Transmission of porcine circovirus genotype type 2 (PCV2) in Russia and genotype association (PCV2d) with porcine dermatitis and nephropathy syndrome (PDNS)

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Abstract. The study of the prevalence of PCV2 genotypes in pig farms of the Russian Federation is presented. It is shown that currently the genotype PCV2d is the most common, which is important in connection with the assumed probability of lack of the immune response to PCV-2d induced by PCV-2a-based vaccines. The main goal of this investigation was to conduct monitoring studies for DNA, immunoglobulins IgG and IgM classes to PCV2 by the polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) assays, respectively, as well as identification of the PCV2 genotype by sequencing of pigs of different ages were carried out at one of the Russian industrial pig farms with PCVAD (Porcine circovirus associated disease) outbreaks that used PCV2a vaccination. The data of this field observation indicates that PCV2d – is a major genotype in Russia. Additionally, it was shown that PCV2d prevalence in the farm correlates with clinical manifestation of PCVAD.

1. Introduction

Porcine circovirus type 2 (PCV2) remains one of the most economically significant pathogen for swine industry. PCV2 is a causative agent of several diseases (porcine circovirus associated diseases, PCVAD): post weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), reproductive disorders, respiratory pathology and a subclinical form. Beginning in the late 2000s, there was many outbreaks of PDNS associated with the new PCV2 genotype, - PCV2d. The predominance of the genotype of PCV2d in the USA, China, Germany, etc. has been demonstrated. In previous study it was find that all major genotypes of PCV2: PCV2a, PCV2b and PCV2d are presented in Russia (2010-2017). Current state of the prevalence of PCV2 genotypes in Russia from 2017 remains unknown.

The prevalence of pathogens, which may cause PDNS such as PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 3 (PCV3) and Pasterella multocida is also high [1-4]. To find correlation between PDNS and PCV-2 an extensive study, such as cross-sectional study, is required.

The aims of this investigation were: to study prevalence of PCV2 genotypes in Russia and establish the correlation between the PCV2d and PDNS in one of the pig farms in Russia.

2. Material and methods
To assess the prevalence of genotypes of PCV2 in Russia, samples of pathological material obtained from piglets from various regions of Russia (Moscow, Vologda, Tyumen, Belgorod, Smolensk, Bryansk, Tomsk, Sverdlovsk, Krasnoyarsk regions, and the Republic of Buryatia) were examined.

2.1 Sequencing
For PCR, LongPCRMix (Alfa-enzyme, Russia) was used according to the manufacturer’s method. The primers for the amplification and sequencing of the PCV2 genome fragments were written using the sequences of the PCV2 published in the GenBank database. As a result, the fragments obtained in PCR blocked the entire genome of the virus. The nucleotide sequence was determined by the Sanger method using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, the USA) using an ABI PRISM 3130 Genetic Analyzer automatic sequencer (Applied Biosystems, the USA) according to the manufacturer’s method.

Analysis of DNA fragments was performed using the electrophoretic technique in agarose gel. The accumulated fragments were cut out and isolated from the gel using a PCR purification kit (“GeneJET PCR Purification Kit, TermoScientific) according to the manufacturer’s method.

2.2 Farm history
The farm is endemic for PRRSV, PCV2, Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae, Pasterella multocida.

No clinical signs of PDNS have been observed on Belgorod farm since 2011 - since the start of routine PCV2a vaccination - pigs are immunized with a recombinant subunit vaccine based on capsid protein of PCV-2a. In 2018, piglets in the fattening period (at the age of 120 days) began to show clinical signs of PDNS: According to veterinary specialists, single spots of purple color appeared in the small of piglets’ backs/tails, then spots appeared on the stomach and back, and, than (3-5 days after first spots appearance), - on the head and ears; death occurs within 24 hours after full skin coverage with spots. No additional clinical signs were detected. The morbidity rate was about 3% among piglets of the age of 120-140 days. Mortality rate was about 100%.

Blood samples were taken piglets of various ages (57, 80, 124, 147 and 197 days) in order to determine the dynamics of changes in the serologic/virologic status of animals relating to PCV2, PCV3, PRRSV. bacteriological studies for Pasterella multocida have been carried out by traditional method. Samples of lungs, kidneys, liver, spleen, lymph nodes and skin were taken from piglets with pathology (and without it).

2.3 Diagnostic tes-kits
To detect the DNA of PCV2, the RNA of PRRSV, test-kits (VETBIOCHEM, Russia) were used. To detect the DNA of PCV3, PCR was used as described previously. To detect the antibodies to PCV2, PRRSV test systems (VETBIOCHEM, Russia; Ingenasa, Spain) were used. The detection of Pasterella multocida was carried out by the bacteriological method.

3. Results
PCV2 identified in all surveyed farms. Phylogenetic analysis (figure 1) of the identified isolates showed that the main genotype is PCV2d.

Comparison of the ORF-2 nucleotide sequences of PCV2a and identified isolates of PCV2d show that this region of the genome has characteristic differences between the genotypes of the virus. It is known that the capsid protein of PCV2 has 4 immunodominant regions (A, B, C and D) located in the area of amino-acid residues: 65-87, 113-139, 169-183, as well as at the end of the C-terminal end [5].

Thus, the differences in nucleotide sequence coding for ORF-2 in the identified PCV2d isolates revealed contain not only the previously described changes — an increase in the size of the PCV2d capsid protein by one amino acid as compared to PCV2a (234 vs. 233, respectively) but also replacements in immunodominant areas.
Previously, it was shown that a single amino acid substitution in the capsid protein of PCV2 affects the probability of a specific interaction with monoclonal antibodies [6].

It is known that the clinical forms of PCVADs are associated with a high proportion of animals with viremia, as well as with a significant viral load. Under experimental conditions, it was shown that the efficacy of vaccines based on PCV2a is lower than vaccines based on PCV2a and PCV2d in the case of using PCV2d as a challenge strain.

Figure 1. Phylogenetic dendrogram built on the basis of the ORF2 of Russian PCV2 isolates.

Nevertheless, the features of the infection dynamics on the farm, as well as the emergence of the new PCV2 genotypes, necessitate a revision of the standard approaches for specific prevention of PCVADs. One of the most effective methods for controlling PSVADs is a prophylactic vaccination. Most vaccination protocols provide for immunization of pigs at 2–3 weeks of age with vaccines based on PCV2a. Using of vaccines based on PCV2a and PCV2d may improve efficacy of specific prevention of PCVAD.
Additionally, the influence of colostral immunity to PCV2 on vaccination of piglets is still ambiguously interpreted. In the case of a high level of maternal antibodies, it is advisable to consider the issue of shifting the beginning of the vaccination period of the pigs for 3-5 weeks. The application of this approach was accompanied by a decrease in viral load and clinical signs of PCAVDs relative to a group of piglets immunized at 3 weeks of age.

Thus, for successful control of the PSAVD on a pig farm, it is necessary to conduct monitoring studies of blood serum, to detect the DNA of PCV2 by PCR; to detect the IgG IgM to PCV2 in ELISA (Figure 2), as well as to identify the PCV2 genotype by the sequencing method. The data obtained can be used to adapt the PCVAD vaccination protocols.

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Figure 2. The percentage of positive animals in different age groups.
