UDC 619:616.98:578:636.4

DOI: 10.29326/2304-196X-2019-3-30-29-33

DETECTION OF PORCINE CIRCOVIRUS TYPE 3 IN RUSSIAN PIG HOLDINGS

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SUMMARY

Diseases associated with porcine circoviruses (mainly with porcine circovirus type 2) have various manifestations, are common in pigs in countries having well-developed pig industry and responsible for significant economic losses. Porcine circovirus type 3 (PCV-3) causing systemic inflammation of unknown etiology in animals was detected the USA in 2015. Later, data on PCV-3 detection in Asia, Europe and South America were published. Analysis of literature data on current epidemic situation on PCV-3 infection in foreign countries as well as the disease clinical manifestations and postmortem lesions are described. Results of molecular and genetic tests of biomaterials collected from pigs in 51 holdings located in 28 regions of the Russian Federation are presented. A total of 280 samples of biological materials of different types (organs, tissues, stillborn piglets) collected from domestic pigs with respiratory, reproductive and neurological disorders, dermatitis and from emaciated pigs were tested and PCV-3 genome was detected in 11 samples from 9 holdings located in 5 regions of the Russian Federation. Porcine circovirus type 3 was detected in lung, bronchial and mediastinal lymph node, spleen tissues from grower and fattening piglets, adult pigs and aborted fetuses. Samples that were positive for PCV-3 DNA when tested with molecular methods (PCR, real-time PCR) were tested for other pathogens. The following pathogens were also detected in 6 out of 11 samples (55%): *Actinobacillus pleuropneumoniae*, *Mycoplasma hyorhinis*, *Streptococcus suis*, *Haemophilus parasuis*, *Mycoplasma hyopneumoniae* and *Pasteurella multocida*. Porcine circovirus type 2 was detected in one sample. Presented test results are indicative of probable combined etiology of respiratory and reproductive disorders in tested pigs that results in various clinical manifestations. Grower and fattening piglets were found to be the most susceptible to PCV-3-associated disease. Further studies are required for identification of actual PCV-3 pathogenicity and its prevalence in the territory of the Russian Federation.

Key words: polymerase chain reaction, porcine circovirus type 3 (PCV-3), pigs.

INTRODUCTION

Circoviruses belong to *Circovirus* genus, *Circoviridae* family. Porcine circovirus infections are endemic in countries with well-developed pig farming (Germany, Canada, US, Ireland, France, Spain). Until recently it was considered that two circovirus types could be found in pig populations: circovirus type 1 (PCV-1) and type 2 (PCV-2). Whereas PCV1 is not pathogenic for the animal species in question, PCV-2 constitute a primary etiological agent of postweaning multisystemic wasting syndrome (PMWS), is associated with nephropathy syndrome and porcine dermatitis (PDNS), linked to porcine reproductive and respiratory syndrome and also can provoke clinical symptoms of diseases affecting gastrointestinal, lymphatic, blood circulatory and nervous systems as well as skin disorders. Currently, antibodies against PCV-2 have been detected in pigs of nearly all establishments and the number of seropositive animals can attain 100% [2, 8].

In 2015, the University of Minnesota Veterinary Diagnostic Laboratory (UMN-VDL), US detected a new circovirus type 3 – PCV-3. Its detection was preconditioned by the results of metagenomic sequencing of tissue samples collected from pigs with multisystemic lesions which were found negative when tested for known pathogens such as PCV-2, influenza A virus, virus of porcine reproductive and respiratory syndrome, classical swine fever virus, pestivirus, FMD virus, porcine parvovirus type 1 and type 2, West Nile virus, encephalomyocarditis virus, *Mycoplasma hyosynoviae*, *Mycoplasma hyopneumoniae* and *Erysipelothrix rhusiopathiae* [8]. Almost simultaneously another American research group used metagenomic sequencing to detect PCV-3 in tissues from sows with chronic reproductive failure as well as in organs of mummified fetuses with signs typical of PDNS from the same sows. R. Palinski et al. conducted retrospective testing of 48 tissue samples of

animals suffering from PDNS in which PCV-2 had not been detected previously. It was established that in 93.8% of cases the samples contained PCV-3 [3].

Later it was demonstrated that the virus was widespread in Asia, notably, China, South Korea, Thailand [7, 13, 15, 16]. Other reference sources indicate presence of the virus in European countries, such as Ireland, Poland, Denmark, Italy, Spain, UK, Germany, Sweden and in South America, namely, Brazil [4, 5, 9–11].

R. Fux et al. carried out phylogenetic analysis of full-genome sequences of PCV-3 strains which were obtained in the course of research work as well as of sequences registered in GenBank database. This allowed them to detect two distinct strain groups which can be considered to be PCV-3 genotypes [11].

Although circovirus type 3 was found in animals of different age with various clinical signs, its role as a possible pathogen in infectious pathology in pigs remains unclear.

The work aimed to address PCV-3 situation in pigs in Russia with the use of molecular and genetic methods.

MATERIALS AND METHODS

Pathological materials. The following materials were used for diagnostic testing: fresh or frozen pieces of lungs and spleen, lymph nodes of pigs with respiratory disorders and signs of emaciation; tissues of animals with nervous disorders and dermatitis; organs of aborted sows, placenta, organs of still-born piglets obtained from Russian pig farms.

Isolation of DNA from 10% suspension of biological material was carried out with the use of 6 M guanidinium isothiocyanate and GF/F glass-fiber filters [1].

PCV-3 was detected by classical polymerase chain reaction (PCR) with the use of a pair of primers specific for the given virus according to the methods described by G. H. Chen in 2017 [6].

Differential diagnosis of viral and bacterial diseases associated with respiratory and reproductive pathologies was conducted by molecular methods (real-time PCR and PCR) simultaneously with the main test.

Data on clinical manifestation of the disease and age of animals from which pathological material samples were taken were obtained from descriptive part of accompanying documents and also in the course of additional survey of veterinarians working at establishments where respiratory and reproductive syndromes were registered in pigs.

Table 1
Detection of PCV-3 genome in pigs with different pathologies

Pathology	Number of samples	
	tested	positive
Respiratory	121	10
Reproductive: still birth	3	–
Reproductive: abortions	26	1
Dermatitis	2	–
Nervous	3	–
Other	125	–

RESULTS AND DISCUSSION

To achieve the goal, we tested 280 samples of pathological materials obtained from various Russian pig establishments. In 2018, the FGBI "ARRIAH" received samples from pigs with lesions of various etiology. The samples were delivered from 51 farms located in 28 Russian regions to be tested for infectious pathogens.

The following materials were tested for DNA of PCV-3: organs and tissues of pigs with respiratory disorders (121 samples), still-born piglets (3 samples), sows with reproductive disorders/abortions (26 samples), pigs with dermatitis (2 samples), nervous system disorders (3 samples) as well as with other clinical signs (125 samples). Main information on the test is presented in Table 1.

PCV-3 genome was detected in 11 samples (4%) received from 9 farms of 5 Russian regions: in lungs, bronchial and mediastinal lymph nodes, spleen and also in organs of aborted fetus.

Samples positive for PCV-3 DNA were also tested for other pathogens by molecular methods (PCR, real time PCR). The test results are shown in Table 2.

We did not detect genetic material of other infectious agents in 5 out of 11 testes samples (45%). However, apart from porcine circovirus type 3 we detected the following in 6 tested samples of pathological material: *Actinobacillus pleuropneumoniae* (two times), *Mycoplasma hyorhinis* (two times), *Streptococcus suis* (two times), *Haemophilus parasuis* (two times), *Mycoplasma hyopneumoniae* and *Pasteurella multocida*. Interestingly, we also detected DNA of porcine circovirus type 2 along with PCV-3 DNA in one of the samples (Table 2).

Foreign publications state that the number of samples in which PCV-3 was detected accounted for a considerable part of tested samples: 73% (South Korea), 35% (China), 22% (Poland), 20% (Ireland), 13% (USA) and 5% (UK) [4, 14, 16].

The virus was detected in different types of pathological material. For example, in 2017 in Poland R. Palinski et al. determined the presence of PCV-3 in breeding sows with skin lesions as well as in mummified fetuses of those breeding sows. Besides, the virus was detected in 13% of samples of lungs, oral fluids and nasal swabs from animals with respiratory diseases [3]. In China PCV-3 was isolated from lungs of piglets with signs of respiratory infection and body temperature above 40 °C [12]. Besides, PCV-3 was detected in breeding sows with reproductive pathologies in 24 out of 35 Chinese farms that were examined [13].

According to S. Hayashi (Japan), PCV-3 is typically detected in lymph nodes, notably, submandibular and mesenteric (33% and 25% of cases respectively), less often – in tissues of kidneys (29%), intestine (12%), lung lymph nodes (8%), lungs (7%), brain (8%), spleen (4%), liver (3%) and organs of fetuses (2%) [9]. X. Ku also found PCV-3 in different organs of pigs. Following the detection of the virus in the fetus organs, like many other foreign authors he suggested that PCV-3 was involved in reproductive pathology with vertical transmission of the virus. Research conducted in the US, China and Thailand confirm the hypothesis [9, 13, 15].

Though PCV-3 genome was found in many internal organs of pigs, foreign researchers failed to determine a specific target organ for infection with the given virus [9]. The role of the virus in the pathogenesis of diseases with different syndromes in pigs remains underexplored. Besides, a variety of factors conducive to pathologies should

Table 2
Results of testing of samples containing DNA of PCV-3 for genetic material of other infectious agents

No.	Region of Russian Federation	Age of animal	Pathology	Organs taken for testing	Other pathogens detected
1	Voronezh Oblast	Fattening piglet	Respiratory	Lungs, spleen, regional lymph nodes	–
2	Belgorod Oblast	Fattening piglet	Respiratory	Lungs, spleen, regional lymph nodes	PCV-2
3	Kursk Oblast	Fattening piglet	Respiratory	Lungs, regional lymph nodes	*Actinobacillus pleuropneumoniae*
4	Lipetsk Oblast	Fattening piglet	Respiratory	Lungs, spleen, regional lymph nodes	–
5	Belgorod Oblast	Fattening piglet	Respiratory	Lungs, regional lymph nodes	*Actinobacillus pleuropneumoniae, Mycoplasma hyopneumoniae*
6	Krasnodar Krai	Grower piglet	Respiratory	Lungs, regional lymph nodes	*Haemophilus parasuis, Mycoplasma hyorhinis, Streptococcus suis*
7	Voronezh Oblast	Grower piglet	Respiratory	Lungs, spleen, regional lymph nodes	*Streptococcus suis*
8	Belgorod Oblast	Grower piglet	Respiratory	Lungs, regional lymph nodes	*Haemophilus parasuis, Mycoplasma hyorhinis, Pasteurella multocida*
9	Belgorod Oblast	Breeding sow	Respiratory	Lungs, regional lymph nodes	–
10	Belgorod Oblast	Breeding sow	Respiratory	Lungs, regional lymph nodes	–
11	Belgorod Oblast	Aborted fetus	Reproductive	Parenchymal organs	–

be taken into consideration (stress, overpopulation, immunodeficiency disorders, poor quality feed).

Accompanying documents for the materials sent for testing to the FGBI "ARRIAH" state that positive samples were taken from grower and fattening piglets (8 samples – 73%), adult animals (2 samples – 18%) and an aborted fetus (1 sample – 9%) (Table 2).

Foreign sources report PCV3 detection in samples taken from piglets aged 14–21 days and 63–70 days and also in aborted fetuses [7]. T. Stadejek et al. state that PCV-3 is most common among weaners, grower piglets and breeding sows but is rarely found in suckling piglets. The authors suggested that the difference in frequency of detecting the pathogen among age groups was conditioned by protection factors of the immune system of breeding sows [10]. Data obtained by S. Hayashi indicates that frequency of PCV-3 detection stood at 7% for aborted fetuses, 13% – for suckling piglets (3–4 weeks), 9% – for weanling piglets (8–10 weeks) and 9% – for fattening piglets and adult pigs [9].

Test results presented in Table 2 suggest possible mixed etiology of respiratory pathologies and reproductive disorders in tested pigs which is supported by the detection of genetic material of other infectious agents in more than a half of samples found positive for PCV-3 genome (*A. pleuropneumoniae*, *M. hyopneumoniae*, *M. hyorhinis*, *S. suis*, *H. parasuis*, *P. multocida*).

Mixed infection PCV-2 + PCV-3 is found quite often abroad (in 46% of the cases). Along with PCV-2 the following co-infection agents are often detected: parvovirus (60%), virus of porcine reproductive and respiratory syndrome (11%) as well as *Staphylococcus suis*, *Escherichia coli* and *Staphylococcus haemolyticus* (quantitative data are unavailable). Proceeding from these data the authors assume that such associations of agents result in a broad spectrum of clinical signs, notably, in systemic manifestations of the infection [9, 14].

Considering the above-mentioned, we see the need for additional testing to study PCV-3 monoinfection and its associations with other pathogens.

Thus, the test results confirm that PCV-3 is present on Russian pig farms. The above-mentioned information suggests that PCV-3 can cause or at least stimulate development of respiratory pathology and reproductive disorders in pigs. It seems reasonable to further research circovirus type 3 propagation in Russia and investigate the influence of the given pathogen and its associated agents of viral and bacterial etiology on development of various pathologies in pigs.

CONCLUSIONS

1. Circovirus type 3 was shown to be endemic on Russian pig farms.

2. Circovirus type 3 was detected in the pathological material taken from animals with respiratory and reproductive pathologies and is a possible etiological agent conditioning their appearance or is conducive to the development of a disease.

3. Circovirus type 3 was detected in the pathological material from grower and fattening piglets, adult animals and an aborted fetus.

4. Along with circovirus type 3 the following was detected: *Actinobacillus pleuropneumoniae*, *Mycoplasma hyorhinis*, *Streptococcus suis*, *Haemophilus parasuis*, *Mycoplasma hyopneumoniae* and *Pasteurella multocida*, which can attest to a mixed etiology of the occurrence (course) of porcine respiratory and reproductive diseases.

Conflict of interest. The authors declare no conflict of interest.

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Submitted on 28.05.19
Approved for publication on 21.08.19