JOURNAL OF CLINICAL MICROBIOLOGY, June 2006, p. 2057–2062 Vol. 44, No. 6
0095-1137/06/$08.00+0 doi:10.1128/JCM.02634-05

Prevalence of Noroviruses and Sapoviruses in Swine of Various Ages Determined by Reverse Transcription-PCR and Microwell Hybridization Assays

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Received 20 December 2005/Returned for modification 9 March 2006/Accepted 24 March 2006

Noroviruses (NoVs) and sapoviruses (SaVs) are emerging enteric pathogens that cause diarrhea in humans and animals. Porcine genogroup II (GII) NoVs replicate in pigs, but their pathogenesis is undefined. The porcine SaV/GIII/Cowden/80/US strain causes diarrhea and intestinal lesions in pigs. Recently, genetically diverse porcine NoVs (genotypes 11, 18, and 19 within GII) and SaVs comprising at least two genogroups (GIII and GVI?/JJ681-like) and two unclassified strains (G?/QW19 and G?/LL26) were identified; however, their prevalence has not been reported. To investigate the prevalence of porcine NoVs and SaVs, 621 fecal samples were collected from swine of various ages from seven swine farms and one slaughterhouse in three states in the United States. Fecal samples were tested by reverse transcription-PCR and microwell hybridization assays with porcine NoV- and SaV-specific primers and probes, respectively. Porcine GII NoVs were detected exclusively from finisher pigs with an overall prevalence of 20%. Porcine GIII SaVs were detected in 62% of pigs, with the highest prevalence in postweaning pigs and lowest in nursing pigs. Porcine GVI?/JJ681-like SaVs and the G?/QW19-like SaVs were detected infrequently in pigs. The G?/LL26-like SaVs were detected mainly in younger pigs. Because some porcine NoVs and SaVs are genetically or antigenically related to human strains and recombinants within NoVs or SaVs occur for human and pig strains, the high prevalence and subclinical infection rate of these viruses in pigs raise questions of whether pigs may be reservoirs for human strains or for the emergence of new human and porcine recombinants.

Members of the *Norovirus* genus within the family *Caliciviridae*, except for the newly identified murine norovirus (NoV) (17), cause gastroenteritis in humans and calves (7, 20). In humans, NoVs are estimated to cause 23 million cases of illness and two-thirds of food-borne illnesses annually in the United States alone (25). They cause more than 90% of nonbacterial gastroenteritis outbreaks worldwide (4, 7, 22). There is no cell culture system available for NoVs, except for the murine MNV-1 strain, and so genetic classification of NoVs based on the complete capsid gene is generally accepted (7, 35). Presently, these genetically highly variable viruses have been classified into five genogroups (GI to -V), with GII the most prevalent in humans but with new variants and recombinants being recognized frequently (1, 4, 21, 24). Genogroup II NoV RNA has been detected in field fecal samples or intestinal contents at slaughter of adult pigs in Japan and Europe and recently by our laboratory in the United States (30, 31, 33). Porcine NoVs replicate in gnotobiotic pigs, but their detailed pathogenesis and prevalence among swine have yet to be defined (33).

Sapoviruses (SaVs) belong to another genus, *Sapovirus*, within the family *Caliciviridae*. Human SaVs cause sporadic cases and outbreaks of gastroenteritis mainly in young children <5 years of age (28). Based on the capsid gene, SaVs have been classified into five genogroups: GI, -II, -IV, and -V infect humans, and GIII infects pigs. Not only intragenogroup but also intergenogroup SaV recombinants have been identified (10, 18, 34). The Po/SaV/GIII/Cowden/80/US strain causes diarrhea and intestinal lesions in gnotobiotic pigs (6, 9). However, detailed studies of the prevalence of SaVs in swine are also lacking.

Recently, genetically diverse and potential recombinant porcine NoVs and SaVs were identified in our studies (33, 34). Porcine NoVs were classified into three genotypes within GII (GII-11, -18, and -19), with the GII-18 porcine NoVs sharing the highest amino acid identity with the Hu/NoV/GII-3/Mexico strain. More interestingly, the porcine GII NoVs were antigenically related to human GII NoVs (5, 33). Porcine SaVs comprise at least two genogroups (GIII and GVI?/JJ681-like) (8, 34). There are two more unclassified but distinct SaV strains (G?/QW19 and G?/LL26), with the QW19 strain sharing the highest amino acid identity with the Hu/SaV/GII/Mc10 strain in a short fragment (95 amino acids) in the RNA-dependent RNA polymerase (RdRp) region.

These results raise questions of whether pigs are reservoirs for emergence of new human NoV and SaV variants or if porcine-human recombinants could emerge. However, no prevalence study has been reported for porcine NoVs and SaVs in pigs. In this study, we collected 621 pig fecal samples from seven swine farms and one slaughterhouse in three states.

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TABLE 1. Number and timing of fecal sample collections from nursing, postweaning, and finisher pigs and sows from different farms

Swine farm or slaughterhouse	Date of sample collection			No. of samples				
	Spring	Summer	Winter	Nursing (1–3 wks)	Postweaning (3–10 wks)	Finisher (20–24 wks)	Sow (>1 yr)	Total
MI farm A			Dec. 2002	NA[a]	NA	61	NA	61
NC farm A	May 2003			NA	NA	8	NA	8
NC farm B		June 2003		NA	NA	21	NA	21
OH farm A	Mar. 2003			NA	NA	10	NA	10
OH farm A	Apr. 2004			14[b]	12[b]	12	13	51
OH farm B	Mar. 2003			NA	30	60	NA	90
OH farm B	May 2004			NA	15[d]	NA	NA	15
OH farm B		June 2004		NA	15	15	NA	30
OH farm B		July 2004		31	NA	NA	15	46
OH farm B			Dec. 2004	30	NA	NA	15	45
OH farm B	Mar. 2005			NA	30[c]	30	NA	60
OH farm C	May 2003			NA	NA	NA	32	32
OH farm C	May 2004			15[c]	12[b]	12	12	51
OH farm D	May 2004			8[b]	10	NA	NA	18
OH slaughterhouse	Apr. 2003			NA	NA	83	NA	83
Total				98	124	312	87	621

[a] NA, not available.
[b] One sample was collected from a diarrheic pig.
[c] Two samples were collected from diarrheic pigs.
[d] Three samples were collected from diarrheic pigs.

They were tested for porcine NoVs and SaVs by five individual reverse transcription (RT)-PCRs followed by corresponding microwell hybridization assays (32).

MATERIALS AND METHODS

Sampling. Fecal samples (n = 621) were collected from December 2002 to March 2005 from seven swine farms and one slaughterhouse in three states (Ohio [OH], Michigan [MI], and North Carolina [NC]) in the United States, referred to as OH farms A, B, C, and D, MI farm A, NC farms A and B, and OH slaughterhouse A (Table 1). The OH farm B and MI farm A had total pig inventories of approximately 50,000 pigs, and the other farms had total farm inventories of approximately 1,000 pigs. The Ohio slaughterhouse received pigs from multiple farms, and the farms were not identified during collection. Four age groups of pigs were included: nursing pigs (1 to 3 weeks), postweaning (nursery) pigs (3 to 10 weeks), finisher pigs (10 to 24 weeks), and sows (>1 year of age). Except for four nursing pigs (one, two, and one pig from OH farms A, C, and D, respectively) and seven postweaning pigs (one, five, and one pig from OH farms A, B, and C) that had diarrhea at sampling, all others were clinically normal at the time of sample collection. Samples collected during 2002 to 2003 were convenience samples from another ongoing project for a prevalence study of *Salmonella*. Later, we sampled from OH farms A, B, C, and D different ages of pigs and in different seasons to investigate whether the prevalence of porcine NoVs and SaVs differed among the age groups and seasons. Fresh fecal samples were collected from individual pigs, placed into sterile specimen containers, and stored frozen at −20°C or −70°C.

RNA extraction. Sample RNA was extracted from 200 μl of 10% fecal suspension by using the TRIzol LS (Invitrogen, Carlsbad, CA) procedure. The RNA pellet was resuspended in 40 μl RNase-free water and stored at −70°C.

RT-PCR to detect porcine GIII SaVs and to monitor for RT-PCR inhibitors. Primers, which were biotinylated at the 5′ end, for RT-PCRs and probes for microwell hybridization assays used in this study were reported previously (Table 2) (32). First, one-step RT-PCR with primer pair PEC66/65 and a competitive internal control (IC) RNA for GIII SaVs was performed to detect GIII SaVs and to monitor for RT-PCR inhibition as described previously (32). The GIII SaV-specific amplicons were tested by microwell hybridization assays with a mixture

TABLE 2. Primers and probes used for the detection of porcine NoVs and SaVs

Porcine NoV or SaV/genogroup or genotype	Primer name (sense) and sequence (5′→3′)	Product size (bp)	Probe name and sequence (5′→3′)
NoV/GII-11, -18, -19	PNV7 (+): AGG TGG TGG CCG AGG AYC TCC T PNV8 (−): TCA CCA TAG AAG GAR AAG CA	211	PoNoroP1A: AGC CAG TGG GCG AAG GAG TTC CAC TGT GAT GTG CA PoNoroP1B: AGC CAA TGC GCT ATG GAG TTC CAC TGT GAT GTG CA PoNoroP1C: AGC CAA TGG GCA AAG GAA TTC CAT TGT GAT GTG CA
SaV/GIII	PEC66 (+): GAC TAC AGC AAG TGG GAT TCC PEC65 (−): ATA CAC ACA ATC ATC CCC GTA	330	PoSapoP1A: ATR ACA CTG GTG AAG GGC ATG CCA GAG GGG AG PoSapoP1B: ATC ACA CTG GTG AAG GGC ATG CCT GAT GGT AA
SaV/GVI?/JJ681	PEC68 (+): CCG CTA TAA ATT TAT TGG GTG PEC67 (−): ACG GGA CCC CAT ATT TTT GG	225	PECP1: AAC TGA GTT GAT CAC ACT GGT GCC TGG CAT TCC AGA
SaV/G?/QW19	PSV6 (+): CGG TCA TTT TGT GTG GAC TG PSV7 (−): ATT GCC CGT ATA AGG CAC A	219	PoSapoP1C: ATG ACG CTG GTG AAT GGC ATG CCA GAG GGA AG
SaV/G?/LL26	PSV11 (+): CAC CCA GAG GTG ATT TCA ACA GCA PSV14 (−): TTC TGC GTA ACA CTG GAG CAC ACA	231	PECP2: GTA AGC CCT TCT GTG TGG GCA ATG AGA TGT CAA ACA

TABLE 3. Prevalence of porcine GII NoVs and GIII SaVs in pigs

Swine farm or slaughterhouse	No. of samples positive for virus/total (%)					
	GII NoVs (finisher)	GIII SaVs				
		Nursing	Postweaning	Finisher	Sow	Total
MI farm A	2/61 (3)	NA[a]	NA	23/61 (37)	NA	23/61 (37)
NC farm A	2/8 (25)	NA	NA	8/8 (100)	NA	8/8 (100)
NC farm B	0/21 (0)	NA	NA	16/21 (76)	NA	16/21 (76)
OH farm A	5/22 (22)	4/14 (28)	12/12 (100)	20/22 (90)	13/13 (100)	49/61 (80)
OH farm B	43/105 (40)	7/61 (11)	71/90 (78)	58/105 (55)	23/30 (76)	159/286 (55)
OH farm C	0/12 (0)	7/15 (46)	12/12 (100)	12/12 (100)	26/44 (59)	57/83 (68)
OH farm D	NA	3/8 (37)	9/10 (90)	NA	NA	12/18 (66)
OH slaughterhouse	12/83 (14)	NA	NA	65/83 (78)	NA	65/83 (78)
Total	64/312 (20)	21/98 (21) C[b]	104/124 (83) A	202/312 (64) B	62/87 (71) B	389/621 (62)

[a] NA, not available.
[b] Values followed by different capital letters (A, B, and C) differed significantly ($P \leq 0.05$).

of two GIII SaV-specific probes (PoSapoP1A and PoSapoP1B). The IC RNA amplicons were detected by agarose gel electrophoresis, stained with ethidium bromide, and visualized using UV light. The IC RNA amplicons are 472 bp, which were readily differentiated from the porcine GIII SaV-specific amplicons (330 bp). When a sample RNA contained RT-PCR inhibitors, tested with primer pair PEC66/65, this sample RNA was diluted 1:10 in RNase-free water until the RT-PCR was no longer inhibited. This freshly diluted RNA was also used for RT-PCR with other primer pairs.

Detection of porcine GII NoVs (32). The RT and PCR were performed separately for the detection of three genotypes (GII-11, -18, and -19) of porcine NoVs at the same time using a pair of degenerate primers, PNV7 and PNV8 (Table 2). The porcine NoV-specific amplicons were confirmed by a microwell hybridization assay with a mixture of three probes (PoNoroP1A, PoNoroP1B, and PoNoroP1C) to detect all three genotypes of porcine NoVs.

Detection of GVI?/JJ681-like, G?/QW19-like, and G?/LL26-like porcine SaVs (32). Three coupled RT-PCR and microwell hybridization assays (primer pair-probe combinations, PEC68/67-PECP1, PSV6/7-PoSapoP1C, and PSV11/14-PECP2) were performed to detect GVI?/JJ681-like, G?/QW19-like, and G?/LL26-like porcine SaVs, respectively (Table 2).

Statistical analysis. Prevalence differences among farms, pig age groups, and collection times were analyzed by a chi-square test (Statistical Analysis System; SAS Institute). Statistical significance was assessed throughout at a P value of ≤ 0.05.

RESULTS

A total of 621 pig fecal samples collected during a 4-year-period from seven U.S. swine farms and one slaughterhouse were tested for porcine NoVs and SaVs by coupled RT-PCR–microwell hybridization assays.

Porcine NoVs were detected exclusively among subclinically infected finisher pigs. The NoVs were detected exclusively from finisher pigs, although samples from other ages of pigs were also collected at the same time from the same farm when the finisher pigs tested NoV positive (Tables 3 and 4). For example, all postweaning pigs ($n = 30$) were NoV negative, whereas 70% (42/60) of finisher pigs were NoV positive in OH farm B in March 2003. Four of six swine farms with samples from finisher pigs and the slaughterhouse were NoV positive. In finisher pigs, the overall NoV prevalence was 20% (range, 3 to 40% among NoV-positive farms).

We also examined whether the NoV prevalence differed among the different collection times. Using OH farm B as an example, we collected 105 fecal samples from finisher pigs at three times (March 2003, $n = 60$; June 2004, $n = 15$; March 2005, $n = 30$) (Table 4). The prevalence of NoVs in March 2003 (42/60; 70%) was significantly higher than those at the other two times, June 2004 (1/15; 7%) and March 2005 (0/30; 0%). It is likely a NoV outbreak occurred in March 2003 among finisher pigs in OH farm B. We also examined if the season (summer [July] versus winter [December] of 2004) influenced the detection of NoVs in nursing pigs and sows, but all swine were NoV negative at both sampling times.

Porcine GIII SaVs were most prevalent among postweaning swine. The GIII SaVs were detected from all farms and from

TABLE 4. Prevalence of porcine GII NoVs and GIII SaVs in pigs at OH farm B

Season and date collected			No. of samples positive for virus/total (%)					
			GII NoVs (finisher)	GIII SaVs				
Spring	Summer	Winter		Nursing	Postweaning	Finisher	Sow	Total
Mar. 2003			42/60 (70)	NA[a]	22/30 (73)	53/60 (88)	NA	75/90 (83)
May 2004			NA	NA	11/15 (73)	NA	NA	11/15 (73)
	June 2004		1/15 (7)	NA	8/15 (53)	5/15 (33)	NA	13/30 (43)
	July 2004		NA	4/31 (13)	NA	NA	9/15 (60)	13/46 (28)
		Dec. 2004	NA	3/30 (10)	NA	NA	14/15 (93)	17/45 (38)
Mar. 2005			0/30 (0)	NA	30/30 (100)	0/30 (0)	NA	30/60 (50)
Total			43/105 (41)	7/61 (11)	71/90 (79)	58/105 (55)	23/30 (77)	159/286 (56)

[a] NA, not available.

TABLE 5. Prevalence of G?/LL26-like porcine SaVs in pigs

Swine farm or slaughterhouse	No. of samples positive for virus/total (%)				
	Nursing (1–3 wks)	Postweaning (3–10 wks)	Finisher (10–24 wks)	Sow (>1 yr)	Total
MI farm A	NA[a]	NA	0/61 (0)	NA	0/61 (0)
NC farm A	NA	NA	0/8 (0)	NA	0/8 (0)
NC farm B	NA	NA	0/21 (0)	NA	0/21 (0)
OH farm A	3/14 (21)	7/12 (58)	0/22 (0)	0/13 (0)	10/61 (16)
OH farm B	15/61 (25)	0/90 (0)	1/105 (1)	0/30 (0)	16/286 (6)
OH farm C	2/15 (13)	1/12 (8)	0/12 (0)	0/44 (0)	3/83 (4)
OH farm D	0/8 (0)	3/10 (30)	NA	NA	3/18 (17)
OH slaughterhouse	NA	NA	0/83 (0)	NA	0/83 (0)
Total	20/98 (20)	11/124 (9)	1/312 (0)	0/87 (0)	32/621 (5)

[a] NA, not available.

all ages of pigs (Table 3). The overall prevalence of porcine GIII SaVs was 62% and ranged from 37 to 100% among farms. The prevalence of GIII SaV in postweaning pigs (83%) was significantly higher than in sows (71%) and finisher pigs (64%), and the prevalence of GIII SaV in nursing pigs (21%) was significantly lower than in any other age groups.

We further examined whether the GIII SaV prevalence in pigs differed among the different collection times. Using OH farm B as an example (Table 4), the GIII SaVs were detected at every sampling time from this farm. At different times, the prevalence of GIII SaVs in finisher pigs and sows differed significantly, whereas that of nursing and postweaning pigs was not significantly different. All 30 finisher pigs were GIII SaV negative in March 2005, whereas 100% (30/30) of postweaning pigs were SaV positive, which differed from the March 2003 results, when both groups had similar percentages (88% and 73%) of SaV-positive pigs.

Porcine GVI?/JJ681-like SaVs and porcine G?/QW19-like SaVs were detected infrequently in pigs. Among 621 samples, only one finisher pig from MI farm A, two nursing pigs from OH farm A, and one postweaning diarrheic pig from OH farm B tested positive for GVI?/JJ681-like SaVs by RT-PCR and microwell hybridization assays. Among 621 samples, one postweaning pig from OH farm D and three sows and one nursing pig from OH farm C tested weakly positive for G?/QW19-like SaVs by RT-PCR and microwell hybridization assays. The RT-PCR products with the primer pair PSV6/7 of these samples did not show a visible band on agarose gel electrophoresis.

Porcine G?/LL26-like SaVs were detected mainly in nursing and postweaning pigs. Except for one positive sample from a finisher pig from OH farm B, all other G?/LL26-like SaV-positive samples were detected only from the nursing and postweaning pigs (Table 5). The prevalence ranged from 0 to 25% and 0 to 58% among nursing and postweaning pigs, respectively.

Coinfection with NoVs and SaVs occurred in finisher pigs. Coinfection of pigs by porcine NoVs and SaVs was found in finisher pigs from three of four NoV-positive farms and the slaughterhouse (Table 6). The coinfection rate (number of coinfection/number of SaV or/and NoV positive) was 27% overall, ranging from 0 to 62% among farms.

Prevalence of RT-PCR inhibitors in field pig fecal samples. We used a competitive IC RNA control to monitor for RT-PCR inhibitors. We found that overall 8% (49/621) of fecal samples contained RT-PCR inhibitors. After 10-fold dilution of extracted RNA, those samples were no longer inhibited in RT-PCRs, and 45% (22/49) of them became NoV or/and SaV positive.

TABLE 6. Prevalence of porcine NoVs, SaVs, and coinfection in finisher pigs

Swine farm or slaughterhouse	No. of samples positive for virus/total (%)		
	NoVs	SaVs	Coinfection[a]
MI farm A	2/61 (3)	23/61 (37)	0/25 (0)
NC farm A	2/8 (25)	8/8 (100)	2/8 (25)
OH farm A	5/22 (22)	20/22 (90)	3/22 (13)
OH farm B	43/105 (40)	58/105 (55)	39/62 (62)
OH slaughterhouse	12/83 (14)	65/83 (78)	6/71 (8)
Total	64/279 (23)	175/279 (63)	50/188 (27)

[a] Coinfection with NoV or SaV or both positive.

DISCUSSION

In this study, we investigated the prevalence of porcine NoVs and SaVs in U.S. swine by RT-PCR and microwell hybridization assays with NoV- and SaV-specific primers and probes targeting the viral RdRp region, respectively (32). Microwell hybridization assays permitted the confirmation of NoV- or SaV-specific RT-PCR amplicons and proved to be sensitive, specific, economic, and less time-consuming assays (32). An IC RNA was coamplified with the GIII SaV RNA to monitor for RT-PCR inhibitors as well as technical errors. As noted by the detection of RT-PCR inhibitors in 8% of the samples, the use of an IC RNA was an important control when testing fecal samples.

Surprisingly, NoVs were detected only from finisher pigs, although pigs of other ages were also tested, including adult sows. We sampled 98 nursing pigs on five different occasions and 124 postweaning pigs at seven different times but still failed to detect porcine NoVs from either of these two age groups. In human NoV infections, NoVs have been detected in young children between 2 months and 2 years of age (27), but no NoV infections were reported in infants less than 2 months of age. In a comparative seroprevalence study of Norwalk virus and rotavirus in infants, children, and adults in the United States (16), investigators found that antibodies to rotavirus are

acquired rapidly during infancy and early childhood and that by 36 months of age over 90% of individuals have rotavirus antibodies, whereas Norwalk virus antibodies are acquired gradually in childhood and then more quickly during the adult years and that by 50 years of age about 50% of individuals have Norwalk virus antibodies. These results suggest that Norwalk virus is not a major cause of illness in infants and young children, and in adults probably only a portion are susceptible to Norwalk virus infection. Recently, researchers found that human NoVs bind to human histo-blood group antigens (HBGAs), which were found to influence the genetic susceptibility of individuals to NoV infections (11–14, 19, 23). Because the HBGAs are developmental antigens (36), they may not be expressed in high levels in the gut of younger children or, similarly, in nursing and postweaning pigs. By analogy, if porcine NoVs bind similarly to similar HBGAs expressed in the gut, perhaps only mature pigs are most readily infected with NoVs. However, why the adult sows were NoV negative in our study is unclear. Possibly, the sows (>1 year) were infected with porcine NoVs when they were younger (10 to 24 weeks) and had acquired immunity to NoV infection. Sampling and testing first-litter swine (gilts) of a similar age as the finisher pigs (<24 weeks) may help to resolve this question. Alternatively, the greater concentration of swine in the finisher facilities as well as differences in housing and management compared to sow facilities may have influenced NoV shedding and infection rates. The finisher barn environment may be more analogous to the confined human populations typical of cruise ships, on which NoV outbreaks are common and difficult to control even by using stringent disinfection measures (15).

On the other hand, GIII SaVs were detected in all ages of pigs, with a high prevalence of 62% overall. The GIII SaV prevalence in nursing pigs (21%) was significantly lower than in any other age group, possibly due to protection of nursing pigs by maternal antibodies to GIII SaVs in the milk. By comparison, the prevalence of GIII SaV shedding in sows was high (71%). It was not surprising that the prevalence of GIII SaVs was the highest in postweaning pigs (83%), because during this period pigs stop receiving maternal antibodies through milk and are under social (mixing) and environmental stresses. In postweaning pigs, the SaV prevalence in normal pigs (97/117; 83%) was lower than that in diarrheic pigs (7/7; 100%), which suggests that GIII SaVs may be involved in causing diarrhea in postweaning pigs. However, there were no statistical differences due to the small number of diarrheic pigs sampled. The ability of porcine GIII SaVs to cause intestinal lesions and diarrhea has been confirmed experimentally using the GIII SaV Cowden strain in studies of SaV-seronegative gnotobiotic pigs (6, 9).

The Po/SaV/GVI?/JJ681/00/US strain was detected previously from a diarrheic postweaning pig from an OH swine farm that was not sampled in this study. Four more samples (JJ672, JJ674, JJ675, and JJ680) collected from diarrheic postweaning pigs from the same farm at the same time also tested GVI?/JJ681-like SaV positive by RT-PCR with the primer pair PEC68/67 (32). In this study, only four samples, including one from a diarrheic postweaning pig, were positive for GVI?/JJ681-like SaVs, possibly due to the very low number of samples collected and tested from diarrheic pigs. Experimental inoculation of SaV-seronegative pigs with GVI?/JJ681-like SaVs is needed to determine whether they cause postweaning pig diarrhea.

Among porcine SaVs, the G?/QW19 strain is genetically most closely related to human GII SaVs (34). In this study, only 5 of 621 pigs were weakly positive for QW19-like SaVs. When RT-PCR with the calicivirus universal primer pair p290/110, which was first used to amplify the QW19 strain (34), and nested PCR with SaV QW19-specific primer pair PSV6/7 were performed for the 60 samples from MI farm A, from which the QW19 strain was detected, only one sample (QW17) tested strongly positive. These results suggest that G?/QW19-like SaVs do not replicate well in the pig host. Whether pigs are alternative hosts for these SaVs needs further investigation, and whether human SaV strains similar to the porcine SaV QW19 strain circulate among people having occupational exposure to pigs is unknown, but such studies could provide information on the origin of the G?/QW19-like porcine SaVs.

The G?/LL26-like SaVs were detected mainly in nursing and postweaning pigs, similar to SaV infections of humans, which are mainly in young children (28). Whether LL26-like SaVs are associated with diarrhea mainly in younger pigs is unknown, due to the low number of diarrheic samples collected and tested.

No obvious clinical signs were observed for the GII NoV- or GIII SaV-positive adult pigs (sows and finisher pigs). Nevertheless, the high subclinical infection rates of NoVs and GIII SaVs in pigs are important for the persistence of these viruses in nature and pose a risk for emergence of strains that may transmit to humans. Instead of being homogeneous, RNA virus populations consist of closely related viral mutants and recombinants, referred to as quasispecies (3). They are subjected to continuous genetic variation, competition, and selection during virus replication. When the virus population size increases, the expected number of viral genomes with more mutation sites also increases. Subsequently, this raises the risk of emergence of "new" variants with changes in host cell tropism or the ability to replicate in a different host species.

Coinfection of pigs by NoVs and SaVs, mainly GIII SaVs, was detected in 27% of 188 finisher pigs. In humans, mixed infections of NoVs and SaVs were reported in 1 of 36 outbreaks (2); mixed infections of NoVs and other enteric viruses, particularly rotaviruses and less frequently adenoviruses and astroviruses, are also common (26, 29). Whether NoVs and SaVs are present as coinfections with other known pig pathogens, such as porcine rotaviruses, and whether such mixed infections exacerbate the clinical severity of diarrhea need to be investigated.

To our knowledge, this is the first study to describe the prevalence of the shedding of NoVs and SaVs in pigs of various ages. Porcine NoVs were detected exclusively in normal adult finisher pigs. Porcine GIII SaVs infected all ages of pigs, with the shedding highest in postweaning pigs and lowest in nursing pigs. Porcine GVI?/JJ681-like and G?/QW19-like SaVs were detected only infrequently in pigs. Porcine G?/LL26-like SaVs mainly infected nursing and postweaning pigs. Mixed infections by NoVs and SaVs were also found in finisher pigs. In summary, the high prevalence of porcine GII NoVs and GIII SaVs in pigs, the close genetic and antigenic relationships between human and porcine NoVs, and the emer-

gence of intergenogroup recombinants of SaVs raise public health concerns for swine as a possible host reservoir for the zoonotic transmission of NoVs and SaVs.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (grant R01 AI 49742), NRI, U.S. Department of Agriculture (CGP grant 1999 02009), and Ohio Agricultural Research and Development Center (OARDC), The Ohio State University (Graduate Student Research Enhancement grant project 2002-114). Salaries and research support were provided by state and federal funds provided to the OARDC.

Special thanks go to Terry Workhemp, Carl Link, Alan Evers, Gary Bowman, and Myung Guk Han for help in sample collection. We thank Ana Azevedo and Veronica Costantini for technical assistance. We also thank Jeffrey LeJeune (Food Animal Health Research Program, The Ohio State University) for critical review of the manuscript and help in sample collection.

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