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Short communication

Distribution of ORF2 and ORF3 genotypes of Porcine Circovirus Type 2 (PCV-2) in wild boars and domestic pigs in Germany

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Abstract

Porcine circovirus 2 (PCV-2), the essential infectious agent in PCVD (porcine circovirus diseases) circulates at high rates among domestic pig and wild boar populations. Wild boars may be viremic and shed the virus with excretions and secretions, and thus serve as a reservoir for domestic pig PCV-2 infection. We hypothesize that PCV-2 strains circulating in wild boars and in domestic pigs are significantly different and thus, partially independent. To prove this hypothesis, the present study investigated by sequence analysis the distribution of ORF2 and ORF3 genotypes of the PCV-2 genome within wild boars (n=40) and domestic pigs (n=60) from overlapping greater areas of Germany. The genotypes were compared with PCV-2 sequences from the Genbank database. The dominating genotype in domestic pigs was PCV-2b (98.4% of infected pigs), while only 4.8% of them were infected with PCV-2a. The corresponding prevalences of PCV-2a and -2b genotypes in wild boars were 58 and 70%, respectively. When also ORF3 genotypes were taken into account, more than 50% of wild boar PCV-2 genotypes were rare among German and European domestic pigs. In conclusion, these data provide evidence for a certain independence of PCV-2 infections in both species and a low chance for domestic pigs to be infected with PCV-2 of wild boar origin. On the other hand, PCV-2 genotypes specific for domestic pigs are also common in wild boars, although at lower frequencies, suggesting the spread of domestic pig PCV-2 to the wild boar population.

Keywords: PCV-2; Wild boars; Genotype; Prevalence

1. Introduction

Porcine circovirus type 2 (PCV-2) is considered the essential infectious agent of PCVD (porcine circovirus diseases) (Segalés et al., 2005) or PCVAD (porcine circovirus associated diseases) (AASV, 2007), including post-weaning multisystemic wasting syndrome (PMWS) (Harding, 1996), respiratory (Pesch et al., 2000) as well as reproductive (Pensaert et al., 2001) disorders, and it has been associated with porcine dermatitis and nephropathy syndrome (PDNS) (Segalés et al., 1998). PCVD is responsible for significant economic losses to the swine industry worldwide (Armstrong and Bishop, 2004). PCV-2 is ubiquitous and distributed worldwide within domestic pig populations. The virus has also been detected by serological studies in wild boars from several countries (Belgium: Sanchez et al., 2001; Spain: Vicente et al., 2004; Ruiz-Fons et al., 2006; Czech Republic: Sedlak et al., 2008), and by direct detection of PCV-2 antigens or DNA (Spain: Vicente et al., 2004; Germany: Knell et al., 2005; Reiner et al., 2010; Hungary: Csagola et al., 2006). Subclinically infected wild boars may be viremic and shed the virus with excretions and secretions (Krakowka et al., 2005). Thus, while wild boars are considered a potential reservoir of viruses for domestic pigs, such as classical swine fever virus and Aujeszky`s disease virus (e.g. Albina et al., 2000), it is still unclear (Ruiz-Fons et al., 2008), if this situation applies also to PCV-2 (Vicente et al., 2004). Because the contact between domestic pigs and wild boars is very limited and PCV-2 is more transmissible by direct contact (Dupont et al., 2009), we hypothesize that PCV-2 strains circulating in wild boars and in domestic pigs are different and not in a direct exchange. To prove this hypothesis, we investigated the distribution of PCV-2 genotypes within wild boar and domestic pig populations from overlapping greater areas of Germany by sequence analysis.

2. Materials and methods

From 531 wild boars, hunted between November 2004 and March 2007, 40 samples from 17 hunting grounds situated in eleven federal states of Germany were randomly chosen for sequence analysis.

Domestic pig samples were taken from 60 apparently healthy (unrestricted at slaughter) animals from 18 provenances located within 12 of the 14 federal states of Germany between April and November 2007.

DNA was extracted from lung, tonsils, spleen, *Ln. bronchialis* and *Ln. mesentericus* samples as described elsewhere (Reiner et al., 2010).

ORF2 and ORF3 genotypes were detected by pyrosequencing on a PyroMark ID (Biotage, Sweden) system and sequences were analyzed with the Pyro-Mark ID 1.0 Software (Biotage, Sweden). PCR was performed in a total volume of 40 µl consisting of 20 µl of Multiplex Mastermix (Qiagen, Hilden, Germany), 4 µl of primermix (ORF2: Pyro-PCV2-F, Pyro-PCV2-R; ORF3: Orf3F, Orf3R; final concetration 0.4 µM; Tab.1), 12 µl of water and 4 µl of template DNA. Cycling conditions were as follows: intitial activation of the DNA polymerase for 15 min at 95°C, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C (ORF2) and 59°C (ORF3), respectively, for 90 seconds and extension at 72°C for 30 seconds, followed by final extension at 72°C for 10 min. Pyrosequencing was performed with 18 µl of PCR product using sequencing primers (Tab. 1) Pyro-PCV2-S (ORF2) and Orf3S (ORF3) as described by the manufacturer.

PCV-2 genotypes were classified based on ORF2 sequences as described by Olvera et al. (2007) and Segalés et al. (2008). ORF3 genotypes were defined from PCV-2 Genbank database entries based on polymorphic sites at positions 310 to 304 of ORF3, which corresponds to genomic positions 362 to 368.

Statistical analysis was performed with the "Statistical Package for Social Sciences" (SPSS, version 15.0). Significances of differences in frequencies were tested with Chi^2-test. Differences between group means were calculated with ANOVA.

3. Results

Two ORF2 (Tab. 2) and nine ORF3 (Tab. 3) genotypes were detected in twelve combinations (Tab. 4) , with significant differences between wild boars and domestic pigs. There was no significant effect of the hunting season. PCV-2b was the most common genotype in both species. Almost 60% of the infected wild boars but only 4.8% of the infected domestic pigs carried PCV-2a. ORF3 genotypes 1 and 3 had the highest prevalence, with ORF3 genotype 1 prevalence differing significantly between wild boars and domestic pigs. ORF3 genotypes 2 and 10 were detected 5 times more frequently in wild boars than in domestic pigs. Low prevalence genotypes 5 and 9 were detected only in domestic pigs, genotypes 4 and 12 in wild boars only. However, the effects of genotypes 2, 4, 10, and 12 were not significant because of their overall low prevalence. Combined ORF2/ORF3 sequences in domestic pigs were dominated by genotype combinations 2b/1 and 2b/3. Other combinations were rare (2b/2, 2b/5, 2b/6, 2b/9, 2b/10, 2a/3 and 2a/10) or missing (2b/4, 2b/12, 2a/1, 2a/2). PCV-2 genotypes of wild boars turned out to be more heterogeneous than those of domestic pigs. Thus, there was a high prevalence for genotype combinations 2a/10, 2a/1, 2a/3, 2a/2, and 2b/2 that were found significantly (except for 2b/2 and 2a/3) less frequently in PCV-2 from domestic pigs. The distribution of PCV-2 genotypes differed significantly ($P<0.05$) between wild boars and domestic pigs from the North, the East, and the Southwest of Germany (statistics not shown) but not from Central Germany. Two different PCV-2 genotypes in one animal were identified from 27% and 4% of wild boars and domestic pigs, respectively ($P<0.001$), and at least two different PCV-2 genotypes were identified from 90% of hunting grounds and 50% of domestic pig provenances with sequence information from at least two animals (P<0.01) (data not shown).

ORF2 and ORF3 genotypes were assigned to 520 PCV-2 sequences extracted from the Genbank database and compared to PCV-2 genotypes identified in this study (Tab. 5). Among the database entries there were only five PCV-2 sequences from German domestic pigs from 1999/2000 which differed significantly from the domestic sequences of the present study and from 124 sequences obtained from different European countries. However, European and domestic pig PCV-2 sequences of the present study are in good agreement with 2b/1 and 2b/3 being the dominant genotypes. All other genotype combinations are infrequent in both groups. Six sequences of European wild boars are available in the Genbank database. Although they are derived from six individuals only, each representing a different genotype, these genotypes agree with the major genotypes 2b/1, 2a/1, 2b/2, 2b/4 and 2a/10 of the wild boars of the present study. Frequencies of genotypes 2b/1 and 2b/3 in wild boars are comparable with those of European domestic pigs. However, genotypes 2a/1, 2b/2, 2a/3 and 2a/10 that add up to a prevalence of 58% in wild boars are very rare in domestic pigs of the present study (9.5%) and in European domestic pigs (8%). Genotypes 2a/1, 2b/2 and 2a/10 are also very rare worldwide (7.5%), and genotype 2a/3 is not present at all in the 520 PCV-2 sequences extracted from the Genbank database.

4. Discussion

The aim of the present study was to improve our understanding of PCV-2 exchange between wild boars and domestic pigs, based on PCV-2 genotypes. Partial sequences of ORF2 and ORF3 were determined from 40 wild boars and 60 domestic pigs from four greater areas of

Germany (North, East, Southwest and Central Germany). Significant differences in genotype frequencies have been obtained. Of course the restricted number of samples cannot provide a fully representative nationwide analysis, but the data is suitable for a good overview, because a large area has been included and both, wild boar and domestic pig samples have been acquired from the same greater areas.

Almost all domestic pigs were shown to carry PCV-2b which is in accordance to studies from other countries (Gagnon et al., 2010; Li et al., 2010), therefore the significantly lower prevalence in wild boars was unexpected. Most of the genotypes correspond well with those from European domestic pig PCV-2 sequences derived from the Genbank database (August 2009; n=124), but they contrast very obviously with the deposited five sequences from German domestic pigs. These five sequences originate from the years 1999/2000. Therefore the differences may indicate the general switch from PCV-2a to genotype PCV-2b after the year 2003 that has been well described by Dupont et al. (2008). The conformity of domestic pig sequences of the present study with European Genbank genotypes documents the generality of the present study.

Differences and conformity of PCV-2 genotypes derived from wild boars and domestic pigs, either from this study or from European Genbank database entries, indicate that wild boar and domestic pig PCV-2 do coexist with some exchange. The higher diversity of PCV-2 genotypes in wild boars may be explained by recombination events between viruses of different genotypes. This assumption if further supported by the fact that the level of coinfection with several genotypes is higher in wild boars. More than 50% of the wild boar PCV-2 genotypes are extremely rare in domestic pigs in Europe (Genbank) and Germany (this study), even worldwide, inferring a certain independence of wild boar PCV-2 genotypes from domestic pig PCV-2 genotypes. On the other hand, genotypes 2b/1 and 2b/3 may indicate a certain exchange. Moreover, the fact that PCV-2a is prevalent in wild boars (57.5%), but rare in domestic pigs (4.8%) and that PCV-2b is the almost exclusive genotype in domestic pigs and also prevalent in wild boars argues for the hypothesis that exchange of PCV-2 between both species emerges primarily from domestic pigs to wild boars and to a much lesser extent in the reverse direction. A possible explanation for this hypothesis lies in the different PCV-2 loads of wild boars and domestic pigs as described by Reiner et al. (2010). Data of Albina et al. (2000) support this hypothesis. They suggested that virus infections of swine have a higher probability to spread from domestic pigs to wild boars rather than the reverse, except for domestic pigs kept outdoor which have increased chances of direct contact to infected wild boars.

5. Conclusion

PCV-2 shows a high variability within and between wild boars and domestic pigs. Some genotypes can be found almost exclusively in wild boars. These findings prove a certain independence of PCV-2 infections in both species and a low chance for domestic pigs to be infected with PCV-2 of wild boar origin. On the other hand, domestic pig specific PCV-2 genotypes are also common in wild boars, although at lower frequencies, suggesting the spread of domestic pig PCV-2 to wild boar populations.

Acknowledgements

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Table 1: PCR and sequencing primers for Pyrosequencing

Name	Sequence (5'-3')	Length [bp]	Position*	Literature
Pyro-PCV2-F	GCCCTGAATTTCCATATGAAATAA	104	964-987	this study
Pyro-PCV2-R	Biotin-5´-ATTTAATCTTAAAGACCCCCCACT-3´		1044-1067	
Pyro-PCV2-S	TTTATCACTTCGTAATGG		1002-1019	
Orf3F	ATTGTTGGCGAGGAGGGTAA	318	180-199	this study
Orf3R	Biotin-5´-CCCGCGGAAATTTCTGAC-3´		480-497	
Orf3S	GTAAAGAAGGCAACTTAC		343-360	

*position in Genbank acc. no. NC_005148

Table 2: Overall variability of PCV-2 genotypes between wild boars and domestic pigs (in %)

PCV-2 genotypes[1]	Sequences (5´-3´)	Wild boars (n=40)	Domestic pigs (n=60)	P[2]
2b	TTAAGGGTTAA	70.0	98.4	<0.001
2a	TTTAGGGTTTA	57.5	4.8	<0.001

[1]: according to Segales et al., 2008

[2]: Significance

Table 3: Overall variability in ORF3 sequences between wild boars and domestic pigs (in %).

ORF3 genotypes	Sequences (5´-3´)	Wild boars (n=40)	Domestic pigs (n=60)	P[1]
1	TGATGGAGTG	31.6	47.2	<0.001
2	TGATGGAATG	21.0	3.8	<0.001
3	TGATCGAGTG	39.5	41.5	n.s.
4	TGATTGAGTG	2.6	0	n.s.
5	TGGTGGAGTG	0	1.9	n.s.
6	TGATAGAGTG	2.6	1.9	n.s.
9	TGATCGAATG	0	1.9	n.s.
10	TTATTGAGTG	26.3	5.7	0.004
12	TTATTGAATG	2.6	0	n.s.

[1]: Significance, n.s. not significant

Table 4: Overall combination of ORF2 and ORF3 genotypes in wild boars and domestic pigs (in %).

ORF2/ORF3 genotypes	Wild boars (n=40)	Domestic pigs (n=60)	P[1]
2b/1	18.4	47.2	0.007
2b/2	10.5	3.8	n.s.
2b/3	26.3	37.7	n.s.
2b/4	2.6	0	n.s.
2b/5	0	1.9	n.s.
2b/6	2.6	1.9	n.s.
2b/9	0	1.9	n.s.
2b/10	5.2	3.8	n.s.
2b/12	2.6	0	n.s.
2a/1	13.2	0	0.011
2a/2	10.5	0	0.028
2a/3	13.2	3.8	n.s.
2a/10	21.1	1.9	0.004

[1]: Significance, n.s. not significant

Table 5: Comparison of the frequencies (%) of PCV-2 genotypes identified in domestic pigs and wild boars from this study and from sequences extracted from the Genbank database[1]

Genotypes			Domestic pigs (DP)									Wild boars (WB)	
			Genbank								This study		Genbank
ORF2	ORF3		Total[2]	AS[2]	SA[2]	AF[2]	AU[2]	NA[2]	EU[2]	Ger[2]	DP	WB	WB
		n	520	308	13	1	8	66	124	5	60	40	6
2b	1		30.2	30.8	23.1			7.6	43.5		47.2	18.4	33.3
2a			1.2	1.3					1.6			13.2	16,7
2b	2		3.6	5.6					1.6		3.8	10.5	16.7
2a			3.5	0.3			75.0	1.5	8.0	20.0			
2b	3		13.1	10.4					29.0		37.7	26.3	
2a											3.8	13.2	
2b	4		7.5	1.0	38.5			43.9	1.6			2.6	16.7
2b	5		0.4		15.4						1.9		
2b	6		0.2	0.0					0.8		1.9	2.6	
2b	7		8.7	14.6									
2b	8		0.2	0.3									
2b	9		6.8	10.9					0.8		1.9		
2a			0.2	0.3									
2b	10		0.8	1.0					0.8		3.8	5.2	
2a			2.7	0.9	7.7		12.5	4.5	4.8	40.0	1.9	21.1	16.7
2b	11		1.0	0.9					1.6				
2b	12		0.4	0.6								2.6	
2a			8.3	3.9	15.4	100.0	12.5	40.9					
2b	13		0.4	0.6									
2a			1.9	3.2									
2a	14		0.2	0.3									
2a	15		0.2					1.5					
2b	16		0.4	0.6									
2b	17		0.2						0.8				
2a	18		0.2						0.8				
2b	19		6.7	11.3									
2a			1.4	0.6					4.0	40.0			

[1]: release August 2009

[2]: Total: all sequences; AS: Asia; SA: South America; AF: South Africa; AU: Australia; NA: North America; EU: Europe; Ger: Germany.