UDC 578.3+582.974

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COMPLETE GENOME SEQUENCE OF PORCINE CIRCOVIRUS TYPE 2 UKRAINIAN ISOLATES

Porcine circovirus type 2 (PCV2) is associated with distinct syndromes and diseases in swine, collectively known as porcine circovirus-associated diseases (PCVAD), which include postweaning multisystemic wasting syndrome (PMWS), PCV2-associated pneumonia as a part of the porcine respiratory disease complex (PRDC), PCV2-associated enteritis, PCV2-associated reproductive failure, and porcine dermatitis and nephropathy syndrome (PDNS) (1–3). PCV2-infection is widespread and essentially all pig herds are infected with PCV2. Porcine circovirus 2 (PCV2), a member of the genus Circovirus in the family Circoviridae, is a very small single-stranded negative-sense DNA virus of approximately 1.7 kb (4). The genome of PCV2 encodes three major open reading frames (ORFs) encoding the replicase proteins (ORF1), the viral capsid protein (ORF2), and a protein with suggested apoptotic activity (ORF3) (5). Previous reports showed that there were five PCV2 genotypes, including PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e (6–9). Here, we report the complete genome sequence of Ukrainian PCV2 isolates from different regions of Ukraine.

Key words: Porcine circovirus type 2, porcine circovirus-associated diseases, multisystemic wasting.

Introduction. Porcine circovirus type 2 (PCV2) was first recognized as a causative agent of postweaning multisystemic wasting syndrome (PMWS), a multi-factorial disease in swine in Canada in 1991 (Harding and Clark, 1997). Subsequently, it has been reported in almost all intensive pig production countries worldwide (Allan and Ellis, 2000; Chae, 2005). PCV2 causes several clinical and pathological conditions in pigs including porcine respiratory disease complex (PRDC), reproductive failures, porcine dermatitis and nephropathy syndrome (PDNS), proliferative and necrotizing pneumonia and congenital tremor (Darwich et al., 2004; Chae, 2005). Currently, these associated diseases and conditions linked to PCV2 are called porcine circovirus associated diseases (PCVAD).

PCV2 belonging to the family Circoviridae, is a smallest mammalian, non-enveloped, single-stranded DNA virus encoding a circular genome about 1.76 kb (Mankertz et al., 1997). The genome of PCV2 contains 3 major open reading frames (ORFs): ORF1, ORF2 and ORF3. The Cap protein is the main structural and major immunogenic protein of PCV2, which is encoded by ORF2. As a result, ORF2 is commonly used for reconstruction of the phylogenetic tree similarity to the whole PCV2 genome study (Olvera et al., 2007) Several studies suggested that PCV2 could be divided into 2 major genotypes (Carman et al., 2006; Cheung et al., 2007; Ma et al., 2007; Takahagi et al., 2008; Kim et al., 2009). Recently, both genotypes were proposed and referred to PCV2a (PCV2- genotype 2) and PCV2b (PCV2-genotype 1). However, PCV2c genotype has been described, but only found in Denmark (Segales et al., 2008). Interestingly, the virulence of PCV2a and PCV2b isolates was similar in the conventional SPF pig model, but the virulence of the isolates within the same cluster differed (Opriessnig et al., 2008). Alternatively, PCV2 can be classified into 8 subgroups 1A to 1C and 2A to 2E (Olvera et al., 2007), but those were not associated with the disease conditions or geographic areas. Recently, a new type of PCV referred to PCV1/2a was reported and later found to be a chimeric virus containing ORF1 of PCV1 and ORF2 of PCV2a in Canada in 2009 (Gagnon et al., 2010).

In Ukraine, PMWS caused by PCV2 has been reported from 2000. However, genetic information about PCV2, spreaded in swine herds of Ukraine has been still unavailable. Therefore, the objective of this study was to determine the genetic characterizations of complete genome of current PCV2 isolates from Ukraine pigs from different regions of Ukraine (included Cherkasy, Kharkiv, Chernigiv, Zaporiggia regions).

Materials and Methods.

Field samples: Clinical samples (serum samples and lymph nodes) from different farms in high pig density provinces of Ukraine submitted to Molecular Diagnostic Laboratory at CVD (Center of Veterinary Diagnostics) during 2014-2015 years were included in this study. These samples were kept at -80°C until performing DNA extraction and PCR. Viral DNA was extracted from lymphoid tissue homogenates and serum samples using NucleoSpin Extract Viral DNA Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions.

PCR amplification: A full-length ORF2 gene of PCV2 was amplified by PCR with forward primer, PCV2-f1 (5'-CCA TGC CCT GAA TTT CCA TA-3') and reverse primer PCV2-r1 (5'-ACA GCG CAC TTC TTT CGT TT-3') published by Takahagi et al. (2008), in a 50 µl reaction mixture. The amplification reaction was performed with an initial step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 1 min and a final extension step at 72°C for 7 min. The PCV2 positive samples of 702 nt were used for DNA sequencing.

Viral sequences and phylogenetic analysis: The PCR products were separated by 1.5% agarose gel electrophoresis and purifiedwith NucleoSpin Extract II (Macherey-Nagel, Düren, Germany) for the sequences. DNA sequencing was carried out with primers used in the previous PCR reaction. A total of 4 sequences from Ukraine pigs were obtained and translated intoamino acid sequences for analysis. The 4 Ukraine PCV2 sequences were analyzed together with representative complete genome sequences reported in GenBank. A phylogenetic trees was constructed by MEGA 6 software (Tamura et al., 2007) using the neighbor-joining (NJ) method with 1000 bootstrapping replicates (Saitou and Nei, 1987).

Results and discussion.

Genetic characterization: All 4 Ukraine of PCV2 sequences in this study had a genome length of 1768 nt and revealed nucleotide identities ranged between 100-95% (Table 1), indicating no significant differences between PCV2 genotype.

***Table* 1. Comparison of Ukrainian isolates (AK and NT sequences of complete genome) with PCV2 reference strains in the same group by nucleotide and deduced amino-acid sequences**

Isolate PCV2	subgroup	AK, %	NT, %
swine_Chernigiv	1A/B	100	100
swine_Zaporigga	1A/B	100	100
swine_Cherkasy	2	87,1	95,2
swine_Kharkiv	2	87,1	94,9
PCV2_Uy_2_Cap	1A/B	91,9	96
PT-15152-03_cap	1A/B	92,7	97,1
NL_Control_1_capsid_protein	1A/B	91,1	96
FJFQ0511_cap	1A/B	91,1	95,7
YZ_cap_protein	1A/B	91,1	95,7
HN1-5_cap	1A/B	91,1	95,7
IL_capsid_protein	1A/B	91,9	96,3
09JX_Cap	1A/B	90,3	95,5
CHST_cap	1A/B	91,1	95,7
09GD_Cap	1A/B	91,9	96,3
C7201-1_capsid_protein	1A/B	90,3	95,2
CH3_capsid_protein	1A/B	100	99,5
PT-34765-06_cap	1A/B	100	99,5
NIVS-5_putative_capsid_protein	1A/B	100	99,5
WB-H-1_capsid_protein	1A/B	100	99,5
ZHZ1_cap_protein	1A/B	100	99,7
DK442case_capsid_protein	1A/B	99,2	99,5
Fd1_capsid	1A/B	100	99,5
AUT5_capsid_protein	1A/B	100	99,7
NL_Control_3_capsid_protein	1A/B	99,2	99,2
Henan_cap_protein	1A/B	100	99,5
SXTY14_ORF2	1A/B	100	99,7
ZHZ1_pig_gi1033208412	1A/B	100	99,7
AUT5_gi37791490	1A/B	100	99,7
SXTY_PCV_gi1031916872	1A/B	100	99,7
1397/2011_Vicenza_36_07/07/2011_	1A/B	100	99,7
SNUVR000463_gi573463974	1A/B	100	99,7
DK558control_gi156193221	1A/B	100	99,7
1C-China	1C	91,1	96,3
PCV2_Gen2-_Hungary	2	89,5	96

Genome consists of at least three ORFs, encoding 2 major proteins, the Rep and Cap proteins. Multiple sequence alignment was completed by means of MEGA6 with other available strains from the GenBank nucleotide database. Ukraine isolates from Chernigiv and Zaporigga shares a 100 % identity to each other and high identity (95% to 99.7%) with the strains from 1A/B subgroup. Ukraine isolates from Cherkasy, Kharkiv shares near 100 % identity to each other and high identity (94% to 96%) with the strains from subgroup 2.

Phylogenetic analyses: The phylogenetic analysis in this study reconstructed from the 4 Ukraine complete sequences from Cherkasy, Kharkiv, Chernigiv, Zaporiggia regions of Ukraine and sequences published in GenBank database representing all PCV2 genotypes shown in Fig. 1.

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.11324677 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method [3] and are in the units of the number of base substitutions per site. The analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 387 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [4].

Two Ukraine isolate PCV2 sequence belonged to genotype 1 (PCV2b) and two Ukraine isolate PCV2 sequence belonged to genotype 2 (PCV2a) according to the classification proposed by Grau-Roma et al. (2008). Based on the subgroup terminology described previously (Olvera et al., 2007), nucleotides 262-267 and amino acids 88-89 of ORF2 were compared and classified. The nucleotide sequences "CCCCGC", "CCCCTC" and "AAAATC" are the signatures motif for PCV2b subgroup 1A/B, 1C and PCV2a, respectively. The amino acid "PR" was enclosed with subgroup1A/B, while the PL and KI were related with subgroup 1C and PCV2a (Cheung et al., 2007). Ukraine isolate PCV2 from Chernigiv and Zaporiggia genotype 1 were divided into 1A/ B subgroups. Ukraine isolate PCV2 from Cherkasy, Kharkiv were divided into genotype 2.

The sequence and phylogenetic analyses performing in this study did not show any evidence of recombination as reported in PCV type 2 isolated in Hong Kong, Korea and USA (Ma et al., 2007; Choi and Chae, 2008; Hesse et al., 2008).

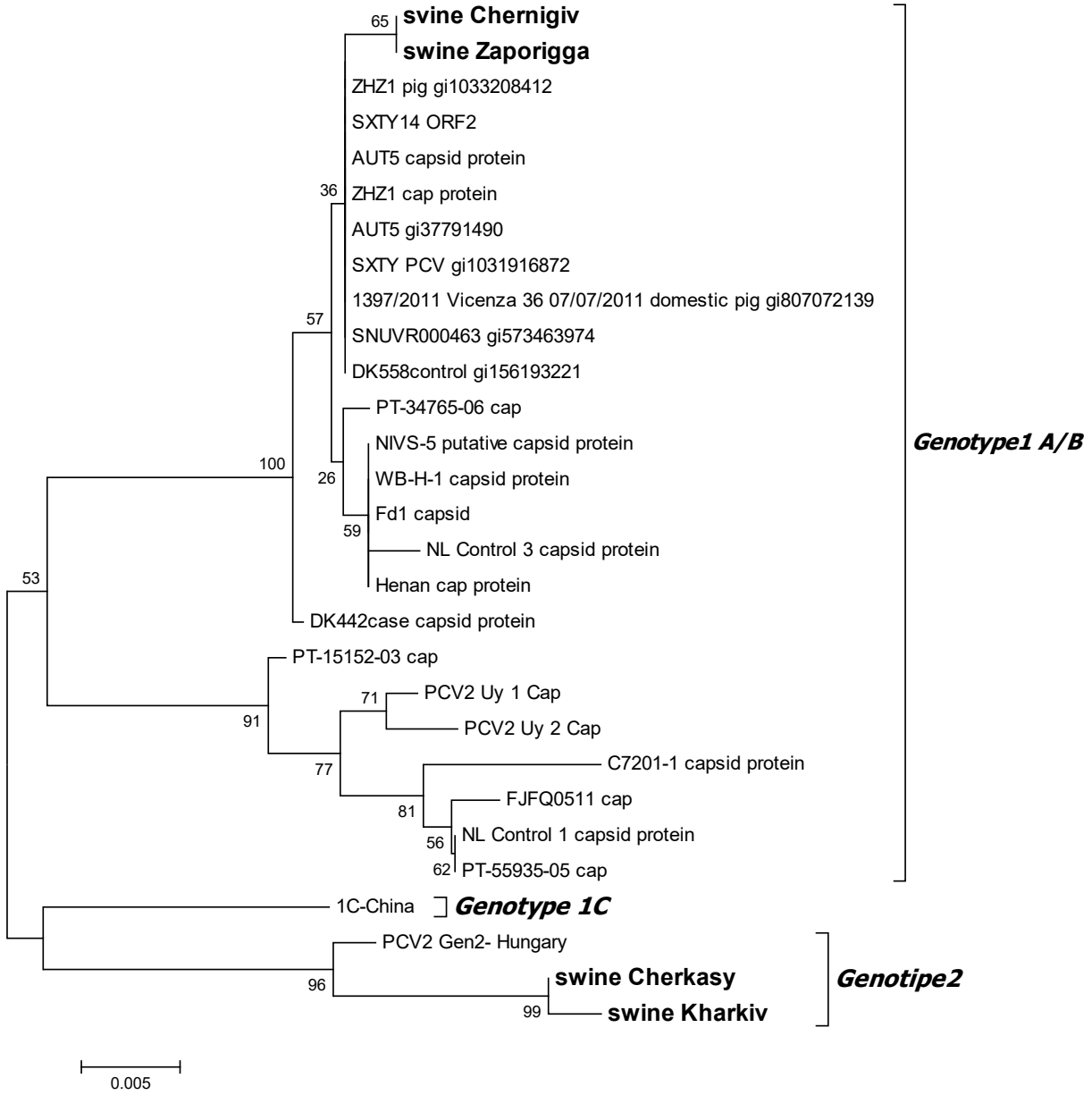

Figure 1. Evolutionary relationships of PCV2

However, a few amino acid replacements among Ukraine sequences in this study were observed. Due to the high nucleotide substitution rate of PCV2 compared to other single-stranded DNA viruses, it was estimated approximately $1.2x10^3$ substitutions/site/year (Firth et al., 2009). Therefore, the emerging of any new PCV2 genotype is possible in the future. Since the samples in this study were collected from the highest pig density provinces, the results yielded in this study can demonstrate at least 2 introductions of PCV2 into Ukraine. Imported swine breeders and semen appear to be the major route or transmission. Another evidence of introducing new virus strain into the swine herds is using improper killed chimeric vaccine in Canada (Gagnon et al., 2010).

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Received to editorial board 06.10.17

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ПОВНОГЕНОМНИЙ СИКВЕНС УКРАЇНСЬКОГО ІЗОЛЯТУ ЦИРКОВІРУСУ СВИНЕЙ 2-ГО ТИПУ

Свинячий цирковірус 2 (PCV2) асоціюється з різними синдромами і хворобами свиней, які відомі під загальною назвою свинячих цирковірус-асоційованих захворювань (PCVAD), які включають синдром мультисистемного розладу (PMWS) PCV2-асоційовані пневмонії (PRDC), PCV2, асоційований з ентеритом, PCV2, асоційований з репродуктивною функцією, а також свинячі дерматит і синдром нефропатії (PDNS) (1-3). PCV2-інфекція широко поширена і по суті всі свині стада заражені PCV2. Свинячий цирковірус 2 (PCV2), член роду* Circovirus *родини Circoviridae. Має ол- ДНК вірусу приблизно 1,7 кб (4). Геном PCV2 кодує три основних відкритих рамок зчитування (ORF,), які кодують репліказу (ORF1), вірусний білок капсида (ORF2), і білок, із запропонованою апоптичною активністю (ORF3) (5). Попередні дані показали, що існує п'ять генотипів PCV2, в тому числі PCV2a, PCV2b, PCV2c, PCV2d і PCV2e (6-9). В нашій роботі ми провели секвенування повного геному генома українськогих ізолятів PCV2з різних регіонів України.

Ключові слова: свинячий цирковірус 2, свинячий цирковірус-асоційованих захворювань, мультисистемний розлад.

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ПОЛНОГЕНОМНЫЙ СИКВЕНС УКРАИНСКОГО ИЗОЛЯТА ЦИРКОВИРУСА СВИНЕЙ 2-ГО ТИПА

Свиной цирковирус 2 (PCV2) ассоциируется с различными синдромами и болезнями свиней которые известны под общим названием свиных цирковирус-ассоциированных заболеваний (PCVAD), которые включают синдром мультисистемных нарушений (PMWS) PCV2-ассоциированной пневмонии, PCV2, ассоциированный с энтеритом, PCV2, ассоциированных с репродуктивной функцией, а также свиные дерматит и синдром нефропатии (PDNS) (1-3). PCV2-инфекция широко распространена и по существу все свиньи стада заражены PCV2. Свиной цирковирус 2 (PCV2), член рода* Circovirus *семейства Circoviridae Геном – очень маленькая оц- ДНК вируса примерно 1,7 кб (4). Геном PCV2 кодирует три основных открытых рамок считывания (ORF,), кодирующие репликазуи (ORF1), вирусный белок капсида (ORF2), и белок, с предложенной апоптотической активностью (ORF 3) (5). Предыдущие данные показали, что существует пять генотипов PCV2, в том числе PCV2a, PCV2b, PCV2c, PCV2d и PCV2e (6-9). Здесь мы сообщаем полную последовательность генома украинских изолятов PCV2из разных регионов Украины.

Ключевые слова: свиной цирковирус 2, свиной цирковирус-ассоциированных заболеваний, синдром мультисистемных нарушений.

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PREVALENCE OF DRUG RESISTANT HIV STRAINS IN HIV-INFECTED PATIENTS OF REPRODUCTIVE AGE

The prevalence of drug resistant HIV strains among HIV-positive reproductive aged persons with ineffective antiretroviral therapy (ART) was assessed. The prevalence of drug resistant strains of HIV was 73.8% in the group of women and 89.29% in the group of men (totally in 80.0% of patients). In the spectrum of drug resistance mutations (DRMs) the most prevalent mutation associated with high-level resistance to nucleoside reverse transcriptase inhibitors was substitution M184V (80.36%); in addition, the high prevalence of K65R (26.79%) was indicated. The most common mutations causing a high-level resistance to non-nucleoside reverse transcriptase inhibitors were G190S/A (57.14%), Y181C (37.50%), K101E (33.93%). The DRMs to protease inhibitors were indicated with significantly less frequent (5.36%).

Key words: HIV, drug resistant mutations, antiretroviral therapy.

Introduction. Antibiotic and antiretroviral resistance has become a major clinical and public health problem nowadays. The selection of resistant forms of pathogens is based on natural processes of microbes adaptation to conditions of environment. Uncontrolled, inappropriate and overabundant using of antimicrobial, antiviral and antifungal drugs, absence of strong clinical protocols of there using, extensive applying of them in agriculture and animal husbandry, the