SciMedCentral

Annals of Virology and Research

Short Communication

Virus Safety of Xenotransplantation: Prevalence of Porcine Circovirus 2 (PCV2) in Pigs

Julian Heinze, Elena Plotzki, and Joachim Denner*

Robert Koch Institute, Berlin, Germany

***Corresponding author**

Joachim Denner, Robert Koch Institute, Berlin, Germany, Tel: 49187542800; Email: DennerJ@rki.de

Submitted: 08 November 2016

Accepted: 02 December 2016

Published: 02 December 2016

Copyright

© 2016 Denner et al.

OPEN ACCESS

Keywords

- Porcine circoviruses
- Xenotransplantation
- Virus safety

Abstract

Porcine circovirus 2 (PCV2) is the causative agent of a whole series of diseases in pigs, called PCV2 diseases (PCVD). The most relevant of them is the systemic disease (PCV2-SD), formerly called post weaning multi systemic wasting syndrome (PMWS). Xenotransplantation using pig cells, tissues and organs is under development to overcome the shortage of human transplants for the treatment of tissue and organ failure. Xenotransplantation requires functional cells, tissues or organs from healthy animals and therefore the donor pigs should be free of PCV2. Selection of PCV2-free animals will also prevent transmission of the virus to the human recipient. Using a PCR method, (i) Göttingen Minipigs, which are well characterised and which were already used in pig to non-human primate xenotransplantations, (ii) newly generated Aachen Minipigs, (iii) genetically modified pigs generated for xenotransplantation, (iv) pigs from a slaughterhouse and (v) pigs from a German farm were screened for PCV2. 50% of the Aachen minipigs and 14% of Göttingen minipigs were PCV2 positive, but the animals were apparently healthy. None of the slaughterhouse animals, the farm animals and the genetically modified animals were positive for PCV2, because they had been vaccinated. The data indicate that PCV2 may be found in healthy pigs even under SPF conditions, and that vaccination is a powerful tool to prevent infection.

ABBREVIATIONS

GAPDH: Glycerinaldehyd-3-phosphat-Dehydrogenase; GGTA1: Alpha-Galactosyltransferase 1; HAR: Hyperacute Rejection; HEV: Hepatitis E Virus; HO1: Heme Oxygenase1; Neu5Gc: N-glycolylneuraminic acid; PCV1: Porcine Circovirus 1; PCV2: Porcine circovirus 2; PCVD: Porcine Circovirus Diseases; PCVAD: Porcine Circovirus Associated Diseases; PCV2-SD: PCV2 Systemic Disease; PCV2-LD: PCV2 Lung Disease; PCV2-ED: PCV2 Enteric Disease; PCV2-SI: PCV2 Subclinical Infection; PDNS: Porcine Dermatitis and Nephropathy Syndrome; PCMV: Porcine Cytomegalovirus; PMWS: Post weaning Multi systemic Wasting Syndrome; CMAH: Cytidine Monophosphate-N-Acetylneuraminic Acid Hydroxylase

INTRODUCTION

Porcine circovirus 2 (PCV2) is considered as the essential infectious agent of a whole series of diseases in swine which are now called porcine circovirus diseases (PCVD) in Europe or porcine circovirus associated diseases (PCVAD) in North America [1]. The diseases associated with PCV2 are PCV2-SD, a systemic disease in piglets, formerly called post weaning multi systemic wasting syndrome (PMWS); PCV2-LD, a lung disease, previously called proliferative and necrotising pneumonia; PCV2-ED, an enteric disease; PCV2-RD, a PCV2-reproductive disease; PCV2-subclinical infection (PCV2-SI), and porcine dermatitis and nephropathy syndrome (PDNS). PCV2-SD disease is characterised by wasting, pale skin, diarrhoea and jaundice, PCV2-LD by respiratory distress, PCV2-ED by diarrhoea, PCV2-RD by stillborn, mummified piglets, and returns to oestrus, PVCV-SI by a reduced growth rate and PDNS by dark red skin patches in hind legs and swollen kidneys. Type and severity of the disease depend on the viral subtype, genetic factors of the host and co-infections [2].

The risk posed by PCV2 for xenotransplantation may be based, first of all, on the reduced quality of the transplant due to infection and replication of the virus and it may be based, second, on the transmission of PCV2 to the human recipient. Viral replication seems to take place in a number of cell types of the pig, mainly epithelial and endothelial cells as well as macrophages [3], so that most organs needed for transplantation may be infected. Since PCV2 suppresses innate immunity, concomitant infections are considered a hallmark in infection and disease development [4], so that in addition to PCV2 other zoonotic microorganisms may be transmitted to the recipient. Whether direct infection of humans with PCV2 poses a risk for the recipient, is still unclear. Whereas human cells can be infected with PCV2 resulting in cytopathogenic effects [5], vaccination of humans with a live attenuated rotavirus vaccine contaminated with PCV1 or PCV2 particles did not result in infection of the vaccinated children [6,7].

The prevalence of PCV2 has been well studied in wild boars and breeding herds, however, considerable differences in the prevalence in pig herds was observed when analysing the literature, ranking from 0.65% to 100% infected (Table 1). Table (1) compiles the results of 11 studies in over eight countries world-wide. Based on these data we were interested to analyse the prevalence of PCV2 in pigs which may be used for xenotransplantation in comparison with pigs produced for meat production.

MATERIALS AND METHODS

Animals

The following pig breeds were analysed: First, Göttingen Minipigs which were well characterised by the breeding company, Ellegaard, Denmark www.minipigs.dk, as well as by our studies [8-10]. Second, Aachen Minipigs, a recently established minipig breed [11]. Third, genetically modified pigs specially produced for xenotransplantation in the Chair of Livestock Biotechnology of the Technical University Munich, Germany [12]. The genetic modifications with the goal to prevent immunological rejection of the xenotransplant included CD55, CD46, and CD59, which are all complement regulatory proteins preventing Hyperacute Rejection (HAR). In addition, these pigs were expressing HO1 (heme oxygenase, an enzyme that catalyses the degradation of heme) and A20. A20, a zinc finger protein, which has a strong antiapoptotic and anti-inflammatory function and over expression of A20 in islet cells increased transplant survival [13]. The knockout of the alpha-galactosyltransferase 1(GGTA1) (GalT-KO) and of the cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) abolished expression of the α-Gal and the N-glycolylneuraminic acid (Neu5Gc) epitopes [12]. The animals were immunised with IngelvacCircoflex (Boehringer Ingelheim). In addition, German landrace hybrid pigs, 7 non-transgenic and 7 expressing INSLEA29Y, which were used in a preclinical trial transplanting their islet cells into marmosets [14] were analysed. Furthermore, three genetically modified cross-breeds of German Landrace and Large White, with a GalT-KO, expressing human CD46 and human thrombomodulin, which had been found negative for the porcine cytomegalovirus (PCMV) and hepatitis E virus (HEV) when tested using sensitive methods and which were used as donors for an orthotopic heart transplantation into a baboon [15,16] were analysed. Fourth, pigs in a slaughterhouse near Berlin and finally, fifth, animals from a farm in Fehmarn, Germany were tested. The Fehmarn animals were German landrace (sow) crossed with Yorkshire (boar). The animals are screened every four and 12 weeks serologically for Actinobacillus pleuropneumonia (APP), all 12 weeks for Porcine reproductive and respiratory syndrome (PRRS) and mycoplasma by ELISA, all 24 weeks for rhinitis, all 12 weeks by PCR for dysentery. The animals are vaccinated against PCV2using a vaccine from Intervet and against Porcine intestinal adenopathy (PIA), caused by Lawsonia intracellularis (Dr. Gripps, ZNVG eG, Neumünster, Germany, personal communication).

DNA isolation

DNA was isolated from either blood, liver or spleen of the animals using DN easy blood and tissue kit (Qiagen, Hilden, Germany) and from sera using the Pure Link Viral RNA/DNA Mini Kit (Life Technologies, Carlsbad, USA). DNA was quantified using a Nano Drop spectrophotometer.

Table 1: Reported prevalence of PCV2 in wild boars and domestic pigs in different countries.

Country	Pig breed	Year	Method	Total number	%	Reference
Bavaria, Germany	domestic	2016	real-time PCR	198 sows 590 piglets	0.65 0	Eddicks et al., 2016 [19]
Europe#	domestic	2016	real-time	202	54	
Spain	domestic		real-time PCR	75 PVC2-SD* 12 PDNS**	92 92	Olvera et al., 2004 [20]
Slovakia	domestic	2011	real-time PCR immunofluorescence		54 35	Csank et al.,2011 [21]
Slovakia	wild boars	2014	real-time PCR immunofluorescence	51	37 49	Bhide et al., 2014 [22]
Brasil	wild boars	2013	immunofluorescence	2305	84.9	Barbosa et al., 2016 [23]
Poland	domestic	2012	real-time PCR	312	75.6	Fabisiak et al., 2012 [24]
Bavaria, Germany	wild boar	2012	real-time PCR ELISA	203	50.7 56	Hammer et al., 2012 [25]
Transylvania	wild boar	2010	real-time PCR	469	13.5	Cadar et al., 2010 [26]
US	domestic	2010	real-time PCR ELISA	125 sows 125 colostrum 125 piglets	47/97*** 40/100 78/29	Shen, et al., 2010 [27]
Germany	wild boar domestic	2010	nested PCR	349 348	63 100	Reiner et al., 2010 [28]
Japan	domestic	2010	real-time PCR	29	100+ 100	Sasaki et al., 2010 [29]

Animals from five European countries, from 3% to 95% in different farms
* Pigs suffering from PCV2 systemic disease (post weaning wasting syndrome)
** Pigs suffering from porcine dermatitis and nephropathy syndrome
*** % DNA positive/ % antibody positive
+ 120 days after weaning

PCR

A PCR was performed as described using the primers specific for PCV1 or PCV2 (Table 2), [17] with 150 ng DNA in a 25 µl volume (1 mM of each primer, 2 mM $MgCl_2$, 10xPCR buffer (Thermo Fisher, Schwerte, Germany), 200 mM dNTPs, 0.75 U AmpliTaq Gold polymerase (Thermo Fisher, Schwerte, Germany), 5% DMSO). Cycling parameters were 95°C for 12 min and 42 cycles of 20 s at 95°C for denaturation optimal annealing temperature of primers for 20s and elongation for 45s at 72°C, followed by a final step at 72°C for 10min.Porcine GAPDH was used as reference gene. The PCR method for the detection of porcine GAPDH (for primers see Table (2)) was performed using the Go Taq Green Master mix (Promega, Fitchburg, USA), 1 µl of each primer (10 µM), 150 ng DNA and water in a total reaction volume of 25 µl. Amplification was performed for 2 min at 95°C and then cycled 45 times through 30s denaturation at 95°C, 30s at 57°C and extension for 40s at 72°C depending, followed by an extension step for 5 min at 72°C. A 1% agarose and the Gene Ruler™ 100 bp DNA ladder (Thermo Scientific, Schwerte, Germany) were used for gel electrophoresis. The sensitivity of the PCR was determined using the PCV2 plasmid PCV2Ori_pcdna3 (courtesy Dr. A. Mankertz, Robert Koch Institute) and estimated as 100 copies.

RESULTS

Prevalence of PCV2 in minipigs

First, DNA isolated from liver samples or sera from Göttingen Minipigs were tested for PCV2 (Figure 1). As expected, a 415bp band was detected in some cases and altogether 14% of the Göttingen Minipigs were found PCV2-positive. Second, DNA isolated from the liver or the spleen from Aachen Minipigs, a new breed founded and described only recently [11] were screened. When the PCV2-specific PCR was performed with DNA from Aachen Minipigs, in addition to the expected specific band

Table 2: Primers used in this study.

Primer/probe	Sequence 5´-3´	Position and accession number	Reference
PCV1 fw (F41) PCV1 rev (B42)	ATACGGTAGTATTGGAAAGGTAGGG ACACTCGATAAGTATGTGGCCTTC	1428-1452(KJ746929.1) 358-335	Mankertz et al., 2000 [17]
PCV2 fw (F66) PCV2 rev (B67)	GGTTTGTAGCCTCAGCCAAAGC GCACCTTCGGATATACTGTCAAGG	567-546 (KT868491.1) 152-175	Mankertz et al., 2000 [17]
pGAPDH fw pGAPDH rev	ACATGGCCTCCAAGGAGTAAGA GATCGAGTTGGGGCTGTGACT	1040-1062 (NM_001206359.1) 1188-1168	Duvigneau et al., 2005 [30]

PCV1: Porcine Circovirus 1; fw: forward, rev: reverse; PCV2, Porcine Circovirus 2; Pgapdh: porcine glyceraldehyde 3-phosphate dehydrogenase.

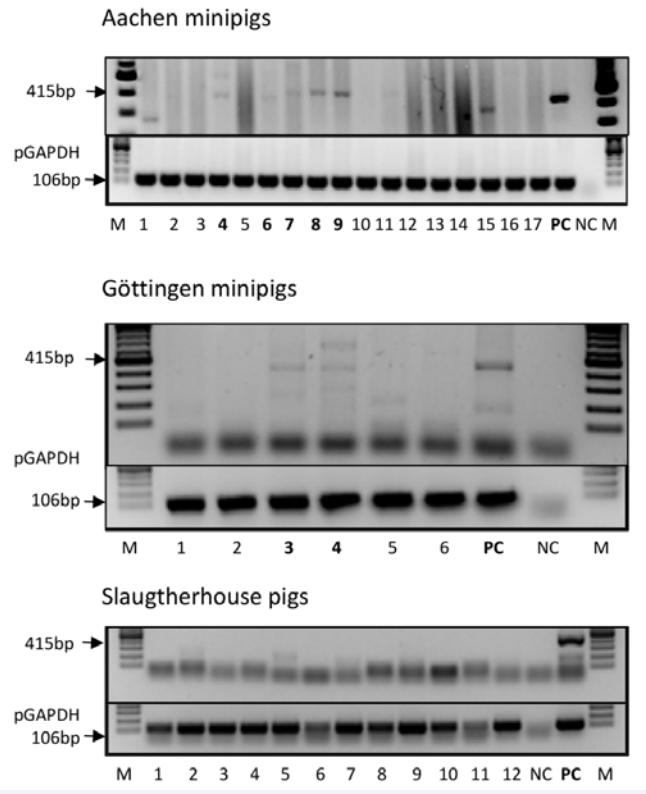

Figure 1 Results of the PCR analysis of DNA from different pigs. The 415 bp bands indicate the positive animals, their number is in bold. The GAPDH control verifies the presence of DNA.

(415bp), additional bands were observed (Figure 1). However, sequencing of the amplicons demonstrated that only the 415bp bands, but not the others, represented PCV2 (Figure 2). Altogether, 50% of the Aachen Minipigs were found PCV2-positive. When the amplicons of the PCR reaction were sequenced, differences between the sequences of the viruses in the Aachen Minipigs and that from the Göttingen minipig were detected. The viruses from the Aachen Minipigs are more closely to an US American isolate, the viruses from the Göttingen Minipigs are more closely to previously sequence German isolates (Figure 2). The sequence of PCV1 is different (Figure 2) and when primers specific for PCV1 were used in a PCR analysis, in none of the tested animals PCV1 was detected (not shown).

Prevalence of PCV2 in genetically modified pigs

When genetically modified pigs generated for application in

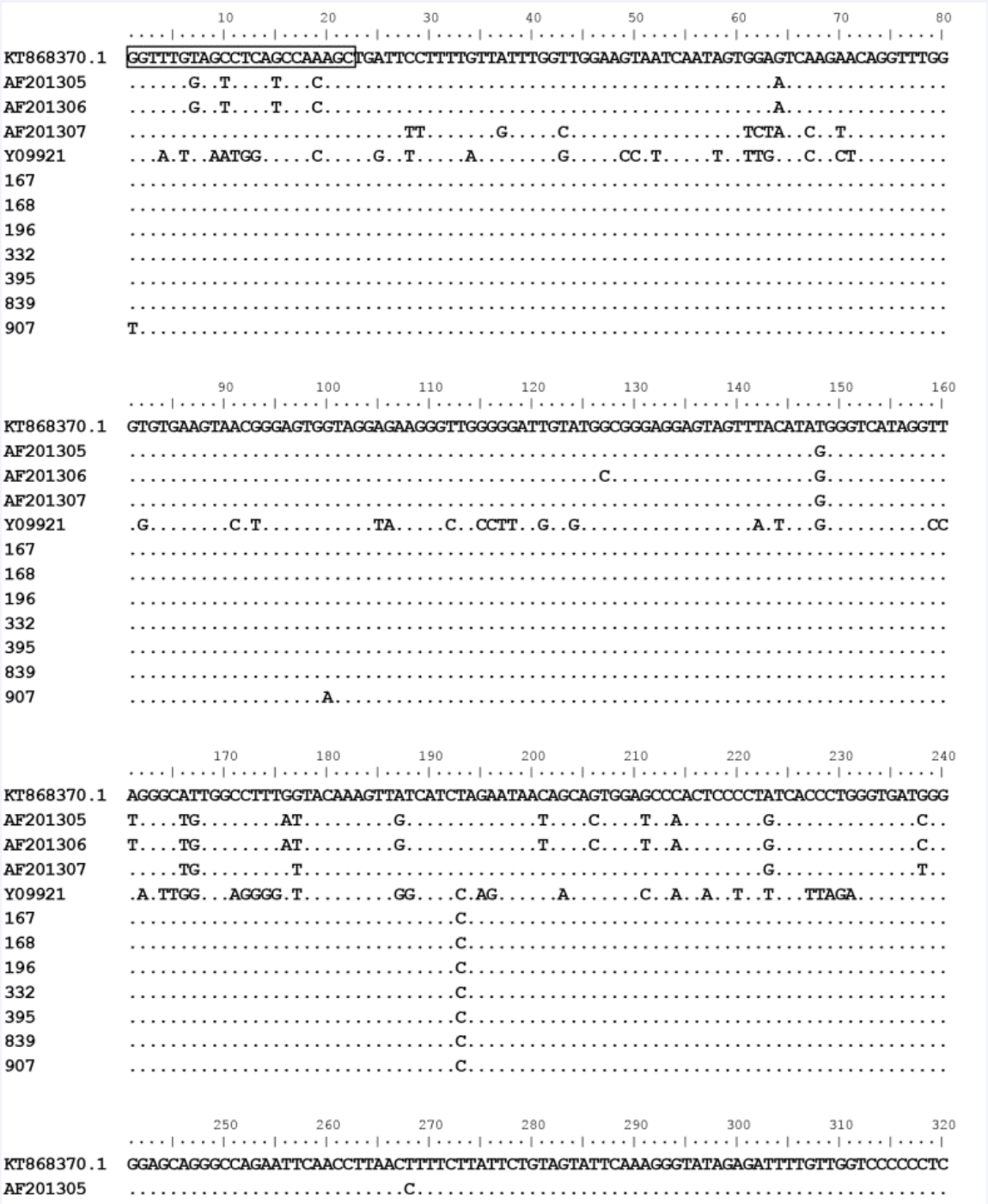

Figure 2 Comparison of PCV2 sequences amplified from DNA from Göttingen/Aachen Minipigs with published sequences. The accession numbers are indicated: KT868370.1, which is isolate USA/OK-003/2005; AF201305, AF201306, and AF201307, which are German isolates (GER1, GER2 and GER3); Y09921 is a German PCV1 isolate. Virus sequences 168,167were from Aachen Minipigs, all others from Göttingen Minipigs. The primer binding sites are framed.

xenotransplantation were screened for PCV2 and of PCV1, these viruses were not detected in all samples tested, including samples from the spleen or from whole blood (Figure 1), (Table 3). The animals were expressing one transgene such as INSLEA29Y in order to prevent rejection of the transplanted islet cells [14], or several transgenes such as the complement regulatory genes CD55, CD46, and CD59, the heme oxygenase HO1 and A20 in combination with knock-outs of enzymes responsible for the expression of α-Gal and Neu5Gc sugar epitopes on the cell surface [12]. Other genetically modified pigs, which had a GalT-KO and were expressing human CD46 and human thrombomodulin, had been used for orthotopic heart transplantation [15,16]. All these animals were free of PCV2 since they were vaccinated.

Prevalence of PCV2 in farm animals

In order to evaluate the prevalence of PCV2 in farm animals for meat production, slaughterhouse pigs and pigs in a German farm were analysed. DNA was isolated from the blood of the animals and tested. Interestingly, all animal were found negative for PCV2, indicating that they were vaccinated. They were also negative for PCV1.

DISCUSSION

Here genetically modified animals generated in Germany for application in xenotransplantation and pigs for meat production were screened for the prevalence of PCV1 and PCV2. Since the genetically modified pigs were vaccinated, no PCV2 was found in these animals, which already had been used in preclinical islet cell transplantations and heart transplantations into non-human primates [14-16]. This is the first report describing screening for PCV2 in genetically modified pigs generated for xenotransplantation. Until now mainly wild boars and domestic pigs in Europe, Japan and Brazil were analysed using different methods and PCV2 was found in 0.65 to 100% of the animals (Table 1).

The fact that Göttingen Minipigs were infected with PCV2 was surprising, since these animals are kept under highly hygienic SPF conditions and were introduced into the facility by Caesarean delivery. However, data showing transplacental transmission of PCV2 [18] may explain how the virus was introduced into the facility. The Aachen minipigs are a newly established breed and are kept still not under SPF conditions. Most importantly, in both strains, Göttingen and Aachen minipigs, PCV2 can be eliminated by vaccination. That vaccination is successful can be seen in the other groups, where vaccination was performed and all animal were PCV2-negative.

Table 3: Prevalence of PCV2 in different pig breeds. All animals were negative for PCV1.

Pig breed	Number of positive / total tested animals	Percentage of positive animals (%)
Aachen minipig	11/22	50
Göttingen minipig	3/21	14
Genetically modified pigs	0/6	0
Genetically modified pigs for islet cell transplantation, non-modified controls	0/7	0
Genetically modified pigs for heart transplantation	0/7	0
Farm pigs	0/3	0
Slaugtherhouse pigs	0/10	0
	0/12	0

Although PCV2 can infect human cells [5], no transmission of PCV was observed when vaccination of children with a rotavirus vaccine contaminated with circoviruses was performed [6,7]. However, it is still unknown what may happen when PCV infect immune suppressed humans.

Therefore, animals produced for xenotransplantation should be vaccinated before entering a SPF facility, animals should be analysed using highly sensitive detection methods and negative animals should be selected. Once the animals are in a SPF facility and PCV2 negative, vaccination will be not be required anymore.

CONCLUSION

The world-wide first screening of genetically modified pigs generated specifically for xenotransplantation showed that the animals were not infected with PCV1 and PCV2, obviously because they were vaccinated. On the other hand, Göttingen Minipigs already used in pig to non-human primate xenotransplantation were infected with PCV2 despite the fact that they are produced in a SPF facility. PCV can be eliminated from pig herds using sensitive detection methods, vaccination and isolation of the animals.

ACKNOWLEDGEMENTS

We would like to thank Dr. A. Mankertz, Robert Koch Institute, for the PCV2Ori_pcdna3 plasmid and for advice.

REFERENCES

1. Opriessnig T, Langohr I. Current state of knowledge on porcine circovirus type 2-associated lesions. Vet Pathol. 2013; 50: 23-38.
2. Segalés J, Kekarainen T, Cortey M. The natural history of porcine circovirus type 2: from an inoffensive virus to a devastating swine disease?. Vet Microbiol. 2013; 165: 13-20.
3. Hamberg A, Ringler S, Krakowka S. A novel method for the detection of porcine circovirus type 2 replicative double stranded viral DNA and nonreplicative single stranded viral DNA in tissue sections. J Vet Diagn Invest. 2007; 19: 135-141.
4. Grau-Roma L, Fraile L, Segalés J. Recent advances in the epidemiology, diagnosis and control of diseases caused by porcine circovirus type 2. Vet J. 2011; 187: 23-32.
5. Hattermann K, Roedner C, Schmitt C, Finsterbusch T, Steinfeldt T, Mankertz A. Infection studies on human cell lines with porcine circovirus type 1 and porcine circovirus type 2. Xenotransplantation. 2004; 11: 284-294.
6. Baylis SA, Finsterbusch T, Bannert N, Blümel J, Mankertz A. Analysis of porcine circovirus type 1 detected in Rotarix vaccine. Vaccine. 2011; 29: 690-697.
7. McClenahan SD, Krause PR, Uhlenhaut C. Molecular and infectivity studies of porcine circovirus in vaccines. Vaccine. 2011; 29: 4745-4753.
8. Semaan M, Rotem A, Barkai U, Bornstein S, Denner J. Screening pigs for xenotransplantation: prevalence and expression of porcine

endogenous retroviruses in Göttingen minipigs. Xenotransplantation. 2013; 20: 148-156.

9. Morozov VA, Morozov AV, Rotem A, Barkai U, Bornstein S, Denner J. Extended Microbiological Characterization of Göttingen Minipigs in the Context of Xenotransplantation: Detection and Vertical Transmission of Hepatitis E Virus. PLoS One. 2016; 10: e0139893.

10. Morozov VA, Plotzki E, Rotem A, Barkai U, Denner J. Extended microbiological characterization of Göttingen minipigs: porcine cytomegalovirus and other viruses. Xenotransplantation. 2016.

11. Plotzki E, Heinrichs G, Kubícková B, Ulrich RG, Denner J. Microbiological characterization of a newly established pig breed, Aachen Minipigs. Xenotransplantation. 2016; 23: 159-167.

12. Fischer K, Kraner-Scheiber S, Petersen B, Rieblinger B, Buermann A, Flisikowska T, et al. Efficient production of multi-modified pigs for xenotransplantation by 'combineering', gene stacking and gene editing. Sci Rep. 2016; 6: 29081.

13. Oropeza M, Petersen B, Carnwath JW, Lucas-Hahn A, Lemme E, Hassel P, et al. Transgenic expression of the human A20 gene in cloned pigs provides protection against apoptotic and inflammatory stimuli. Xenotransplantation. 2009; 16: 522-534.

14. Plotzki E, Wolf-van Buerck L, Knauf Y, Becker T, Maetz-Rensing K, Schuster M, et al. Virus safety of islet cell transplantation from transgenic pigs to marmosets. Virus Res. 2015; 204: 95-102.

15. Abicht JM, Mayr TA, Reichart B, Plotzki E, Güthoff S, Falkenau A, et al. Hepatic Failure After Pig Heart Transplantation Into a Baboon: No Involvement of Porcine Hepatitis E Virus. Ann Transplant. 2016; 21:12-26.

16. Morozov VA, Abicht JM, Reichart B, Mayr T, Guethoff S, Denner, J. Active Replication of Porcine Cytomegalovirus (PCMV) Following Transplantation of a Pig Heart into a Baboon Despite Undetected Virus in the Donor Pig. Ann Virol Res. 2016; 2:1018.

17. Mankertz A, Domingo M, Folch JM, LeCann P, Jestin A, Segalés J, et al. Characterisation of PCV-2 isolates from Spain, Germany and France. Virus Res. 2000; 66: 65-77.

18. Sarli G, Morandi F, Panarese S, Bacci B, Ferrara D, Bianco C, et al. Reproduction in porcine circovirus type 2 (PCV2) seropositive gilts inseminated with PCV2b spiked semen. Acta Vet Scand. 2012; 54: 51.

19. Eddicks M, Koeppen M, Willi S, Fux R, Reese S, Sutter G, et al. Low prevalence of porcine circovirus type 2 infections in farrowing sows and corresponding pre-suckling piglets in southern German pig farms. Vet Microbiol. 2016; 187: 70-74.

20. Olvera A, Sibila M, Calsamiglia M, Segalés J, Domingo M. Comparison of porcine circovirus type 2 load in serum quantified by a real time PCR in postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome naturally affected pigs. J Virol Methods. 2004; 117: 75-80.

21. Csank T, Pistl J, Polláková J, Holoda E, Harvan M. Prevalence of porcine circovirus 2 infection in pig population in Slovakia. Acta Virol. 2011; 55: 267-271.

22. Bhide K, Csank T, Pistl J, Ciberej J. Prevalence of porcine circovirus 2 and virus-specific antibodies in wild boars (Sus scrofa) in Slovakia. Acta Virol. 2014; 58: 386-388.

23. Barbosa CN, Martins NR, Freitas TR, Lobato ZI. Serological Survey of Porcine circovirus-2 in Captive Wild Boars (Sus scrofa) from Registered Farms of South and South-east Regions of Brazil. Transbound Emerg Dis. 2016; 63: 278-280.

24. Fabisiak M, Szczotka A, Podgórska K, Stadejek T. Prevalence of infection and genetic diversity of porcine circovirus type 2 (PCV2) in wild boar (Sus scrofa) in Poland. J Wildl Dis. 2012; 48: 612-618.

25. Hammer R, Ritzmann M, Palzer A, Lang C, Hammer B, Pesch S, et al. Porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 infections in wild boar (Sus scrofa) in southwestern Germany. J Wildl Dis. 2012; 48: 87-94.

26. Cadar D, Cságola A, Spinu M, Dán A, Ursu K, Lorincz M, et al. Prevalence of porcine circoviruses in Transylvanian wild boars, detected by real-time PCR-short communication. Acta Vet Hung. 2010; 58: 475-481.

27. Shen H, Wang C, Madson DM, Opriessnig T. High prevalence of porcine circovirus viremia in newborn piglets in five clinically normal swine breeding herds in North America. Prev Vet Med. 2010; 97: 228-233.

28. Reiner G, Bronnert B, Hohloch C, Fresen C, Haack I, Willems H, et al. Qualitative and quantitative distribution of PCV2 in wild boars and domestic pigs in Germany. Vet Microbiol. 2010; 145: 1-8.

29. Sasaki K, Tsukahara T, Taira O, Tsuchiya K, Itoh M, Ushida K. Prevalence of porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 in piglets after weaning on a commercial pig farm in Japan. Anim Sci J. 2010; 81: 135-141.

30. Duvigneau JC, Hartl RT, Groiss S, Gemeiner M. Quantitative simultaneous multiplex real-time PCR for the detection of porcine cytokines. J Immunol Methods. 2005; 306: 16-27.

Cite this article

Heinze J, Plotzki E, Denner J (2016) Virus Safety of Xenotransplantation: Prevalence of Porcine Circovirus 2 (PCV2) in Pigs. Ann Virol Res 2(3): 1023.