminator Ready Reaction Cycle Sequencing (Applied Biosystems) in the automatic sequencer ABI 3500 Genetic Analyzer (Applied Biosystems). The sequence was matched and deposited (GenBank MZ883094) in the GenBank database using BLAST on the NCBI server[4], showing 99.61% similarity with the FPV (MF541125 GenBank) and phylogenetic analysis corroborated this sequence as FPV group. Phylogenetic analysis was performed by comparing g nucleotide sequences with other parvoviruses using software (Muscle) and phylogenetically analyzed using PhyML[5] and tree design in iTol[6] (Fig.4).

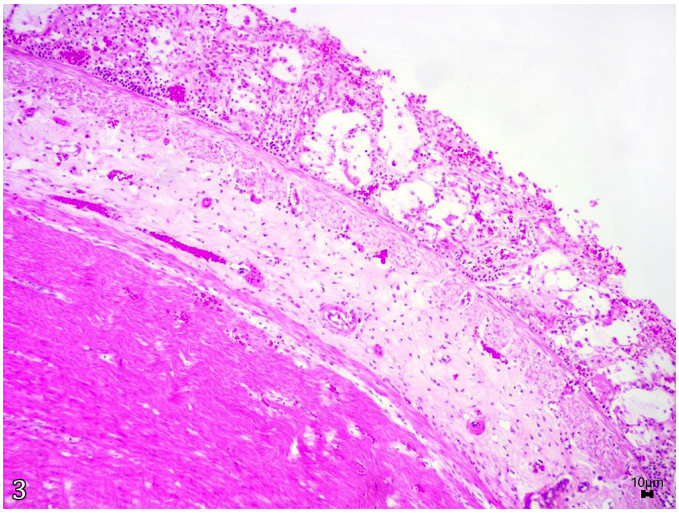

Fig.3. Intestine. Severe depletion of mucosa-associated lymphoid tissue (MALT), diffuse and marked necrotic enteritis with necrosis of crypt cells, fusion and flattening of villi and submucosal edema. HE, obj.10x.

DISCUSSION AND CONCLUSION

The clinicopathological correlation of the histopathological findings associated with the detection of FPV by complementary exam (PCR) was compatible with an infection of FPV. Several clinical and serological studies have confirmed that large felids are more susceptible to FPV. The animal was approximately six months old in the present case, corroborating the literature that mentions that FPV affects young domestic and wild felids (Duarte et al. 2009, Castro et al. 2014, Barrs 2019).

The gastrointestinal clinical signs, anorexia, and blood count findings the day before death are also consistent with the literature, as described by Castro et al. (2014) and Stuetzer & Hartmann (2014), who reported that young infected animals manifest the classic form of the disease, presenting with depletion of intestinal crypts and bone marrow cells, leading to diarrhea, anorexia, vomiting, and depletion of white cells. According to Truyen et al. (2009), PCR can be conducted utilizing blood or feces, with the preference for blood usage in cats lacking diarrhea symptoms. For the current research, the decision was made to employ a rectal swab for PCR, given the recent manifestation of distinct diarrhea symptoms in the animal.

Necropsy revealed pathological changes suggestive of parvovirus infection in the intestine, as described in previous literature (Demeter et al. 2010, Stuetzer & Hartmann 2014). It was not possible to determine the specific etiology of laminar polioencephalomalacia. However, this alteration may be directly or indirectly associated with thiamine deficiency in carnivores, although lesions were not observed in anatomical locations specific to this etiology, such as in histological sections of the caudal colliculus (Sykes 2013, Maxie 2016, Zachary 2017, Terio et al. 2018).

Vaccines have reduced the frequency of FPV infection in urban domestic cats, but the virus persists in the outskirts

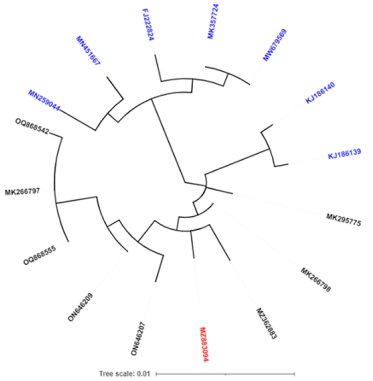

Fig.4. Phylogenetic analysis based on the nucleotide sequence of the VP2 gene of feline panleukopenia virus – FPV (black) and canine parvovirus – CPV (blue) based Muscle alignment software, PhyML[5] and tree design in iTol[6]. The Jaguar sequence is red.

[4] Available at <http: www.ncbi.nlm.nih.gov/BLAST> Accessed on Oct. 24, 2022.

[5] Available at <www.phylogeny.fr> Accessed on Aug. 17, 2023.

[6] Available at <itol.embl.de> Accessed on Aug. 17, 2023.

due to strays and limited resources. Wild cats are at risk when interacting with strays from these areas (Day et al. 2016). Wild felids are at a higher risk of exposure when they meet stray animals from urban outskirts, as in the study of Duarte et al. (2009), where a phylogenetic analysis of FPV strains from Lisbon domestic cats demonstrated striking similarity to strains found in tigers and lions.

In Brazil, cases of FPV occasionally occur in institutions that keep wild felids in captivity, with only a presumptive clinical diagnosis being made (Filoni 2006). Molecular diagnosis is essential because of its high sensitivity and specificity. Canine parvovirus (CPV) can be isolated from healthy and diseased cats (Stuetzer & Hartmann 2014). However, only 5% of feline panleukopenia cases are caused by CPV variants, specifically CPV-2a, b, and c (Barrs 2019).

The surveillance of roadkill and collected wildlife serves as an indicator of instances involving and exposing wild animals to the FPV and its variants. Such occurrences can arise due to the encroachment of domestic animals into natural habitats, rendering these creatures more vulnerable to diseases and vehicle collisions.

This study's primary limitations involve presenting findings from a single case of a wild animal, from which we procured and analyzed merely a partial sequence. The sequenced fragment spanned 1,022bp and was localized within the VP2 capsid protein region of the parvovirus, impeding a more precise depiction of the prevalence and influence of FPV in these creatures. Furthermore, there was no data on *Protoparvovirus 1* molecular characterization of stray animals (dog and cat) in the city, which hampered a complete association with the infection source.

Another issue is the infection timeline since clinical signs begin after rehabilitation enclosure, but infection during hospitalization cannot be ruled out. These environments must implement rules to avoid direct and indirect contact with domestic animals that may carry infectious agents and transmit them to wild animals undergoing treatment. It is necessary to implement stricter measures to prevent possible contact between these animals and potential carriers of FPV.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

REFERENCES

Barrs V.R. 2019. Feline panleukopenia: A re-emergent disease. Vet. Clin. N. Am., Small Anim. Pract. 49(4):651-670. <https://dx.doi.org/10.1016/j.cvsm.2019.02.006> <PMid:30967253>

Castro N.B., Rolim V.M., Hesse K.L., Oliveira E.C., Almeida P.R., Wouters A.T.B., Driemeier D. & Sonne L. 2014. Achados patológicos e imuno-histoquímicos em felinos domésticos com panleucopenia felina. Pesq. Vet. Bras. 34(8):770-775. <https://dx.doi.org/10.1590/S0100-736X2014000800011>

Cotmore S.F., Agbandje-McKenna M., Canuti M., Chiorini J.A., Eis-Hubinger A.-M., Hughes J., Mietzsch M., Modha S., Ogliastro M., Pénzes J.J., Pintel D.J., Qiu J., Soderlund-Venermo M., Tattersall P. & Tijssen P. 2019 Ictv Report Consortium. ICTV virus taxonomy profile: Parvoviridae. J. Gen. Virol. 100(3):367-368. <https://dx.doi.org/10.1099/jgv.0.001212> <PMid:30672729>

Day M.J., Horzinek M.C., Schultz R.D. & Squires R.A. 2016. Diretrizes para a vacinação de cães e gatos. J. Small Anim. Pract. 57:699-706. Available at <chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://wsava.org/wp-content/uploads/2020/01/WSAVA-vaccination-guidelines-2015-Portuguese.pdf> Accessed on Aug. 2, 2023.

Decaro N., Buonavoglia C. & Barrs V. 2020. Canine parvovirus vaccination and immunisation failures: are we far from disease eradication? Vet. Microbiol. 247:108760. <https://dx.doi.org/10.1016/j.vetmic.2020.108760> <PMid:32768213>

Demeter Z., Palade E.A. & Rusvai M. 2010. Feline panleukopenia virus infection in various species from Hungary. Lucr. St. Med. Vet. 43(1):73-81.

Duarte M.D., Barros S.C., Henriques M., Fernandes T.L., Bernardino R., Monteiro M. & Fevereiro M. 2009. Fatal infection with feline panleukopenia virus in two captive wild carnivores (*Panthera tigris* and *Panthera leo*). J. Zoo Wildl. Med. 40(2):354-359. <https://dx.doi.org/10.1638/2008-0015.1> <PMid:19569486>

Filoni C. 2006. Exposição de felídeos selvagens a agentes infecciosos selecionados. Tese de Doutorado, Universidade de São Paulo (USP), São Paulo. 126p. <https://dx.doi.org/10.11606/T.10.2006.tde-26012007-171939>

Furtado M.M. & Filoni C. 2008. Diseases and their role for jaguar conservation. Cat News Special Issue (4):35-40.

Martins Del Barrio M.A. 2016. Doenças infectocontagiosas, p.693-698. In: Mazzotti G.A. & Roza M.R. (Eds), Medicina Felina Essencial. Equalis, Brasil.

Maxie M.G. 2016. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol. 1. 6th ed. Elsevier, St. Louis.

Mira F., Purpari G., Di Bella S., Colaianni M.L., Schirò G., Chiaramonte G., Gucciardi F., Pisano P., Lastra A., Decaro N. & Guercio A. 2019. Spreading of canine parvovirus type 2c mutants of Asian origin in southern Italy. Transbound. Emerg. Dis. 66(6):2297-2304. <https://dx.doi.org/10.1111/tbed.13283> <PMid:31254456>

Porfirio G. 2019. Etnozoologia e conservação da onça-pintada (*Panthera onca*) no Brasil. Interações, 20(2):559-574. <https://dx.doi.org/10.20435/inter.v0i0.1717>

Rice J.K. 2017. Successful treatment of feline panleukopenia: a guideline for rescuers and veterinarians, part I. J. Vet. Sci. Med. Diagn. 6(2). <https://dx.doi.org/10.4172/2325-9590.1000223>

Santana W.O., Silveira V.P., Wolf J.M., Kipper D., Echeverrigaray S., Canal C.W., Truyen U., Lunge V.R. & Streck A.F. 2022. Molecular phylogenetic assessment of the canine parvovirus 2 worldwide and analysis of the genetic diversity and temporal spreading in Brazil. Infect. Genet. Evol. 98:105225. <https://dx.doi.org/10.1016/j.meegid.2022.105225> <PMid:35101636>

Silveira M.M., Candido S.L., Dutra V., Miyazaki S.S. & Nakazato L. 2018. Detection of noroviruses in free-ranging jaguars (*Panthera onca*) in the Pantanal, Mato Grosso, Brazil. Arch. Virol. 163(7):1961-1963. <https://dx.doi.org/10.1007/s00705-018-3789-7> <PMid:29516245>

Stuetzer B. & Hartmann K. 2014. Feline parvovirus infection and associated diseases. Vet. J. 201(2):150-155. <https://dx.doi.org/10.1016/j.tvjl.2014.05.027> <PMid:24923754>

Sykes J.E. 2013. Canine and Feline Infectious Diseases. Elsevier Health Sciences. p.187-194. <https://dx.doi.org/10.1016/C2009-0-41370-9>

Tegegne D., Tsegaye G., Faustini G. & Franzo G. 2022. First genetic detection and characterization of canine parvovirus type 2 (*Carnivore protoparvovirus 1*) in southwestern Ethiopia. Vet. Res. Commun. 47(2):975-980. <https://dx.doi.org/10.1007/s11259-022-10027-4> <PMid:36322244>

Terio K.A., McAloose D. & Leger J.St. 2018. Pathology of Wildlife and Zoo Animals. Elsevier, p.263-283. <https://dx.doi.org/10.1016/C2015-0-01586-6>

Truyen U., Addie D., Belák S., Boucraut-Baralon C., Egberink H., Frymus T., Gruffydd-Jones T., Hartmann K., Hosie M.J., Lloret A., Lutz H., Marsilio F., Pennisi M.G., Radford A.D., Thiry E. & Horzinek M.C. 2009. Feline panleukopenia. ABCD guidelines on prevention and management. J. Feline Med. Surg. 11(7):538-546. <https://dx.doi.org/10.1016/j.jfms.2009.05.002> <PMid:19481033>

Yoon S.H., Jeong W., Kim H.-J. & An D.-J. 2009. Molecular insights into the phylogeny of canine parvovirus 2 (CPV-2) with emphasis on Korean isolates: A Bayesian approach. Arch. Virol. 154(8):1353-1360. <https://dx.doi.org/10.1007/s00705-009-0444-3> <PMid:19590812>

Zachary J.F. 2017. Pathologic Basis of Veterinary Disease Expert Consult. 6th ed. Elsevier. 1408p. (E-book ISBN: 978-0-323-35775-3).