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# THE APPLICATION OF PCR BASED METHODS IN DIAGNOSTICS OF SOME VIRAL INFECTIONS OF SWINE

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Review paper

**Abstract:** Viral infections of swine cause significant economic losses in swine husbandry. They manifest in death of infected animals of different ages or in decreased productivity during the manufacturing process. Having that in mind, rapid and reliable diagnostics of viral infections is crucial in the prevention of disease transmission in herds of swine. Today, virological laboratories all over the world use different diagnostic methods such as isolation of virus in cell lines, ELISA, virus neutralization test, direct and indirect immunofluorescence and hemagglutination and hemagglutination inhibition tests. Virus isolation, virus neutralization test and some other standard virological methods are time consuming and rather expensive, therefore, molecular methods such as conventional PCR, RT - PCR, real-time PCR and direct sequencing methods are applied worldwide as fast and reliable. Their application is especially necessary for the detection of viruses which cannot be identified by using standard virological methods.

**Key words**: swine, virus, PCR, real-time PCR

## Introduction

Viral infections of swine cause significant economic losses in swine husbandry. They manifest in death of infected animals of different ages or in decreased productivity during the manufacturing process. Having that in mind, rapid and reliable diagnostics of viral infections is crucial in the prevention of disease transmission in herds of swine.

Today, virological laboratories all over the world use different diagnostic methods such as isolation of virus in cell lines, ELISA, virus neutralization test, direct and indirect immunofluorescence and hemagglutination and hemagglutination inhibition tests (*Nišavić et al., 2006; Nišavić et al., 2007; Nišavić*

*et al., 2008; Nišavić et al., 2013*). Virus isolation, virus neutralization test and some other standard virological methods are time consuming and rather expensive, therefore, molecular methods such as conventional PCR, RT - PCR, real-time PCR and direct sequencing methods are applied worldwide as fast and reliable (*Milić et al., 2010; Nišavić et al., 2010; Veljović et al., 2013*).

Porcine circovirus 2 (PCV2) belongs to the family *Circoviridae* and genus *Cyrcovirus*. PCV2 is single-stranded with circular DNA and without outer envelope (*Segales et al., 2012*). The viral genome consists of 1767-1768 nucleotides and contains three ORF regions (*Larochelle et al., 2002*). The ORF1 region (rep gene) is responsible for the synthesis of proteins involved in the replication process, ORF2 (cap gene) region provides the synthesis of capsid proteins, whilst the third region - ORF3 encodes proteins that are likely involved in virus induced cell apoptosis. Based on the results of phylogenetic analysis of the whole genome of porcine circovirus type 2, there are four genotypes: PCV2a, PCV2b, PCV2c and PCV2d (*Liu et al., 2006; Cheung et al., 2007; Hesse et al., 2008; Wang et al., 2009*).

Depending on the localization of the pathological process and ages of pigs, PCV2 causes different kinds of disease. Today, the disease caused by PCV2 is best known as post-weaning multisystemic wasting syndrome (PMWS). This disease is manifested by the appearance of stunting, pallor of the skin, respiratory distress, and sometimes diarrhea and jaundice in weaned piglets for fattening (*Segales et al., 2005*). Infection of young animals, fattening pigs and adult pigs caused by PCV2 is also manifested as dermatitis and nephropathy syndrome or PDNS (*Rose et al., 2012*). Furthermore, porcine circovirus 2 may cause reproductive disorders and abortion (*O' Conor et al., 2001*), pneumonia (*Kim et al., 2003; Segales et al., 2004*) and enteritis (*Kim et al., 2002*).

Porcine parvovirus belongs to the family *Parvoviridae*. This is a non-enveloped single-stranded virus with linear DNA. Viral capsid consists of 32 capsomeres. The course and outcome of porcine parvovirus infection of swine depends on the immune status of infected animals and the stage of pregnancy at the time of infection. Infection of pregnant pigs causes fetal death and mummification (*Mengeling et al., 1975*). Fetuses infected before the 70th day of pregnancy usually die, while fetuses infected later synthesize specific antibodies against parvovirus and usually survive.

Aujeszky's disease virus (ADV) or pseudorabies virus (PrV) belongs to the family *Herpesviridae*, subfamily *Alphaherpesuirinae* and genus *Varicellovirus*. The viral genome consists of double-stranded DNA and the virus possesses an outer envelope.

Pseudorabies virus primarily causes disease in pigs, but it also occurs in cattle, sheep, goats, dogs, cats and wild boars which are thought to be the reservoir of the virus. Virus is transmitted among animals by direct and indirect contact (*Verpoest et al., 2014; Moreno et al., 2015*).

Aujeszky's disease virus causes neurological symptoms, respiratory distress and in some cases reproductive disorders in infected swine (*Pol et al., 2013; Moreno et al., 2015*). One of the most significant characteristics of pseudorabies infection in swine is the establishment of virus latency in olfactory bulb or trigeminal ganglia. The virus is reactivated as a result of stress or immunosuppressive factors leading to the appearance of clinical symptoms of the disease (*Huang et al., 2004; Steinringl et al., 2012*).

Based on available literature data, it can be concluded that molecular methods such as conventional PCR, RT-PCR, real-time PCR or direct sequencing are used worldwide in laboratory diagnostics of infections of swine caused by PCV2, PPV and PrV. Ogawa et al., 2009 used multiplex PCR and multiplex RT-PCR for the detection of PCV2, PrV, PPV, PRRS virus, Japanese encephalitis virus, porcine rotavirus, porcine epidemic diarrhea virus, and TGE virus from different kinds of samples such as feces, aborted fetuses or internal organs. The results of the investigation showed the presence of PCV2 in 32 samples and PPV and PRRS virus in 9 samples. The detection of PCV2, PPV and PRRS virus was performed by *Jiang et al. (2010)*. Seventy –six samples taken from pigs of 4 to 12 weeks of age and 27 aborted foetuses from 11 farms in Zhejiang Province in China, were examined by multiplex PCR. The presence of PCV2 was detected in 7 samples whilst PRRS virus and PPV were identified in 3 samples. Mixed infections caused by PCV2 and PPV as well as by PCV2 and PRRS virus were detected in 2 and 14 samples respectively, whilst 34 samples were negative for the presence of nucleic acids of the abovementioned viruses. *Opriessnig et al. (2014)* investigated the presence of porcine parvovirus and porcine circovirus type 2 in tissue samples from pigs originating from different parts of North America. The study included 586 blood serum samples and 164 samples of lung tissue collected from pigs in the period from 1996 to 2013. The presence of antibodies against PCV2 was found in 27.7% (162/586) samples, whilst the presence of PPV specific antibodies was found in 48.8% (286/586) of the blood sera. The presence of PCV2 was determined in 78.7% (129/164) of samples of lung tissue, while 56.7% (93/164) of lung samples were positive for the presence of porcine parvovirus. Mixed infections caused by PCV2 and PPV were discovered in 14.3% (84/586) of samples of sera and in 49.4% (81/164) of samples of lung tissue. Additionally, the prevalence of PPV DNA is significantly higher in tissues containing a large amount of PCV2 DNA in comparison to tissue samples that do not originate from animals with clinical symptoms of systemic circovirus infection. Lukač et al., 2016 used PCR for the detecion of PCV2 and PPV in swine from Republika Srpska. The presence of PCV2 was detected in 6 samples (6/80), while PPV was recovered from 5 samples (5/80). It should be noted that the pigs whose samples showed the presence of the aforementioned viruses did not express clinical signs of infection. The simultaneous detection of PCV2, PPV and PRRS virus was performed by Liu et al., 2013 using multiplex PCR. In total, fifty-eight samples of lung, tonsils,

lymph nodes and spleen and 24 samples of aborted fetuses were examined during the investigation. The presence of PRRS virus was detected in 12.19% of samples, while 21.95% of the samples were positive for PRRS and PCV2 viruses. Huang et al. 2013 used multiplex PCR method for the detection of PCV2, PCV1 and PrV. In total, 58 samples of lymph nodes, tonsils and lungs from pigs of 4 to 8 weeks of age were examined. The presence of PCV2 was observed in 30 samples, PCV in 2 samples, whilst the presence of Aujeszky's disease virus was found in only one sample. Mixed infection with PCV1 and PCV2 was detected in eight samples, whilst the presence of PCV2 and PrV was confirmed in six samples. Wilhelm et al., 2006 used the method of real - time PCR for the detection of the presence of PPV, PCV2, PrV and PRRS virus in samples of heart muscle, kidney, lungs, spleen, duodenum, jejunum, thymus and lymph nodes of swine. The obtained results confirmed the validity of real time PCR for fast and reliable routine diagnostics of parvovirus infections in swine as well as infections caused by other aforementioned viruses. Duplex real-time PCR was used by Zeng et al., 2013 for the detection of PPV and PCV2 in different samples collected from pigs. The presence of PCV2 and PPV was detected in 18 samples. Thirty-seven out of 72 samples of boar semen were positive for the presence of PPV, whilst 35 samples were positive for the presence of PCV2. *Larochelle et al. (2002)* performed the molecular characterization and phylogenetic analysis of 34 PCV2 strains identified in swine from eastern parts of Canada. The nucleotide sequences of those strains were compared with analogous sequences of 36 strains of PCV2 published in GenBank database and the results showed a high level of similarity (96% to 100%) with other PCV2 strains identified in Western Canada, the USA, Europe and Asia. The results of this investigation also demonstrated that the ORF1 region of the viral genome is highly conserved in all examined PCV2 strains. Ramos et al. 2013 analysed PCV2 strains from Uruguay. The molecular analysis of the PCV2 cap gene showed a nucleotide similarity of 99.7% among Uruguayan isolates and with two of the Brazilian isolates included in this study. Uruguayan isolates shared a nucleotide and amino acid identity of 99.1– 99.5% with Argentinean strains, which were in turn more closely related to isolates from France, Cuba, Canada and USA. Phylogenetic analysis revealed that Uruguayan PCV2 strains belong to PCV2a genotype. Molecular characterization of PCV2 strains identified in pigs in South Korea was carried out by *Chae et al, (2012)*. From a total of 21 strains of the virus, 17 belonged to the genotype PCV2b, whilst others belonged to genotypes PCV2a and PCV2c. During the extensive examination conducted by *Lukač et al. (2016)* eighty samples from non-vaccinated pigs from the territory of the Republic of Srpska were examined for the presence of PCV2 and PPV. Porcine circovirus 2 identified in this study belonged to PCV2c genotype and had a high level of similarity with some Italian, German and Chinese strains of the virus, while the identified PPV viruses were similar to viruses identified in UK, USA and China. *Cadar et al. (2012)* examined tissue samples and organs originating from 842 wild

boars that were collected in the period from 2006 to 2011 in the western regions of Romania. In addition to these samples, 120 samples collected from domestic pigs were also examined. The results showed that porcine parvovirus mostly diverged in the last 20 to 60 years and that the strains of the virus identified in wild boars have greater genetic diversity regarding to the strains of porcine parvovirus identified in domestic pigs. *Xiofen et al. (2013)* examined the evolutionary development and phylogeny of porcine parvovirus. During this investigation authors used 46 nucleotide sequences of the virus originating from the genetic database. The results showed that a common ancestor of all PPV strains existed about 250 years ago. *Xu et al. (2013)* compared the nucleotide sequences of VP2 gene of PPV - NE / 09 PPV virus and other PPV strains in China. The results showed a high level of similarity between the PPV strain NE / 09 and other PPV strains identified in China and that NE / 09 represents a mutant strain of the existing strains of porcine parvovirus, which has the highest prevalence of infection in pigs in China. *Serena et al. (2011)* compared the nucleotide sequences of Argentinean PrV isolates with the nucleotide sequences of PrV reference strains available at GenBank. A high percentage of nucleotide similarity was demonstrated between genotype I Argentinean strains (CL/7, CL/15, TL/92 and A/94) and the American strains Rice and Becker. The other genotype I Argentinean strains (CL/96, CL/98, CLP/98-10P and RC/79) had 99.7% identity with the reference strains Becker and Rice. Furthermore, the Argentinean genotype I strains showed high similarity with Brazilian genotype I strains (99.0– 99.4). *Verpoest et al. (2014)* performed the molecular characterization of Belgian pseudorabies virus isolates from domestic swine and wild boar. The results showed that one isolate from domestic pig had a sequence identical to the Kaplan reference strain of PrV.

## Conclusion

Regarding available literature data it can be concluded that the application of molecular methods based on PCR is crucial for fast and precise detection of viral infections of swine. The application of these methods is very important during the outbreaks of infection with high mortality rate when it is necessary to conduct fast and reliable diagnostics in order to prevent further dissemination of the infectious agent in animal population. Besides that, their application is especially necessary for the detection of viruses which cannot be identified by using standard virological methods.

## Primena molekularnih metoda zasnovanih na lančanoj reakciji polimeraze u dijagnostici nekih infekcija svinja

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# Rezime

Virusne infekcije izazivaju značajne ekonomske gubitke u svinjarskoj proizvodnji. One se ispoljavaju kako kroz pojavu uginuća životinja, tako i kroz smanjenje produktivnosti. U cilju otkrivanja i sprečavanja širenja virusnih oboljenja svinja danas se u svetu primenjuju standardne i molekularne metode virusološke dijagnostike. Od standardnih metoda dijagnostike u upotrebi su metode izolacije virusa u kulturi ćelija, zatim ELISA, direktna i indirektna imunofluorescencija, kao i hemaglutinacija i inhibicija hemaglutinacije. Primena navedenih metoda podrazumeva duže vreme potrebno za dobijanje rezultata ispitivanja od najmanje 5 do 7 dana. Međutim, primena savremenih molekularnih metoda virusološke dijagnostike kao što su PCR, real-time PCR, odnosno metoda direktnog sekvenciranja, podrazumeva kraće vreme potrebno za dobijanje rezultata, odnosno omogućava preciznu dijagnostiku oboljenja u kraćem vremenskom periodu. Pored ovoga, značaj primene ovih metoda se ogleda i u otkrivanju virusa čije se prisustvo u uzorcima na drugi način, odnosno primenom standardnih metoda virusološke dijagnostike ne može detektovati.

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# References

CADAR D., CSAGOLA A., LORINZ M., TOMBASZ K., SPINU M., TUBOLY T. (2012): Detection of natural inter-and intra-genotype recombination events revealed by cap gene analysis and decreasing prevalence of PCV2 in wild boars. Infection, Genetics and Evolution, 12, 2, 420-427.

CHEUNG A., LAGER K., KOHUTYUK O.I., VINCENT A.L., HENRY S.C., BAKER R.B., DUNHAM A.G. (2007): Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. Archives of virology, 152, 5, 1035-1044.

HESSE R., KERRIGAN M., ROWLAND R. (2008): Evidence for recombination between PCV2a and PCV2b in the field. Virus research, 132, 1, 201-207.

HUANG C., HUNG J., WU C., CHIEN M. (2004): Multiplex PCR for rapid detection of pseudorabies virus, porcine parvovirus and porcine circoviruses. Veterinary Microbiology, 101, 209-214.

HUNG J., WU C., CHIEN M. (2004): Multiplex PCR for rapid detection of pseudorabies virus, porcine parvovirus and porcine circoviruses. Veterinary Microbiology, 101, 209-214.

JIANG Y. SHANG H., XU H., ZHU L., CHEN W., ZHAO L., FANG L. (2010): Simultaneous detection of porcine circovirus type 2, classical swine fever virus, porcine parvovirus and porcine reproductive and respiratory syndrome virus in pigs by multiplex polymerase chain reaction. The Veterinary Journal, 183, 172-175.

KIM J., CHUNG H. K., JUNG T., CHO W. S., CHOI C., CHAE C. (2002): Postweaning multisystemic wasting syndrome of pigs in Korea: prevalence, microscopic lesions and coexisting microorganisms. Journal of Veterinary Medical Science, 64, 1, 57-62.

KIM J., CHUNG H.K., CHAE C. (2003): Association of porcine circovirus 2 with porcine respiratory disease complex. The Veterinary Journal, 166, 3, 251-256.

LAROCHELLE R., MAGAR R., D ALLAIRE S. (2002): Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. Virus research, 90, 1, 101-112.

LIU J., CHEN I., DU Q., CHUA H., KWANG J. (2006): The ORF3 protein of porcine circovirus type 2 is involved in viral pathogenesis in vivo. Journal of Virology, 80, 10, 5065-5073.

LIU J.K., WEI C.H., YANG X.Y., DAI A.L., XIAO –HUA L. (2013): Multiplex PCR for the simultaneous detection of porcine reproductive and respiratory syndrome virus, classical swine fever virus, and porcine circovirus in pigs. Molecular and Cellular Probes, 27, 149-152.

LUKAĆ B., KNEŽEVIĆ A., MILIĆ N., KRNJAIĆ D., VELJOVIĆ LJ., MILIĆEVIĆ V., ZORIĆ A., ĐURIĆ S., STANOJEVIĆ M., NIŠAVIĆ J. (2016): Molecular detection of PCV2 and PPV in pigs in Republic of Srpska, Bosnia and Herzegovina, Acta Veterinaria-Beograd, 66, 1, 51-60.

MENGELING W.L., CUTLIP R.C. (1975): Pathogenesis of in utero infection: experimental infection of five-week-old porcine fetuses with porcine parvovirus. American journal of veterinary research, 36, 8, 1173-1177.

MILIĆ, N., NIŠAVIĆ J., AŠANIN R., KNEŽEVIĆ A., AŠANIN J., VIDANOVIĆ D., ŠEKLER M. (2010): Primena lančane reakcije polimeraze (PCR) i metode Real-Time PCR u brzoj identifikaciji goveđeg herpesvirusa 1, Veterinarski Glasnik, 64, 3-4, 159-167.

MORENO A., SOZZI E., GRILLI G., GIBELLI L.R., GELMETTI D., LELLI D., CHIARI M., PRATI P., ALBORALI G.L., BONIOTTI M.B., LAVAZZA A., CORDIOLI P. (2015): Detection and molecular analysis of Pseudorabies virus strains isolated from dogs and a wild boar in Italy. Veterinary Microbiology, 177: 359-365.

NIŠAVIĆ J., MILIĆ N. (2006): Examination of the activity of glycoprotein HN and F antigens of the outer envelope of the parainfluenza virus type 3 by using fusional, hemolytic and hemagglutination tests, in vitro. Acta veterinaria, 56, 5-6, 431-36.
NIŠAVIĆ J., MILIĆ N., VELJOVIĆ Lj. (2007): Examination of the activity of glycoprotein HN and F antigens of the outer envelope of Newcastle disease virus by using fusional, hemolytic, hemagglutination and hemadsorption tests, in vitro. Acta veterinaria, 57, 1, 3-10.
NIŠAVIĆ J., KNEŽEVIĆ A., MILIĆ N. (2008): Primena lančane reakcije polimeraze (PCR) u identifikaciji izolovanih sojeva goveđeg herpesvirusa tip 1, VI Kongres medicinske mikrobiologije, Beograd, juni 2008, Zbornik kratkih sadržaja, 227-228.
NIŠAVIĆ J., MILIĆ N., KNEŽEVIĆ A., JOVANOVIĆ T. (2010): The application of polymerase chain reaction in detection of bovine herpesvirus 1 in clinical samples. Acta Veterinaria, 60, 1, 39-48.
NIŠAVIĆ J., MILIĆ N., VIDANOVIĆ D., ŠEKLER M., LAZIĆ S., PETROVIĆ T. (2013): Primena standardnih i molekularnih metoda u dijagnostici virusnih infekcija, 34. Seminar za inovaciju znanja veterinara, 8.februar, Beograd, 45-63.
O̓ CONOR B., GAUVREAU H., WEST K., BOGDAN J., AYROUD M., CLARK E., ELLIS J.A. (2001): Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. The Canadian Veterinary Journal, 42,7, 551.
OGAWA H., TAIRA O., HIRAI T., TAKEUCHI H., NAGAO A., ISHIKAWA Y., TUCHIYA K., NUNOYA T., UEDA S. (2009): Multiplex PCR and multiplex RT-PCR for inclusive detection of major swine DNA and RNA viruses in pigs with multiple infections. Journal of Virological Methods, 160, 210-214.
OPRIESSING T., XIAO C.T., GERBER P.F., HALBUR P.G. (2014): Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease. Veterinary microbiology, 173, 1, 9-16.
POL F., DEBLANC C., OGER A., LE DIMNA M., SIMON G., LE POTIER M.F. (2013): Validation of a commercial real-time PCR kit for specific and sensitive detection of Pseudorabies. Journal of Virological Methods, 187: 421-423.
RAMOS N., MIRAZO S., CASTRO G., ARBIZA J. (2013): Molecular analysis of Porcine Circovirus Type 2 strains from Uruguay: Evidence for natural occurring recombination. Infection Genetics and Evolution, 19, 23-31.
ROSE N., OPRIESSNIG T., GRASLAND B., JESTIN A. (2012): Epidemiology and transmission of porcine circovirus type 2 (PCV2). Virus research, 164, 1, 78-89.
SEGALES J., ALLAN G.M., DOMINGO M. (2005): Porcine circovirus diseases. Animal Health Research Reviews, 6, 2, 119-142.

SEGALES J., KEKARAINEN T., CORTEY M. (2013): The natural history of porcine circovirus type 2: from an inoffensive virus to a devastating swine disease?.Veterinary microbiology, 165, 1, 13-20.
SERENA M., METZA G., MORTOLA E., ECHEVERNA M. (2011): Phylogenetic analysis of Suid Herpesvirus 1 isolates from Argentina. Veterinary Microbiology, 154, 78-85.
STERINRIGL A., REVILLA – FERNANDEZ S., KOLODZIEJEK J., WODAKA E., BAGO Z., NOWOTNY N., SCHMOLL F., KOFER J. (2012): Detection and molecular characterization of Suid herpesvirus type 1 in Austrian wild boar and hunting dogs. Veterinary Microbiology, 157:276-284.
VELJOVIĆ LJ., KNEŽEVIĆ A., MILIĆ N., NIŠAVIĆ J. (2013): Primena molekularnih metoda u identifikaciji izolovanih sojeva virusa parainfluence 3, 9. Kongres mikrobiologa Srbije, 30. maj – 1.jun, Beograd, 1.
VERPOEST S., CAY A., REGGE N. (2014): Molecular characterization of Belgian pseudorabies virus isolates from domestic swine and wild boar. Veterinary Microbiology, 172, 72-77.
VERPOEST S., CAY A.B., REGGE N.D. (2014): Molecular characterization of Belgian pseudorabies virus isolates from domestic swine and wild boar. Veterinary Microbiology, 172, 72-77.
WANG F., GUO X., GE X., WANG Z., CHEN Y., CHA Z., YANG H. (2009): Genetic variation analysis of Chinese strains of porcine circovirus type 2. Virus research, 145, 1, 151-156.
WILHELM S., ZIMMERMANN P., SELBITZ H.J., TRUYEN U. (2006): Real-time PCR protocol for the detection of porcine parvovirus in field samples. Journal of Virological Methods 2006, 134: 257–260.
XIOFENG R., TAO Y., CUI J., SUO S., CONG Y., TIJSSEN P. (2013): Phylogeny and evolution of porcine parvovirus. Virus research, 178, 2, 392-397.
XU Y., WANG H., HUO G., LI S. (2013.): Characterization of the capsid protein VP2 gene of a virulent strain NE/09 of porcine parvovirus isolated in China. Research in Veterinary Science, 94, 219-224.
ZHENG L., WANG Y., LI M., CHEN H., GUOB X., GENGA J., WANG Z., WEIA Z., CUI B. (2013): Simultaneous detection of porcine parvovirus and porcine circovirus type 2 by duplex real-time PCR and amplicon melting curve analysis using SYBR Green. Journal of Virological Methods, 187, 15-19.

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