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RAPID COMMUNICATION

Emergence of novel circoviruses in humans and pigs and their possible importance for xenotransplantation and blood transfusions

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Abstract

Background: As sequencing is becoming more broadly available, virus discovery continues. Small DNA viruses contribute to up to 60% of the overall virus load in pigs. Porcine circoviruses (PCVs) are small DNA viruses with a single-stranded circular genome. They are common in pig breeds and have not been properly addressed for their potential risk in xenotransplantation. Whereas PCV1 is non-pathogenic in pigs, PCV2 has been associated with various disease manifestations. Recently two new circoviruses have been described, PCV3 and PCV4. While PCV4 is currently present mainly in Asia, PCV3 is widely distributed, and has been identified in commercial pigs, wild boars, and pigs generated for xenotransplantation. In one case PCV3 was transmitted by pigs to baboons via heart transplantation. PCV3 pathogenicity in pigs was controversial initially, however, the virus was found to be associated with porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, and multisystemic inflammation. Inoculation studies with PCV3 infectious clones confirmed that PCV3 is pathogenic. Most importantly, recently discovered human circoviruses (CV) are closely related to PCV3.

Methods: Literature was evaluated and summarized. A dendrogram of existing circoviruses in pigs, humans, and other animal species was created and assessed at the species level.

Results: We found that human circoviruses can be divided into three species, human CV1, CV2, and CV3. Human CV2 and CV3 are closest to PCV3.

Conclusions: Circoviruses are ubiquitous. This communication should create awareness of PCV3 and the newly discovered human circoviruses, which may be a problem for blood transfusions and xenotransplantation in immune suppressed individuals.

KEYWORDS
blood transfusions, circovirus, humans, pigs, xenotransplantation

Abbreviations: CRESS DNA, Circular Rep-encoding single-stranded DNA; CV, Circovirus; DNA, Deoxyribonucleic acid; HuACV, Human associated circovirus; Human CV or HCV or HCirV, Human circovirus; PCV, Porcine circovirus; PDNS, Porcine dermatitis and nephropathy syndrome; Polymerase chain reaction, PCR; Porcine kidney cell line 15, PK-15 cell line; Rep, Replicase associated gene; ssDNA, Single-stranded DNA; TSE, Transmissible spongiform encephalopathies; U.S., United States of America.

1 | INTRODUCTION

Circoviruses—single stranded, small, circular arranged DNA viruses—have been emerging in recent years in many animal species. The *Circoviridae* family includes the smallest (10–20 nm) autonomously replicating animal viral pathogens[1] and has two genera: *Circovirus* and *Cyclovirus*.[2] Within each genus, the species demarcation threshold is 80% genome-wide nucleotide sequence identity.[2] Previously, cycloviruses have been found in 7%–17% of human stools and 3%–55% of meat samples tested, in both humans and animals.[3]

The family *Circoviridae* belongs to the order *Cirlivirales* and the phylum *Cressdnaviricota*.[4] The term CRESS DNA virus was selected in 2012 to refer to the group of ssDNA viruses encoding a replication-associated protein (Rep) that appears to be descended from a common ancestor. "CRESS DNA" stands for circular, rep-encoding ssDNA and encompasses both prokaryotic and eukaryotic viruses, although the Reps of each of these groups have distinct characteristics.[5]

CRESS DNA viruses are ubiquitous viruses with small genomes, present in all environments, with a diverse host range. There appears to be an increasing number of newly discovered CRESS viruses which highlights their diversity and helps to improve the classification of this virus group. The phylum Cressdnaviricota includes several orders, among them the order *Cirlivirales* with the family *Circoviridae*. Other families in the phylum are *Bacilladnaviridae*, *Geminiviridae*, *Nanoviridae*, *Genomoviridae*, and *Smacoviridae*.[4] Little is currently known about these viruses. In a study published in 2020[6] it has been indicated that millions of distinct CRESS virus species likely exist, while only approximately 9000 were catalogued in GenBank's RefSeq database at that time. Furthermore, it is estimated that hundreds of circular DNA elements do not encode any discernible similarities to previously characterized sequences. More recently, efforts have focused on using conserved genes such as the *rep* genes for identifying novel viruses. ssDNA viruses are highly diverged, representing polyphyletically originated groups that can infect the different members of all three domains—archaea, bacteria, and eukarya. These viruses can be linear (minority), or circular arranged (majority).[6]

TABLE 1 Selected Genbank numbers used to create a representative dendogram of circoviruses in pigs, selected other animals and humans.

Human CV1	GQ404856
Human CV2	0N226770
Human CV3	ON677309, ON526744
Human cyclovirus	GQ404857, KF031466,
PCV1	AY184287, JN398656, GU722334
PCV2	AF055392, JX535296, JQ181592, JX099786
PCV3	KX458235, MH367849, KT869077
PCV4	NC055580, MT769268, MT882410
Bat CV clade 1	KJ641724, KJ641723, KJ641727, KJ641711
Bat CV clade 2	KJ641741, KJ641742
Other bat CVs	JX863737, KT783484
Barbel CV (a fish)	GU799606
Dog CV	JQ821392
Goose CV	AF536939
Mink CV	KJ020099
Elk CV	MN585201
TSE-associated circular DNA	HQ444405, HQ44404

TABLE 2 Results of a basic Pubmed search on circoviruses in pigs and humans.

Circovirus investigated	Abbreviation[a]	Full name[b]	Circovirus+ Human
PCV1	247	994	54
PCV2	2061	1737	145
PCV3	288	762	63
PCV4	58	522	54
Human CV	N/A[c]	360	N/A[c]

[a]Search results using the abbreviatd name: For example: PCV1.
[b]Search results uing the full name for example: Porcine circovirus type 1.
[c]Not applicable.

Initially, circoviruses were associated with infection of various bird species including canary circovirus,[7] gull circovirus,[8,9] penguin circovirus,[10] raven circovirus,[11] starling circovirus,[12] swan circovirus,[13] zebra finch circovirus,[14] and others. The first mammalian host known to harbor a circovirus was the pig, in 1974, when researchers noticed an unknown virus as a contaminant of a permanent porcine kidney cell line PK-15[15] and hence the corresponding virus was called porcine circovirus or PCV.[16] Studies performed in the 1980s and 1990s indicated wide spread of the virus in pig farms[17] but association of the virus with disease in pigs was not demonstrated.[18,19] However, in 1997 a novel PCV was identified[20–22] which quickly was associated with widespread disease outbreaks in pigs. Genomic analysis revealed differences in the two viruses and hence they were named PCV1 and PCV2. As of today, two additional pig associated circoviruses have been identified and are termed PCV3, discovered in 2016[23,24] and PCV4, discovered in 2019.[25] PCV4 is present mainly in Asia, but meanwhile the virus was also detected in Europe in wild boars and domestic pigs.[26]

Circoviruses have also been identified in many other mammalian species, some of which develop disease; including dogs,[27] foxes,[28] cats,[29] and others. In addition, circoviruses have also been detected in fish,[30] shellfish,[31,32] amphibia,[33] reptiles,[34] and insects.[35] Circoviruses in bats[36] can be divided into two major clades: Bat-clade-1 and Bat-clade 2.[37] It has been shown that PCV1 and PCV2 are similar to Bat-clade-2 whereas PCV3 is more similar to Bat clade 1. The most recently discovered circovirus in pigs, PCV4, clusters with mink circovirus.[37]

PCV3 can be found in most pigs on a global basis including wild boars and domestic pigs with or without clinical signs and are often associated with various other coinfections.[38] Interestingly, it has also been

TABLE 3 Sequence identities in % between the three represent human circovirus (Human CV) species and porcine circovirus 3 (PCV3).

	Human CV3 ON526744 Human hepatitis CV			Human CV3 ON677309 HuCV1-Paris			Human CV2 ON226770 Human-associated CV2-YN09			Human CV1 GQ404856 Human stool-associated CV		
	Genome	*Rep*[a]	*Cap*[a]	*Genome*	*Rep*[a]	*Cap*[a]	*Genome*	*Rep*[a]	*Cap*[a]	*Genome*	*Rep*[a]	*Cap*[a]
Human CV2 ON677309-HuCV1-Paris	100[b]	99.7	100									
Human CV2 (ON226770-Human-associated CV2-YN09)	80.2	87.4	65.7	80.2	89.2	65.7						
Human CV1 (GQ404856-Human stool-associated CV)	45.4	43.1	21.8	45.3	43.8	21.8	45.2	43.0	23.2			
PCV3 (MH367849)	56.5	62.2	34.3	56.1	61.8	34.3	57.1	65.2	35.8	47.1	44.1	20.5

[a]Indicates the identities of amino acids of Rep and Cap proteins as a percentage.
[b]99.95.

associated with unusual syndromes including erythema multiform in a Greek pig[39] and dippity pig syndrome in Göttingen minipigs.[40] It should be mentioned that PCV3 can be found in invertebrates, in ticks and mosquitoes[41,42] which could indicate that these species may serve as a potential transmission vector in the life-cycle of PCV3.

To better understand the pathogenicity of PCV3, inoculation studies involving PCV3 infectious clones have been conducted. Rescued virus from cells transfected with an infectious DNA clone of a PCV3 derived in China was able to induce PDNS with anorexia, coughing, sneezing, diarrhea, enlarged lymph nodes, hepatitis, and splenitis in specific pathogen-free piglets.[43] The levels of proinflammatory cytokines and chemokines in the PCV3 infected pigs were significantly upregulated.[43] Another infectious PCV3 DNA clone induced changes in alveolar and cardiac tissues of infected mice[44,45] Recently, experimental inoculation of a PCV3 positive tissue homogenate resulted in vascular lesions and localization of PCV3 in lymph nodes in addition to PCV3 viremia approximately from week 1 to 4.[46] Isolation of PCV3 is generally considered difficult but was achieved from different tissues from weak piglets, stillborn and mummified fetuses into PK15 cells which were free of PCV1 and PCV2.[47] Today is commonly accepted that PCV3 can be associated with reproductive disease including late abortions, malformations, mummified, stillborn and weak piglets, cardiac and multisystemic inflammation, in addition to PCV3 systemic disease characterized by wasting, weight loss, ill thrift, poor doers and possible also neurological signs.[23,24,48]

PCV3 has importance for xenotransplantation[49] as the virus was found in genetically modified pigs generated for xenotransplantation and PCV3 was, in a few cases, transmitted to baboons after pig heart transplantation. Interestingly the baboons to which PCV3 was transmitted had the longest survival times following the xenotransplant.[50] It is well known that PCV2 causes immunosuppression, leading to secondary infections with other pathogens[51,52]; however, whether PCV3 can cause immunosuppression needs further clarification.[53] Nevertheless, PCV3 is commonly found in healthy animals indicating that it could attribute to a seemingly undetected and uncontrolled circulation of PCV3.[54] It may be speculated that some pig breeds have viral restriction factors preventing disease or that pathogenicity depends on the virus load.

Recently, human circoviruses have been identified in two instances and in two geographic locations France and China. The report from France described circovirus infection in a 61-year-old woman who had undergone heart and lung transplantation 18 years previously. In March 2023 she was hospitalized due to acute hepatitis.[55] Numerous coinfections including cytomegalovirus colitis, parvovirus B19 bicytopenia, and aspergillus bronchitis were identified. A shotgun metagenomics sequencing approach identified a novel human circovirus which initially was called *Circovirus parisii* or HCirV-1.[55] There was a temporal association between hepatitis and a high load of human circovirus[56] in the liver and blood of the patient. Furthermore, a novel human circovirus was also identified in intravenous drug users in Yunnan, China.[57] That virus was named HuCV2.[57]

A circovirus in humans was initially discovered in 2010, using human stools samples from Nigeria,[3] that virus was discovered without association with any disease. The authors suggested the virus could have been present due to contamination of the food.[3] With the detection of the first human circovirus associated with increased virus load and clinical signs it is likely that additional human circoviruses will follow soon.

Based on extensive tests in pigs, we know that circoviruses are very thermostable.[58] For instance, it has been shown that a virus stock maintained at 120°C for 30 min only resulted in 1 log reduction in virus titre.[58] Circoviruses are also resistant to many disinfectants.[59] In a source animals barrier facility for generating designated pathogen-free pigs to serve as donor animals in xenotransplantation two outbreaks with PCV2 were recorded and the eradication of the virus from the facility was very difficult.[60] The first outbreak was due to

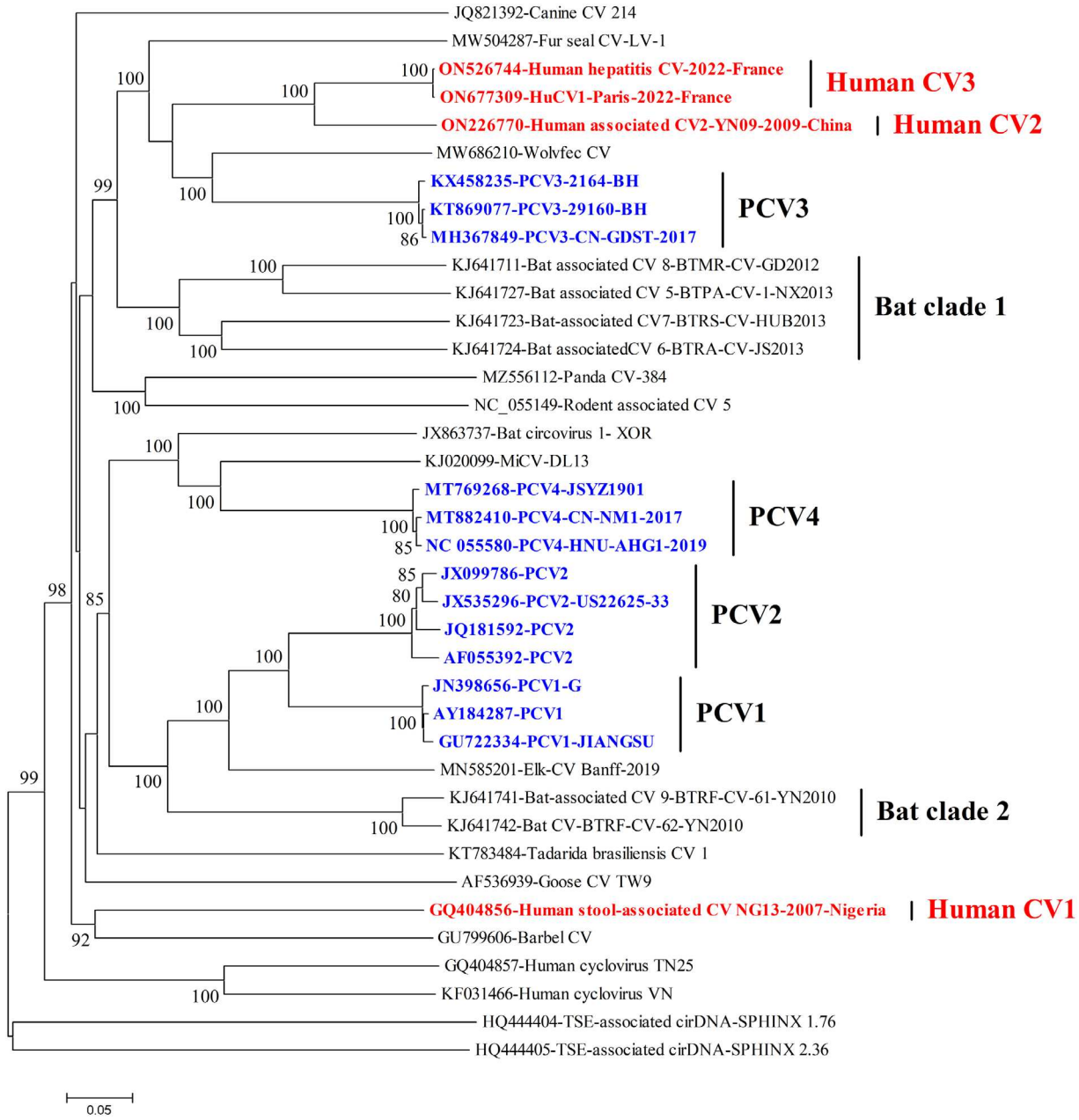

FIGURE 1 The Neighbor-Joining tree constructed based on the genomes of human circoviruses and other represent circoviruses, using the *p*-distance model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (only >70 are shown). Evolutionary analyses were conducted in MEGA7.[65] Circoviruses in pigs were shown in blue font and transmissible encephalopathy (TSE)-associated circular DNAs were used as an outgroup. The classification of bat circoviruses (clade 1 and clade 2) was reported previously.[68]

newly installed steel penning, the second by employee activities. The room and penning were foamed with potassium peroxymonosulfate, sterilized with chlorine dioxide fog, and disinfected with potassium peroxymonosulfate fog and glutaraldehyde ammonium foam.[60] In a similar case in the U.S., PCV2 was eliminated by using VirkonS and a protocol of exposing equipment to ultraviolet light via natural sun light exposure.[61]

The fact that PCV3 is pathogenic in some pigs, but non-pathogenic in others is of great interest. Clinically healthy pigs generated for xenotransplantation transmitted PCV3 to baboons.[50] Most interestingly, two of the baboons receiving the virus had the longest survival time (195 and 182 days) ever observed in this set of experiments.[62] The virus was detected in all analyzed organs of the baboon (spleen, liver, lung, kidney, skin, and muscle) as well as in the explanted pig heart. The virus load was correlated with survival time of the recipient, indicating that the virus was replicating. However, it is unclear whether PCV3 infected baboon cells or whether the virus was replicating in the pig transplant and disseminated pig cells in the body of the baboons

as seen in the case of porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV).[63]

In 2004 the idea of searching for a human circoviruses was first introduced.[64] However, the authors found no evidence of such a virus despite screening 1101 samples and concluded that the results rendered the existence of a human circovirus unlikely.[64] Nevertheless, in 2023 two independent reports were published almost simultaneously and both reported a novel human circovirus[55] which, based on our analysis, appear to belong to different species. Three different species including human CV1, human CV2, and human CV3 have been identified to date. This probably implies that there may be additional human circoviruses in circulation and waiting for discovery.

The objective of this work was to summarize and combine findings of human circoviruses and cycloviruses in different geographic areas and to contrast the human circoviruses to their counterparts in pigs, as pigs are commonly used for xenotransplantation.

2 | MATERIAL AND METHODS

2.1 | Selected GenBank numbers

The Genbank numbers selected for this study are summarized in Table 1.

2.2 | PubMed search

To assess the current focus on specific circoviruses in pigs and humans, PubMed searches (search date: September 8, 2023) were performed using the keywords "circovirus", "humans", "PCV1", "PCV2", "PCV3", and "PCV4" in various combinations.

2.3 | Analysis

The Neighbor-Joining tree method was used in addition to the *p*-distance model. Evolutionary analyses were conducted in MEGA7.[65]

3 | RESULTS AND DISCUSSION

3.1 | PubMed search outcomes

To get an idea of the level of interest in publishing on certain circovirus in pigs or humans we did a basic PubMed search. The results are presented in Table 1. It would appear most publications have been focused on PCV2, followed by PCV3, PCV4, and finally human circoviruses (See Table 2).

3.2 | Phylogenetic and genetic distances of the human circoviruses and other representative circoviruses

A human circovirus, GQ404856, was discovered initially in a human stool sample in 2007 from Nigerian children with no further information provided.[3] It is of interest that initially that virus was classified as cyclovirus[3] Only recently two studies focusing on taxonomy of the family *Circoviridae* were published and GQ404856 from 2010 was clearly classified as circovirus in 2017.[2,66] Human CV1 has a similar genomic structure as other circoviruses with which it clusters.

The more recent human circoviruses from France, both from the same patient but in different publications,[55,56] are, as expected, almost identical except for a single nucleotide difference, resulting in a single amino acid difference in the *rep* gene. In contrast, the human circovirus from China is substantially different from the French strains with genome identities of ca. 80% (Table 3, Figure 1) which appears sufficient to define a new circovirus species[2] hence we classified them into different species (human CV1, CV2, and CV3). It also would appear that human circoviruses are closely related to PCV3. In fact, there are few more similar circovirus species in comparisons between human CV2, human CV3 and PCV3 based on searching from GenBank sequences. The exception is a newly discovered circovirus recovered in four wolverine fecal samples collected during 2018–2019. In Figure 1 we included one of the sequences, MW686210, from a Wolverine (*Gulo gulo*) which belongs to the Mustelidae family.[67] This sequence is closer in identity to PCV3 (63%) than to human circovirus 2 or 3 (approximately 56%). Hence it might be possible that human circoviruses emerged from pigs or vice versa. However, as the genome identity between them is less than 60%, there may be other circoviruses from other species (such as the Wolverine circovirus) which have not been discovered yet.

4 | CONCLUSION

Human circoviruses have been recently discovered for the first time. It is likely that more findings will be occurring soon, and it is reasonable to believe that human CVs may be widely distributed, mainly resulting in subclinical infections. It seems imperative to quickly develop serological assays and PCR kits so that people undergoing blood transfusions but also organ transplantations can be tested beforehand. It also may be necessary to test pigs for presence of these viruses to ensure a safe xenotransplantation.

AUTHOR CONTRIBUTIONS

All authors contributed substantial to the data acquisition, analysis, and interpretation of the data. All authors also contributed to the drafting of the manuscript, and all approved the final version. We acknowledge participation in the Transplant Peer Review Network and complied with the journal's author guidelines and policies.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest.

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REFERENCES

1. Capozza P, Lanave G, Diakoudi G, et al. Diversity of CRESS DNA viruses in squamates recapitulates hosts dietary and environmental sources of exposure. *Microbiol Spectr*. 2022;10(3):e0078022.
2. Breitbart M, Delwart E, Rosario K, Segalés J, Varsani A, ICTV Report C. ICTV virus taxonomy profile: circoviridae. *J Gen Virol*. 2017;98(8):1997-1998.
3. Li L, Kapoor A, Slikas B, et al. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. *J Virol*. 2010;84(4):1674-1682.
4. Krupovic M, Varsani A, Kazlauskas D, et al. Cressdnaviricota: a virus phylum unifying seven families of rep-encoding viruses with single-stranded, circular DNA genomes. *J Virol*. 2020;94(12):e00582-e00620.
5. Koonin EV, Ilyina TV. Computer-assisted dissection of rolling circle DNA replication. *Bio Systems*. 1993;30(1-3):241-268.
6. Tisza MJ, Pastrana DV, Welch NL, et al. Discovery of several thousand highly diverse circular DNA viruses. *eLife*. 2020;9:e51971.
7. Todd D, Weston J, Ball NW, et al. Nucleotide sequence-based identification of a novel circovirus of canaries. *Avian Pathol*. 2001;30(4):321-325.
8. Smyth JA, Todd D, Scott A, et al. Identification of circovirus infection in three species of gull. *Vet Rec*. 2006;159(7):212-214.
9. Twentyman CM, Alley MR, Meers J, Cooke MM, Duignan PJ. Circovirus-like infection in a southern black-backed gull (Larus dominicanus). *Avian Pathol*. 1999;28(5):513-516.
10. Morandini V, Dugger KM, Ballard G, et al. Identification of a novel adélie penguin circovirus at cape crozier (Ross Island, Antarctica). *Viruses*. 2019;11(12):1088.
11. Stewart ME, Perry R, Raidal SR. Identification of a novel circovirus in Australian ravens (Corvus coronoides) with feather disease. *Avian Pathol*. 2006;35(2):86-92.
12. Johne R, Fernández-de-Luco D, Höfle U, Müller H. Genome of a novel circovirus of starlings, amplified by multiply primed rolling-circle amplification. *J Gen Virol*. 2006;87(Pt 5):1189-1195.
13. Halami MY, Nieper H, Müller H, Johne R. Detection of a novel circovirus in mute swans (Cygnus olor) by using nested broad-spectrum PCR. *Virus Res*. 2008;132(1-2):208-212.
14. Rinder M, Schmitz A, Peschel A, Korbel R. Complete genome sequence of a novel circovirus from zebra finch. *Genome Announc*. 2015;3(3):e00560-e00615.
15. Tischer I, Rasch R, Tochtermann G. Characterization of papovavirus- and picornavirus-like particles in permanent pig kidney cell lines. *Zentralbl Bakteriol Orig A*. 1974;226(2):153-167.
16. Tischer I, Gelderblom H, Vettermann W, Koch MA. A very small porcine virus with circular single-stranded DNA. *Nature*. 1982;295(5844):64-66.
17. Tischer I, Bode L, Peters D, Pociuli S, Germann B. Distribution of antibodies to porcine circovirus in swine populations of different breeding farms. *Arch Virol*. 1995;140(4):737-743.
18. Tischer I, Mields W, Wolff D, Vagt M, Griem W. Studies on epidemiology and pathogenicity of porcine circovirus. *Arch Virol*. 1986;91(3-4):271-276.
19. Allan GM, McNeilly F, Cassidy JP, et al. Pathogenesis of porcine circovirus; experimental infections of colostrum deprived piglets and examination of pig fetal material. *Vet Microbiol*. 1995;44(1):49-64.
20. Morozov I, Sirinarumitr T, Sorden SD, et al. Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. *J Clin Microbiol*. 1998;36(9):2535-2541.
21. Hamel AL, Lin LL, Nayar GP. Nucleotide sequence of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. *J Virol*. 1998;72(6):5262-5267.
22. Meehan BM, McNeilly F, Todd D, et al. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. *J Gen Virol*. 1998;79 (Pt 9):2171-2179.
23. Palinski R, Piñeyro P, Shang P, et al. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. *J Virol*. 2017;91(1):e01879-e01916.
24. Phan TG, Giannitti F, Rossow S, et al. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. *Virol J*. 2016;13(1):184.
25. Zhang HH, Hu WQ, Li JY, et al. Novel circovirus species identified in farmed pigs designated as Porcine circovirus 4, Hunan province, China. *Transbound Emerg Dis*. 2020;67(3):1057-1061.
26. Holgado-Martín R, Arnal JL, Sibila M, et al. First detection of porcine circovirus 4 (PCV-4) in Europe. *Virol J*. 2023;20(1):230.
27. Decaro N, Martella V, Desario C, et al. Genomic characterization of a circovirus associated with fatal hemorrhagic enteritis in dog, Italy. *PLoS ONE*. 2014;9(8):e105909.
28. Bexton S, Wiersma LC, Getu S, et al. Detection of circovirus in foxes with meningoencephalitis, United Kingdom, 2009–2013. *Emerg Infect Dis*. 2015;21(7):1205-1208.
29. Payne N, Kraberger S, Fontenele RS, et al. Novel circoviruses detected in feces of sonoran felids. *Viruses*. 2020;12(9):1027.
30. Lőrincz M, Cságola A, Farkas SL, Székely C, Tuboly T. First detection and analysis of a fish circovirus. *J Gen Virol*. 2011;92(Pt 8):1817-1821.
31. Krog JS, Larsen LE, Schultz AC. Enteric porcine viruses in farmed shellfish in Denmark. *Int J J Food Microbiol*. 2014;186:105-109.
32. Myrmel M, Berg EM, Rimstad E, Grinde B. Detection of enteric viruses in shellfish from the Norwegian coast. *Appl Environ Microbiol*. 2004;70(5):2678-2684.
33. Tarján ZL, Pénzes JJ, Tóth RP, Benkő M. First detection of circovirus-like sequences in amphibians and novel putative circoviruses in fishes. *Acta Vet Hung*. 2014;62(1):134-144.
34. Altan E, Kubiski SV, Burchell J, Bicknese E, Deng X, Delwart E. The first reptilian circovirus identified infects gut and liver tissues of black-headed pythons. *Vet Res*. 2019;50(1):35.
35. da Silva Ferreira R, de T Aquino da Cruz LC, de Souza VJ, et al. Insect-specific viruses and arboviruses in adult male culicids from Midwestern Brazil. *Infect Genet Evol*. 2020;85:104561.
36. Ge X, Li J, Peng C, et al. Genetic diversity of novel circular ssDNA viruses in bats in China. *J Gen Virol*. 2011;92(Pt 11):2646-2653.
37. Wang D, Mai J, Yang Y, Xiao CT, Wang N. Current knowledge on epidemiology and evolution of novel porcine circovirus 4. *Vet Res*. 2022;53(1):38.
38. Prinz C, Stillfried M, Neubert LK, Denner J. Detection of PCV3 in German wild boars. *Virol J*. 2019;16(1):25.

39. Halecker S, Papatsiros V, Psalla D, Krabben L, Kaufer B, Denner J. Virological characterization of pigs with erythema multiforme. *Microorganisms*. 2022;10(3):652.
40. Jhelum H, Grand N, Jacobsen KR, et al. First virological and pathological study of Göttingen Minipigs with Dippity Pig Syndrome (DPS). *PLoS ONE*. 2023;18(6):e0281521.
41. Franzo G, Grassi L, Tucciarone CM, et al. A wild circulation: high presence of Porcine circovirus 3 in different mammalian wild hosts and ticks. *Transbound Emerg Dis*. 2019;66(4):1548-1557.
42. Ha Z, Li JF, Xie CZ, et al. First detection and genomic characterization of porcine circovirus 3 in mosquitoes from pig farms in China. *Vet Microbiol*. 2020;240:108522.
43. Jiang H, Wang D, Wang J, et al. Induction of porcine dermatitis and nephropathy syndrome in piglets by infection with porcine circovirus type 3. *J Virol*. 2019;93(4):e02045-e020118.
44. Jiang Z, Wu J, Jiang M, et al. A novel technique for constructing infectious cloning of type 3 porcine circovirus. *Front Microbiol*. 2020;11:1067.
45. Tan CY, Lin CN, Ooi PT. What do we know about porcine circovirus 3 (PCV3) diagnosis so far?: A review. *Transbound Emerg Dis*. 2021;68(6):2915-2935.
46. Hayashi S, Sato T, Ono H, et al. Experimental inoculation of a tissue homogenate containing porcine circovirus type 3 obtained after two in vivo passages in NIBS miniature pigs. *Vet Microbiol*. 2023;281:109740.
47. Mora-Díaz JC, Magtoto R, Houston E, et al. Detecting and monitoring porcine hemagglutinating encephalomyelitis virus, an underresearched betacoronavirus. *mSphere*. 2020;5(3):e00199-e001920.
48. Saporiti V, Franzo G, Sibila M, Segalés J. Porcine circovirus 3 (PCV-3) as a causal agent of disease in swine and a proposal of PCV-3 associated disease case definition. *Transbound Emerg Dis*. 2021;68(6):2936-2948.
49. Denner J, Mankertz A. Porcine circoviruses and xenotransplantation. *Viruses*. 2017;9(4):83.
50. Krüger L, Längin M, Reichart B, et al. Transmission of Porcine Circovirus 3 (PCV3) by xenotransplantation of pig hearts into baboons. *Viruses*. 2019;11(7):650.
51. Allan GM, McNeilly F, Ellis J, et al. Experimental infection of colostrum deprived piglets with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. *Arch Virol*. 2000;145(11):2421-2429.
52. Harms PA, Sorden SD, Halbur PG, et al. Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. *Vet Pathol*. 2001;38(5):528-539.
53. Chen S, Zhang L, Li X, Niu G, Ren L. Recent progress on epidemiology and pathobiology of porcine circovirus 3. *Viruses*. 2021;13(10):1944.
54. Franzo G, He W, Correa-Fiz F, et al. A shift in porcine circovirus 3 (PCV-3) history paradigm: phylodynamic analyses reveal an ancient origin and prolonged undetected circulation in the worldwide swine population. *Adv Science (Weinh)*. 2019;6(22):1901004.
55. Rodriguez C, Boizeau L, Soulier A, et al. Unknown circovirus in immunosuppressed patient with Hepatitis, France, 2022. *Emerg Infect Dis*. 2023;29(5):1051-1054.
56. Pérot P, Fourgeaud J, Rouzaud C, et al. Circovirus hepatitis infection in heart-lung transplant patient, France. *Emerg Infect Dis*. 2023;29(2):286-293.
57. Li Y, Zhang P, Ye M, et al. Novel circovirus in blood from intravenous drug users, Yunnan, China. *Emerg Infec Dis*. 2023;29(5):1015-1019.
58. Welch J, Bienek C, Gomperts E, Simmonds P. Resistance of porcine circovirus and chicken anemia virus to virus inactivation procedures used for blood products. *Transfusion*. 2006;46(11):1951-1958.
59. Patterson AR, Opriessnig T. Epidemiology and horizontal transmission of porcine circovirus type 2 (PCV2). *Anim Health Res Rev*. 2010;11(2):217-234.
60. Noordergraaf J, Schucker A, Martin M, et al. Pathogen elimination and prevention within a regulated, Designated Pathogen Free, closed pig herd for long-term breeding and production of xenotransplantation materials. *Xenotransplantation*. 2018;25(4):e12428.
61. Patterson A, Madson DM, Schalk SD, Halbur PG, Opriessnig T. Establishment and maintenance of a porcine circovirus type 2 (PCV2)-free breeding herd on a site that experienced a natural outbreak of PCV2-associated reproductive disease. *J Swine Health Prod*. 2011;19(3):165-174.
62. Denner J, Längin M, Reichart B, et al. Impact of porcine cytomegalovirus on long-term orthotopic cardiac xenotransplant survival. *Sci Rep*. 2020;10(1):17531.
63. Fiebig U, Abicht JM, Mayr T, et al. Distribution of porcine cytomegalovirus in infected donor pigs and in baboon recipients of pig heart transplantation. *Viruses*. 2018;10(2):66.
64. Hattermann K, Maerz A, Slanina H, Schmitt C, Mankertz A. Assessing the risk potential of porcine circoviruses for xenotransplantation: consensus primer-PCR-based search for a human circovirus. *Xenotransplantation*. 2004;11(6):547-550.
65. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis Version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;33(7):1870-1874.
66. Rosario K, Breitbart M, Harrach B, et al. Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. *Arch Virol*. 2017;162(5):1447-1463.
67. Bandoo RA, Bautista J, Lund M, et al. Identification of novel circovirus and anelloviruses from wolverines using a non-invasive faecal sampling approach. *Infect Genet Evol*. 2021;93:104914.
68. Li G, He W, Zhu H, et al. Origin, genetic diversity, and evolutionary dynamics of novel porcine circovirus 3. *Adv Sci (Weinh)*. 2018;5(9):1800275.

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