RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Noroviruses in finisher swine: prevalence and relatedness to human noroviruses - **NPB #08-183**

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Industry Summary:

Noroviruses (NoVs) are the leading cause of foodborne illness. A potential exists for interspecies transmission of NoVs or the emergence of new epidemic human strains from porcine NoVs. However, an extensive sequence database is lacking for porcine NoVs. Without knowing the diversity of swine NoVs and their relatedness to human strains, it is impossible to pinpoint the possible origin (swine or human) of NoV contaminants reported in pork. The objectives of this study are: 1) to study the prevalence of porcine NoVs in finisher swine barns; 2) to identify newly emerged porcine NoVs and determine the genetic relatedness between porcine and human NoVs. We tested 313 pooled samples from 1436 individual finisher pig fecal samples collected from 33 barns belonging to 9 farms and 3 production systems (BC1-3) in North Carolina. First, we screened for known porcine NoVs. Then, porcine NoV-negative samples were further screened for new NoVs and other caliciviruses. We did not identify human NoVs or new porcine NoVs, but a new porcine enteric calicivirus, St-Valerien-like virus WGP93C strain. The complete genome of the first US St-Valerien-like virus WGP93C strain was characterized to expand the porcine enteric calicivirus sequence database. Finally, a new assay for the detection of St-Valerien-like viruses was developed and used to perform the first prevalence study of these new viruses.

The prevalence of porcine NoVs was 18.5% (range of 5.0-51.7% among farms). It was significant higher in BC2 (32.3%) than BC1 (15.1%) and BC3 (9.2%) production systems. The St-Valerien-like viruses were also endemic in NC farms with a prevalence of 21.1% (range of 2.6-80.0% among farms). Pigs of production system BC1 shed St-Valerien-like viruses in significantly higher frequencies (34.5%) than in the other two production systems (7.3% and 18.4%, respectively). Overall there was no significant difference in NoV or St-Valerien-like virus prevalence among the treatments (three biocides Biosentry, Synergize, Virkon-S and hot water control). The complete genome of WGP93C strain was sequenced and our data supports that the St-Valerien-like viruses represent a new genus within *Caliciviridae.*

The differences among the three production systems are mainly the “origin” of the pigs. Each of the production systems is fully independent and they have their own genetics/breeding units, farrowing sites where the sampled pigs originated, etc. Our finding suggests that breed differences or infection incidence in earlier production stages might affect incidence at the sampled finisher stage. Genetic differences in human blood types are known to affect susceptibility to human NoVs. Whether genetic factors affect pig susceptibility to porcine NoVs requires further investigation. There is no report that porcine NoVs cause clinical signs in

finisher pigs. However, like most RNA viruses, NoVs undergo constant genetic mutation during viral replication and new variants emerge constantly that may have altered host and tissue tropism and/or disease patterns. Nevertheless, because some porcine and human NoV strains are genetically and antigenically related and frequent recombination results in new variants, a potential may exist for the zoonotic emergence of new endemic human NoV strains from porcine NoVs. Therefore, continuously monitoring the prevalence and diversity of NoVs in pigs will provide important information to identify or refute swine or pork products as sources for possible human NoV transmission and infection. Finally, the biocides did not affect the prevalence of porcine NoVs or St-Valerien-like viruses. These results are consistent with the fact that NoVs are extremely stable in the environment and resistant to many disinfectants. Whether the St-Valerien-like viruses cause disease in pigs, including younger pigs or occur in other species, including humans, requires further investigation.

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Keywords: porcine, calicivirus, norovirus, St-Valerien-like viruses, zoonotic

Scientific Abstract.

Noroviruses (NoVs) are the leading cause of foodborne illness. A potential exists for interspecies transmission of NoVs or the emergence of new epidemic human strains by genetic recombination of human and porcine NoVs. However, an extensive sequence database is lacking for porcine NoVs. Without knowing the diversity of swine NoVs and their relatedness to human strains, it is impossible to pinpoint the possible origin (swine or human) of NoV contaminants reported in pork. The objectives of this study are: 1) to study the prevalence of porcine NoVs in finisher swine barns; 2) to identify newly emerged porcine NoVs and determine the genetic relatedness between porcine and human NoVs. We tested 313 pooled samples from 1436 individual finisher pig fecal samples collected from 33 barns belonging to 9 farms and 3 production systems (BC1-3) in North Carolina. A reverse transcription (RT)-PCR coupled hybridization assay was used to screen for known porcine NoVs. Two more RT-PCR assays were performed to detect genetically diverse NoVs and other caliciviruses. Sequence analyses of representative strains did not identify human NoVs or new porcine NoVs, but revealed a new porcine enteric calicivirus, St-Valerien-like virus (WGP93C strain). We further characterized the first US St-Valerien-like virus WGP93C strain to expand the porcine enteric calicivirus sequence database. Finally, a real time RT-PCR for the detection of St-Valerien-like viruses was developed and used to perform the first prevalence study of these new viruses.

The prevalence of porcine NoVs was 18.5% (range of 5.0-51.7% among farms). It was significant higher in BC2 (32.3%) than BC1 (15.1%) and BC3 (9.2%) production systems. The St-Valerien-like viruses were also endemic in NC farms with a prevalence of 21.1% (range of 2.6-80.0% among farms). Pigs of production system BC1 shed St-Valerien-like viruses in significantly higher frequencies (34.5%) than in the other two production systems (7.3% and 18.4%, respectively). Overall there was no significant difference in NoV or St-Valerien-like virus prevalence among the treatments (three biocides Biosentry, Synergize, Virkon-S and hot water control). The complete genome of WGP93C strain was sequenced and our data supports that the St-Valerien-like viruses represent a new genus within *Caliciviridae*.

The differences among the three production systems are mainly the “origin” of the pigs. Each of the production systems is fully independent and they have their own genetics/breeding units, farrowing sites where the sampled pigs originated, etc. Our finding suggests that breed differences or infection incidence in earlier production stages might affect incidence at the sampled finisher stage. Genetic differences in human blood types are known to affect susceptibility to human NoVs. Whether genetic factors affect pig susceptibility to porcine NoVs requires further investigation. There is no report that porcine NoVs cause clinical signs in finisher pigs. However, like most RNA viruses, NoVs undergo constant genetic mutation during viral replication and new variants emerge constantly that may have altered host and tissue tropism and/or disease patterns. Nevertheless, because some porcine and human NoV strains are genetically and antigenically related

and frequent recombination results in new variants, a potential may exist for the zoonotic emergence of new endemic human NoV strains from porcine NoVs. Therefore, continuously monitoring the prevalence and diversity of NoVs in pigs will provide important information to identify or refute swine or pork products as sources for possible human NoV transmission and infection. Finally, the biocides did not affect the prevalence of porcine NoVs or St-Valerien-like viruses. These results are consistent with the fact that NoVs are extremely stable in the environment and resistant to many disinfectants. Whether the St-Valerien-like viruses cause disease in pigs, including younger pigs or occur in other species, including humans, requires further investigation.

Introduction. Noroviruses (NoVs) are the leading cause of foodborne illness (5). Genogroup II (GII) NoVs are the most commonly detected in human outbreaks and new GII genotype 4 (GII.4) variants of increased virulence have emerged, but of unknown origin (2). Previously GII NoVs were detected worldwide from pigs and those porcine GII NoVs are genetically and antigenically related to human GII NoVs (15). Recently, GII.4 NoV RNA was detected from a retail raw pork sample (9) and both human and porcine NoVs were detected from raw market oysters (3) providing food vehicles for potential interspecies transmission of NoVs or generation of recombinants in humans. Also, pigs can be infected experimentally with a GII.4 human NoV strain. These results suggest the potential for interspecies transmission of NoVs or the emergence of new epidemic human strains by genetic recombination of human and porcine NoVs. However, there are only a few small prevalence studies of NoVs in swine. Also an extensive sequence database is lacking for pig NoVs to be able to compare them genetically to human strains. Without knowing the diversity of swine NoVs and their relatedness to human strains, it is premature to test pork products since there is insufficient data to pinpoint the possible origin (swine or human) of the NoV contaminants. In this study, we used pooled finisher pig fecal samples collected as part of a USDA-funded biocide-salmonella project, from 33 barns belonging to 9 farms and 3 production systems, to determine the relationship between NoV prevalence and biocide treatments (barns), farms and production systems. Also, new porcine enteric caliciviruses, St-Valerien-like viruses, were identified and characterized to expand the porcine enteric calicivirus sequence database. Finally, a real time reverse transcription (RT)-PCR for the detection of St-Valerien-like viruses was developed and used to perform the first prevalence study of these new viruses in US swine.

Objectives.

1) To study the prevalence of porcine noroviruses (NoVs) in finisher swine barns.

Our hypothesis is that there will be differences in the prevalence of NoVs between farms or production systems.

2) To identify newly emerged porcine NoVs and determine the genetic relatedness between porcine and human NoVs.

Our hypothesis is that new swine NoVs have emerged and they are genetically similar (within the same genogroup, even the same genotype) to human strains or they may represent recombinants between human and porcine strains.

Materials & Methods.

Experimental design. Known porcine NoVs (GII.11, 18 and 19) were detected by RT-PCR with primer pair PNV7/8 and microwell hybridization assays with a mixture of 3 probes (PoNoroP1A, PoNoroP1B and PoNoroP1C) (14). For samples that tested negative with the above assays, two more RT-PCR assays were performed. To detect human GII.4-like NoVs from pigs, RT-PCR with the degenerate primer pair NLV431/433) (1) was performed. To detect genetically diverse caliciviruses, including newly emerged variants, RT-PCR with calicivirus universal primer pair p290/110 was performed (6). Representative positive samples by RT-PCR with NLV431/433 and/or p290/110 were selected from different barns, farms and production systems for sequencing to examine the genetic diversity of porcine NoVs and other caliciviruses and their relatedness to human strains. A potentially new genus of porcine caliciviruses, St-Valerien-like viruses (8), was identified and its full length genome was characterized. Finally, a new real time RT-PCR assay was developed for the new porcine caliciviruses and used to study their prevalence in swine.

Fecal samples. Our samples were from a major time-nested longitudinal USDA-funded project (Gebreys, PI: "Biocide and heavy metal interventions in swine production and association with multi-drug resistant *Salmonella"*) in finisher pigs. A total of 1436 pig fecal samples were collected (Table 1) during May-October, 2009 from 3 different production systems, 3 farms per system, and 2-4 barns per farm, located in North Carolina (NC). Each barn was treated with one of the three biocides [Biosentry (BIO), Synergize (SYN), Virkon-S (VIR)], or hot water (H2O) as a control. One barn has 25-48 pens. The pigs of each barn were sampled at one week before marketing, collecting one sample per pen in the barn. Fecal samples were collected from selected pigs (based on convenience) using sterile gloves and cups to prevent contamination from environment and between samples. Samples were stored at –20°C until use. In the laboratory, 4-5 individual samples (unpooled aliquots retained) from the same barn were pooled for RNA extraction and RT-PCR screening, obtaining 6-10 pooled samples per barn for a total of 313 pooled fecal samples across all three production systems.

RNA extraction. RNA was extracted from 10% fecal suspensions using 5x MagMAX-96 Viral 1 kit (Applied Bioststems) and stored at -20 or -70 ºC.

RT-PCR to detect porcine NoVs and other caliciviruses. RT and PCR were performed separately using primer pairs PNV7/8 and p290/110 as previously reported (16). To detect GII.4 NoVs from pigs, a single one-step RT-PCR was performed with the degenerate primer pair NLV431 and 433 (1).

Microwell hybridization assays. Microwell hybridization assays for the detection of porcine NoV-specific PNV7/8 amplicons were performed as previously described (14).

Real time RT-PCR for the detection of St-Valerien-like viruses. A TaqMan real-time RT-PCR assay for the detection of St-Valerien-like viruses was developed in our lab. Forward primer (WGP93-polF1, 5'-TCTAAAGCGTGCACTCTGGGTCAT-3', WGP93C genome 3712-3735nt), reverse primer (WGP93-polR1, 5'-ACCCTTTCTCCACCAGGAACTTCT-3', WGP93C genome 3839-3816nt) and probe (WGP93-polP1, FAM-ACGAGTTTGTGGACTTCCTCTCGCA-BHQ, WGP93C genome 3765-3789nt) were designed based on the RNA-dependent RNA polymerase (RdRp) region of both WGP93C and the Canadian AB90, AB104 and F15-10 strains. The assay was performed using QIAGEN OneStep RT-PCR Kit and a real time thermocycler (RealPlex, Eppendorf). The RT was performed at 50 °C for 30 min followed by denaturing at 95 °C for 15 min and 45 cycles of PCR cycles (95°C for 15 sec and 59°C for 60 sec). The detection limit of this assay is 10 genomic copy equivalents (GE) per reaction determined based on the standard curve generated using serially diluted plasmid DNA carrying WGP93C-specific p290/110 amplicons. Other porcine enteric pathogens including porcine rotavirus group C, porcine sapovirus strains (Cowden, QW270, QW19, JJ681 and LL26), porcine NoV strains (QW48, QW101, QW126 and QW218) and human NoV GII.4 HS66 strain were not detected. Thus this real-time RT-PCR is sensitive and specific for the detection of St-Valerien-like viruses.

Sequence analysis of the complete genome of St-Valerien-like virus, WGP93C strain.
The p290/110 amplicon was sequenced directly and a short fragment sequence (265 nt) of RdRp was obtained. Then two WGP93C-specific primers were designed based on it and used to get the 3'-end, about 2.7 kb genomic sequence as described previously (11) and the internal 2.5 kb genomic sequence with primer p1210 as described by L'Homme et al. (8). Finally, the 5'-end about 1.5 kb genomic sequence was obtained using 5'-RACE protocol (12). The cDNA was synthesized using SuperScript III First-Strand cDNA synthesis kit (Invitrogen), and PCR was performed using TaKaRa Ex Taq™ polymerase (TaKaRa Mirus Bio, Madison, WI).

Cloning and sequencing. The RT-PCR products of representative strains were purified by QIAquick Gel Extraction kit (Qiagen Inc., Valencia, CA). The purified RT-PCR products were sequenced directly using forward and reverse primers or were cloned into pCR2.1 (T/A) vector (for <1 kb insertion) or pCR-XL-TOPO vector (for

>1 kb insertion) (Invitrogen) for sequencing. DNA sequencing were performed using BigDye Terminator Cycle chemistry (Applied Biosystems, Foster, CA) and automated sequencer ABI Prism 3100XL (Applied Biosystems).

Sequence analysis. Sequence editing was performed by DNASTAR Lasergene software package (version 7) (DNASTAR Inc., Madison, WI). Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov) was used to find homologous hits. Multiple sequence alignment was done by ClustalW (version 1.83) at DDBJ (http://www.ddbj.nig.ac.jp). Phylogenetic analysis (Neighbor-Joining) with bootstrap (1,000 replicates) was conducted using MEGA v 4 (13).

Statistical analysis. Observed prevalence rates of porcine NoVs and St-Valerien-like viruses at the different levels of sampling were calculated. The relationship and differences in prevalence among the various treatments (barns), farms and production systems were assessed using Chi-square and Binomial proportion test (Statistical Analysis System, SAS). Statistical significance was assessed throughout at $P < 0.05$.

Results.

I. The prevalence of porcine NoVs in finisher swine barns.

All nine swine farms were positive for porcine NoVs (Tables 1 & 2). The overall prevalence was 18.5% (range of 5.0-51.7% among farms). We also examined whether the NoV prevalence differed among different production systems, farms and treatments (barns). The prevalence of NoVs in production system BC2 [31/96, (32.3%)] was significantly higher than that in the other 2 production systems [18/119 (15.1%) and 9/98 (9.2%), respectively] (Table 2). At the farm level, the prevalence of NoVs in farm DC [15/29, (51.7%)] was significantly higher than that in six of eight farms. Overall there was no significant difference in NoV prevalence among the treatments (three biocides and the water control) (Table 3). However, within each farm, only farm RW showed a significant difference for NoV prevalence among different treatments (Table 1), highest for SYN (7/10, 70%), moderate for BIO (3/10, 30%) and lowest for VIR (0/10, 0%).

Table 1. The prevalence of porcine NoVs and St-Valerien-like viruses in finisher swine barns in North Carolina

Prod. Sys.	Farm	Txt (barn)	Sampling time	No. of individual samples	No. of pooled samples	NoVs		St-Valerien-like viruses		Mixed Infection of NoVs & St-Valerien-like viruses
						Positive pooled samples	Prevalence (%)	Positive pooled samples	Prevalence (%)	
BC1	BH	H2O	Aug, 2009	48	10	0	0	4	40	0
BC1	BH	BIO	Aug, 2009	48	10	2	20	2	20	0
BC1	BH	SYN	Aug, 2009	48	10	1	10	0	0	0
BC1	BH	VIR	Aug, 2009	48	10	1	10	2	20	0
BC1	EW	H2O	July, 2009	42	10	2	20	0	0	0
BC1	EW	BIO	July, 2009	39	9	0	0	0	0	0
BC1	EW	SYN	July, 2009	47	10	0	0	1	10	0
BC1	EW	VIR	July, 2009	48	10	1	10	0	0	0
BC1	WL	H2O	May, 2009	47	10	2	20	10	100	2
BC1	WL	BIO	May, 2009	47	10	1	10	10	100	1
BC1	WL	SYN	May, 2009	46	10	4	40	2	20	1
BC1	WL	VIR	May, 2009	48	10	4	40	10	100	4
BC2	DC	H2O	July, 2009	25	6	3	50	1	16.7	0
BC2	DC	BIO	July, 2009	35	8	4	50	1	12.5	1
BC2	DC	SYN	July, 2009	29	7	4	57.1	0	0	0
BC2	DC	VIR	July, 2009	36	8	4	50	1	12.5	0
BC2	FF	H2O	Oct, 2009	46	7	2	28.6	0	0	0
BC2	FF	BIO	Oct, 2009	48	10	0	0	1	10	0
BC2	FF	SYN	Oct, 2009	40	10	2	20	0	0	0
BC2	FF	VIR	Oct, 2009	48	10	2	20	1	10	0
BC2	RW	BIO	May, 2009	45	10	3	30	0	0	0
BC2	RW	SYN	May, 2009	48	10	7	70	2	20	2
BC2	RW	VIR	May, 2009	46	10	0	0	0	0	0
BC3	GO	BIO	Aug, 2009	45	10	1	10	1	10	0
BC3	GO	VIR	Aug, 2009	46	10	0	0	2	20	0
BC3	TE	H2O	July,2009	47	10	1	10	4	40	0
BC3	TE	BIO	July,2009	38	9	1	11.1	2	22.2	0
BC3	TE	SYN	July,2009	40	10	0	0	4	40	0
BC3	TE	VIR	July,2009	47	10	2	20	2	20	0
BC3	TT	H2O	July,2009	42	10	0	0	1	10	0
BC3	TT	BIO	May,2009	45	10	2	20	0	0	0
BC3	TT	SYN	July,2009	38	9	2	22.2	2	22.2	0
BC3	TT	VIR	May,2009	46	10	0	0	0	0	0
Total				1436	313	58	18.5	66	21.1	11

Table 2. The prevalence of porcine NoVs among farms and production systems

Production system	BC1			BC2			BC3		
Farm	BH	EW	WL	DC	FF	RW	GO	TE	TT
Positive/Total (%)	4/40 (10.0)^{B}	3/39 (7.7)^{B}	11/40 (27.5)^{AB}	15/29 (51.7)^{A}	6/37 (16.2)^{B}	10/30 (33.3)^{AB}	1/20 (5.0)^{B}	4/39 (10.3)^{B}	4/39 (10.3)^{B}
Subtotal	18/119 (15.1)^{b}			31/96 (32.3)^{a}			9/98 (9.2)^{b}		

Percentage with different letters differ significantly (P< 0.05, Binomial proportion test).

Table 3. The prevalence of NoVs and St-Valerien-like viruses among treatment groups

	NoVs				St-Valerien-like viruses			
	H2O	BIO	SYN	VIR	H2O	BIO	SYN	VIR
Positive/Total (%)	10/63 (15.9)	14/86 (16.3)	20/76 (26.3)	14/88 (15.9)	20/63 (31.8)	17/86 (19.8)	11/76 (14.5)	18/88 (20.5)

P>0.05, Chi-square test.

2. Sequence analyses of Mon431/433 and p290/110 amplicons.

The RT-PCR amplicons of primer sets Mon431/433 and p290/110 of representative strains based on barn level were sequenced. All of them were known porcine NoVs or sapoviruses (data not shown) except for the p290/110 amplicons of pooled sample WGP93 that is a St-Valerien-like virus. We tested the individual five samples (WGP93A, B, C, D and E) that were pooled to form WGP93 and found that WGP93C had the highest virus titer. So the WGP93C fecal sample was used for further genomic characterization of the first US St-Valerien-like virus.

3. Genomic characterization of St-Valerien-like virus WGP93C strain.

The US WGP93C strain is genetically similar to the Canadian St-Valerien-like viruses AB90, AB104 and F15-10 strains (8), sharing 89.3-89.7% overall genomic nucleotide identity. Strains representing different genera in *Caliciviridae* were used to build the phylogenetic tree (Fig. 1). We included caliciviruses, and viruses detected in WGP93C strain was grouped together AB104 and F15-10 strains to form a new genus within *Caliciviridae*.

Like the Canadian St-Valerien-like AB90, AB104 and F15-10 strains (8), genome of WGP93C is composed of reading frames (ORF) (Fig. 2). ORF1 amino acids (aa) long and encodes a polyprotein containing the putative nonstructural proteins (N-terminal, P19, Vpg, protease and polymerase) major capsid protein VP1 (516 aa). encodes the putative minor structural VP2 (150 aa). All putative nonstructural proteins except for protease, VP1 and VP2 share the highest aa identity with Tulane virus (4).

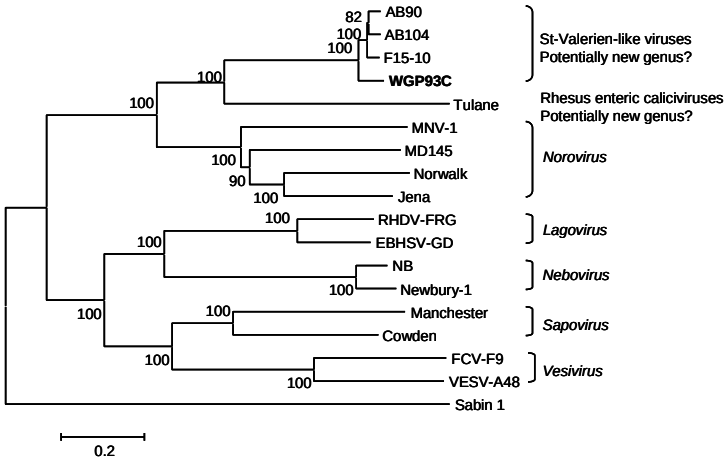

Fig. 1. Neighbor-Joining phylogenetic tree based on the complete genomes of strains representing *Norovirus, Sapovirus, Lagovirus, Vesivirus and Nebovirus* genera, and two potentially new genera, respectively, in *Caliciviridae*. The newly identified St-Valerien-like virus WGP93C strain is in bold. Poliovirus Sabin 1 strain (Genbank # V01150) was used as an out-group control.

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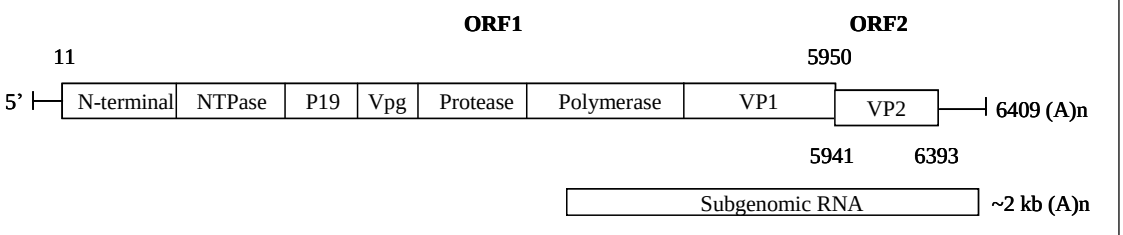

Fig. 2. The genome organization, open reading frames (ORF) and subgenomic RNA are shown for St-Valerien-like virus WGP93C strain (Porcine/St-Valerien-like virus/WGP93C/2009/US, GenBank accession number GU592498). The numbers alone indicate nucleotide position. A poly(A) tail is shown as (A)n.

4. The prevalence of St-Valerien-like viruses in NC finisher swine barns.
All nine swine farms were positive for St-Valerien-like viruses (Tables 1 & 5). The overall prevalence was 21.1% (range of 2.6-80.0% among farms). The prevalence of St-Valerien-like viruses in production system BC1 [41/119, (34.5%)] was significantly higher than that in the other 2 production systems [7/96 (7.3%) and 18/98 (18.4%), respectively] (Table 4). At the farm level, the prevalence of St-Valerien-like viruses in farm WL [32/40, (80.0%)] was significantly higher than that at the other eight farms. Overall there was no significant difference for St-Valerien-like virus prevalence among the treatments (three biocides and the water control) (Table 3).

Table 4. The prevalence of porcine St-Valerien-like viruses among farms and production systems

Production system	BC1			BC2			BC3		
Farm	BH	EW	WL	DC	FF	RW	GO	TE	TT
Positive/Total (%)	8/40 (20.0)^{BC}	1/39 (2.6)^{C}	32/40 (80)^{A}	3/29 (10.3)^{BC}	2/37 (5.4)^{C}	2/30 (6.7)^{C}	3/20 (15)^{BC}	12/39 (30.8)^{B}	3/39 (7.7)^{C}
Subtotal	41/119 (34.5)^{a}			7/96 (7.3)^{b}			18/98 (18.4)^{b}		

Percentage with different letters differ significantly ($P < 0.05$, Binomial proportion test).

5. Mixed infection of porcine NoVs and St-Valerien-like viruses in NC finisher swine barns. There were 11 mixed infections of NoVs and St-Valerien-like viruses in three (WL, DC and RW) of the nine farms (Table 1).

Discussion.
We found that the prevalence of porcine NoVs was significantly higher ($P<0.05$) in BC2 (32.3%) than BC1 (15.1%) and BC3 (9.2%) production systems (Table 2). All three production systems are of the "conventional type" with overall similar management systems. The differences among the three production systems are mainly the "origin" of the pigs. Each of the three production systems is fully independent and they have their own genetics/breeding units, farrowing sites where the sampled pigs originated etc. There might be other variations among the three systems but it is hard to capture or pinpoint specific differences. However this finding suggests that breed differences or infection incidence in earlier production stages might affect incidence at the sampled finisher stage. Genetic differences in human blood types are known to affect susceptibility to human NoVs (5). Whether genetic factors affect pig susceptibility to porcine NoVs needs further investigation.

We did not identify new porcine NoVs or human NoVs by sequence analyses of the RT-PCR amplicons of representative strains using primer sets Mon431/433 and p290/110. Recently, a Japanese group (10) detected GII.4 NoVs from pigs. We will use their primer set G2SKF/R that detected GII.4 NoVs in Japanese swine to screen our pig fecal samples to further investigate if there are GII.4-like NoVs circulating in US swine. If no

GII.4 NoV is detected from US pig samples, it suggests that pork GII.4 NoV contaminants likely are from human but not pig sources.

At present, there is no report that porcine NoVs cause clinical signs in finisher pigs. However, like most RNA viruses, NoVs undergo constant genetic mutation during viral replication because the viral RNA polymerase lacks proof reading, new variants emerge constantly and they may change host and tissue tropism and/or disease patterns. Nevertheless, because some porcine and human NoV strains are genetically and antigenically related and frequent recombination results in new variants (16), a potential may exist for the zoonotic emergence of new endemic human NoV strains from porcine NoVs. Therefore, continuous monitoring of the prevalence and diversity of NoVs in pigs will provide important information to identify or refute swine or pork as sources for possible human NoV transmission and infection.

Our data support that of L'Homme et al. (8) who propose that the St-Valerien-like viruses represent a new genus within *Caliciviridae*. The WGP93C is the first St-Valerien-like virus strain that was identified in US. The St-Valerien-like viruses were endemic in all the nine NC farms tested, with a prevalence of 21.1% (66/313). Pigs of production system BC1 shed St-Valerien-like viruses in significantly higher frequencies than in the other two production systems. The prevalence on farm WL [32/40, (80.0%)] was significantly higher than that on the other eight farms, suggesting that an outbreak occurred at this farm during sampling. Our results indicate that St-Valerien-like viruses are prevalent in NC swine farms. Whether they cause disease in pigs, including younger pigs, or occur in other species, including humans, requires further investigation. Mixed infections by porcine NoVs and St-Valerien-like viruses occurred but were less common.

Finally, the biocides did not affect the prevalence of porcine NoVs or St-Valerien-like viruses. These results are consistent with the fact that NoVs are extremely stable in the environment and resistant to many disinfectants and even the chlorine concentrations used in drinking water treatment do not inactivate human NoVs (5, 7).

Publications and presentations.

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