Immunohistochemical Method for Detection PCV-2 Antigen in Pigs

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Abstract. Immunohistochemical (IHC) identification of the porcine circovirus type 2 (PCV-2) in organs and tissues was applied for better diagnostics of porcine circovirus disease. The IHC provides an additional confirmation to the diagnosis and allows to access cell and tissue tropism of the virus in pig organs. The research was conducted in animal tissues obtained during the disease outbreaks in pig farms using own monoclonal antibodies to the virus capsid protein. The results of IHC study have provided an insight into etiological role of the PCV-2 in pathological lesions found in all observed parenchymal organs, most of them localized in the lungs and lymph nodes. Accumulation of the virus has been found in the alveolar macrophages and lymph nodes. The lesions detected in other parenchymal organs have not been associated with PCV-2 accumulation.

1. Introduction

Porcine circovirus type 2 (PCV2) is a DNA virus infecting pigs and belongs to the family *Circoviridae*. According to the latest data from the International Committee on Taxonomy of Viruses, the *Circoviridae* family consists of two genera of *Circovirus* and *Cyclovirus*. These viruses have circular, single-stranded DNA genomes (Zaberezhny AD et al., 2017). Cycloviruses were discovered in 2010, they had a high degree of kinship with circoviruses, however, they had genomic and phylogenetic differences, that led to the creation of their own genus within the family *Circoviridae* (Rosario K et al., 2017). Circoviruses are currently the smallest viruses found in animals. The diameter of their virions ranges from 13 to 25 nanometres. The virion contains 60 capsid protein subunits, which form the dodecahedron unit. Their genomes depending on the virus type, have the following number of nucleotides: PCV-1 – 1758 to 1760, PCV-2 – 1766 to 1769, PCV-3 – 1999 to 2001, CVS-4 - 1770 (Afolabi KO et al., 2017, Klaumann F et al., 2018). PCV2 is currently regarded as the essential factor responsible for causing porcine circovirus-associated diseases (PCVADs) in pigs (Bolin SR., et al., 2001), which include postweaning multisystemic wasting syndrome (PMWS) (Segales J et al., 1997), porcine dermatitis and nephropathy syndrome (PDNS), respiratory and reproductive dysfunction (Afolabi KO et al., 2017, Madson DM, 2011). The PCVADs cause significant financial losses for the all world swine industry (Darwich L, 2004, Segales J, 2012, Segales J et al., 2013). Since middle 2000, commercial vaccination has been carried out in piglets against this virus (Raev SA, 2014). An ELISA has been developed to detect PCV-2-specific antibodies in blood serum, as well as PCR to detect viral genome in variety of biological samples. The animals with PCVAD – like clinical signs and healthy animals could be positive for PCV-2 in ELISA and PCR. Thus, there is a
need in additional confirmation of the etiological role of PCV-2 in the disease. In the present study we have applied IHC for postmortem detection of PCV-2 antigen in pig parenchymal organs.

2. Materials and methods
The study was carried out according to protocols approved by «Guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care, international». Animals. The probes with pathologic lesions were collected from pigs which showed problems in reproductive function and clinical signs for PCVAD in 4 farms from different regions of Russian Federation.

2.1. Histological examination
Pathological probes were collected from lungs (a section of lung was collected from each of the 5 anteroventral lung regions, which included the right cranial lobe, right middle lobe, the accessory lobe, and a section from each portion of the divided left cranial lobe), trachea, lymph nodes, spleen, heart, liver and kidney. From the obtained pathological material, samples of organs were cut, fixed in 10% buffered formalin. Paraffin filling of samples was performed in a carousel-type automatic installation. Paraffin blocks were cut to a thickness of 3 microns and stained routinely (hematoxylin-eosin). Routine staining of slices was performed in a linear automatic installation (Thermo Scientific company, Germany).

2.2. Immunohistochemical examination
Same organs were used for IHC as for paraffin method. After pathologoanatomic revision, the probes were frozen for IHC cryotomic method and sliced to 3 micron thickness. Slices were incubated with 0.9% NaCl and Isoprep (BioVitrum, Russia), ratio 1:3, at 4°C for 60 min. Endogenous peroxidase activity was suppressed by 3% hydrogen peroxide in PBS for 20 min at room temperature. Non-specific binding was inhibited by incubation with a skimmed milk powder for 40 min at 37°C in moist chamber. Then the slices were incubated with peroxidase conjugated anti-PCV-2 monoclonal antibody to PCV-2 capsid protein (D.I. Ivanovski Virology Institute, Russia) followed by incubation with dimethylformamid and 3-amino-9-etilcorbazol solution for visualization of peroxidase staining.

3. Results and discussion
We examined more than 10 probes from each (40 pigs) 100-130-day-old pigs. In our study we compared pathoanatomy, pathomorphology observations and IHC data.

3.1. Clinical signs
The cough, slimming, sometime apathy were noticed in pigs. The abortions in sows were observed sometime.

3.2. Pathoanatomic changes
Changes were observed in all organs. Mostly they were represented by wide areas of hemorrhages (lung, lymph node, trachea and kidney) which were characterized by lobular blood fillings in the lungs, enlarged spleen and lymph nodes and deformed (with blunt edge) spleen and liver. In some organs such as spleen and kidney a necrotic foci were observed.

3.3. Microscopy data
Pathomorphology studies revealed damages in all pigs from 4 farms. The hearts had cardiomyopathy, fibrinous deposits in the pericardium. The kidneys were with areas of hemorrhage and tubules epithelial necrosis. In histological study of the spleen, necrosis areas were identified along with violation of histoarchitectonics of lymphoid follicles, the central blood vessels were found with obturated lumen and with hyperemia and peri-vascular swelling. The spleen was impregnated with eosinophilic cells excessively. In the liver, numerous lymphocytic foci and peri-vascular inflammation were observed. Inflammatory process was detected in the trachea as well as necrosis of structural
elements, and edema in different locations. In lung, (Fig. 1) we observe areas of emphysema with damage of alveoli wall. Pathologic lumen was accompanied by alveoli disruption, presence of erythrocytes, and hematoxiline-stained homogenous substance. These areas contained hemorrhagic foci and were adjacent to fields of ruptured alveoli. An edemic process was observed in lung pleura. The lymph node (Fig. 2) contained necrotic foci, hemorrhagic areas and structural damage of follicles.

**Figure 1.** Lung. 1 visceral leaf; 2 rupture of the alveoli wall; 3 alveoli atelectasis (arrow). H&E, x 100.

**Figure 2.** Lymph node. 1 lymphatic follicle; 2 necrosis of parenchymal cells; 3 hemorrhage. H&E, x 100.

Despite similar anatomic and histologic clinical signs in pigs from all observed farms, situation at one of the 4 farms was significantly milder, and subsequently, no PCV-2 was found at that farm.

### 3.4. Immunohistochemical research

We used cryotomic method for the IHC to avoid fixation of tissues that may influence the consistency of the results. In all slices of each probe various antibody concentration (1:50-1:800) were used. The PCV-2 antigen was found in lung and lymph nodes in the 3 of 4 examined farms as a result. In all farms the PCV-2 antigen was not found in probes from other organs such is heart, trachea, spleen, liver, kidney. The study of pathological material from piglets with strongest anatomic and histologic deviations shows PCV-2 antigen positive lesions in lung (Figure 3) and lymph node (Figure 4) slices.

**Figure 3.** Identification of capsid protein of PCV-2 in lung tissue. The arrows indicate peroxidase staining of alveolar macrophages in the background of additional Hematoxylin Mayer staining. X400

**Figure 4.** Identification of capsid protein of PCV-2 in lymph node tissue. The arrows indicate peroxidase staining of alveolar macrophages in the background of additional Hematoxylin Mayer staining. X400
Thus, PCV-2 antigen was detected in 60 of 440 probes of pigs, despite that pathologic signs were found in all organs. Since the virus was detected in lungs and lymph nodes, we suggest that pathology in other parenchymal organs can be caused by virus toxic activity. Most of PCV-2 positive areas are concentrated in mononuclear cells of alveoli in atelectasis leading to tissue damages in lung. Lymph nodes positive in IHC reaction were mostly detected in necrotic regions and in inflammatory tissue with massive proliferation of lymphocytes.

4. Conclusion
The IHC study is a reliable method for direct confirmation of the PCV-2 role in swine diseases. The results of this study may provide an added value to epizootiology data by revealing subclinical forms of PCV-2 infection after slaughter.

5. References
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