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CLINICAL AND PATHOLOGICAL ASPECTS, DIAGNOSIS, PROPHYLAXIS AND CONTROL IN PORCINE CIRCOVIRUS TYPE 3 INFECTION IN NEWBORN, WEANED, OR FATTENING PIGLETS – a literature review

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Abstract

PCV-3 infection causes a wide variety of clinical signs in affected pigs, ranging from dermato-nephrotic syndrome, similar to that caused by PCV2, to reproductive, digestive, and nervous disorders and mortality in piglets. Researchers investigating this virus have observed that these manifestations can be caused or aggravated by association with other viral or bacterial pathogens.

Key words: clinical aspects, diagnosis, prophylaxis, control, PCV-3, pigs

Introduction

Although the virus was discovered almost nine years ago and is believed to have spread undetected among pig populations worldwide over the last 20-30 years, not all aspects of its pathogenesis are yet known. Molecular and genetic characterization has been achieved, but the mechanisms by which PCV3 causes the observed lesions and clinical signs have not yet been identified in detail.

The macroscopic and, especially, microscopic lesion patterns have been described in detail more in studies that experimentally infected young piglets with PCV3 than in analyses of naturally infected animals. These studies also provided important data on the dynamics of viremia, antibody titers, and disease progression.

Last but not least, the techniques and methods used to identify the virus have been developed as protocols, for the most part, in the laboratories where they were used. Several researchers point out that standardized techniques (e.g., immunohistochemistry) will need to be developed in the future for detecting the virus in tissue samples.

Clinical aspects

The clinical signs associated with PCV3 are very diverse, sometimes similar to those produced by PCV2, and vary depending on the age and production category of the affected animals. Several studies identify respiratory symptoms such as dyspnea, interstitial pneumonia, bronchitis, or various bronchopneumonia in newborn, weaned, or fattening piglets (Phan T.G. *et al*, 2016; Palinski R. *et al*, 2017; Zhai S.L. *et al*, 2017; Shen H. *et al*, 2018; Kedkovik R. *et al*, 2018; Kim S.H. *et al,* 2018; Qi S. *et al*, 2019).

Other clinical signs associated with PCV3 infection are digestive in nature and include diarrhea, vomiting, and dehydration, especially in newborn or suckling piglets (Zhai S.L. *et al*, 2017; Qi S. *et al*, 2019).

Pregnant sows can be affected by this virus and may experience various reproductive disorders (Figure 2), ranging from abortion at different stages of embryo development, retention of dead fetuses, delivery of dead fetuses, or even death (Ku X. *et al*, 2017; Palinski R. *et al*, 2017; Faccini S. *et al*, 2017; Zou Y. *et al*, 2018; Tochetto C. *et al*, 2018; Dal Santo A.C., *et al* 2020).

During pregnancy and breastfeeding, PCV3 can cause death in newborns or infants, the birth of underdeveloped newborns or newborns with myocardial and nervous system pathologies (Kim S.C. *et al*, 2018a; Deim Z. *et al*, 2019; Arruda B. *et al*, 2019). For piglets during the suckling period, the clinical signs associated with PCV3 infection can be varied. Some authors have reported congenital or acquired tremors of neurological origin (monocytic encephalitis, encephalomyelitis, vasculitis, gliosis), weakness, arrhythmias (caused by myocarditis), and reduced growth rate (Phan T.G. *et al*, 2016; Chen D. *et al*, 2017; Arruda B. *et al*, 2019; Williamson S. *et al*, 2021).

In addition, clinical signs associated with PDNS (dermato-nephrotic syndrome), such as

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erythema, alopecia, skin erosions, skin necrosis, renal failure, and proteinuria, were observed in piglets and sows (Palinski R. *et al*, 2017; Arruda B. *et al*, 2019).

In young or newborn piglets, cardio-respiratory clinical signs associated with myocarditis and periarteritis, which can be caused by PCV3 infection, as well as joint changes (arthrogryposis) have also been observed (Phan T.G. *et al*, 2016; Williamson S. *et al*, 2021). An experimental study published in 2025 tracked and illustrated how PCV2, PCV3, and PCV4 infections affect infected piglets differently (Zheng J. *et al*, 2025). The results are similar to those previously reported, but a number of other aspects regarding clinical signs are also mentioned. All three viruses caused fever, but it did not exceed 38-40^{o} C. Infection with PCV3, as well as PCV4, caused erythematous dermatitis approximately 4-8 days post-infection (dPI), which then disappeared at approximately 20 dPI. Dyspnea, respiratory noises, loss of appetite, and lethargy were observed, symptoms that disappeared at approximately 14-22 dPI (Zheng J. *et al*, 2025).

Pathological aspects

A series of studies have established a strong correlation between the presence of PCV3 (identified by immunohistochemistry as the sole pathogen in the tissues tested) and morphopathological lesions in naturally infected animals (Phan T.G. *et al*, 2016; Palinski R. *et al*, 2017; Arruda B. *et al*, 2019).

PCV3 was identified in macrophages, cardiomyocytes, and leukocytes of the cardiac arteries and correlated with lesions such as myocarditis, anorexia, and progressive weight loss (Phan T.G. *et al*, 2016; Arruda B. *et al*, 2019). Palinski localizes the virus in the skin, in the cytoplasm of dermal, follicular, and perifollicular lymphocytes, but also in the kidneys, especially in the epithelium of the urinary tubules and in the interstitium, in pigs with PDNS. Associated lesions were necrotizing vasculitis, vascular fibrinoidosis, lymphohistiocytic arteritis, multifocal granulomatous lymphadenitis, and glomerulonephritis (Palinski R. *et al*, 2017; Vargas-Bermudez D.S. *et al*, 2021).

Respiratory PCV3 was identified in alveolar macrophages and lymphocytes and associated with lesions such as lymphocytic perivascular and peribronchiolar sleeves, thickening of interalveolar septa, interstitial pneumonia, necrotic pneumonia, and bronchiolitis (Kim S.C. *et al*, 2018; Kedkovid R. *et al*, 2018; Tan C.Y. *et al*, 2020; Vargas-Bermudez D.S. *et al*, 2021).

PCV3 can also affect the nervous system, both white and gray matter, causing encephalitis and the formation of perivascular sleeves made up of lymphocytes and glial cells (Arruda, 2019).

Last but not least, PCV3 causes damage to the reproductive system (congestion, necrosis, various types of inflammation), being identified in trophoblasts, placenta, live or even mummified fetuses (Arruda B. *et al*, 2019; Vargas-Bermudez D.S. *et al*, 2021).

In 2025, Zheng and colleagues succeeded in illustrating a very detailed anatomopathological picture of PCV2, PCV3, and PCV4 infection in a comparative experimental study (Figures 3). In this study, tissue samples were collected from various organs (heart, liver, spleen, lungs, kidneys, intestines, lymph nodes) at 35 zPI.

In the case of PCV2 and PCV3 infection, the piglets were found to be affected by dermatitis and hemorrhages in the ears. In the case of PCV3 and PCV4 infection, pleural and peritoneal effusions were found. In the heart, deformities of the apex were observed in the case of PCV3 infection.

In the liver, the effects of PCV3 caused an irregular appearance in terms of color and texture, as well as jaundice.

Lungs affected by PCV3 infection showed fibrous inflammation, while PCV 2 and 4 caused less severe lesions.

The kidneys from pigs infected with PCV3 were discolored and had whitish spots of necrosis on their surface.

Discrete lesions were observed in the intestine, such as the presence of vacuoles in the mucosa and serosa, as well as petechiae.

From a histopathological point of view, the microscopic lesions observed were consistent with those observed during the necropsy. In the heart, PCV3 caused edema of the cardiac muscle fibers, edema of the interstitial space, and the presence of a predominantly mononuclear inflammatory infiltrate.

The liver of pigs infected with PCV3 showed areas of focal necrosis located at the periphery of the centro-lobular veins, pyknosis and karyorrhexis in the hepatocyte, and the presence of a predominantly mononuclear inflammatory infiltrate.

At the splenic level, a reduction in cellularity was observed in the red pulp and white pulp (lymphopenia, lymphoclasia).

The lungs showed degeneration of the alveolar and bronchial epithelium, the presence of an inflammatory infiltrate in the interstitial space,

which also caused thickening and stiffening of the interalveolar septa.

The kidneys showed glomerular atrophy in the cortex, interstitial vasodilation, and steatosis of the proximal convoluted tubules.

PCV3 also caused intestinal lesions, manifested by desquamation of the mucosal epithelium and changes in the tissue architecture of the glands in the mucosa and submucosa.

The lymph nodes showed vacuolization of lymphocytes in the paracortical area, as well as lymphocyte depletion in the germinal centers (Zheng J. *et al*, 2025).

Using immunohistochemistry, the same study showed PCV3 in cardiomyocytes and alveolar pneumocytes (Zheng J. *et al*, 2025).

In another experimental study conducted in 2019, Jian and colleagues observed similar lesions in pigs infected with PCV3. The lungs were collapsed, presenting lobular pneumonia, bronchopneumonia, multifocal hemorrhage, or localized consolidation. The lymph nodes were enlarged, 2-3 times their normal size, and their consistency was increased.

The liver was congested, with light-colored nodules and areas of necrosis. The kidneys were enlarged and dotted with petechiae. The spleen was enlarged, with marginal hemorrhagic foci (Jiang S. *et al*, 2019).

From a histopathological point of view, pulmonary lesions were represented by lymphoplasmacytic interstitial bronchopneumonia or mixed inflammatory infiltrate (neutrophils/histiocytes, epithelial cells, macrophages, lymphocytes, and plasma cells), perivascular and peribronchiolar predominantly located. Thickening of the bronchial submucosa and interalveolar septa was observed, as well as accumulation of purulent inflammatory exudate in the bronchial lumen and alveolar cavity.

In the tracheobronchial lymph nodes, the cortical and medullary blood vessels were ectatic, and areas of lymphocytic necrosis and hemorrhage were present. The inguinal lymph nodes showed cortical lymphopenia and epithelial cell hyperplasia. The lymph nodes showed lymphocytic necrosis, lymphoid depletion, abundant hemosiderin deposition, and clusters of natural killer cells, as well as abundant eosinophilic infiltrates (in the tracheobronchial, mesenteric, and inguinal lymph nodes).

In the spleen, lymphocytosis was observed in the white pulp, rarefaction of lymphoid follicles, hemorrhages, and necrosis at the margins of lymphoid follicles. In the red pulp, dilatation of blood sinuses and necrosis of reticular cells were observed.

The kidneys showed dilation of the convoluted tubules, with partial degeneration and regeneration of their epithelium, inflammatory infiltrate (lymphocytes, macrophages) at the interstitial and glomerular levels.

At the hepatic level, sinusoidal capillary ectasia and hepatocyte degeneration were observed.

At the cardiac level, the cardiac muscle fibers were swollen, in myolysis and with pyknotic nuclei, and the interstitium showed edema and focal hemorrhages. The epicardium was thickened, with areas of necrosis and an eosinophilic and lymphocytic infiltrate.

The small intestine showed mucosal de-epithelialization, local pyknosis of enterocytes, necrosis, and lymphocytic and eosinophilic infiltrate in the submucosa.

Another study from 2021, conducted by Temeeyasen and colleagues, highlighted a series of histological lesions similar to those already mentioned in pigs experimentally infected with PCV3. Among these, the most important were lymphoplasmacytic encephalitis with perivasculitis, multifocal lymphoplasmacytic hepatitis, lymphoplasmacytic interstitial nephritis, and lymphoplasmacytic periarteritis and myocarditis. Lymphoplasmacytic periarteritis and arteritis were found in the peritoneal cavity and in the serosa of the intestine and spleen (Temeeyasen G. *et al*, 2021).

Similar results were obtained by Saporiti in 2021 in a study conducted on weaned piglets naturally infected with PCV3, which showed slowed growth. After necropsy, tissue samples were collected from the cerebellum, brain, kidneys, lungs, spleen, large and small intestines, liver, and nasal turbinates.

Microscopically, the pigs showed moderate to severe multiorgan lymphoplasmacytic periarteritis and arteritis in the mesenteric arteries, heart, kidneys, spleen, portal arterioles, meninges, lungs, and/or stomach. The lesion appeared as circumferential or segmental lymphoplasmacytic inflammation affecting the periarteriolar connective tissue and adventitia, or the tunica media and intima. Vacuolization was observed in the smooth muscle cells of the tunica media and detachment of endothelial cells. The central nervous system presented mild lymphoplasmacytic meningoencephalitis, characterized by diffuse gliosis and the formation of discrete multifocal perivascular sleeves. Generalized lymphoplasmacytic myocarditis, mild lymphoplasmacytic and histiocytic interstitial pneumonia, mild lymphoplasmacytic nephritis, and mild lymphoplasmacytic periportal hepatitis were

also observed. The nasal cones were examined in only 2 pigs, which presented mild lymphoplasmacytic rhinitis and mild lymphoplasmacytic periarteritis (Saporiti V., 2021).

Diagnosis

In animals affected by natural infection, the best methods for establishing a clear causal relationship between the presence of PCV3 and the clinical signs or lesions observed are in situ hybridization (ISH) and immunohistochemistry (IHC).

Firstly, the authors of various studies investigating PCV3 infection argue that only this virus should be identified in the tissues tested, and not other co-infecting pathogens. PCV3 should ideally be identified from areas of lesions in the various affected tissues and organs. In situ hybridization (ISH) is preferred by some authors because there are no standardized antibodies for immunohistochemical reactions (Palinski R. *et al*, 2017; Li X. *et al*, 2018). PCV3 was identified by ISH in affected cardiac cells in a study by Phan in 2016. Palinski and colleagues were able to localize the virus in affected cells in the skin, kidneys, and lymphoid tissue of pigs affected by PDNS. Last but not least, Arruda, in 2019, also used ISH to identify PCV 3 in different tissues from fetuses, newborn piglets, or weaned piglets (Arruda B. *et al*, 2019; Saporiti V., 2021).

In addition to identifying PCV-3 in lesion tissues, viral DNA has also been detected in serum (Klaumann F. *et al*, 2018a; Kedkovid R. *et al*, 2018a; Tochetto C. *et al*, 2020), oral secretions (Klaumann F. *et al*, 2018a), feces (Collins P.J. *et al*, 2017), semen (KuX. *et al*, 2017), colostrum (Kedkovid R. *et al*, 2018a), and a range of different tissue samples, with or without lesions (thymus, lymph nodes, spleen, lungs, kidneys, liver, intestine, CNS, placenta, uterus, fetuses) (Palinski R. *et al*, 2017; Klaumann F. *et al*, 2018b, c; Wang W. *et al*, 2019; Woźniak A. *et al*, 2020), most likely due to systemic circulation via the bloodstream.

Techniques can be divided into detection of the infectious agent or its genetic material and detection of antibodies. Among the former, the most commonly used methods are molecular techniques such as PCR and qPCR. Sanger sequencing and NGS are used for phylogenetic studies (Phan T.G. *et al*, 2016; Palinski R. *et al*, 2017; Ku X. *et al*, 2017; Fux, 2018; Ye, 2018; Yuzhakov A.G. *et al*, 2018; Wen S. *et al*, 2018; Arruda B. *et al*, 2019; Qi S. *et al*, 2019; Wang W. *et al*, 2019).

The serological tests used to date are very limited. ELISA and reverse ELISA, based on targeting recombinant and purified PCV-3 cap protein, most often produced in the laboratory where the research is conducted, have been used by several researchers (Palinski R. *et al*, 2017; Deim Z. *et al*, 2018; Mora-Diaz J. *et al*, 2020) to detect and evaluate the reactivity of specific anti-PCV3 antibodies.

Techniques such as ISH and IHC have been adapted and used to detect the PCV-3 genome or antigen in FFPE (formalin-fixed paraffin-embedded) tissues. In situ hybridization localizes the viral genome in tissues using a probe (Kedkovid R. *et al*, 2018). A special type of ISH, called RNA scope, can also be used to identify the presence of PCV-3 in the cells in which it replicates, with the reaction being visualized by a color marker (Phan T.G. *et al*, 2016; Arruda B. *et al*, 2019; Mora-Díaz J. *et al*, 2020; Vargas-Bermúdez D.S. *et al*, 2021).

The RNAscope in situ hybridization used by Mora-Diaz in 2020 involved the use of probes designed based on the 270–1357 region of the PCV2 capsid genes for detection, in order to exclude contamination or co-infection, as well as the 2–1049 region for specific detection of PCV3. For in vitro detection, PK-15 cells were inoculated into chamber slides and infected with virus.

Cell smears were hybridized with PCV2- and PCV3-specific probes, washed, and subjected to an amplification (amp) sequence as follows: amp 1 for 30 minutes at 40 °C; Amp. 2 for 15 min at 40 °C; Amp. 3 for 30 min at 40 °C; Amp. 4 for 15 min at 40 °C; Amp. 5 for 30 min at room temperature (RT); Amp. 6 for 15 min at RT.

Red signals were formed to indicate the presence of PCV3 labeled with peroxidase by incubating the slides with a freshly prepared red solution for 10 min, also at RT. Green signals were formed to identify alkaline phosphatase-labeled PCV2 by applying a freshly prepared green solution for 10 min at RT. The slides were counterstained with hematoxylin (Mora-Diaz J. *et al*, 2020).

Immunohistochemistry is used to detect cells infected with viral antigen, usually using a monoclonal antibody (Mab) for the PCV3-Cap protein (Palinski R. *et al*, 2017; Li X. *et al*, 2018). The technique has been applied, among other things, to FFPE PCV-3 qPCR-positive lymphoid tissues in an attempt to standardize it, but it has not provided clear and conclusive results regarding consistent viral detection in tissues (Saporiti V., 2021).

Real-time PCR (qPCR)

Real-time PCR was used to demonstrate and monitor PCV3 replication *in vitro* (cell culture) and *in vivo* (viremia). Viral DNA extractions from

viral culture supernatant, serum, nasal swabs, and tissues were performed using the MagMAX-96 Pathogen RNA/DNA Kit (Applied Biosystem™, Waltham, MA, USA) with the KingFisher™ Flex 96 Deep-Well Magnetic Particle Processor (Thermo Fisher Scientific) following the manufacturer's instructions. The DNA extracts were used to detect the conserved region of open reading frame 2 (ORF2 or capsid gene) of PCV3 and a highly conserved region of ORF1 of PCV to exclude contamination with PCV1/PCV2 using TaqMan™ Fast Virus 1-step Master Mix (Thermo Fisher). Internal control RNA (Xeno™, Applied Biosystem™) was included in the master mix to monitor PCR amplification and detect PCR inhibition. qPCR was performed with the following cycling conditions: one cycle at 50 °C for 5 minutes, one cycle at 95 ◦C for 20 minutes, 40 cycles at 95 ◦C for 3 seconds and 60 ◦C for 30 seconds (Mora-Diaz J. *et al*, 2020).

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Prevention and control

High levels of inflammatory cytokines and chemokines may be responsible for the clinical signs produced by PCV3 and for tissue damage caused by the migration of inflammatory cells, including neutrophils, eosinophils, and macrophages, to local tissues and organs. The symptoms observed are similar to those of PCV2 infection. Therefore, elucidating the mechanisms underlying cytokine/chemokine imbalance may help to devise a strategy to stimulate the host's immune response against PCV3 infection (Jiang S. *et al*, 2019).

Currently, there are no strategies for combating and preventing PCV3 infection in pig farms, partly because the pathogenesis of the virus is not well understood. However, it can be assumed that measures applicable to other viruses, especially circoviruses, may be highly effective. In addition, since PCV3 has also been found in other animal species, a form of prophylaxis may be to prevent contact between farm pigs and wild pigs, as well as to eliminate possible parasites (ticks) (Klaumann F. *et al*, 2019).

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Table 1

Types of tests that can be used to identify the PCV3 genome or specific antibodies (according to different authors) and the primers/probes used (Saporiti V., 2021)

Method		Target		Reference
PCR		Rep gene	F - TGCTACGAGTGTCCTGAAGAT R - CTTCTCCGCAACTTCAGTCAG	Fu et al., 2017
			F - AAAGCCCGAAACACAGGTGGTGT R - TTTTCCCGCATCCTGGAGGACCAAT	Franzo et al., 2018b
			F - CGAGAATTCCGAGATTGGCGAAGATTCC R - CGAGAATTCTCTCGAGGTAAT CCCCCTCT	Ye et al., 2018
			F - GGGCACACAGCCATAGAT R - TTCCGGGAC ATAAATGCT	Chen et al., 2017
			F - TTACTTAGAGAACGGACTTGTAACG R - AAATGAGACACAGAGCTATATTCAG	Ku et al., 2017
		Cap gene	F - GTGCCGTAGAAGTCTGTCATT R - TACACTCAGCCCTGTAATTTCT	Sun et al., 2017
			F - CGAGAATTCGCATAAGGG TCGTCTTGGAG R - CGAGAATTCTATGCG GAAAGTTCCACTCG	Ye et al., 2018
			F - ATGAGACACAGAGCTATATT R - TTAGAGAACGGACTTGTAAC	Guo et al., 2019
			F - TTACTTAGAGAACGGACTTGTAACG R - AAATGAGACACAGAGCTATATTCAG	Wang et al., 2019
qPCR	Taqman	Cap gene	F - AGT GCT CCC CAT TGA ACG R - ACA CAG CCG TTA CTT CAC Probe- FAM–ACCCCATGG–Zen-CTCAACACATATGACC– BHQ1	Palinski et al., 2017
		Rep gene	F – TGACGGAGACGTCGGGAAAT R -CGGTTTACCCAACCCCATCA- Probe -FAM-GGGCGGGGTTTGCGTGATTT-BHQ1	Franzo et al., 2018
	SYBER Green	Cap gene	F - GTGCCAGGG CTTGTTATTCT R - CTATTCATTAGGAGGCCCACAG	Jiang et al., 2019
			F - AACGGTGGGGTCATATGTGTTG R - AGACGACCCTTATGCGGAAA	Tochetto et al., 2020
	Taqman	Cap gene of PCV-3 and PCV-2	PCV-3 F - CGGTGGGGTCATATGTGTTG PCV-3 R - CACAGCCGTTACTTCACC PCV-3 Probe - ROX-CTTTGTCCTGGGTGAGCGCTGGTAG-BHQ2 PCV-2 F - CCAGGAGGGCGTTSTGACT PCV-2 R - CGYTACCGYTGGAGAAGGAA PCV-2 Probe - FAM-AATGGCATCTTCAACACCCGCCTCT-TAMRA	Kim et al., 2017
Multiplex PCR		Cap gene of PCV-3 and Rep gene of PCV- 2	PCV-3 F - GGTGAAGTAACGGCTGTGTTTT PCV-3 R - ACACTTGGCTCCARGACGAC PCV-3 Probe -FAM- ATGCGGAAAGTTCCACTCGK-BHQ1 PCV-2 F- GARACTAAAGGTGGAACTGTACC PCV-2 R- TCCGATARAGAGCTTCTACAGC PCV-2 Probe - VIC- AGGAGTACCATTCCAACGGGG-BHQ1	Wang et al., 2019
	SYBER Green	Rep gene of PCV-3 and Cap gene of PCV- 4	PCV-3 F - CGACCGAGTGGGAATCTA PCV-3 R - AGGCATCTTCTCCGCAAC PCV-4 F - CCACATAGTCTCCATCCAGTTG PCV-4 R - TACAGCCTCCCATTTGCATATTA	Hou et al., 2021
iELISA	Mab	Anti-Cap antibody	NA	Palinski et al., 2017
	Type of Ab NI	Anti-Cap antibody	NA	Deng et al., 2018
	Pab	IgM / IgG	NA	Mora-Diaz et al., 2020
iIFA	PAb	Anti-PCV-3 whole viral particle antibody	NA	Mora-Diaz et al., 2020
IHC	Mab	Antigen (Cap protein)	NA	Palinski et al., 2017;
	Mab	Antigen (Cap protein)	NA	Li et al., 2018
ISH	RNAscope	mRNA	RNAscope probe (catalog number not informed)	Phan et al., 2016
			RNAscope probe (catalog number 463961 or 530431)	Arruda et al., 2019
			RNAscope probe (catalog number not informed)	Mora-Díaz et al., 2020
			RNAscope probe (catalog number not informed)	Vargas-Bermúdez et al., 2021
	PCR DIG		RNAscope probe (catalog number not informed)	Kim et al., 2018a
	PCR DIG	DNA fragment of Rep gene	PCR DIG with primers targeting ORF1: 1- ATACTGCAGGCATCTTCTCCG 2 - TATTGTGGAGTGTGGAGGCAGT	Kedkovid et al., 2018b

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