別紙様式第2-1

No. 28

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 4. 26	該当なし	
一般的名称	人全血液		研究報告の公表状況	W. M. Switze, N. D. Wolfe, J. K. Carr, A. D. Garcia, V. Shanmugam, U. Tamoufe, J. Torimiro, A. T. Prosser, M. LeBreton, E. Mpoudi-Ngole, F. E. McCutchan, D. L. Birx, T. M. Folks, D. S. Burke, W. Heneine. International Conference on Emerging Infectious Diseases; 2006 Mar 19-22; Atlanta, Georgia.	公表国 米国	
販売名(企業名)	人全血液CPD「日赤」(日本赤十字社) 照射人全血液CPD「日赤」(日本赤十字社)					

研究報告の概要

○中央アフリカの猟師における新たなヒトTリンパ球向性ウイルス(HTLV)の出現

背景:HTLV-1の多様性は、サルTリンパ球向性ウイルス(STLV)-1の多系交雑種の伝播から生じたと思われる。しかし、ヒトと、既感染のヒト以外の霊長類(NHP)との接触が新たなHTLVの発生に寄与しているかどうかについてはほとんど判っていない。

方法:狩猟、屠殺、霊長類ペットの飼育などで、NHPの血液との接触が報告されている中央アフリカ人930例におけるHTLVの多様性を調査した。血漿検体はEIA測定法およびウエスタンブロット測定法を用いて血清学的に検査を行った。複数のウイルス領域の配列を、WB陽性例のPBL DNAからPCR増幅し、その後、既知の霊長類Tリンパ球向性ウイルス(PTLV)による系統発生解析を行った。

結果:プロウイルスの配列はWB陽性13例からPCRで増幅し決定した。系統発生解析によって猟師2名が新ウイルスに感染していることが明らかになり、このウイルスはHTLV-3およびHTLV-4と名づけられた。HTLV-3はSTLV-3と同属で、これまでヒトにおいては検出されていなかった。HTLV-4は既知のHTLV/STLVとは異なっており、遺伝的に等距離で、新たな系統を形成するものだった。11名は、これまでヒトにおいて認められていなかったマンドリルからのSTLV-1(n=3)および他のSTLV-1変異株(n=2)を始めとするHTLV-1と同属の様々なウイルスに感染していた。

結論:この個体群に2つの新しいHTLVが特定され、これまで知られていたものよりも幅広いHTLVの多様性が示される。こうした知見もまた、NHPへの曝露がHTLV出現に関与することを示唆するものである。

報告企業の意見	今後の対応
既感染のヒト以外の霊長類の血液と接触のあった中央アフリカ地域の人において、幅広い多様性をもつ新たな遺伝系統のHTLVが特定されたとの報告である。	今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

人全血液CPD「日赤」
照射人全血液CPD「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

④

coral trout and trevally (5 outbreaks each). There were 6 outbreaks of oily stools (keriorrhoea) from eating Escolar fish.

Conclusions: Seafood is responsible for approximately 1 in 5 identified foodborne outbreaks in Australia, although the median number of people affected is low. To prevent these outbreaks people should avoid eating certain fish or shellfish harvested from contaminated waters.

Slide Session 13

Epidemiology of Emerging Zoonotic Diseases I

Marquis I

Monday, March 20, 2006, 1:15 pm - 2:45 pm

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Emergence of Novel Human T-lymphotropic Viruses Among Central African Hunters

W. M. Switzer[1], N. D. Wolfe[2], J. K. Carr[3], A. D. Garcia[1], V. Shanmugam[1], U. Tamoufe[4], J. Torimiro[2], A. T. Prosser[1], M. LeBreton[2], E. Mpoudi-Ngole[4], F. E. McCutchan[3], D. L. Birx[1], T. M. Folks[1], D. S. Burke[2], W. Heneine[1];

[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Johns Hopkins University, Baltimore, MD, [3]Henry M. Jackson Foundation, Rockville, MD, [4]Army Health Research Center, Yaounde, CAMEROON.

Background: HTLV-1 diversity appears to have resulted from multiple cross-species transmissions of STLV-1. However, little is known whether contact between humans and infected nonhuman primates (NHPs) continues to contribute to the emergence of novel HTLVs.

Methods: We investigated HTLV diversity among 930 central Africans reporting contact with NHP blood through hunting, butchering, and keeping primate pets. Plasma samples were tested serologically using EIA and WB assays. Sequences from several viral regions were PCR amplified from PBL DNA of WB reactive persons followed by phylogenetic analysis with known PTLVs.

Results: Proviral sequences were PCR-amplified from 13 WB reactive persons. Phylogenetic analysis revealed infection of two hunters with novel viruses we designated HTLV-3 and HTLV-4. HTLV-3 falls within the genetic diversity of STLV-3, a virus not seen before in humans. HTLV-4 is distinct and genetically equidistant from all known HTLVs/STLVs and formed of a new phylogenetic lineage. Eleven persons were also infected with a broad diversity of HTLV-1, including STLV-1 from mandrills (n=3) and other STLV-1 variants (n=2) not previously seen in humans.

Conclusions: We identify in this population two new HTLVs and demonstrate greater HTLV diversity than previously recognized. These findings also suggest that NHP exposure contributes to HTLV emergence.

(characters w/ spaces =1381; 199 words)

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From Civet Cats to Horseshoe Bats: Tracing the Origin of SARS

L. Wang[1], Z. Shi[2], S. Zhang[3], H. Field[4], P. Daszak[5], B. T. Eaton[1];

[1]CSIRO Australian Animal Health Laboratory, Geelong, AUSTRALIA, [2]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CHINA, [3]Institute of Zoology, Chinese Academy of Sciences, Beijing, CHINA, [4]Department of Primary Industries and Fisheries, Brisbane, AUSTRALIA, [5]Consortium for Conservation Medicine, New York, NY.

Background: Severe acute respiratory syndrome (SARS) emerged in 2002-3 in southern China. Civets and other small mammals in the wildlife markets of Guangdong province were implicated in the transmission cycle. However, the true wildlife reservoir of the etiological agent of SARS, the SARS coronavirus (SARS-CoV), remained elusive. Prevention of future SARS outbreak will be difficult, if not impossible, to achieve without knowing the natural reservoir and the mechanism of spill over.

Methods: During March to December of 2004, 408 bats representing 9 species, 6 genera and 3 families, from four locations in China (Guangdong, Guangxi, Hubei and Tianjin) were sampled by trapping in their native habitat. Blood and fecal and throat swabs were collected; serum samples along with cDNA from fecal or throat samples were independently analysed, double-blind, using different methods by groups in China and Australia.

Results: Among six genera of bat species, three species from the genus *Rhinolophus* (horseshoe bats) demonstrated a high SARS-CoV antibody prevalence. The serological findings were corroborated by PCR analyses using primer pairs derived from the SARS-CoV. Three different SARS-like viruses (SL-CoV), were detected. Genome analysis demonstrated that SL-CoVs have an identical genome organization with that of SARS-CoV. SL-CoVs display greater genetic variation than SARS-CoVs isolated from humans or civets. SARS-CoVs nestle phylogenetically within the spectrum of SL-CoVs, indicating that the virus responsible for the SARS outbreak was a member of this new coronavirus group, tentatively named the SARS cluster of coronaviruses (SCCoVs).

Conclusions: Serologic and molecular surveys revealed the presence of SCCoVs in different species of horseshoe bats covering a wide geographic area in China. Bats are found in live animal markets across China, are eaten by people, and their feces and other body parts used in traditional medicine. We hypothesize that SCCoVs spilled over from this natural reservoir to civets and other immunologically naïve species in the wild animal markets, leading to a cycle of infection in traded wildlife, and thence to humans.

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Echinococcosis in Tibetan Populations of Western Sichuan Province, China

T. Li;

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Background: Human cystic echinococcosis (CE), caused by infection with the larval stage of *Echinococcus granulosus*, and alveolar echinococcosis (AE), caused by infection with the larval stage of *E. multilocularis*, are two of the most pathogenic zoonotic parasitic helminthic infections of humans in the Northern Hemisphere. Human CE occurs worldwide in association with livestock herding, within which the main dog-sheep cycle for *E. granulosus* is transmitted. Human AE is a much rarer parasitic infection and is primarily transmitted between foxes and small mammals in wildlife cycle.

Methods: We screened 3,199 people from Shiqu County, Ganze Tibetan Autonomous Prefecture, Sichuan Province, China, for abdominal echinococcosis (hydatid disease) by portable ultrasound combined with specific serodiagnostic tests.

Results: Both CE and AE were co-endemic in these populations with the highest village co-endemic prevalence values recorded anywhere in the world: 12.9% were infected with one or the other form (6.8% CE and 6.1% AE). The prevalence of both CE and AE was significantly higher in females than in males and increased with the age of the person screened. Pastoral Tibetan herdsmen were at highest risk for infection (prevalence 19.0%). Prevalence of CE varied in 5 townships from 0% to 12.1%, while AE prevalence ranged from 0% to 14.3%. Risk factors associated with both infections included the number of owned dogs, frequency of contact with dogs, and sources of drinking water.

Conclusions: Both CE and AE disease should be considered as a public health priority in the pastoral communities of the eastern Tibetan Plateau.

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No. 22

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 3. 25	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Wang QH, Han MG, Cheetham S, Souza M, Funk JA, Saif LJ. Emerg Infect Dis. 2005 Dec;11(12):1874-81.	公表国
販売名（企業名）	解凍赤血球濃厚液「日赤」（日本赤十字社） 照射解凍赤血球濃厚液「日赤」（日本赤十字社）			米国

研究報告の概要

○ブタ・ノロウイルスはヒト・ノロウイルスに関連していた

日本及びヨーロッパにおける成体ブタからの遺伝子型IIグループ（GII）ノロウイルス（NoV）のRNA検出及び米国のブタからのGII NoV抗体の検出は、米国のブタでNoVが検出されていないにも関わらず、ブタNoVのヒトへの感染に関して公衆衛生上の懸念をもたらしている。ブタNoVを検出し、ウイルスの遺伝的多様性及びヒトNoVとの関連性を調査するため、健常な米国の成体ブタ由来の糞便275サンプルを、カリシウイルスuniversal primerを用いた逆転写ポリメラーゼ連鎖反応によってスクリーニングした。6検体がNoV陽性であった。ブタNoVの5検体の3'末端における3kbの配列解析に基づき、GIIの遺伝子型3種と組換型と思われる1種が同定された。ブタNoVの遺伝子型のうち種は、遺伝的抗原的にヒトNoVと関連性があり、ノトバイオートのブタで複製された。こうした結果は、無症候感染の成体ブタが新種のヒトNoVの宿主となりうるか、あるいはブタ/ヒトのGII組換え型が出現する可能性を示唆するものである。

報告企業の意見	今後の対応
ブタNoVの遺伝子型のうち1種は、遺伝的抗原的にヒトNoVと関連性があったとの報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(5)

RESEARCH

Porcine Noroviruses Related to Human Noroviruses

Qiu-Hong Wang,* Myung Guk Han,* Sonia Cheetham,* Menira Souza,*
Julie A. Funk,† and Linda J. Saif*

Detection of genogroup II (GII) norovirus (NoV) RNA from adult pigs in Japan and Europe and GII NoV antibodies in US swine raises public health concerns about zoonotic transmission of porcine NoVs to humans, although no NoVs have been detected in US swine. To detect porcine NoVs and to investigate their genetic diversity and relatedness to human NoVs, 275 fecal samples from normal US adult swine were screened by reverse transcription–polymerase chain reaction with calicivirus universal primers. Six samples were positive for NoV. Based on sequence analysis of 3 kb on the 3′ end of 5 porcine NoVs, 3 genotypes in GII and a potential recombinant were identified. One genotype of porcine NoVs was genetically and antigenically related to human NoVs and replicated in gnotobiotic pigs. These results raise concerns of whether subclinically infected adult swine may be reservoirs of new human NoVs or if porcine/human GII recombinants could emerge.

Noroviruses (NoVs) (family *Caliciviridae*, genus *Norovirus*) cause diarrhea in humans and animals (1–3). The NoV genome is 7.3–7.7 kb long with 3 open reading frames (ORFs) encoding a polyprotein that undergoes protease processing to produce several nonstructural proteins, including an RNA-dependent RNA polymerase (RdRp), a major capsid protein (VP1, capsid), and a minor capsid protein (VP2) (1,4,5). The capsid protein contains a conserved shell (S) and hypervariable protruding (P) domains (6). Noroviruses are genetically diverse and make up 27 genotypes within 5 genogroups, GI/1–8, GII/1–17, GIII/1–2, GIV, and GV, based on the capsid genes of 164 strains (7). Human NoVs cause an estimated 23 million cases of illness annually in the United States (8) and >90% of nonbacterial epidemic gastroenteritis worldwide (1). The low infectious dose, environmental resistance, strain diversity, shedding from asymptomatic persons, and varied transmission vehicles render human NoVs highly contagious.

Norovirus RNA was detected by reverse transcription–polymerase chain reaction (RT-PCR) in 4 of 1,017 normal slaughtered pigs in Japan (9) and in 2 of 100 pooled pig fecal samples in the Netherlands (10). These porcine NoVs (Sw43/97/JP, Sw918/97/JP, and 34/98/NET) are genetically similar and are classified into GII (9,10), like most epidemic human NoVs (11–13). Also, the virus-like particles (VLPs) of Sw918 strain cross-react with antibodies against human GII but not GI NoVs (14). The close genetic and antigenic relationships between human and porcine NoVs raise public health concerns regarding their potential for zoonotic transmission and as reservoirs for emergence of new epidemic human strains.

Farkas et al. (14) reported that US swine sera react with Po/NoV/GII/Sw918 strain, but no direct detection of NoV from US swine has been reported. To detect porcine NoVs and assess their genetic diversity and relatedness to human NoVs, we screened 275 pig fecal samples from US swine by RT-PCR with a calicivirus universal primer pair p290/110 targeting the RdRp region (15,16), followed by sequencing the 3 kb on the 3′ end of the genome for 5 NoV strains. Gnotobiotic pigs were inoculated with porcine NoVs to examine their infectivity and to produce convalescent-phase antiserum for antigenic analysis.

Materials and Methods

Fecal samples (N = 275) were collected from December 2002 to June 2003 from finisher (10–24 weeks of age) pigs and gestating sows (≥1 year of age) from 3 Ohio swine farms (10, 60, and 32 samples), 1 Ohio slaughterhouse (83 samples), 1 Michigan swine farm (61 samples), and 2 North Carolina swine farms (8 and 21 samples). Fresh fecal samples were collected from individual pigs, placed into sterile containers, and stored frozen.

Sample RNA was extracted from 10% to 20% of fecal suspensions in sterile Eagle minimal essential medium

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(EMEM, Invitrogen, Carlsbad, CA, USA) by using Trizol LS (Invitrogen). For some samples, RNA was concentrated and purified by using QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA, USA).

RT-PCR was performed separately by using primer pair p290 (5′-GATTACTCCAAGTGGGACTCCAC-3′) (15) and p110 (5′-ACDATYTCATCATCACCATA-3′) (16) as previously described (15) but at 48°C for annealing (317 bp for NoV or 329 bp for sapovirus). To amplify the 3-kb 3′ end fragment, cDNA was synthesized by SuperScript III First-Strand cDNA synthesis kit (Invitrogen) with primer VN_3T_{20} (5′-GAGTGACCGCGGCCGCT$_{20}$-3′). PCR was then performed with TaKaRa Ex Taq polymerase (TaKaRa Mirus Bio, Madison, WI, USA) with primers p290 and VN_3T_{20}. Quantitative (endpoint titration) RT-PCR (17) was performed with primer pair PNV7 (5′-AGGTGGTGGCCGAGGAYCTCCT-3′) and PNV8 (5′-TCACCATAGAAGGARAAGCA-3′) targeting the RdRp (211 bp) of QW101 strain.

RT-PCR products were purified with the QIAquick Gel Extraction kit (Qiagen) before cloning into pCR2.1-TOPO (T/A) or PCR XL cloning kit (Invitrogen). Five clones of each sample were sequenced. DNA sequencing was performed with BigDye Terminator Cycle and 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Sequence editing was performed by Lasergene software package (v5, DNASTAR Inc., Madison, WI, USA). The Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/BLAST) was used to find homologous hits. Multiple sequence alignment was performed with ClustalW (v1.83) at DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp). Phylogenetic and bootstrap (1,000 replicates) analyses were conducted by using MEGA (v2.1) (18). Identification of recombinants was performed by using the Recombinant Identification Program (RIP, http://hivweb.lanl.gov/RIP/RIPsubmit.html) (19). The classification and GenBank accession numbers of NoVs are listed in Table 1.

Four gnotobiotic pigs were maintained and euthanized as previously described (25,26). The inoculate was a 20% fecal filtrate (0.2 μm) in EMEM of the QW126 or QW144 (QW101-like, GII-18) strains or EMEM only (2 negative control pigs). One pig was inoculated with QW126 orally and intranasally at 9 days of age, and convalescent-phase antiserum LL616 was collected at postinoculation day (PID) 26. A second pig was inoculated with QW144 orally at 35 days of age and euthanized at PID 5.

Immune electron microscopy (IEM) was performed as described previously (27). For enzyme-linked immunosorbent assay (ELISA), the recombinant baculovirus-expressed human NoV VLPs and rotavirus VP2 and VP6 (2/6)-VLPs (negative control) (28) were CsCl-gradients purified. We coated 96-well microplates with VLPs (200 ng/well) in carbonate buffer (pH 9.6) and blocked with 5% nonfat dry milk in phosphate-buffered saline (PBS)-Tween 20 (0.05%). Serially diluted serum samples that included positive and negative controls were added to duplicate positive- and negative-coated wells, and the plates were incubated. After washing, horseradish peroxidase (HRP)-labeled goat anti-pig immunoglobulin G (IgG) (H + L) for pig sera or goat anti-human IgG + IgA + IgM (H + L) (KPL, Gaithersburg, MD, USA) for human serum was added. After incubation and washing, the substrate 3,3′,5,5′-tetramethylbenzidine was added. The cutoff value was the mean absorbance of the negative coatings multiplied by 2.

Western blot was performed as described previously (29). Nitrocellulose membranes were incubated with pig convalescent-phase antiserum LL616 against porcine GII-18 NoV or negative control serum in PBS containing 4% nonfat dry milk followed by goat anti-pig IgG (H + L)-HRP conjugate.

Results

Porcine NoVs were classified into 3 genotypes within GII based on the complete capsid sequences: 1 genotype with prototype Japanese strains Sw43 and Sw918 and 2 new genotypes. A total of 19 of 275 samples showed a potential positive band after agarose gel electrophoresis of the RT-PCR products of primer pair p290/110. Fourteen samples representative of each potentially positive farm or the slaughterhouse were sequenced. After performing BLAST search, we identified 6 NoVs (QW48, Michigan farm A; QW101, QW125, and QW126, Ohio farm B; and QW170 and QW218, Ohio slaughterhouse), 3 sapoviruses, and 5 sequences that had no significant hit in the database. Because the QW126 shared 99% nucleotide (nt) identity with the QW101 and QW125 strains in the 274-nt RdRp region, it was not sequenced further.

We sequenced the 3-kb 3′ end of the genome containing the partial RdRp, VP1 and VP2 genes, and the 3′ untranslated region of the 5 strains. The porcine NoVs represented 3 distinct clusters: 1) Sw43, Sw918, and QW48; 2) QW101 and QW125; and 3) QW170 and QW218, on the basis of the size of each gene and the ORF1-ORF2 overlap region (Table 2). Across the 3 kb, the QW101 and QW125 strains and the QW170 and QW218 strains shared 99% nt identity.

The amino acid identity of the predicted complete and S and P domains of the capsid protein of the 5 porcine NoVs, the previously reported porcine NoVs (Sw43 and Sw918), and representative human, bovine, and murine NoV strains is summarized in Table 3. In the complete capsid, the QW48 strain was most closely related to the porcine NoV prototype Sw43 strain (98% amino acid identity); the QW170 and QW218 strains shared the highest

amino acid identities (81%) to porcine Sw43 and Sw918 strains; the QW101 and QW125 strains showed the highest amino acid identity to human GII-3/Mexico (71.4%), then to human GII-6/Baltimore (71.0%), porcine QW218 (71.0%), and porcine Sw43 (70.6%) strains. The S and P domains of these NoVs showed similar relationships. A neighbor-joining phylogenetic tree based on the amino acid sequences of the complete capsids (Figure 1) showed that QW48 grouped with Sw43 and Sw918 strains into GII-11 and that QW170 and QW218 formed a new

Table 1. Classification and GenBank accession numbers of norovirus (NoV) strains used for sequence analysis*

Strain	Genus/genogroup-genotype	Abbreviation	GenBank accession no.
Hu/Norwalk/68/US	NoV/GI-1	Norwalk	M87661
Hu/Hawaii/71/US	NoV/GII-1	Hawaii	U07611
Hu/Melksham/89/UK	NoV/GII-2	Melksham	X81879
Hu/Snow Mountain/76/US	NoV/GII-2†	Snow Mountain	AY134748
Hu/Mexico/89/MX	NoV/GII-3	Mexico	U22498
Hu/Toronto/91/CA	NoV/GII-3	Toronto	U02030
Hu/SaitamaU18/97-99/JP	NoV/GII-3	SaitamaU18	AB039781
Hu/SaitamaU201/98/JP	NoV/GII-3	SaitamaU201	AB039782
Hu/Arg320/ARG	NoV/GII-3†	Arg320	AF190817
Hu/Camberwell/101922/94/AUS	NoV/GII-4	Camberwell	AF145896
Hu/Lordsdale/93/UK	NoV/GII-4	Lordsdale	X86557
Hu/Bristol/93/UK	NoV/GII-4	Bristol	X76716
Hu/MD145-12/87/US	NoV/GII-4	MD145	AY032605
Hu/Farmington Hills/02/US	NoV/GII-4	Farmington Hills	AY502023
Hu/Langen1061/02/DE	NoV/GII-4	Langen	AY485642
Hu/Hillingdon/93/UK	NoV/GII-5	Hillingdon	AJ277607
Hu/New Orleans 306/94/US	NoV/GII-5	New Orleans	AF414422
Hu/Baltimore/274/1993/US	NoV/GII-6	Baltimore	AF414408
Hu/SaitamaU3/97/JP	NoV/GII-6	SaitamaU3	AB039776
Hu/SaitamaU4/97/JP	NoV/GII-6	SaitamaU4	AB039777
Hu/SaitamaU16/97/JP	NoV/GII-6	SaitamaU16	AB039778
Hu/SaitamaU17/97/JP	NoV/GII-6	SaitamaU17	AB039779
Hu/Seacroft/90/UK	NoV/GII-6†	Seacroft	AJ277620
Hu/Leeds/90/UK	NoV/GII-7	Leeds	AJ277608
Hu/Gwynedd/273/94/US	NoV/GII-7	Gwynedd	AF414409
Hu/Amsterdam/98-18/98/NET	NoV/GII-8	Amsterdam	AF195848
Hu/SaitamaU25/97-99/JP	NoV/GII-8	SaitamaU25	AB039780
Hu/VA97207/97/US	NoV/GII-9‡	VA97207	AY038599
Hu/NLV/Erfurt/546/00/DE	NoV/GII-10	Erfurt	AF427118
Hu/Mc37/00-01/THA	NoV/GII-10†	Mc37	AY237415
Po/Sw43/97/JP	NoV/GII-11	Sw43	AB074892
Po/Sw918/97/JP	NoV/GII-11	Sw918	AB074893
Po/MI-QW48/02/US	**NoV/GII-11**	**QW48**	**AY823303**
Hu/Gifu/96/JP	NoV/GII-12‡	Gifu	AB045603
HU/Wortley/90/UK	NoV/GII-12†	Wortley	AJ277618
Hu/SaitamaU1/97-99/JP	NoV/GII-12†	SaitamaU1	AB039775
Hu/Fayetteville/98/US	NoV/GII-13	Fayetteville	AY113106
Hu/M7/99/US	NoV/GII-14	M7	AY130761
Hu/J23/99/US	NoV/GII-15	J23	AY130762
Hu/Tiffin/99/US	NoV/GII-16	Tiffin	AY502010
Hu/Neustrelitz260/00/DE	NoV/GII-16	Neustrelitz	AY772730
Hu/CS-E1/02/US	NoV/GII-17	CS-E1	AY502009
Po/OH-QW101/03/US	**NoV/GII-18**	**QW101**	**AY823304**
Po/OH-QW125/03/US	**NoV/GII-18**	**QW125**	**AY823305**
Po/OH-QW170/03/US	**NoV/GII-19‡**	**QW170**	**AY823306**
Po/OH-QW218/03/US	**NoV/GII-19‡**	**QW218**	**AY823307**
Bo/Newbury-2/76/UK	NoV/GIII-2	Newbury-2	AF097917
Hu/Alphatron/98-2/98/NET	NoV/GIV	Alphatron	AF195847
Mu/MNV-1/03/US	NoV/GV	MNV-1	AY228235

*Classification is based on the capsid gene sequences. The 5 porcine NoV strains sequenced in this study are in boldface.
†Previously reported recombinants (20–24).
‡Potential recombinants found in this study.

Table 2. Sizes of the putative capsid protein VP1 and the minor capsid protein VP2, the overlap regions, and the 3′ UTR of GII NoV*

Species/genogroup-genotype/strain	ORF1-ORF2 overlap (nt)	VP1 (aa)	ORF2-ORF3 overlap (nt)	VP2 (aa)	3′ UTR (nt)
Po/GII-11/Sw43	17	547	NA	NA	NA
Po/GII-11/Sw918	17	547	NA	NA	NA
Po/GII-11/QW48	17	547	1	253	57
Po/GII-18/QW101	20	557	1	275	48
Po/GII-18/QW125	20	557	1	275	48
Po/GII-19/QW170	17	548	1	254	51
Po/GII-19/QW218	17	548	1	254	51
Hu/GII-1/Hawaii	20	535	1	259	42
Hu/GII-2/Snow Mountain	20	542	1	259	45
Hu/GII-3/SaitamaU18	20	548	1	254	37
Hu/GII-4/MD145	20	539	1	268	46
Hu/GII-5/New Orleans	20	540	1	258	35
Hu/GII-6/SaitamaU3	20	550	1	259	54
Hu/GII-7/Gwynedd	20	540	1	257	68
Hu/GII-8/SaitamaU25	20	537	1	257	53
Hu/GII-9/VA97207	20	537	1	257	51
Hu/GII-10/Mc37	20	548	1	258	34
Hu/GII-12/SatamaU1	20	535	1	259	50
Hu/GI-1/Norwalk	17	530	1	212	66

*UTR, untranslated region; NoV, norovirus; ORF, open reading frame; nt, nucleotide; aa, amino acid; NA, not available.

genotype (GII-19), which was closer to porcine than to human strains. However, QW101 and 125 formed a new genotype (GII-18) between human and porcine GII NoVs.

Further analysis of the predicted C-terminal ≈260 amino acids of the RdRp region (Figure 2) showed similar grouping results for QW48, QW101, and QW125 strains but different for QW170 and QW218 strains, which were in the same cluster (GII-11) as Sw43, Sw918, and QW48 in the RdRp region. This finding suggested that a recombination event occurred between QW170/218-like and Sw43-like NoVs. The complete VP2 sequences of representative strains were also analyzed (data not shown). Results were similar to those of the capsid sequence classification.

A potential recombination event occurred between QW170/218-like and Sw43-like strains. To examine where the recombination occurred, we performed RIP analysis by placing the 3′-end RdRp and the capsid sequence of QW170 or QW218 as a query sequence and the corresponding sequences of Sw43 and QW101 as background sequences. The resulting diagram (Figure 3A) showed that QW170 had high similarity to Sw43 in the RdRp but not in the capsid region. This abrupt change happened in the RdRp-capsid junction region. Therefore, we performed sequence alignments of the RdRp-capsid junction of NoVs, including the calicivirus genomic-subgenomic conserved 18-nt motif (20) (Figure 3B). Between Sw43, QW170, and QW218, all 18 nt were identical, but identities decreased downstream of this motif. QW170 and QW218 grouped with Sw43 with a high bootstrap value of 95 in the RdRp tree (Figure 2), whereas they segregated from Sw43 with the highest bootstrap value of 100 in the capsid tree (Figure 1). We could not clarify which was the parent or progeny strain.

The porcine NoVs replicated in gnotobiotic pigs. Two pigs were inoculated with QW101-like GII-18 porcine NoVs (QW126 and QW144 strains) to verify their replication in pigs as confirmed by quantitative RT-PCR and IEM and to produce convalescent-phase serum to examine antigenic reactivity with human NoVs. These 2 strains were confirmed as QW101-like porcine NoVs in both the RdRp (169-nt) and the capsid S domain (363-nt) regions by sequence analysis of the RT-PCR products (Q.H. Wang and L.J. Saif, unpub. data). They shared 99% and 100% amino acid identities to the QW101 strain in the 2 regions, respectively. Porcine NoV shedding, assessed by quantitative RT-PCR with primer pair PNV7/8, was detected at PID 3–5 (euthanized) after QW144 exposure, coincident

Table 3. Percentage amino acid identities of noroviruses within the capsid region

	Complete capsid (S domain, P domain)					
Strain	Po/GII*	Hu/GII†	Hu/GI/ Norwalk	Bo/GIII/ Newbury-2	Hu/GIV/ Alphatron	Mu/GV/MNV-1
QW48	96–98 (100, 94–97)	63–71 (77–85, 53–63)	43 (59, 36)	45 (62, 36)	53 (71, 42)	39 (58, 29)
QW101, QW125	70–70.6 (83, 63)	61–71.4 (77–86, 51–64)	42 (59, 35)	45 (62, 38)	54 (71, 44)	39 (58, 28)
QW170, QW218	81 (90, 74)	62–69 (77–82, 52–62)	43 (59, 36)	45 (61, 37)	53 (72, 40)	39 (60, 27)

*Includes Sw43 and Sw918 strains.
†Includes Hawaii, Snow Mountain, Mexico, MD145, New Orleans, Baltimore, Gwynedd, Amsterdam, VA97207, Erfurt, Gifu, Fayetteville, M7, J23, and Neustrelitz strains.

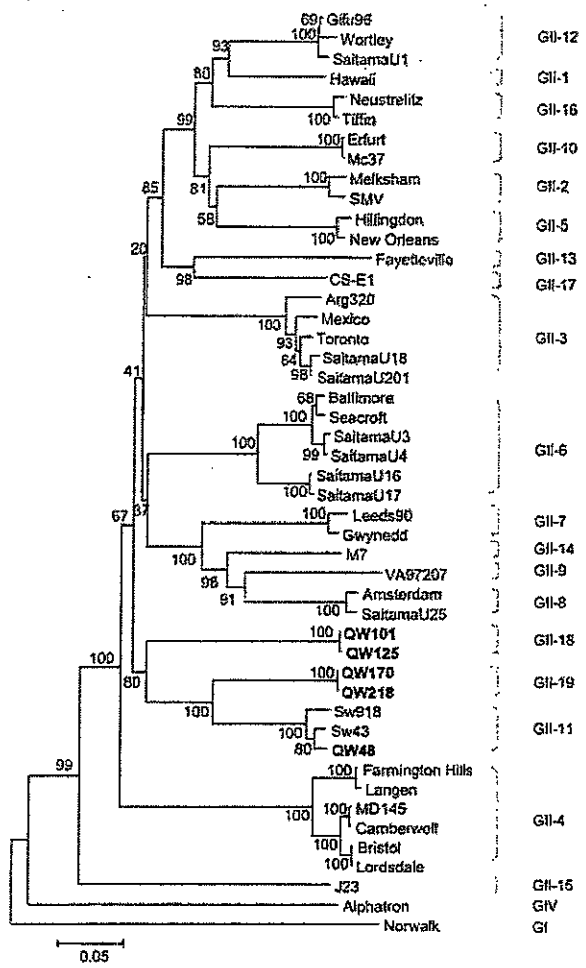

Figure 1. Neighbor-joining phylogenetic tree of genogroup II noroviruses (NoVs) based on the complete capsid region. The 5 newly identified porcine NoV strains are in **boldface**. Genogroups (G) and genotypes (numbers after G) are indicated. The human NoV GI-1/Norwalk and GIV/Alphatron strains were used as outgroup controls.

with mild diarrhea. The RT-PCR–detectable units of the rectal swab RNA increased from negative at PID ≤ 2, 10^3 at PID 3–4, and 10^4 at PID 5 (large intestinal contents). Norovirus shedding was detected only at PID 5 without diarrhea after QW126 exposure. Examination of the intestinal contents of the pig inoculated with QW144 by IEM with pig convalescent-phase antiserum LL616 showed clumps of ≈32-nm NoV particles (Figure 4). The 2 control pigs had no virus shedding or diarrhea. Detailed studies of the pathogenesis of porcine NoVs in gnotobiotic pigs are in progress (S. Cheetham and L.J. Saif, unpub. data).

Antisera to QW101-like (QW126) porcine NoVs cross-reacted with VLPs of human GII NoVs in ELISA and Western blot. In ELISA (Table 4), the pig convalescent-phase antiserum (LL616) to QW101-like porcine NoV QW126 strain showed higher titers (1:400–1:800) to GII-3/Toronto, GII-4/MD145, GII-4/HS66, and GII-6/Florida strains; a lower titer (1:100) to GII-1/Hawaii strain; and lowest titer (1:10) to GI-3/Desert Shield strain. In Western blot (Figure 5), the capsid proteins (59–60 kDa) of Toronto, MD145, HS66, and Florida strains, but not the Hawaii and Desert Shield strains, were detected by pig antiserum LL616 but not the negative control serum (data not shown). Thus, 1-way antigenic cross-reactivity exists between human NoV antigens and porcine NoV (GII-18) antiserum, with moderate cross-reactivity to human NoVs GII-3, 4, and 6; low cross-reactivity to GII-1; and very low cross-reactivity to GI-3.

Discussion

All porcine NoVs were detected from pigs without clinical signs (9,10). Subclinically infected pigs may be natural reservoirs for NoVs, and because porcine GII NoVs are genetically and antigenically related to human NoVs, concerns exist about their zoonotic potential. Whether human NoV strains similar to the QW101-like porcine NoVs circulate among people with occupational exposure to pigs is

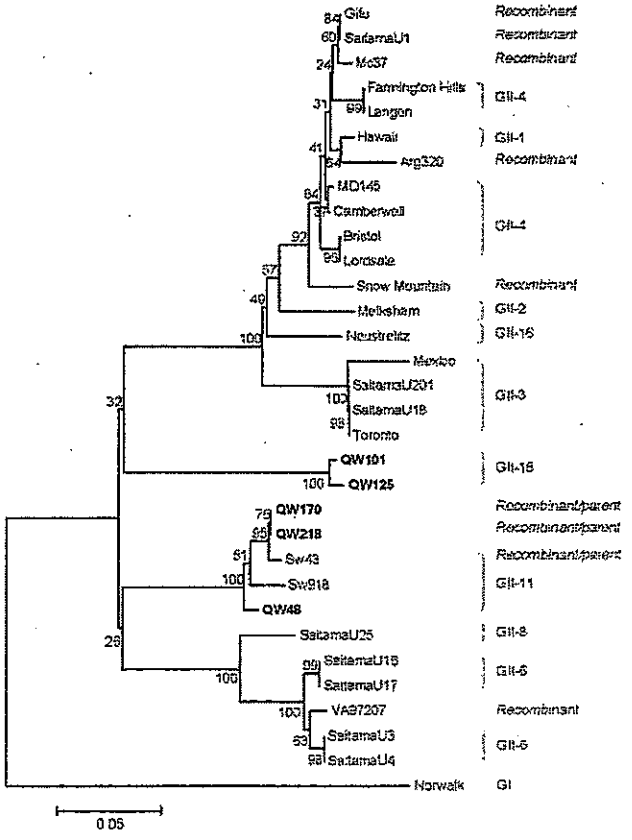

Figure 2. Neighbor-joining phylogenetic tree of genogroup II noroviruses (NoVs) based on the partial RNA-dependent RNA polymerase region (C-terminal 260–266 amino acids). The 5 newly identified porcine NoV strains are in **boldface**. Genogroups (G) and genotypes (numbers after G) are indicated. The human NoV GI-1/Norwalk and strain was used as outgroup control.

unknown, but such studies could provide information on the zoonotic potential of these porcine NoVs.

The RdRp-capsid junction region of NoVs contains a highly conserved 18-nt motif in genomic and subgenomic RNA that is believed to be a transcription start signal (1,20). All 18 nt were identical within each genogroup except for the Hu/GII/J23, Po/GII/QW101, and Po/GII/QW125 strains (Figure 3B, sequence alignments on other GI and GIII strains are not shown). This finding suggests that homologous recombination may occur within this motif between NoVs of different genotypes within the same genogroup. Recombinant human GII NoVs have been reported previously (20–24). To our knowledge, this study is the first identification of a potential recombinant between pig NoVs. At present, NoV recombinants have been detected exclusively between viruses within the same genogroup and within the same host species, but few animal NoVs have been sequenced (RdRp and capsid) for comparative analysis, especially those from animals in developing countries, where humans and animals may be in close contact.

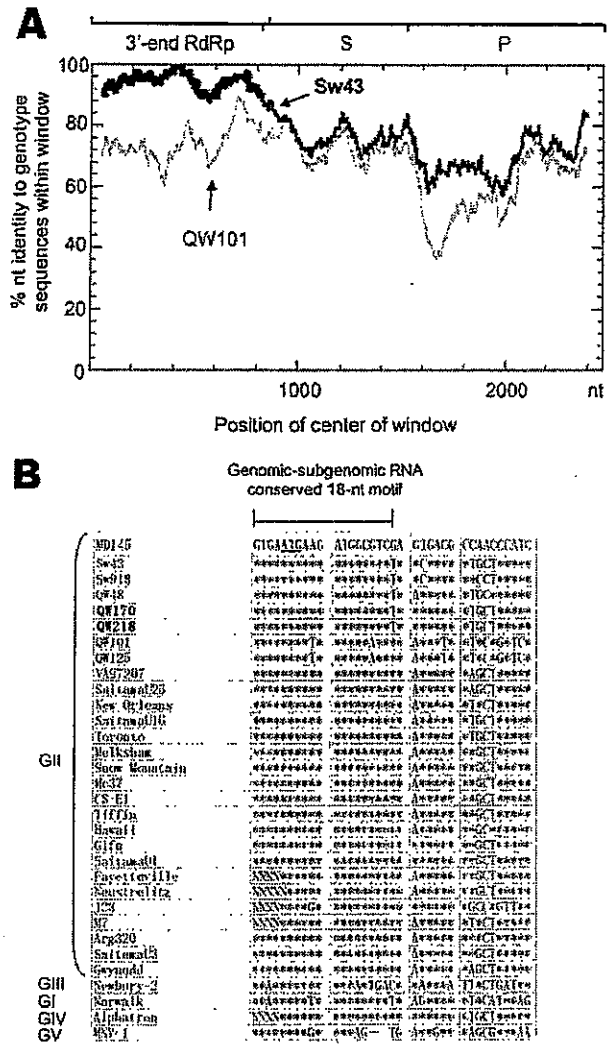

Figure 3. Identification of a potential recombination event between QW170 and Sw43 strains. A) Recombination Identification Program analysis of QW170 strain. At each position of the window, the query sequence (QW170) was compared to each of the background genotype representatives (GII-11/Sw43 and GII-18/QW101). When the query sequence is similar to the background sequences, the homologous regions are indicated as thick lines on the plot. Analysis parameters were window size of 100 and significance of 90%. The nucleotide positions of the 3′-end RNA-dependent RNA polymerase (RdRp) and the shell (S) and protruding (P) domains of the capsid protein are indicated. B) Sequence alignments of the RdRp-capsid junction region of noroviruses (NoVs). The genomic and subgenomic conserved 18-nucleotide (nt) motif is indicated by a horizontal line with 2 vertical bars. Asterisks indicate the identical residues to the sequence of the first line. Dashes represent gaps. The letter N indicates missing data on the residue. The start codon of open reading frame ORF 2 is underlined. Five NoV genogroups are indicated.

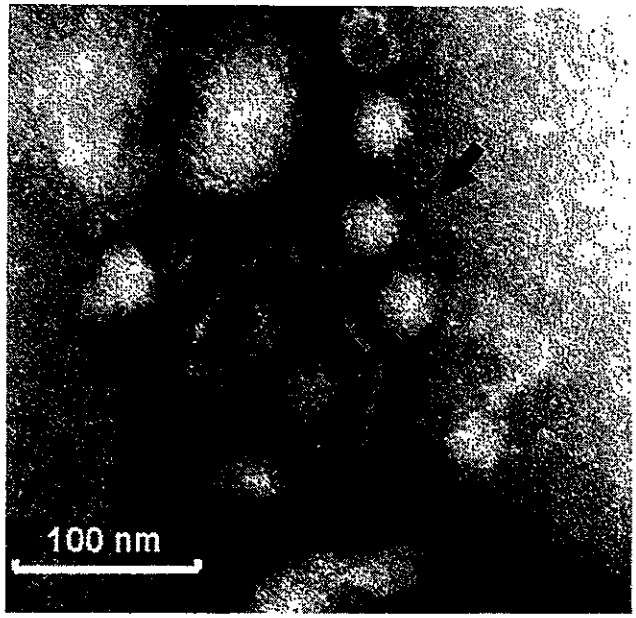

Figure 4. Immune electron micrograph of porcine noroviruses (NoVs). The diluted intestinal contents of a gnotobiotic pig euthanized on postinoculation day 5 to QW101-like porcine NoVs (QW144) were incubated with convalescent-phase serum LL616 from another gnotobiotic pig inoculated with QW101-like porcine NoVs (QW126) and visualized by negative staining with 3% phosphotungstic acid. The arrow indicates a small clump of NoV-like particles.

The QW101-like porcine NoVs replicated in gnotobiotic pigs with fecal shedding, documented by quantitative RT-PCR and IEM. No cell culture system or animal disease models are available for human NoVs, which impedes the study of their pathogenesis, replication strategies, host immune responses, and preventive approaches. The infection of pigs with porcine NoVs may provide a new infection or disease model to study NoV infections.