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研究報告の概要

○アフリカ南部で発見された新規出血熱関連アレナウイルス、Lujoウイルスの遺伝子検出と特徴

Lujoウイルス(LUJV)はアレナウイルス科の新たなウイルスで、過去30年間で初めて発見された出血熱関連旧世界アレナウイルスである。LUJVは南アフリカにおけるヒト疾患のアウトブレイク中に分離され、院内感染とこれまでにない高い死亡率(4/5例、80%)が特徴である。アウトブレイクの死亡患者から採取した血漿及び組織由来のRNA抽出物の無作為パイロシークエンスにより、検体受領から72時間以内で同定と詳細な系統発生学的特徴の分析ができた。LUJVの全ゲノム分析では、かなり昔に旧世界アレナウイルスから分岐して独特の配列を持っていることが判明した。ウイルスのG1糖タンパク質シークエンスは、他の旧世界/新世界アレナウイルスとは大きく異なって両者のほぼ中間に位置し、特徴的なレセプター親和性を持っていた。LUJVは系統的に独立した新しい高病原性アレナウイルスである。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
南アフリカにおいて、系統的に独立した新しい高病原性アレナウイルスのLujoウイルスが検出されたとの報告である。 アレナウイルスは脂質膜を持つ比較的大型のRNAウイルスである。これまで、本剤によるアレナウイルス感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考える。	念のため今後も情報収集に努める。なお、日本赤十字社では帰国(入国)後4週間は献血不適とし、輸入感染症の防止に努めている。

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Genetic Detection and Characterization of Lujo Virus, a New Hemorrhagic Fever–Associated Arenavirus from Southern Africa

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Abstract

Lujo virus (LUJV), a new member of the family *Arenaviridae* and the first hemorrhagic fever–associated arenavirus from the Old World discovered in three decades, was isolated in South Africa during an outbreak of human disease characterized by nosocomial transmission and an unprecedented high case fatality rate of 80% (4/5 cases). Unbiased pyrosequencing of RNA extracts from serum and tissues of outbreak victims enabled identification and detailed phylogenetic characterization within 72 hours of sample receipt. Full genome analyses of LUJV showed it to be unique and branching off the ancestral node of the Old World and New World arenaviruses. The virus G1 glycoprotein sequence was highly diverse and almost equidistant from that of other Old World and New World arenaviruses, consistent with a potential distinctive receptor tropism. LUJV is a novel, genetically distinct, highly pathogenic arenavirus.

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Competing Interests: SKH and ME are employees of 454 Life Sciences, Inc., a Roche Company.

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☯ These authors contributed equally to this work.

Introduction

Members of the genus *Arenavirus*, comprising currently 22 recognized species (http://www.ictvonline.org/virusTaxonomy.asp?version=2008), are divided into two complexes based on serologic, genetic, and geographic relationships [1,2]: the New World (NW) or Tacaribe complex, and the Old World (OW) or Lassa-Lymphocytic choriomeningitis complex that includes the ubiquitous arenavirus type-species *Lymphocytic choriomeningitis virus* (LCMV; [3]). The RNA genome of arenaviruses is bi-segmented, comprising a large (L) and a small (S) segment that each codes for two proteins in ambisense coding strategy [4,5]. Despite this coding strategy, the *Arenaviridae* are classified together with the families *Orthomyxoviridae* and *Bunyaviridae* as segmented single-strand, negative sense RNA viruses.

The South American hemorrhagic fever viruses Junin (JUNV; [6,7]), Machupo (MACV; [8]), Guanarito (GTOV; [9]) and Sabia virus (SABV, [10]), and the African Lassa virus (LASV [11]), are restricted to biosafety level 4 (BSL-4) containment due to their associated aerosol infectivity and rapid onset of severe disease. With the possible exception of NW Tacaribe virus (TCRV; [12]), which has been isolated from bats (*Artibeus* spp.), individual arenavirus species are commonly transmitted by specific rodent species wherein the capacity for persistent infection without overt disease suggests long evolutionary adaptation between the agent and its host [1,13–16]. Whereas NW arenaviruses are associated with rodents in the *Sigmodontinae* subfamily of the family *Cricetidae*, OW arenaviruses are associated with rodents in the *Murinae* subfamily of the family *Muridae*.

Humans are most frequently infected through contact with infected rodent excreta, commonly via inhalation of dust or aerosolized virus-containing materials, or ingestion of contaminated foods [13]; however, transmission may also occur by inoculation with infected body fluids and tissue transplantation [17–19]. LCMV, which is spread by the ubiquitous *Mus musculus* as host species and hence found world-wide, causes symptoms in humans that range from asymptomatic infection or mild febrile illness to meningitis and encephalitis [13]. LCMV infection is only rarely fatal in immunocompetent adults; however, infection during pregnancy bears serious risks for mother and child and frequently results in congenital abnormalities. The African LASV, which has its reservoir in rodent species of the *Mastomys* genus, causes an estimated 100,000–500,000 human infections per year in West African countries (Figure 1). Although Lassa fever is typically subclinical or associated with mild febrile illness, up to 20% of cases may have severe systemic disease culminating in fatal outcome [20,21]. Three other African arenaviruses are not known to cause human disease: Ippy virus (IPPYV; [22,23]), isolated from

Author Summary

In September and October 2008, five cases of undiagnosed hemorrhagic fever, four of them fatal, were recognized in South Africa after air transfer of a critically ill index case from Zambia. Serum and tissue samples from victims were subjected to unbiased pyrosequencing, yielding within 72 hours of sample receipt, multiple discrete sequence fragments that represented approximately 50% of a prototypic arenavirus genome. Thereafter, full genome sequence was generated by PCR amplification of intervening fragments using specific primers complementary to sequence obtained by pyrosequencing and a universal primer targeting the conserved arenaviral termini. Phylogenetic analyses confirmed the presence of a new member of the family *Arenaviridae*, provisionally named Lujo virus (LUJV) in recognition of its geographic origin (Lusaka, Zambia, and Johannesburg, South Africa). Our findings enable the development of specific reagents to further investigate the reservoir, geographic distribution, and unusual pathogenicity of LUJV, and confirm the utility of unbiased high throughput pyrosequencing for pathogen discovery and public health.

Arvicanthis spp. and Mobala virus (MOBV; [24]) isolated from *Praomys* spp. in the Central African Republic (CAR); and Mopeia virus (MOPV) that like LASV is associated with members of the genus *Mastomys*, and was reported from Mozambique [25] and Zimbabwe [26], although antibody studies suggest that MOPV and LASV may also circulate in CAR [27] where the geographies of these viruses appear to overlap (Figure 1). Up to present, there have been no published reports of severe human disease associated with arenaviruses isolated from southern Africa.

In September 2008 an outbreak of unexplained hemorrhagic fever was reported in South Africa [28]. The index patient was airlifted in critical condition from Zambia on September 12 to a clinic in Sandton, South Africa, after infection from an unidentified source. Secondary infections were recognized in a paramedic (case 2) who attended the index case during air transfer from Zambia, in a nurse (case 3) who attended the index case in the intensive care unit in South Africa, and in a member of the hospital staff (case 4) who cleaned the room after the index case died on September 14. One case of tertiary infection was recorded in a nurse (case 5) who attended case 2 after his transfer from Zambia to Sandton on September 26, one day before barrier nursing was implemented. The course of disease in cases 1 through 4 was fatal; case 5 received ribavirin treatment and recovered. A detailed description of clinical and epidemiologic data, as well as immunohistological and PCR analyses that indicated the presence of an arenavirus, are reported in a parallel communication (Paweska et al., Emerg. Inf. Dis., submitted). Here we report detailed genetic analysis of this novel arenavirus.

Results/Discussion

Rapid identification of a novel pathogen through unbiased pyrosequencing

RNA extracts from two post-mortem liver biopsies (cases 2 and 3) and one serum sample (case 2) were independently submitted for unbiased high-throughput pyrosequencing. The libraries yielded between 87,500 and 106,500 sequence reads. Alignment of unique singleton and assembled contiguous sequences to the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank) using the Basic Local Alignment Search Tool (blastn and blastx; [29]) indicated coverage of approximately 5.6 kilobases (kb) of sequence distributed along arenavirus genome scaffolds: 2 kb of S segment sequence in two fragments, and 3.6 kb of L segment sequence in 7 fragments (Figure 2). The majority of arenavirus sequences were obtained from serum rather than tissue, potentially reflecting lower levels of competing cellular RNA in random amplification reactions.

Full genome characterization of a newly identified arenavirus

Sequence gaps between the aligned fragments were rapidly filled by specific PCR amplification with primers designed on the pyrosequence data at both, CU and CDC. Terminal sequences were added by PCR using a universal arenavirus primer, targeting the conserved viral termini (5′-CGC ACM GDG GAT CCT AGG C, modified from [30]) combined with 4 specific primers positioned near the ends of the 2 genome segments. Overlapping primer sets based on the draft genome were synthesized to facilitate sequence validation by conventional dideoxy sequencing. The accumulated data revealed a classical arenavirus genome structure with a bi-segmented genome encoding in an ambisense strategy two open reading frames (ORF) separated by an intergenic stem-loop region on each segment (Figure 2) (GenBank Accession numbers FJ952384 and FJ952385).

Our data represent genome sequences directly obtained from liver biopsy and serum (case 2), and from cell culture isolates obtained from blood at CDC (case 1 and 2), and from liver biopsies at NICD (case 2 and 3). No sequence differences were uncovered between virus detected in primary clinical material and virus isolated in cell culture at the two facilities. In addition, no changes were detected between each of the viruses derived from these first three cases. This lack of sequence variation is consistent with the epidemiologic data, indicating an initial natural exposure of the index case, followed by a chain of nosocomial transmission among subsequent cases.

Lujo virus (LUJV) is a novel arenavirus

Phylogenetic trees constructed from full L or S segment nucleotide sequence show LUJV branching off the root of the OW arenaviruses, and suggest it represents a highly novel genetic lineage, very distinct from previously characterized virus species and clearly separate from the LCMV lineage (Figure 3A and 3B). No evidence of genome segment reassortment is found, given the identical placement of LUJV relative to the other OW arenaviruses based on S and L segment nucleotide sequences. In addition, phylogenetic analysis of each of the individual ORFs reveals similar phylogenetic tree topologies. A phylogenetic tree constructed from deduced L-polymerase amino acid (aa) sequence also shows LUJV near the root of the OW arenaviruses, distinct from characterized species, and separate from the LCMV branch (Figure 3C). A distant relationship to OW arenaviruses may also be inferred from the analysis of Z protein sequence (Figure S1). The NP gene sequence of LUJV differs from other arenaviruses from 36% (IPPYV) to 43% (TAMV) at the nucleotide level, and from 41% (MOBV/LASV) to 55% (TAMV) at the aa level (Table S1). This degree of divergence is considerably higher than both, proposed cut-off values within (<10–12%), or between (>21.5%) OW arenavirus species [31,32], and indicates a unique phylogenitic position for LUJV (Figure 3D). Historically, phylogenetic assignments of arenaviruses have been based on portions of the NP gene [1,33], because this is the region for which most sequences are known. However, as more genomic sequences have become available, analyses of full-length GPC sequence have revealed evidence of possible relationships between OW and NW

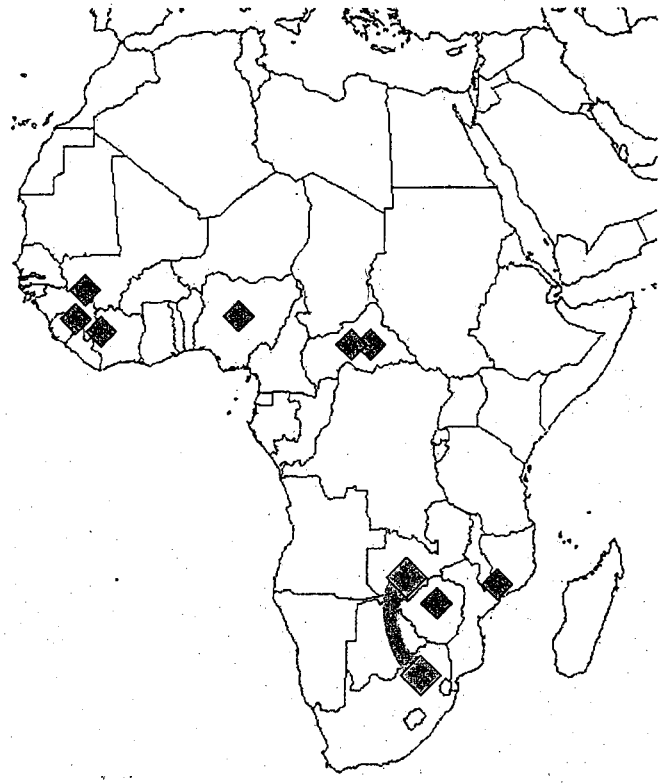

Figure 1. Geographic distribution of African arenaviruses. MOBV, MOPV, and IPPYV (blue) have not been implicated in human disease; LASV (red) can cause hemorrhagic fever. The origin of the LUJV index and secondary and tertiary cases linked in the 2008 outbreak are indicated in gold.
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arenaviruses not revealed by NP sequence alone [34]. Because G1 sequences are difficult to align some have pursued phylogenetic analyses by combining the GPC signal peptide and the G2 sequence for phylogenetic analysis [16]. We included in our analysis the chimeric signal/G2 sequence (Figure 3E) as well as the receptor binding G1 portion (Figure 3F); both analyses highlighted the novelty of LUJV, showing an almost similar distance from OW as from NW viruses.

Protein motifs potentially relevant to LUJV biology

Canonical polymerase domains pre-A, A, B, C, D, and E [35–37] are well conserved in the L ORF of LUJV (256 kDa, pI = 6.4; Figure 4). The Z ORF (10.5 kDa, pI = 9.3) contains two late domain motifs like LASV; however, in place of the PTAP motif found in LASV, that mediates recognition of the tumor susceptibility gene 101, Tsg101 [38], involved in vacuolar protein sorting [39,40], LUJV has a unique Y_{77}REL motif that matches the YXXL motif of the retrovirus equine infectious anemia virus [41], which interacts with the clathrin adaptor protein 2 (AP2) complex [42]. A Tsg101-interacting motif, P_{90}SAP, is found in LUJV in position of the second late domain of LASV, PPPY, which acts as a Nedd4-like ubiquitin ligase recognition motif [43]. The RING motif, containing conserved residue W_{44} [44], and the conserved myristoylation site G_2 are present [45–47] (Figure 4). The NP of LUJV (63.1 kDa, pI = 9.0) contains described aa motifs that resemble mostly OW arenaviruses [48], including a cytotoxic T-lymphocyte (CTL) epitope reported in LCMV (GVYMGNL; [49]), corresponding to G_{122}VYRGNL in LUJV, and a potential antigenic site reported in the N-terminal portion of LASV NP (RKSKRND; [50]), corresponding to R_{55}KDKRND in LUJV (Figure 4).

The GPC precursor (52.3 kDa, pI = 9.0) is cotranslationally cleaved into the long, stable signal peptide and the mature glycoproteins G1 and G2 [51–54]. Based on analogy to LASV [55] and LCMV [56], signalase would be predicted to cleave between D_{58} and S_{59} in LUJV. However, aspartate and arginine

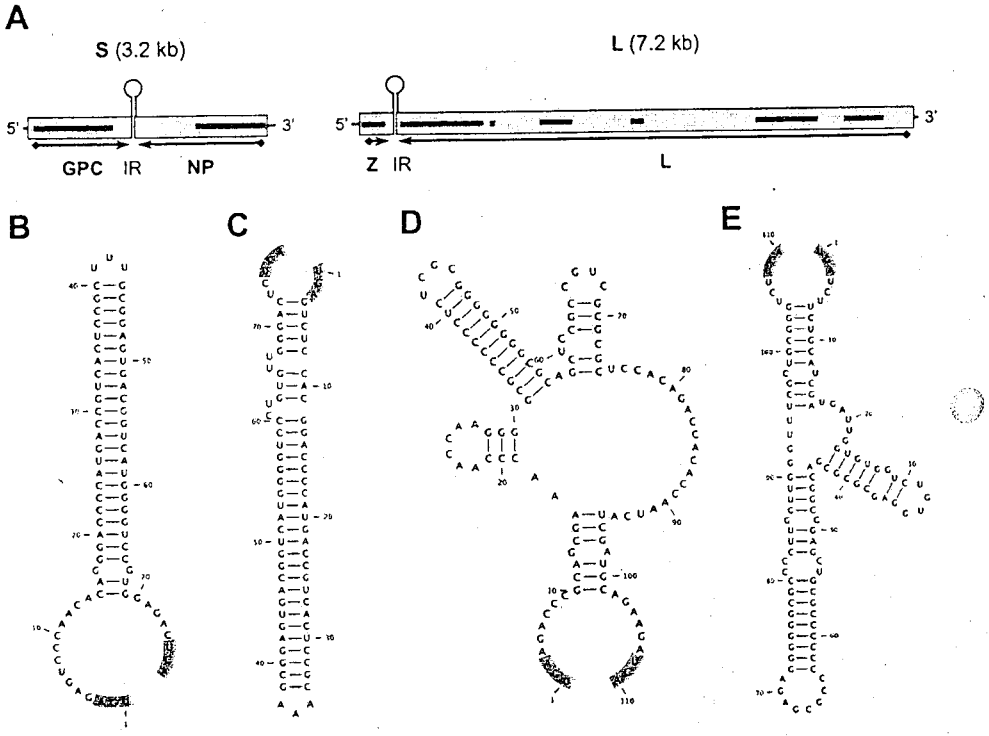

Figure 2. LUJV genome organization and potential secondary structure of intergenic regions. Open reading frames (ORF) for the glycoprotein precursor GPC, the nucleoprotein NP, the matrix protein analog Z, and the polymerase L, and their orientation are indicated (A); blue bars represent sequences obtained by pyrosequencing from clinical samples. Secondary structure predictions of intergenic regions (IR) for S (B, C) and L segment sequence (D, E) in genomic (B, D) and antigenomic orientation (C, E) were analyzed by mfold; shading indicates the respective termination codon (opal, position 1), and its reverse-complement, respectively.
doi:10.1371/journal.ppat.1000455.g002

residues in the −1 and −3 positions, respectively, violate the (−3,−1)-rule [57]; thus, cleavage may occur between S_{59} and S_{60} as predicted by the SignalP algorithm. The putative 59 aa signal peptide of LUJV displays a conserved G_2, implicated in myristoylation in JUNV [58], however, it is followed in LUJV by a non-standard valine residue in position +4, resembling non-standard glycine residues found in Oliveros virus (OLVV [59]) and Latino virus (LATV; http://www2.ncid.cdc.gov/arbocat/catalog-listing.asp?VirusID = 263&SI = 1). Conservation is also observed for aa residues P_{12} (except Amapari virus; AMAV [60]), E_{17} [61](except Pirital virus; PIRV [62]), and N_{20} in hydrophobic domain 1, as well as I_{32}KGVFNLYK$_{40}$SG, identified as a CTL epitope in LCMV WE (I_{32}KAVYNFATCG; [63]) (Figure 4).

Analogous to other arenaviruses, SKI-1/S1P cleavage C-terminal of $RKLM_{221}$ is predicted to separate mature G1 (162 aa, 18.9 kDa, pI = 6.4) from G2 (233 aa, 26.8 kDa, pI = 9.5) [52,53,64]. G2 appears overall well conserved, including the strictly conserved cysteine residues: 6 in the luminal domain, and 3 in the cytoplasmic tail that are included in a conserved zinc finger motif reported in JUNV [65] (Figure 4). G2 contains 6 potential glycosylation sites, including 2 strictly conserved sites, 2 semi-conserved sites N_{335} (absent in LCMVs and Dandenong virus; DANV [19]) and N_{352} (absent in LATV), and 2 unique sites in the predicted cytoplasmic tail (Figure 4). G1 is poorly conserved among arenaviruses [16], and G1 of LUJV is no exception, being highly divergent from the G1 of the other arenaviruses, and shorter than that of other arenaviruses. LUJV G1 contains 6 potential glycosylation sites in positions comparable to other arenaviruses, including a conserved site N_{93}HS (Figure 4), which is shifted by one aa in a motif that otherwise aligns well with OW arenaviruses and NW arenavirus clade A and C viruses. There is no discernable homology to other arenavirus G1 sequences that would point to usage of one of the two identified arenavirus receptors; Alpha-dystroglycan (α-DG) [66] that binds OW arenaviruses LASV and LCMV, and NW clade C viruses OLVV and LATV [67], or transferrin receptor 1 (TfR1) that binds pathogenic NW arenaviruses JUNV, MACV, GTOV, and SABV [68] (Figure S2).

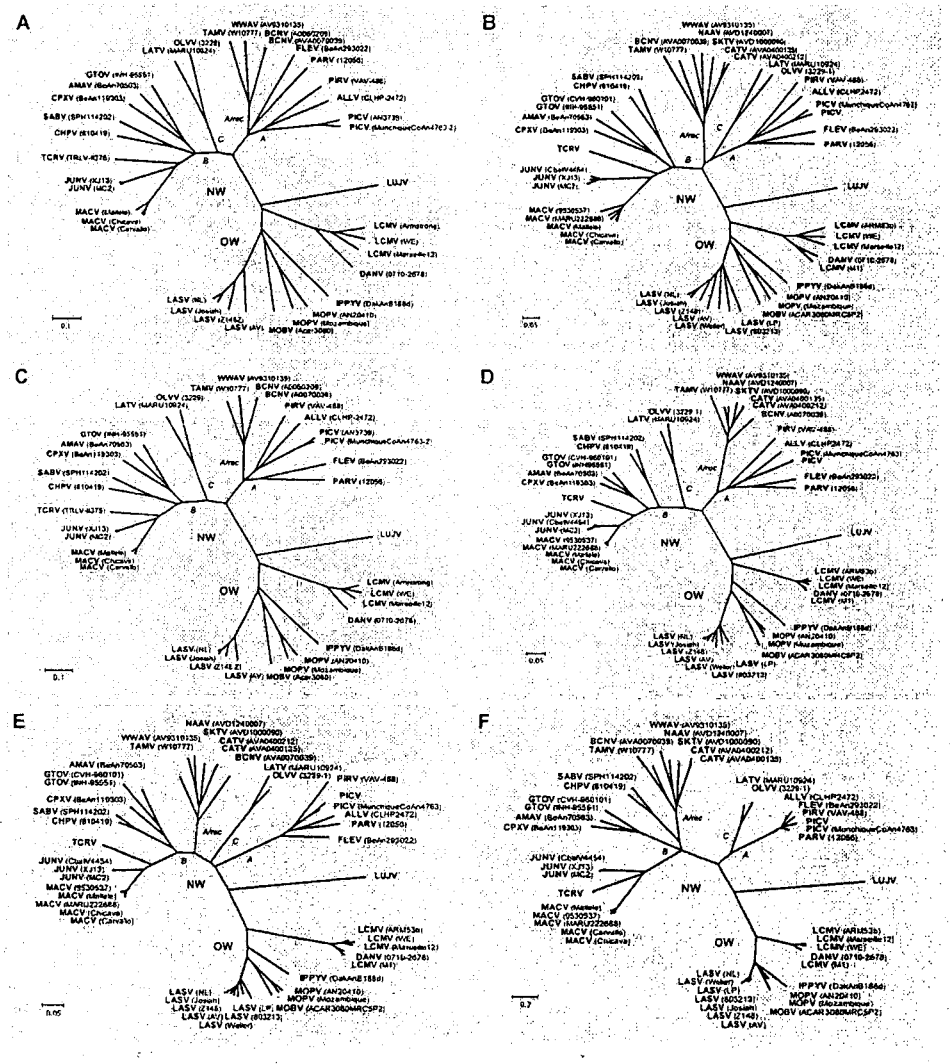

Figure 3. Phylogenetic analyses of LUJV. Phylogenetic relationships of LUJV were inferred based on full L (A) and S segment nucleotide sequence (B), as well as on deduced amino acid sequences of L (C), NP (D), Signal/G2 (E) and G1 (F) ORF's. Phylogenies were reconstructed by neighbor-joining analysis applying a Jukes-Cantor model; the scale bar indicates substitutions per site; robust boostrap support for the positioning of LUJV was obtained in all cases (>98% of 1000 pseudoreplicates). GenBank Accession numbers for reference sequences are: ALLV CLHP2472 (AY216502, AY012687); AMAV BeAn70563 (AF512834); BCNV AVA0070039 (AY924390, AY922491), A0060209 (AY216503); CATV AVA0400135 (DQ865244), AVA0400212 (DQ865245); CHPV 810419 (EU, 260464, EU260463); CPXV BeAn119303 (AY216519, AF512832); DANV 0710-2678 (EU136039, EU136038); FLEV BeAn293022 (EU627611, AF512831); GTOV INH-95551 (AY358024, AF485258), CVH-960101 (AY497548); IPPYV DakAnB188d (DQ328878, DQ328872); JUNV MC2 (AY216507, D10072), XJ13 (AY358022, AY358023), CbalV4454 (DQ272266); LASV LP (AF181853), 803213 (AF181854), Weller (AY628206), AV (AY179171, AF246121), Z148 (AY628204, AY628205), Josiah (U73034, J043204), NL (AY179172, AY179173); LATV MARU10924 (EU627612, AF485259); LCMV Armstrong (AY847351), ARM53b (M20869), WE (AF004519, M22138), Marseille12 (DQ286932, DQ286931), M1 (AB261991); MACV Carvallo (AY619642, AY619643), Chicava (AY624354, AY624355), Mallele (AY619644, AY619645), MARU222688

(AY922407), 9S30537 (AY571959); MOBV ACAR3080MRC5P2 (DQ328876, AY342390); MOPV AN20410 (AY772169, AY772170), Mozambique (DQ328875, DQ328874); NAAV AVD1240007 (EU123329); OLVV 3229-1 (AY216514, U34248); PARV 12056 (EU627613, AF485261); PICV (K02734), MunchiqueCoAn4763 (EF529745, EF529744), AN3739 (AF427517); PIRV VAV-488 (AY216505, AF277659); SABV SPH114202 (AY358026, U41071); SKTV AVD1000090 (EU123328); TAMV W10777 (EU627614, AF512828); TCRV (J04340, M20304); WWAV AV9310135 (AY924395, AF228063).
doi:10.1371/journal.ppat.1000455.g003

In summary, our analysis of the LUJV genome shows a novel virus that is only distantly related to known arenaviruses. Sequence divergence is evident across the whole genome, but is most pronounced in the G1 protein encoded by the S segment, a region implicated in receptor interactions. Reassortment of S and L segments leading to changes in pathogenicity has been described in cultured cells infected with different LCMV strains [69], and between pathogenic LASV and nonpathogenic MOPV [70]. We find no evidence to support reassortment of the LUJV L or S genome segment (Figure 3A and 3B). Recombination of glycoprotein sequence has been recognized in NW arenaviruses [14,16,33,34,71–73], resulting in the division of the complex into four sublineages: lineages A, B, C, and an A/recombinant lineage that forms a branch of lineage A when NP and L sequence is considered (see Figure 3C and 3D), but forms an independent branch in between lineages B and C when glycoprotein sequence is considered (see Figure 3D). While recombination cannot be excluded in case of LUJV, our review of existing databases reveals no candidate donor for the divergent GPC sequence. To our knowledge is LUJV the first hemorrhagic fever-associated arenavirus from Africa identified in the past 3 decades. It is also the first such virus originating south of the equator (Figure 1). The International Committee on the Taxonomy of Viruses (ICTV) defines species within the *Arenavirus* genus based on association with a specific host, geographic distribution, potential to cause human disease, antigenic cross reactivity, and protein sequence similarity to other species. By these criteria, given the novelty of its presence in southern Africa, capacity to cause hemorrhagic fever, and its genetic distinction, LUJV appears to be a new species.

Materials and Methods

Sequencing

Clinical specimens were inactivated in TRIzol (liver tissue, 100 mg) or TRIzol LS (serum, 250 µl) reagent (Invitrogen, Carlsbad, CA, USA) prior to removal from BSL-4 containment. Total RNA extracts were treated with DNase I (DNA-free, Ambion, Austin, TX, USA) and cDNA generated by using the Superscript II system (Invitrogen) and 100–500 ng RNA for reverse transcription primed with random octamers that were linked to an arbitrary, defined 17-mer primer sequence [74]. The resulting cDNA was treated with RNase H and then randomly amplified by the polymerase chain reaction (PCR; [75]); applying a 9:1 mixture of a primer corresponding to the defined 17-mer sequence, and the random octamer-linked 17-mer primer, respectively [74]. Products >70 base pairs (bp) were selected by column purification (MinElute, Qiagen, Hilden, Germany) and ligated to specific linkers for sequencing on the 454 Genome Sequencer FLX (454 Life Sciences, Branford, CT, USA) without fragmentation of the cDNA [19,76,77]). Removal of primer sequences, redundancy filtering,

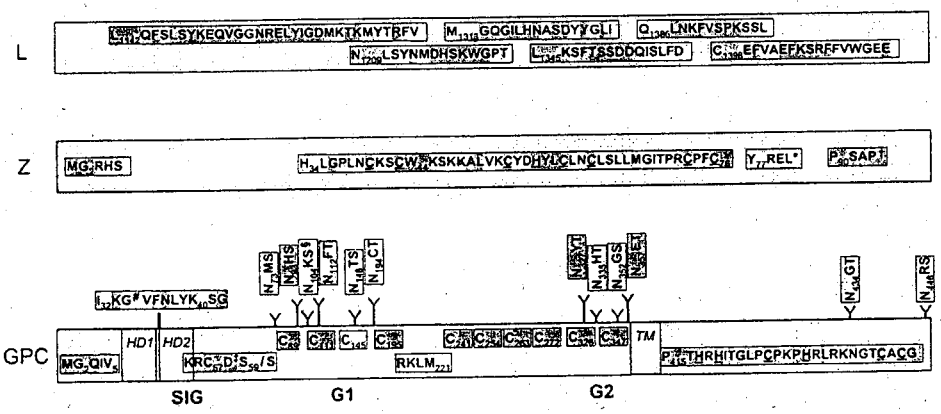

Figure 4. Schematic of conserved protein motifs. Conservation of LUJV amino acid motifs with respect to all other (green highlight), to OW (yellow highlight), or to NW (blue highlight) arenaviruses is indicated; grey highlight indicates features unique to LUJV. Polymerase motifs pre-A (L_{1142}), A (N_{1209}), B (M_{1313}), C (L_{1345}), D (Q_{1386}), and E (C_{1398}) are indicated for the L ORF; potential myristoylation site G_2, the RING motif H_{34}/C_{76} and potential late domains YXXL an PSAP are indicated for the Z ORF; and myristoylation site G_2, posttranslational processing sites for signalase (S_{59}/S_{60}) and S1P cleavage ($RKLM_{221}$), CTL epitope (I_{32}), zinc finger motif P_{415}/G_{440}, as well as conserved cysteine residues and glycosylations sites (Y) are indicated for GPC. * late domain absent in NW viruses and DANV; †:PSAP or PTAP in NW viruses, except in PIRV and TCRV (OW viruses: PPPY); # G in all viruses except LCMV (=A); ‡ D in NW clade A only; § conserved with respect to OW, and NW clade A and C; *HD*, hydrophobic domain; *TM*, transmembrane anchor.
doi:10.1371/journal.ppat.1000455.g004

and sequence assembly were performed with software programs accessible through the analysis applications at the GreenePortal website (http://156.145.84.111/Tools).

Conventional PCRs at CU were performed with HotStar polymerase (Qiagen) according to manufacturer's protocols on PTC-200 thermocyclers (Bio-Rad, Hercules, CA, USA): an enzyme activation step of 5 min at 95°C was followed by 45 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 to 3 min depending on the expected amplicon size. A two-step RT-PCR protocol was also followed at CDC using Invitrogen's Thermoscript RT at 60 degrees for 30 min followed by RNase H treatment for 20 min. cDNA was amplified using Phusion enzyme with GC Buffer (Finnzymes, Espoo, Finland) and 3% DMSO with an activation step at 98°C for 30 sec, followed by the cycling conditions of 98°C for 10 sec, 58°C for 20 sec, and 72°C for 1 min for 35 cycles and a 5 min extension at 72°C. Specific primer sequences are available upon request. Amplification products were run on 1% agarose gels, purified (MinElute, Qiagen), and directly sequenced in both directions with ABI PRISM Big Dye Terminator 1.1 Cycle Sequencing kits on ABI PRISM 3700 DNA Analyzers (Perkin-Elmer Applied Biosystems, Foster City, CA).

Sequence analyses

Programs of the Wisconsin GCG Package (Accelrys, San Diego, CA, USA) were used for sequence assembly and analysis; percent sequence difference was calculated based on Needleman-Wunsch alignments (gap open/extension penalties 15/6.6 for nucleotide and 10/0.1 for aa alignments; EMBOSS [78]), using a Perl script to iterate the process for all versus all comparison. Secondary RNA structure predictions were performed with the web-based version of mfold (http://mfold.bioinfo.rpi.edu); data were exported as .ct files and layout and annotation was done with CLC RNA Workbench (CLC bio, Århus, Denmark). Protein topology and targeting predictions were generated by employing SignalP, and NetNGlyc, TMHMM (http://www.cbs.dtu.dk/services), the web-based version of TopPred (http://mobyle.pasteur.fr/cgi-bin/portal.py?form=toppred), and Phobius (http://phobius.sbc.su.se/). Phylogenetic analyses were performed using MEGA software [79].

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Supporting Information

Figure S1 Phylogenetic tree based on deduced Z amino acid sequence. In contrast to phylogenetic trees obtained with the other ORFs (Figure 2), poor bootstrap support (43% of 1,000 pseudoreplicates) for the branching of LUJV off the LCMV clade was obtained with Z ORF sequence. For GenBank accession numbers see Figure 2.
Found at: doi:10.1371/journal.ppat.1000455.s001 (0.44 MB TIF)

Figure S2 Pairwise sliding-window distance analysis of GPC sequence. LUJV and members of the OW (LASV, MOPV, IPPYV, LCMV, DANV) and NW (GTOV, CPXV, BNCV, PIRV, OLVV, SABV, MACV) arenavirus complex were compared using LASV NL (A) or GTOV CVH (B) as query (10 aa step; 80 aa window).
Found at: doi:10.1371/journal.ppat.1000455.s002 (4.21 MB TIF)

Table S1 Pairwise nucleotide and amino acid differences between LUJV and other OW and NW arenaviruses. * NAAV, North American arenavirus. † Values <30% (amino acid) or <33% (nucleotide) are highlighted in green.
Found at: doi:10.1371/journal.ppat.1000455.s003 (0.20 MB DOC)

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Author Contributions

Conceived and designed the experiments: TB WIL. Performed the experiments: TB JTP LKM SKH GP MLK JW. Analyzed the data: TB LKM SKH CS GP MLK ME STN WIL. Contributed reagents/materials/analysis tools: JTP CS JW BS ME. Wrote the paper: TB JTP BS STN WIL.

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別紙様式第2-1

No. 16

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	総合機構処理欄
				2009. 8. 11	該当なし	
一般的名称	乾燥濃縮人血液凝固第Ⅷ因子					
販売名(企業名)	クロスエイトM250(日本赤十字社) クロスエイトM500(日本赤十字社) クロスエイトM1000(日本赤十字社) クロスエイトM静注用250単位(日本赤十字社) クロスエイトM静注用500単位(日本赤十字社) クロスエイトM静注用1000単位(日本赤十字社)	研究報告の公表状況	ProMED 20090806.2782, 2009 Aug 6. 情報源: Portal Amazonia, 2009 Aug 5.		公表国 ブラジル	使用上の注意記載状況・その他参考事項等 クロスエイトM250 クロスエイトM500 クロスエイトM1000 クロスエイトM静注用250単位 クロスエイトM静注用500単位 クロスエイトM静注用1000単位

研究報告の概要

○オロポーチ熱—ブラジル(アマパー州)

8月4日、ブラジル、アマパー州南部の都市マザガオの当局は、過去3ヶ月間にオロポーチ熱に感染した人は657名以上になると発表した。このうち29名が検査によって感染を確認された。患者は当初マラリアやデング熱を疑われていたが、検査によって初めてオロポーチ熱であることが判明した。アマパー州では長い間報告されていなかった、オロポーチ熱は艾カカ(Cicoides)によって媒介される疾患で、症状はデングやマラリアによく似ており、発熱、頭痛、全身の筋肉痛などが認められる。2009年の初発例は3月に発生し、4月、5月には600例を超えた。

オロポーチウイルスは、ブラジル国内で2番目に多いアルボウイルス性発熱疾患の原因ウイルスで、ブラジルでは過去30年間で約50万例以上の同疾患患者が発生しており、パナマ、ペルー、スリナム、トリニダード・トバゴでも発生が確認されている。感染流行の発生はアマゾン地域に限られている。

報告企業の意見	今後の対応
ブラジル、アマパー州南部の都市マザガオで、オロポーチ熱のアウトブレイクが発生したとの報告である。オロポーチウイルスは脂質膜を持つ比較的大型のRNAウイルスで、これまで本製剤によるロポーチ熱発症の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考える。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。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。

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

(15)

Back

Archive Number 20090806.2782
Published Date 06-AUG-2009
Subject PRO/AH/EDR> Oropouche fever - Brazil: (AP)

OROPOUCHE FEVER - BRAZIL: (AMAPA)
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Wed 5 Aug 2009
Source: Portal Amazonia [in Portuguese, trans. Mod.TY, edited]
<http://portalamazonia.globo.com/pscript/noticias/noticias.php?idN=89739>

In the prefecture, about 657 cases of Oropouche fever have been reported
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The Municipality of Mazagao (PMMZ) yesterday (4 [Aug 2009]) released a report of around 657 cases of oropouche [virus] infection with fever in the municipality in last 3 months. Of these, 29 were [laboratory] confirmed by the Instituto Evandro Chagas (IEC). The IEC found that the disease was caused by biting midges [_Culicoides_].

According to the secretary of health of Mazagao, Jose Monteiro, the 1st [disease] suspected was malaria followed by dengue, and only afterward was oropouche diagnosed by the IEC. The disease has not been reported in Amapa for a long time. The symptoms are very similar to those of dengue and malaria: fever, headache, generalized myalgia. Biting midges, common in the region, are one of the vectors of the virus.

The 1st cases of oropouche fever appeared in March 2009; in April and May this year there was an tremendous increase of notifications, more than 600, in Mazagao Velho and Carvao localities. We are taking several steps, such as a service for cleaning and spraying in the city, to eliminate the outbreak of the disease, said Jose Monteiro.

The oropouche virus is the 2nd most frequent cause of arbovirus fever in Brazil. According to the Ministry of Health (MoH), about half a million cases of fever have occurred in Brazil in the last 30 years, there are records of events in Panama, Peru, Suriname and Trinidad and Tobag

Outbreaks of oropouche fever have been recorded only in the Amazon. Global warming of the planet, deforestation and consequent redistribution of insect vectors and animal reservoirs are some factors.

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[Mazagao is located next to Manga, just to the southwest, near the mouth of the Amazon River. Its population does not exceed 15 000 inhabitants, providing an incidence of 4380 fever cases per 100 000 inhabitants overall. Oropouche is a virus of the Bunyaviridae family. It was isolated for the 1st time in 1960. It is transmitted by _Culicoides spp_ and is one of the most common causes of undifferentiated fever in northern and central-west Brazil. The disease caused by the virus and essentialkly is benign, presenting no great (health or mortality) risk. - Mod.LJS]

[Oropouche fever cases have also occurred in Peru, where it was

initially confused with dengue virus infections (see the ProMED archive below). Some recent reports of oropouche virus infections in Brazil include:

Ref: Sporadic oropouche virus infection, acre, Brazil.
Emerg Infect Dis 15:348-50.
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2657612>

Authors: Bernardes-Terzian AC, de-Moraes-Bronzoni RV, Drumond BP, Da Silva-Nunes M, da-Silva NS, Urbano-Ferreira M, Speranca MA, Nogueira ML. 20

Ref: Oropouche fever epidemic in Northern Brazil: epidemiology and molecular characterization of isolates.
J Clin Virol, 44:129-33.
<http://www.journalofclinicalvirology.com/article/S1386-6532%2808%2900399-5> (abstract)

Authors: Vasconcelos HB, Azevedo RS, Casseb SM, Nunes-Neto JP, Chiang JO, Cantuaria PC, Segura MN, Martins LC, Monteiro HA, Rodrigues SG, Nunes MR, Vasconcelos PF. 2009.

A map showing the location of Mazagão in the Amazon River delta can be accessed at:
<http://www.maplandia.com/brazil/amapa/mazagao/sao-tome/register/>

A HealthMap/ProMED-mail interactive map of Brazil can be accessed at:
<http://healthmap.org/promed/en?v=-10.8,-53.1,4>
- Mod. TY]

[See also:
1995

Oropouche fever - Peru 19950328.0167
.....ty/ljs/ejp/dk

別紙様式第2-1

No. 14

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	総合機構処理欄
				2009. 7. 21	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況		公表国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)			山中篤志, 菊池郁夫, 姫路大輔, 井上靖, 上田章. 第83回日本感染症学会総会学術講演会; 2009 Apr 23-24; 東京.	日本	

研究報告の概要

○新興感染症コウモリオルソレオウイルス感染症による急性上気道炎を発症した国内初症例

2007年に初めて報告された新興感染症コウモリオルソレオウイルス(別名マラッカウイルス)感染による急性上気道炎を経験した。患者は38歳男性で2007年11月に新婚旅行にて2週間インドネシア・バリ島に滞在した。帰国数日前より発熱、関節痛が出現し、帰国時も症状持続のため他院を受診し、抗菌薬、解熱剤を処方され帰宅したが、症状が悪化し救急外来に搬送された。簡易インフルエンザキットにて陰性であったが、高熱、関節痛及び上気道炎症状が強く、現地の鶏との濃厚接触歴があったため鳥インフルエンザ感染疑い例としての患者対応を開始した。翌日には鳥インフルエンザ感染は否定され隔離解除、約1週間後には軽快退院し、以後症状を認めなかった。後日、ウイルス分離、血清抗体価よりオルソレオウイルス感染症であったことが判明した。

レオウイルス科のコウモリオルソレオウイルスによる急性上気道炎は、2007年に初症例がマレーシアにて報告された。このウイルスはコウモリを自然宿主とするとされ、水平感染の可能性が示唆されている。本患者は渡航先でコウモリとの直接の接触はなかったが、発症数日前に上気道症状を有する現地住民との接触歴を有していた。感染判明後直ちに本患者及び接触者を対象に血清学的検査が行われ、本患者では回復期に抗体が検出されたが、他の対象者は全て陰性であった。本症例は国内初症例であるとともに、国際的にも1例目の報告以降に未だ報告を確認できていない。今回は日本人旅行者の多い旅行地での短期滞在で感染しており、水平感染の可能性も示唆されていることから、輸入感染症として今後わが国でも大変危惧される感染症である。加えて、症状がインフルエンザの症状と大変似ていることから鳥インフルエンザ、新型インフルエンザ感染症の擬似症例としても今後重要になってくると考える。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
インドネシア・バリ島から帰国後コウモリオルソレオウイルスによる上気道炎を発症し、ヒトーヒト感染の可能性が考えられた国内初の症例である。 オルソレオウイルスは脂質膜を持つRNAウイルスである。これまで、本剤によるオルソレオウイルス感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考える。	念のため今後も情報収集に努める。なお、日本赤十字社では帰国(入国)後4週間は献血不適とし、輸入感染症の防止に努めている。

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JRC2009T-040

224

O-171 新興感染症コウモリオルソレオウイルス感染症による急性上気道炎を発症した国内初症例

県立宮崎病院内科
○山中篤志, 菊池郁夫, 姫路大輔, 井上 靖, 上田 章

今回, 我々は2007年に初めて報告された新興感染症コウモリオルソレオウイルス(別名マラッカウイルス)感染による急性上気道炎を経験したので報告する.

症例は38歳男性で2007年11月に新婚旅行にて2週間インドネシア・バリ島に滞在した. 帰国数日前より発熱, 関節痛が出現し, 帰国時も症状持続のため他院を受診し抗菌薬, 解熱剤を処方され帰宅したが症状増悪し救急外来に搬送された. 簡易インフルエンザキットにて陰性であったが, 高熱, 関節痛及び咳嗽, 咽頭痛などと上気道炎症状強く, 問診にて現地の鶏との濃厚接触歴があったため鳥インフルエンザ感染疑い例としての患者対応を開始した. 翌日には鳥インフルエンザ感染は否定され隔離解除, 約1週間後には軽快退院し, 以後症状を認めなかった. 後日, ウイルス分離, 血清抗体価よりオルソレオウイルス感染症であったことが判明した.

レオウイルス科のコウモリオルソレオウイルスによる急性上気道炎は2007年に初症例がマレーシアにて報告された. このウイルスはコウモリを自然宿主とするとされ, 水平感染の可能性が示唆されている. 本患者は渡航先でコウモリとの直接の接触はなかったが, 発症数日前に上気道症状を有する現地住民との接触歴を有していた. 感染判明後直ちに本患者及び接触者を対象に血清学的検査が行われ, 本患者では回復期に抗体が検出されたが, 他の対象者は全て陰性であった.

本症例は国内初症例であるとともに国際的にも1例目の報告以降に未だ報告を確認できていない. 今回は日本人旅行者の多い旅行地での短期滞在で感染しており, また水平感染の可能性も示唆されていることから輸入感染症として今後わが国でも大変危惧される感染症である. 加えて, 症状がインフルエンザの症状と大変似ていることから鳥インフルエンザ, 新型インフルエンザ感染症の擬似症例としても今後重要になってくると考える.

O-172 国内初の新興感染症「ヒトアナプラズマ症」2症例について

静岡県立大・食品栄養科学・微生物[1], 高知県衛生研究所[2], 宝戸病院[3], 愛媛県立中央病院[4], 岐阜大学[5], 国立感染症研究所細菌第一部[6], 国立感染症研究所ウイルス第一部[7], ○大橋典男[1], 千屋誠治[2], 船戸恭孝[3], 塩沢正明[4], 高野 愛[5], 川端寛樹[6], 安藤秀二[7], 岸本寿男[7]

近年, マダニを介してヒトに発熱症状を起こす新興感染症「ヒトアナプラズマ症」が欧米で問題となっている. 今回, 2002~2003年に高知県で日本紅斑熱が疑われた患者18名の保存血清を解析したところ, 2名からヒトアナプラズマ(Anaplasma phagocytophilum: Ap)に特異的なp44/msp2遺伝子が検出され, ヒトアナプラズマ症の国内における存在を初めて確認した. 1名はヒトアナプラズマ症で, もう1名はApと日本紅斑熱リケッチア(*Rickettsia japonica*: Rj)の混合感染症例であった. 【症例1】61歳, 男性, 農業. 2003年1月5日より39℃台の発熱が出現. 1月6日に近医受診. 体幹中心に紅斑を認める. セフェム系抗菌薬が無効で, 1月8日にA病院に紹介. 日本紅斑熱疑いで入院. 入院時, 体幹中心の紅斑と, 右臀背部に刺し口様の所見あり. WBC正常, CRP上昇, 軽度肝機能障害. 入院後MINO200mg/日の点滴で徐々に解熱し, 紅斑, 全身倦怠感も改善し, 1月17日に退院となるも農業診断は不明であった. 今回, 保存血清からApおよびRj遺伝子が検出され, ヒトアナプラズマ症と診断された. 【症例2】73歳, 男性, 森林業. 2002年8月29日より発熱と発疹が出現, 9月2日に近医受診し日本紅斑熱疑いで入院. 入院時38℃の発熱と, 全身の発疹, 右大腿部に刺し口様の出血跡あり. WBC正常, CRP上昇, 中等度肝機能障害. 入院後MINO200mg/日の点滴で徐々に解熱し, 発疹も改善し, 9月22日退院. 今回, 保存血清からApおよびRj遺伝子が検出され, 両者の混合感染症例と診断された. 今後リケッチア症を疑う患者では, ヒトアナプラズマ症も考慮すべきである. [非会員共同研究者: 島田図, 高畑(静岡県立大), 川森文彦(静岡県環衛研), 福永和俊(高知衛研), 浜宇津良治(中芸クリニック), 中島秀樹(高知大)]

別紙様式第2-1

No. 29

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	総合機構処理欄
				2009. 7. 21	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	大橋典男, 千屋誠造, 船戸豊彦, 塩尻正明, 高野愛, 川端寛樹, 安藤秀二, 岸本寿男. 第83回日本感染症学会総会学術講演会; 2009 Apr 23-24; 東京.	公表国 日本	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)					

研究報告の概要

○国内初の新興感染症「ヒトアナプラズマ症」2症例について

近年、マダニを介してヒトに発熱症状を起こす新興感染症「ヒトアナプラズマ症」が欧米で問題となっている。今回、2002～2003年に高知県で日本紅斑熱が疑われた患者18名の保存血液を解析したところ、2名からヒトアナプラズマ(*Anaplasma phagocytophlum*: *A. p*)に特異的なp44/msp2遺伝子が検出され、ヒトアナプラズマ症の国内における存在を初めて確認した。1名はヒトアナプラズマ症で、もう1名は*A. p*と日本紅斑熱リケッチア(*Rickettsia japonica*: *R. j*)の混合感染症例であった。【症例1】61歳男性農業。2003年1月5日より39℃台の発熱が出現、1月6日に近医受診。体幹中心に紅斑を認める。セフェム系抗菌薬が無効で、1月8日にA病院に紹介、日本紅斑熱疑いで入院。入院時、体幹中心の紅斑と、右肩背部に刺し口様の所見あり。WBC正常、CRP上昇、軽度肝機能障害。入院後ミノマイシン200mg/日の点滴で徐々に解熱し、紅斑、全身倦怠感も改善し、1月17日に退院となるも最終診断は不明であった。今回、保存血液から*A. p*遺伝子が検出され、ヒトアナプラズマ症と診断された。【症例2】73歳男性森林業。2002年8月29日より発熱と発疹が出現、9月2日に近医受診し日本紅斑熱疑いで入院。入院時38℃ の発熱と、全身の発疹、右大腿部に刺し口様の出血跡あり。WBC正常、CRP上昇、中等度肝機能障害。入院後ミノマイシン200mg/日の点滴で徐々に解熱し、発疹も改善し、9月22日退院。今回、保存血液から*A. p*遺伝子と、*R. j*遺伝子が検出され、両者の混合感染症例と診断された。今後リケッチア症を疑う患者では、ヒトアナプラズマ症も考慮すべきである。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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

報告企業の意見	今後の対応
2002～2003年に高知県で日本紅斑熱が疑われた患者18名の保存血液を解析したところ、2名からヒトアナプラズマに特異的なp44/msp2遺伝子が検出され、日本におけるヒトアナプラズマ症の存在が初めて確認されたとの報告である。	日本赤十字社では、発熱などの体調不良者を献血不適としている。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。

(17)

JRC2009T-041

224

O-171 新興感染症コウモリオルソレオウィルス感染症による急性上気道炎を発症した国内初症例

県立宮崎病院内科
○山中篤志, 菊池郁夫, 姫路大輔, 井上 靖, 上田 章

今回, 我々は2007年に初めて報告された新興感染症コウモリオルソレオウィルス(別名マラッカウィルス)感染による急性上気道炎を経験したので報告する.

症例は38歳男性で2007年11月に新婚旅行にて2週間インドネシア・バリ島に滞在した. 帰国数日前より発熱, 関節痛が出現し, 帰国時も症状持続のため他院を受診し抗菌薬, 解熱剤を処方され帰宅したが症状増悪し救急車にて当院救急外来に搬送された. 簡易インフルエンザキットにて陰性であったが, 高熱, 関節痛及び咳嗽, 咽頭痛などと上気道炎症状強く, 問診にて現地の鶏との濃厚接触歴があったため鳥インフルエンザ感染疑い例としての患者対応を開始した. 翌日には鳥インフルエンザ感染は否定され隔離解除, 約1週間後には軽快退院し, 以後症状を認めなかった. 後日, ウィルス分離, 血清抗体価よりオルソレオウィルス感染症であったことが判明した.

レオウィルス科のコウモリオルソレオウィルスによる急性上気道炎は2007年に初症例がマレーシアにて報告された. このウィルスはコウモリを自然宿主とするとされ, 水平感染の可能性が示唆されている. 本患者は感染先でコウモリとの直接の接触はなかったが, 発症数日前に上気道症状を有する現地住民との接触歴を有していた. 感染判明後直ちに本患者及び接触者を対象に血清学的検査が行われ, 本患者では回復期に抗体が検出されたが, 他の対象者は全て陰性であった.

本症例は国内初症例であるとともに国際的にも1例目の報告以降に未だ報告を確認できていない. 今回は日本人旅行者の多い旅行地での短期滞在で感染しており, また水平感染の可能性も示唆されていることから輸入感染症として今後わが国でも大変危惧される感染症である. 加えて, 症状がインフルエンザの症状と大変似ていることから鳥インフルエンザ, 新型インフルエンザ感染症の疑似症例としても今後重要になってくると考える.

O-172 国内初の新興感染症「ヒトアナプラズマ症」2症例について

静岡県立大・食品栄養科学・微生物[1],
高知県衛生研究所[2],
室戸病院[3],
愛媛県立中央病院[4],
岐阜大学[5],
国立感染症研究所細菌第一部[6],
国立感染症研究所ウイルス第一部[7]
○大橋典男[1], 千屋誠造[2], 船戸豊彦[2], 塩尻正明[2], 高野 愛[3], 川端寛樹[4], 安藤秀二[5], 岸本寿男[6]

近年, マダニを介してヒトに発熱症状を起こす新興感染症「ヒトアナプラズマ症」が欧米で問題となっている. 今回, 2002～2003年に高知県で日本紅斑熱が疑われた患者18名の保存血液を解析したところ, 2名からヒトアナプラズマ(Anaplasma phagocytophilum: Ap)に特異的なp44/msp2遺伝子が検出され, ヒトアナプラズマ症の国内における存在を初めて確認した. 1名はApとR日本紅斑熱リケッチア(*Rickettsia japonica*: Rj)の混合感染症例であった. 【症例1】61歳 男性 農業. 2003年1月5日より39℃台の発熱が出現. 1月6日に近医受診. 体幹中心に紅斑を認める. セフェム系抗菌薬が無効で, 1月8日にA病院に紹介, 日本紅斑熱疑いで入院. 入院時, 体幹中心の紅斑と, 右肩背部に刺し口様の所見あり. WBC正常, CRP上昇, 軽度肝機能障害. 入院後MINO200mg/日の点滴で徐々に解熱し, 紅斑, 全身倦怠感も改善し, 1月17日に退院となるも最終診断は不明であった. 今回, 保存血液からApの遺伝子が検出され, ヒトアナプラズマ症と診断された. 【症例2】73歳 男性 森林業. 2002年8月29日より発熱と発疹が出現. 9月2日に近医受診し日本紅斑熱疑いで入院. 入院時38℃ の発熱と, 全身の発疹, 右大腿部に刺し口様の出血跡あり. WBC正常, CRP上昇, 中等度肝機能障害. 入院後MINO200mg/日の点滴で徐々に解熱し, 発疹も改善し, 9月22日退院. 今回, 保存血液からApの遺伝子と, Rjの遺伝子が検出され, 両者の混合感染症例と診断された. 今後リケッチア症を疑う患者では, ヒトアナプラズマ症も考慮すべきである. 【非会員共同研究者: 鳥日図, 高桂(静岡県立大), 川森文彦(静岡県環衛研), 福永和俊(高知衛研), 浜宇津良治(中芸クリニック), 中島秀樹(高知大)】

別紙様式第2-1

No. 4

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	総合機構処理欄
				2009. 6. 15	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	平力造, 伊藤綾香, 五井薫, 後藤直子, 百瀬俊也, 日野学. 第57回日本輸血・細胞治療学会総会; 2009 May 28-30; 大宮.	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○輸血関連感染(疑)報告症例の現状と解析(2008年)
【はじめに】日本赤十字社では、薬事法に基づき全国の医療機関より収集した副作用・感染症報告を独立行政法人医薬品医療機器総合機構へ報告している。2008年に報告された輸血関連感染(疑)症例149例の現状と解析結果について報告する。
【対象と方法】調査対象がウイルスに起因する場合は当該製剤の保管検体等による個別NATにより、細菌の場合は当該製剤(使用済みバッグ)又は同一製造番号の血漿の細菌培養試験等により調査を行い解析した。
【結果と考察】149例の病原体別内訳はHBV61例(41%)、HCV38例(26%)、細菌46例(31%)、HEV2例、HIV1例、CMV1例であった。日赤調査によりHBV4例、HEV2例及び細菌2例の献血者検体に病原体を検出した。HBV4例、HEV1例は患者ウイルスとの塩基配列比較により因果関係が高いと評価した。残るHEV1例(輸血後患者ウイルス陰性)は、症状・輸血前後の血清学的検査結果により因果関係が高いと評価した。このHEV2例は、血漿分画製剤の製造販売業者からの献血後情報を発端により判明した事例であった。細菌2例は当該製剤(血小板製剤)からStaphylococcus aureus及びStreptococcus dysgalactiae ssp. equisimilisが同定され、各々患者菌株との遺伝子型別試験等の結果から因果関係が高いと評価した。輸血後B型肝炎の1例の受血者は、その後劇症肝炎により死亡した。輸血後感染症は種々の安全対策及び医療機関による適正使用の推進により減少傾向にある。日赤では安全性をこれまで以上に向上させる目的で2008年8月より血清学的検査を凝集法から化学発光酵素免疫法へ変更し、また、NATについてもより感度の高い新NATシステムによる検査を導入した。ヘモビジランスの一環として輸血関連感染症の動向を今後も注視し、安全対策の効果を検証し、解析結果をフィードバックし、更なる血液製剤の安全性向上に資することとしたい。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
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赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見

2008年に全国の医療機関から報告された輸血関連感染症例の現状とその解析結果についての報告である。
日本赤十字社では、血清学的検査に加え、HBV、HCV、HIVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、「血液製剤等に係る遡及調査ガイドライン」(平成20年12月26日付薬食発第1226011号)に基づき、輸血感染症の調査を行っている。

今後の対応

輸血感染症に関する新たな知見等について今後も情報の収集に努める。検査精度向上のため、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた新NATシステムを導入した。

(18)

JRC2009T-033

O-053 輸血関連感染(疑)報告症例の現状と解析(2008年)

日本赤十字社血液事業本部安全管理課
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【はじめに】日本赤十字社では，薬事法に基づき全国の医療機関より収集した副作用・感染症報告を独立行政法人医薬品医療機器総合機構へ報告している．2008年に報告された輸血関連感染(疑)症例149例の現状と解析結果について報告する．【対象と方法】調査対象がウイルスに起因する場合は当該製剤の保管検体等による個別NATにより，細菌の場合は当該製剤(使用済みバッグ)又は同一製造番号の血漿の細菌培養試験等により調査(日赤調査)を行い解析した．【結果と考察】149例の病原体別内訳はHBV 61例(41%)，HCV 38例(26%)，細菌46例(31%)，HEV 2例，HIV 1例，CMV 1例であった．日赤調査によりHBV 4例，HEV 2例及び細菌2例の献血者検体に病原体を検出した．HBV 4例，HEV 1例は患者ウイルスとの塩基配列比較により因果関係が高いと評価した．残るHEV1例(輸血後患者ウイルス陰性)は，症状・輸血前後の血清学的検査結果により因果関係が高いと評価した．このHEV 2例は，血漿分画製剤の製造販売業者からの献血後情報を発端により判明した事例であった．細菌2例は当該製剤(血小板製剤)からStaphylococcus aureus及びStreptococcus dysgalactiae ssp. equisimilisが同定され，各々患者菌株との遺伝子型別試験等の結果から因果関係が高いと評価した．輸血後B型肝炎の1例の受血者は，その後劇症肝炎により死亡した．輸血後感染症は種々の安全対策及び医療機関による適正使用の推進により減少傾向にある．日赤では安全性をこれまで以上に向上させる目的で2008年8月より血清学的検査を凝集法から化学発光酵素免疫法へ変更し，また，NATについてもより感度の高い新NATシステムによる検査を導入した．ヘモビジランスの一環として輸血関連感染症の動向を今後も注視し，安全対策の効果を検証し，解析結果をフィードバックし，更なる血液製剤の安全性向上に資することとしたい．

O-054 20プールNAT導入後，初めて輸血後HCV感染を確認された再生不良性貧血の一例

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小池史泰[1]，坂野章吾[1,2]，石田高司[2]，越知則予[1]，村瀬幸雄[1]，尾関一輝[1]，溝上雅史[1]，楠本 茂[2]，
小松弘和[2]，上田龍三[2]，神谷 忠[3]，柚木久雄[4]
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輸血後HCV感染はHBV感染に比べて，感染リスクの推定が困難な程，非常に少ない．今回，20プールNAT検査導入後，はじめて，輸血後HCV感染が成立した症例を経験した．54歳，女性．最重症再生不良性貧血．輸血前感染症検査でHCV抗体陰性，HCVコア蛋白陰性．2007年6月20日に初回輸血．ATG，CyA治療は効果なく，2007年10月1日の同種骨髄移植前の感染症検査で肝機能正常，HCV抗体(CLEIA)陰性であったがHCVコア蛋白が陽性(28183.1fmol/L)を認めた．血液センターに副作用報告し，当院の輸血前凍結保存血清でHCV-RNA(PCR)陰性を確認した．輸血に使用された54本(RCCまたはPC)すべての保管検体のHCV個別NATを実施し，1検体(8月17日，RCC輸血)のHCV-RNA陽性検体が特定できた．この血液の分画原料用血漿を用い，患者，献血者のHCVコア領域(196bp)，およびコア～E1～E2領域(1279bp)の核酸配列をRT-PCR direct sequence，分子系統樹により比較解析した．両者の核酸配列が一致し，輸血によるHCV感染と考えられた．2007年10月17日に骨髄移植を施行し，2008年3月30日に肺炎のため死亡された．HCV混入血の輸血から約7ヶ月の経過でHCV抗体が陽性になることはなく，AST/ALTの上昇もほとんどなかったが，HCVコア蛋白値は5000fmol/L以上であった．20プールNAT陰性献血血液由来の血液製剤からのHCV感染の報告は本邦では初めてであり，本例は非常に微量なHCVが，宿主の免疫能低下により，感染が成立したこと．肝機能異常がなく，HCV抗体陰性であり，HCVコア蛋白が測定されなければ，最後までHCV感染は不明あり，移植後免疫能が回復したときに肝炎発症した可能性がある．血液疾患など宿主の免疫能により，極めて微量のHCVにより，感染が成立し，輸血後感染症検査の重要性，HCVコア蛋白測定の必要性を示唆している．

別紙様式第2-1

No. 11

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	総合機構処理欄
				2009. 5. 18	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. N Engl J Med. 2009 May 22.	公表国 世界各国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○ヒトにおけるブタ由来の新型インフルエンザA型ウイルスの発現

背景:米国において、2009年4月15日と4月17日に、疫学的に無関係な患者2例の検体でブタ由来の新型インフルエンザA型(H1N1)ウイルス(S-OIV)が同定された。同一のウイルス株が、メキシコ、カナダ、その他の地域で検出された。我々は、急速に拡大しつつある米国のアウトブレイクで確定されたヒトS-OIV感染症例642例を報告する。

方法:米国でsubtype分類されていないヒトのインフルエンザA型感染の強化サーベイランスが実行された。検体は、RT-PCR法によるS-OIVの確認検査を行うために、米国疾病対策センターに送られた。

結果:4月15日から5月5日までの期間に、S-OIV感染確定例合計642症例が41の州で確認された。患者の年齢は3ヵ月齢～81歳の範囲で、60%は18歳以下であった。データのある患者のうち、18%にメキシコへの最近の渡航歴があり、16%はS-OIVアウトブレイクが発生した学校で特定された。最も好発した症状は、発熱(患者の94%)、咳(92%)、咽喉炎(66%)であった。25%の患者は下痢を呈し、25%に嘔吐があった。入院の状況が判明した患者399名のうち、36名(9%)が入院を必要とした。データを有する22名の入院患者のうち、12名は季節性インフルエンザ重症化の高いリスクを示す特徴があり、11名は肺炎をきたし、8名は集中治療室での治療を要し、4名は呼吸不全を起こし、2名は死亡した。S-OIVは、今まで同定されていない固有のゲノム組成を持つと判断された。

結論:ブタ由来の新型インフルエンザA型ウイルスは、軽症から重症疾患まで引き起こす、発熱性呼吸器感染症アウトブレイクの原因として特定された。確認された症例数は、実際の発現症例よりも少ない可能性が高い。

使用上の注意記載状況・その他参考事項等

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血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
2009年4月以降、米国において急速に拡大したブタ由来の新型インフルエンザA型(H1N1)アウトブレイクの確定症例642例の報告である。 インフルエンザウイルスは脂質膜を持つRNAウイルスである。本剤によるインフルエンザウイルス感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているため、本剤の安全性は確保されていると考える。	日本赤十字社では、問診で発熱などの体調不良者を献血不適としている。更に、平成21年5月18日付薬食血発第0518001号「新型インフルエンザの国内発生に係る血液製剤の安全性確保について」に基づき、新型インフルエンザの患者又は罹患の疑いのある患者と7日以内に濃厚な接触があった人の献血を制限するほか、献血後に新型インフルエンザと診断された場合には当該血漿の使用を禁止している。新型インフルエンザが流行した場合、献血者減少につながることも予想されることから、今後も引き続き情報の収集に努める。

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Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans

Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team*

ABSTRACT

BACKGROUND

On April 15 and April 17, 2009, novel swine-origin influenza A (H1N1) virus (S-OIV) was identified in specimens obtained from two epidemiologically unlinked patients in the United States. The same strain of the virus was identified in Mexico, Canada, and elsewhere. We describe 642 confirmed cases of human S-OIV infection identified from the rapidly evolving U.S. outbreak.

METHODS

Enhanced surveillance was implemented in the United States for human infection with influenza A viruses that could not be subtyped. Specimens were sent to the Centers for Disease Control and Prevention for real-time reverse-transcriptase–polymerase-chain-reaction confirmatory testing for S-OIV.

RESULTS

From April 15 through May 5, a total of 642 confirmed cases of S-OIV infection were identified in 41 states. The ages of patients ranged from 3 months to 81 years; 60% of patients were 18 years of age or younger. Of patients with available data, 18% had recently traveled to Mexico, and 15% were identified from school outbreaks of S-OIV infection. The most common presenting symptoms were fever (94% of patients), cough (92%), and sore throat (66%); 25% of patients had diarrhea, and 25% had vomiting. Of the 399 patients for whom hospitalization status was known, 36 (9%) required hospitalization. Of 22 hospitalized patients with available data, 12 had characteristics that conferred an increased risk of severe seasonal influenza, 11 had pneumonia, 8 required admission to an intensive care unit, 4 had respiratory failure, and 2 died. The S-OIV was determined to have a unique genome composition that had not been identified previously.

CONCLUSIONS

A novel swine-origin influenza A virus was identified as the cause of outbreaks of febrile respiratory infection ranging from self-limited to severe illness. It is likely that the number of confirmed cases underestimates the number of cases that have occurred.

The members of the writing group (Fatimah S. Dawood, M.D., Epidemic Intelligence Service, Office of Workforce and Career Development, and Seema Jain, M.D., Lyn Finelli, Dr.P.H., Michael W. Shaw, Ph.D., Stephen Lindstrom, Ph.D., Rebecca J. Garten, Ph.D., Larisa V. Gubareva, M.D., Ph.D., Xiyan Xu, M.D., Carolyn B. Bridges, M.D., and Timothy M. Uyeki, M.D., M.P.H., M.P.P., Influenza Division, National Center for Immunization and Respiratory Diseases — all at the Centers for Disease Control and Prevention, Atlanta) assume responsibility for the overall content and integrity of the article. Address reprint requests to Dr. Dawood at the Influenza Division, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, MS A-32, Atlanta, GA 30333, or at fdawood@cdc.gov; or to Dr. Shaw at the Influenza Division, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, MS G-16, Atlanta, GA 30333, or at mshaw1@cdc.gov.

*The members of the Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team are listed in the Appendix.

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N Engl J Med 2009;360:2605-15.

TRIPLE-REASSORTANT SWINE INFLUENZA viruses, which contain genes from human, swine, and avian influenza A viruses, have been identified in swine in the United States since 1998,[1,2] and 12 cases of human infection with such viruses were identified in the United States from 2005 through 2009.[3] On April 15 and April 17, 2009, the Centers for Disease Control and Prevention (CDC) identified two cases of human infection with a swine-origin influenza A (H1N1) virus (S-OIV) characterized by a unique combination of gene segments that had not been identified among human or swine influenza A viruses. As of May 5, 2009, cases of human infection with the same novel virus have also been identified in Mexico, Canada, and elsewhere. We report the first 642 confirmed cases of human infection with this virus in the United States.

METHODS

PATIENTS IN OUTBREAK

On March 30, 2009, in San Diego County, California, a 10-year-old boy with asthma (Patient 1) had an onset of fever, cough, and vomiting. On April 1, he was evaluated in an urgent care clinic, where he received treatment for his symptoms. He recovered from the illness within approximately 1 week. An influenza A virus that could not be sub-typed was identified from a nasopharyngeal specimen that was collected from Patient 1 as part of a clinical trial to evaluate an experimental diagnostic test. As specified by the study protocol, the specimen was then sent to a reference laboratory for further testing and was found to be positive for influenza A virus but negative for both human H1 and H3 subtypes, with the use of real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) testing. On April 15, the CDC received the clinical specimen and identified a novel influenza A (H1N1) virus of swine origin. On the same day, the CDC notified the California Department of Public Health, and an epidemiologic investigation was initiated by state and local health department officials and animal health officials. A viral isolate was found to contain genes from triple-reassortant swine influenza viruses that were known to circulate among swine herds in North America and two genes encoding the neuraminidase and matrix proteins that were most closely related to genes of viruses obtained from ill pigs in Eurasia, according to results available in GenBank.

On March 28, 2009, in Imperial County, California, a 9-year-old girl (Patient 2) without an epidemiologic link to Patient 1 had an onset of cough and fever. Two days later, she was taken to an outpatient clinic that was participating in an influenza surveillance project. A nasopharyngeal swab was collected at the clinic. Patient 2 was treated with amoxicillin–clavulanate, and she had an uneventful recovery. The nasopharyngeal specimen was sent to the Naval Health Research Center in San Diego, where an influenza A virus that could not be subtyped was identified. The specimen was shipped to the CDC, where it was received on April 17, and a novel influenza A (H1N1) virus of swine origin was identified. The genotype of the virus was similar to that of the virus isolated from the sample obtained from Patient 1. On April 17, both cases were reported to the World Health Organization (WHO), according to the provisions of the International Health Regulations.

Epidemiologic investigation of Patients 1 and 2 revealed that neither patient had a recent history of exposure to swine. According to protocol, the identification of these two epidemiologically unlinked patients with novel S-OIV infection prompted the CDC to notify state and local health departments, which initiated case investigations and implemented enhanced surveillance for influenza A viruses that could not be subtyped. The CDC issued recommendations to clinicians, asking that they consider the diagnosis of S-OIV infection in patients with an acute febrile respiratory illness who met the following criteria: residence in an area where confirmed cases of human infection with S-OIV had been identified, a history of travel to such areas, or contact with ill persons from these areas in the 7 days before the onset of illness. If S-OIV infection was suspected in a patient, clinicians were asked to obtain a nasopharyngeal swab from the patient and to contact their state and local health departments in order to facilitate initial testing of the specimen by RT-PCR assay at the state public health laboratory. State public health laboratories were asked to send all specimens identified as influenza A viruses that could not be subtyped to the CDC for further investigation. Additional cases were identified with the use of a nationally standardized case definition of confirmed swine influenza A (H1N1) virus infection, which was defined as an acute febrile respiratory illness with the presence of S-OIV confirmed by real-time RT-PCR, viral culture, or both.

This report was exempt from the requirement for institutional review, and the privacy rule of the Health Insurance Portability and Accountability Act did not apply since it was a public health investigation.

REAL-TIME RT-PCR

The CDC has developed a real-time RT-PCR assay to detect seasonal influenza A, B, H1, H3, and avian H5 serotypes. This assay has been approved by the Food and Drug Administration (FDA) and was distributed in December 2008 through U.S. Public Health laboratories and the WHO's Global Influenza Surveillance Network. Primers and probes specific for swine influenza A (H1 and H3 subtypes) were recently developed and tested for use in a modified version of this assay for the detection of human infection with swine influenza viruses. These previously developed reagents allowed the CDC to quickly modify the existing assay for specific detection of S-OIV. Technical details on this assay have been published on the WHO Global Influenza Programme Web site at www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428.pdf.

NUCLEOTIDE SEQUENCING AND PHYLOGENETIC ANALYSIS

A total of 49 viral isolates from specimens obtained from patients with confirmed S-OIV infection in 13 states in the United States were grown in MDCK cell cultures. Amplicons for sequencing were generated by reverse transcription, followed by PCR amplification to generate overlapping double-stranded DNA amplicons covering each of eight segments of the influenza virus genome. Primers were designed to bind approximately every 200 to 250 nucleotides along the genome with degenerate bases to allow for sequence variation (for details, see the Supplementary Appendix, available with the full text of this article at NEJM.org).

Sequencing reactions were performed on a standard high-throughput sequencing system with the use of BigDye Terminator, version 3.1 (Applied Biosystems) with 1 mm³ of template double-stranded DNA. Sequence data were assembled and contiguous sequences were generated with the Sequencher software package, version 4.7 (Gene Codes). All sequence data that were used in this study are available from GenBank (see the Supplementary Appendix for details).

PHYLOGENETIC ANALYSIS

Phylogenetic trees were inferred with the use of the maximum-likelihood method in the GARLI 0.96b7 package. All phylogenetic analyses were visualized in TreeView, version 1.6.6.

RESULTS

PATIENTS

From April 15 through May 5, 2009, a total of 642 confirmed cases of human infection with the outbreak strain of S-OIV were identified in 41 states (Fig. 1 and 2). Cases of human infection with the outbreak strain of S-OIV were also reported in Mexico, Canada, and other countries.[4] Among 381 U.S. patients for whom data were available, 18% reported having traveled to Mexico within 7 days before the onset of illness; of these patients, 7 were subsequently hospitalized.

Four clusters of confirmed S-OIV infection were identified early in the investigation in schools and universities in South Carolina (7 students), Delaware (22 students), Texas (5 students), and New York (70 students, school staff, and contacts of students). Some students attending the school in New York where the cluster of confirmed cases occurred and who did not have confirmed infection were reported to have traveled to Mexico during the week preceding the cluster of illnesses. In addition to the confirmed cases that were identified in the four school outbreaks, respiratory illnesses for which samples were not obtained occurred among household and school contacts of patients with confirmed S-OIV infection.

DEMOGRAPHIC AND CLINICAL FEATURES

The age of patients with confirmed S-OIV infection ranged from 3 months to 81 years (Table 1). A total of 40% of patients were between the ages of 10 and 18 years, and only 5% of patients were 51 years of age or older. Among the patients for whom clinical information was available, the most common symptoms were fever (94%), cough (92%), and sore throat (66%). In addition, 25% of patients had diarrhea, and 25% had vomiting.

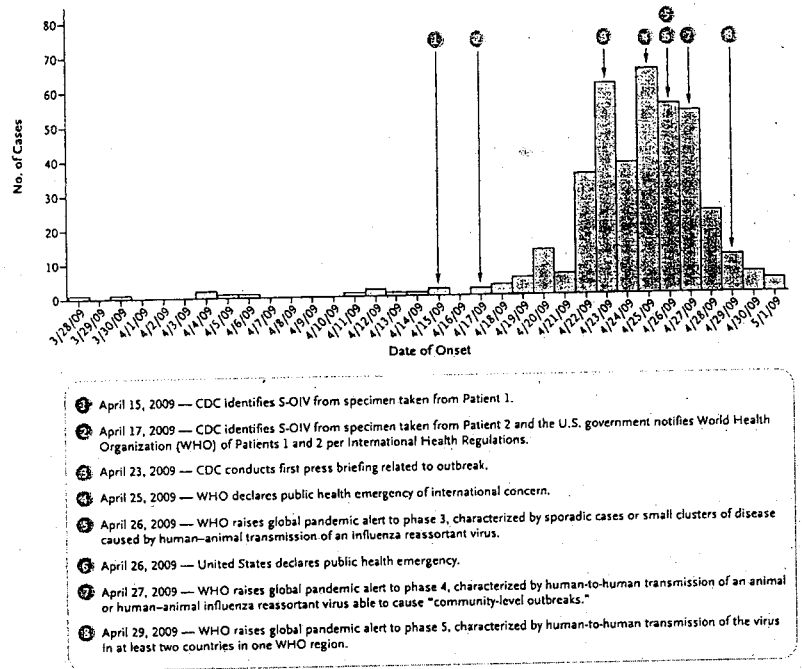

Figure 1. Epidemiologic Curve of Confirmed Cases of Human Infection with Swine-Origin Influenza A (H1N1) Virus with Known Date of Illness Onset in the United States (March 28–May 5, 2009).

Data regarding the date of onset of illness were available for 394 patients. This epidemiologic curve does not reflect all cases of infection with S-OIV from March 28 through May 5, 2009, because of the lag in case reporting and laboratory confirmation.

Of the 399 patients with confirmed S-OIV infection for whom hospitalization status was known, 36 (9%) required hospitalization. The age of hospitalized patients ranged from 19 months to 51 years. Of the 22 hospitalized patients for whom data were available, 4 (18%) were children under the age of 5 years, and 1 patient (4%) was pregnant. Nine patients (41%) had chronic medical conditions: a 41-year-old woman with autoimmune disease treated with multiple immunosuppressive agents; a 35-year-old man with Down's syndrome and a history of congenital heart disease; a 33-year-old woman who was 35 weeks pregnant and who had been in relatively good health with a history of mild asthma and psoriasis that were not being treated with medications; a 22-month-old child with a history of neonatal myasthenia gravis, a ventriculoseptal defect, swallowing dysfunction, and chronic hypoxia; and five patients with asthma alone. Seven patients (32%) reported having traveled to Mexico within 7 days before the onset of illness. Eleven patients (50%) had radiologically confirmed pneumonia, including one patient who had pneumomediastinum, one patient who had necrotizing pneumonia, and one patient who had an empyema that was surgically drained, with no growth from culture of empyema fluid. Eight patients (36%) required admission to an intensive care unit, and four patients (18%) had respiratory failure requiring

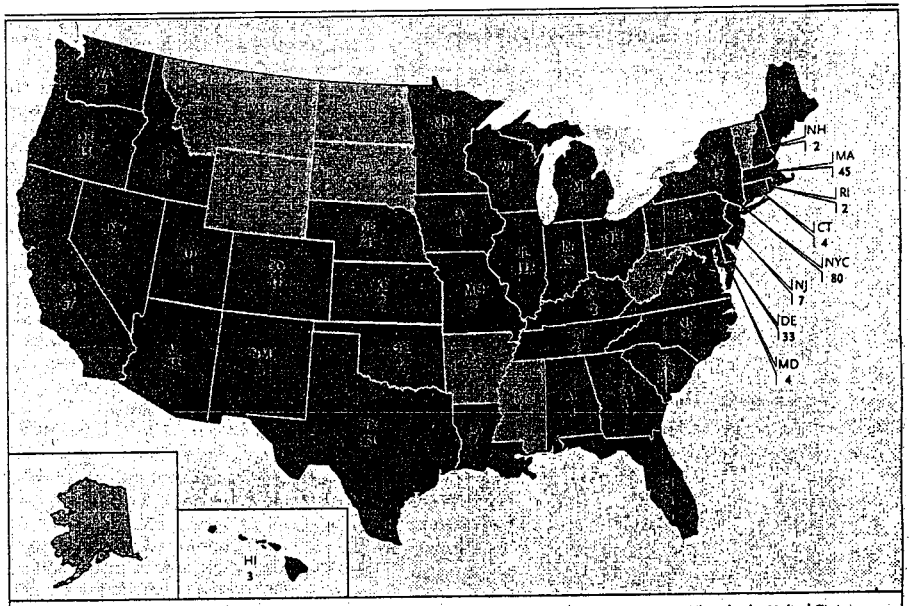

Figure 2. Distribution of 642 Confirmed Cases of Human Infection with Swine-Origin Influenza A (H1N1) Virus in the United States (May 5, 2009).

There were no cases in the District of Columbia. One case involving a resident of Kentucky occurred in Georgia.

mechanical ventilation. Fourteen patients (74%) were treated with oseltamivir after admission to the hospital. As of May 5, 18 of the 22 patients (82%) had recovered from the acute illness; 2 patients — a previously healthy 23-month-old child and a previously healthy 30-year-old woman — remained critically ill with respiratory failure, and the 22-month-old child with neonatal myasthenia gravis and the 33-year-old woman who was pregnant when she became ill died.

LABORATORY ANALYSES

Original clinical samples that were obtained from all 642 patients with confirmed infection and that were received by the CDC were tested with the use of real-time RT-PCR assays for swine influenza, and all the samples were confirmed to be positive for S-OIV. Among the 49 S-OIV isolates from 13 states in the United States that were sequenced at the CDC as of May 5, 2009, all were 99 to 100% identical in all genes. Phylogenetic analysis of sequences of all genes of A/California/04/2009, the virus isolated from Patient 1, showed that its genome contained six gene segments (PB2, PB1, PA, HA, NP, and NS) that were similar to ones previously found in triple-reassortant swine influenza viruses circulating in pigs in North America (Table 2). The genes encoding neuraminidase (*NA*) and M protein (*M*) were most closely related to those in influenza A viruses circulating in swine populations in Eurasia (Fig. 3). This particular genetic combination of influenza virus segments had not been seen before in the United States or elsewhere. Previous North American triple-reassortant swine influenza A (H1) viruses were known to be composed of the hemagglutinin (*HA*), nucleoprotein (*NP*), *NA*, *M*, and nonstructural protein (*NS*) genes, originating from classic swine influenza A viruses; the polymerase PB2 (*PB2*) and polymerase (*PA*) genes from avian influenza viruses from the North American lineage; and the polymerase PB1 (*PB1*) gene from human influenza A viruses.

Table 1. Characteristics and Symptoms of the 642 Patients with Confirmed Swine-Origin Influenza A (H1N1).

Characteristic	Value
Male sex — no./total no. (%)	302/592 (51)
Age	
Median — yr	20
Range — yr	3 mo to 81 yr
Age group — no./total no. (%)	
0–23 mo	14/532 (3)
2–4 yr	27/532 (5)
5–9 yr	65/532 (12)
10–18 yr	212/532 (40)
19–50 yr	187/532 (35)
≥51 yr	27/532 (5)
Student in school outbreak — no./total no. (%)	104/642 (16)
Recent history of travel to Mexico — no./total no. (%)*	68/381 (18)
Clinical symptoms — no./total no. (%)	
Fever	371/394 (94)
Cough	365/397 (92)
Sore throat	242/367 (66)
Diarrhea	82/323 (25)
Vomiting	74/295 (25)
Hospitalization — no./total no. (%)	
Total	36/399 (9)
Had infiltrate on chest radiograph	11/22 (50)
Admitted to intensive care unit	8/22 (36)
Had respiratory failure requiring mechanical ventilation	4/22 (18)
Treated with oseltamivir	14/19 (74)
Had full recovery	18/22 (82)
Vaccinated with influenza vaccine during 2008–2009 season	3/19 (16)
Died	2/36 (6)

* A recent history was defined as travel to Mexico no more than 7 days before the onset of illness.

Although the *HA* of S-OIV belongs to the same lineage as the gene found in recent human cases of triple-reassortant influenza A (H1) virus infection, the two genes differ by approximately 20 to 30 amino acids in the HA1 regions alone (Fig. 1 in the Supplementary Appendix). Among viral isolates from the current epidemic, there were up to five nucleotide changes resulting in four amino acid changes in *HA*.

The *NA* of S-OIV has the closest homology to the Eurasian lineage of swine influenza viruses, such as A/swine/Belgium/1/83 H1N1 (Fig. 2 in the Supplementary Appendix). In contrast, the H1N1 triple-reassortant swine influenza virus in the recent human infections contains *NA* from the North American swine lineage.[3] The *NA* genes from the Eurasian and North American swine influenza virus lineages are highly divergent, with more than 77 differences in amino acids between these lineages. There are two differences in nucleotides and one difference in amino acids between the viruses isolated from specimens taken from Patients 1 and 2. Data from both genetic sequencing and functional neuraminidase-inhibition assays indicate that all S-OIVs that have been examined are susceptible to both oseltamivir and zanamivir, two antiviral medications approved for the prevention and treatment of influenza in the United States (Table 3).

Like *NA*, the *M* gene of A/California/04/2009 has the closest homology to the *M* gene in the Eurasian lineage of swine influenza viruses (Fig. 3 in the Supplementary Appendix). Analyses of the *M* gene from all samples from the current epidemic showed a serine 31-to-asparagine mutation that confers resistance to M2 blockers (adamantanes), including amantadine and rimantadine. This phenotype is typical for recent Eurasian lineage swine influenza viruses but has not previously been seen in American swine viruses.

Sequences of the *PB1*, *PB2*, *PA*, *NP* (replication complex), and *NS* genes obtained from samples from the current epidemic have the closest homology to the genes in the swine influenza viruses that have been recently isolated in the United States from the North American swine lineage. These sequences were 99 to 100% identical at the amino acid level (data not shown; sequences are available from GenBank).

DISCUSSION

As of May 5, 2009, a total of 642 cases of human infection with a novel swine-origin influenza A (H1N1) virus have been identified in the United States, and additional cases have been identified in Mexico, Canada, and elsewhere.[4] On April 25, the WHO declared a public health emergency of international concern, and on April 26, the United States declared a public health emergency. On April 29, the WHO raised the pandemic influenza phase from 4 to 5, indicating that human-to-human transmission of the virus was occurring in at least two countries in one WHO region. The emergence of S-OIV infection among humans presents the greatest pandemic threat since the emergence of influenza A (H3N2) virus in 1968.

In the United States to date, most confirmed cases of S-OIV infection have been characterized by self-limited, uncomplicated febrile respiratory illness and symptoms similar to those of seasonal influenza (cough, sore throat, rhinorrhea, headache, and myalgia), but approximately 38% of cases have also involved vomiting or diarrhea, neither of which is typical of seasonal influenza. However, some patients have been hospitalized with more severe disease, and two patients have died. The observation that 60% of patients were 18 years of age or younger suggests that children and young adults may be more susceptible to S-OIV infection than are older persons or that because of differences in social networks, transmission to older persons has been delayed. It is also possible that elderly persons may have some level of cross-protection against S-OIV infection from preexisting antibodies against other influenza A (H1N1) viruses, as suggested by serologic studies of the 1976 swine influenza vaccine.[5,6] A potential case-ascertainment bias may also exist, with more young people being tested as part of outbreaks of S-OIV infection in schools[7] and fewer older persons being tested for influenza. However, the epidemic is evolving rapidly, and the number of confirmed cases is an underestimate of the number of cases that have occurred.

Continued identification of new cases in the United States and elsewhere indicates sustained human-to-human transmission of this novel influenza A virus. The modes of transmission of influenza viruses in humans, including S-OIV, are not known but are thought to occur mainly through the dissemination of large droplets and possibly small-particle droplet nuclei[8] expelled when an infected person coughs. There is also potential for transmission through contact with fomites that are contaminated with respiratory or gastrointestinal material.[9,10] Since many patients with S-OIV infection have had diarrhea, the potential for fecal viral shedding and subsequent fecal–oral transmission should be considered and investigated. Until further data are available, all potential routes of transmission and sources of viral shedding should be considered.

The incubation period for S-OIV infection appears to range from 2 to 7 days; however, addi-

Table 2. Phylogenetic Analysis of Sequences of all Genes Identified in A/California/04/2009.*

Gene	Nucleotide Length	NCBI Number	Strain	Lineage	Subtype	Identities	Additional Information
HA	1701	AF455600.1	A/Swine/Indiana/P12439/00	North American swine	H1N2	1621/1701 (95%)	
NA	1410	AJ412690.1	A/Swine/Belgium/1/83	Eurasian swine	H1N1	1302/1410 (92%)	
M	972	AJ293925.1	A/Hong Kong/1774/99	Eurasian swine	H3N2	945/972 (97%)	Human case of H3N2 Eurasian swine influenza
PB2	2264	EU3011777.2	A/swine/Korea/JNS06/2004	North American swine	H3N2	2186/2264 (96%)	
PB1	2274	AF342823.1	A/Wisconsin/10/98	North American swine	H1N1	2203/2274 (96%)	
PA	925	AF455717.1	A/Swine/North Carolina/93523/01	North American swine	H1N2	877/925 (94%)	
NP	1497	AF251415.2	A/Swine/Iowa/533/99	North American swine	H3N2	1449/1497 (96%)	
NS	838	AF153262.1	A/Swine/Minnesota/9088-2/98	North American swine	H3N2	809/838 (96%)	

* Data were derived from the Human Genome Project with the use of the Basic Local Alignment Search Tool (BLAST) algorithm (www.ncbi.nlm.nih.gov).

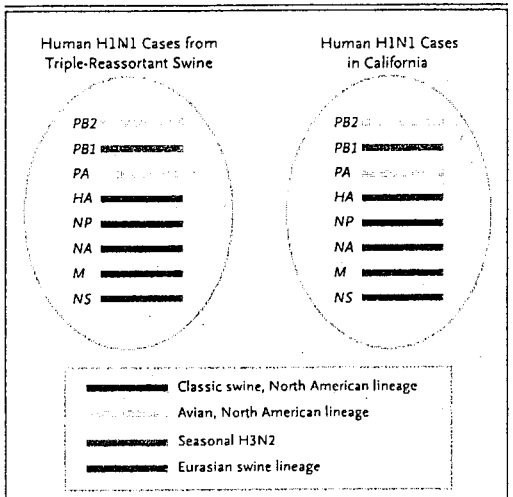

Figure 3. Comparison of H1N1 Swine Genotypes in Recent Cases in the United States.

The triple-reassortant strain was identified in specimens from patients with infection with triple-reassortant swine influenza viruses before the current epidemic of human infection with S-OIV. *HA* denotes the hemagglutinin gene, *M* the *M* protein gene, *NA* the neuraminidase gene, *NP* the nucleoprotein gene, *NS* the nonstructural protein gene, *PA* the polymerase PA gene, *PB1* the polymerase PB1 gene, and *PB2* the polymerase PB2 gene.

tional information is needed. On the basis of data regarding viral shedding from studies of seasonal influenza, most patients with S-OIV infection might shed virus from 1 day before the onset of symptoms through 5 to 7 days after the onset of symptoms or until symptoms resolve; in young children and in immunocompromised or severely ill patients, the infectious period might be longer.[11] Studies of viral shedding to define the infectious period are under way. The potential for persons with asymptomatic infection to be the source of infection to others is unknown but should be investigated.

The clinical spectrum of novel S-OIV infection is still being defined, but both self-limited illness and severe outcomes, including respiratory failure and death, have been observed among identified patients — a wide clinical spectrum similar to that seen among persons infected with earlier strains of swine-origin influenza viruses[3] and seasonal influenza viruses.[12] The severe illness and deaths associated with seasonal influenza epidemics are in large part the result of secondary complications, including primary viral pneumonia, secondary bacterial pneumonia (particularly with group A streptococcus, *Staphylococcus aureus*, and *Streptococcus pneumoniae*),[13-15] and exacerbations of underlying chronic conditions.[16] These same complications may occur with S-OIV infection. Patients who are at highest risk for severe complications of S-OIV infection are likely to include but may not be limited to groups at highest risk for severe seasonal influenza: children under the age of 5 years, adults 65 years of age or older, children and adults of any age with underlying chronic medical conditions, and pregnant women.[17,18] Of the 22 hospitalized patients with confirmed S-OIV infection who have been identified thus far and for whom data are available, 12 had characteristics (pregnancy, chronic medical conditions, or an age of less than 5 years) that conferred an increased risk of severe seasonal influenza, although none of the patients were 65 years of age or older.

Human infection with novel S-OIV emerged in the United States at a time when seasonal influenza A and B virus activity was decreasing. The cocirculation of human influenza A (H1N1) virus, influenza A (H3N2) virus, or influenza B virus in areas where human cases of S-OIV infection are being identified presents diagnostic and treatment challenges for clinicians. Clinicians should consider the diagnosis of S-OIV infection in patients with febrile respiratory illness seeking care in affected areas or in those who have traveled to affected areas. The CDC has developed a Swine Influenza Virus Real-Time RT-PCR Detection Panel. Under the Project Bioshield Act of 2004, the FDA has issued an emergency-use authorization, allowing for the use of this assay by state public health laboratories to respond to the current outbreak.[19] If S-OIV infection is suspected and diagnostic testing is indicated, clinicians should obtain a nasopharyngeal specimen, notify their local public health department, and arrange for specimens to be tested for S-OIV by Swine Influenza Virus Real-Time RT-PCR Detection Panel, according to local and state public health guidance and after consideration of local laboratory capacity for diagnostic testing.

Two classes of antiviral medication are available for the treatment of seasonal human influenza: neuraminidase inhibitors (oseltamivir and zanamivir) and adamantanes (rimantadine and amantadine). During the 2008–2009 influenza season, almost all circulating human influenza A (H1N1) viruses in the United States were resistant to oseltamivir.[20] However, genetic and phenotypic analyses indicate that S-OIV is susceptible to oseltamivir and zanamivir but resistant to the adamantanes.[21] At this time, the clinical effectiveness of antiviral treatment for S-OIV infection is unknown. As of May 5, 2009, the CDC has recommended that given the severity of illness observed among some patients with S-OIV infection, therapy with neuraminidase inhibitors should be prioritized for hospitalized patients with suspected or confirmed S-OIV infection and for patients who are at high risk for complications from seasonal influenza. As recommendations are updated, they will be posted on the CDC's Web site at www.cdc.gov/h1n1flu/recommendations.htm. The FDA has issued an emergency-use authorization that approves the use of oseltamivir to treat influenza in infants under the age of 1 year (treatment that is normally approved for those 1 year of age or older) and for chemoprophylaxis in infants older than 3 months of age (chemoprophylaxis that is normally approved for children 1 year of age or older).[19]

Prevention and control measures for S-OIV are based on our understanding of seasonal human influenza[22] and consideration of potential modes of transmission. As of May 5, 2009, the CDC has recommended that health care workers who provide direct care for patients with known or suspected S-OIV infection should observe contact and droplet precautions, including the use of gowns, gloves, eye protection, face masks, and fit-tested, disposable N95 respirators. In addition, patients with confirmed or suspected S-OIV infection should be placed in a single-patient room with the door kept closed, and airborne-infection isolation rooms with negative-pressure handling should be used whenever an aerosol-generating procedure is being performed. Frequent hand washing with soap and water may reduce the risk of infection and transmission.[23] As recommendations are updated, they will be posted at www.cdc.gov/h1n1flu/guidelines_infection_control.htm. Because the novel S-OIV strain is antigenically distinct from the influenza A (H1N1) strain represented in the 2008–2009 influenza vaccine, seasonal influenza vaccination during the 2008–2009 influenza season is not anticipated to provide protection against novel S-OIV infection. A strain of S-OIV has been identified as a potential egg-derived candidate strain for S-OIV vaccine development and has been sent to partner laboratories for evaluation and further development.

Given the rapidly evolving nature of this outbreak, the CDC's recommendations are likely to change as more information becomes available. Clinicians are advised to monitor the H1N1 Influenza Center (NEJM.org) and the CDC Web site (www.cdc.gov/h1n1flu/) for changes in guidance for testing, treatment, and infection control.

In conclusion, we report an outbreak of human infection with a novel influenza A (H1N1) virus of swine origin in the United States, which is spreading through sustained human-to-human transmission in multiple countries. The identification of human S-OIV infection in geographically dispersed countries and across continents demonstrates the ease with which infection can be spread and facilitated by air and land travel and community networks and gatherings. As enhanced surveillance for S-OIV infection is implemented globally, additional cases are expected to be identified. The cases of infection with S-OIV described in this report may provide guidance for clinicians with respect to presenting symptoms and outcomes of infection with this novel virus.

Table 3. Susceptibility of 37 Isolates of Swine-Origin Influenza A (H1N1) Virus to Neuraminidase Inhibitors.*

Variable	Oseltamivir		Zanamivir	
	IC_{50}	R/S	IC_{50}	R/S
	nM		*nM*	
Mean	0.57	S	0.59	S
Median	0.54		0.59	
Seasonal control				
Known susceptibility	0.63	S	0.60	S
Known resistance	265.27	R	1.27	S

* Susceptibility was analyzed with the use of chemiluminescent neuraminidase inhibition assay with the NAStar Kit (Applied Biosystems). IC_{50} denotes inhibitory concentration of 50%, R resistant, and S susceptible.

No potential conflict of interest relevant to this article was reported.

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the CDC.

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APPENDIX

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CLINICAL TRIAL REGISTRATION

The *Journal* requires investigators to register their clinical trials in a public trials registry. The members of the International Committee of Medical Journal Editors (ICMJE) will consider most clinical trials for publication only if they have been registered (see N Engl J Med 2004;351:1250-1). Current information on requirements and appropriate registries is available at www.icmje.org/faq.pdf.

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医薬品
医薬部外品　研究報告　調査報告書
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一般的名称	乾燥人フィブリノゲン		研究報告の公表状況	THE CANADIAN PRESS/2009/09/16	公表国 カナダ	
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研究報告の概要

オーストラリアの研究者は、ブタインフルエンザに感染する人々の一部が生命を脅かす疾患にかかる理由について手掛かりを発見した可能性がある。それが正しい場合、パンデミックの死亡者を減らす助けになる治療の武器が存在する。グループは、新型H1N1ウイルスで重症になった妊婦がウイルスを退けて、身体がワクチンに反応するのを助けることが知られている特定の抗体が低レベルにあることを発見した。

このチームは、Dr. Claire Gordonが抗体レベル（クラスだけではなくサブクラスまで見る検査）をオーダーした時に発見を実現した。サブタイプの低下が特に急速だった重症患者が現れた際にチームが召集され、免疫グロブリン（提供された血液から得られる抗体含有血液製剤）が役に立つかどうか検討した。試験は入院患者がIgG2抗体レベルが低いことを示した。彼らはICUの全てのブタインフルエンザ患者の検査を指示し始めた。「ICUが必要な全ての患者でIgG2が不十分であることを発見した」と、彼はサンフランシスコからのインタビューで言った。データはICAAC（米国微生物学会年次会議）で示された。

重症例は、軽症だった人々で検出される約3分の1のIgG2濃度であった。

2～20%の人々がいくつかの抗体欠損があるが、それらの人々全員がIgG2欠損であるわけではないことが知られている。危篤状態であった患者の4人のうち、3人が免疫グロブリン治療で生存した。

グループの仕事はまだ仮説を証明していないものの、ブタインフルエンザ患者を看護している北半球の医師はIgG2レベルを確認して、細菌感染症でしばしば重病の人々に与えられる免疫グロブリンを使用することを考慮すべきであるとチームリーダーのDr. Graysonは語った。

報告企業の意見	今後の対応
新聞情報ではあるが、オーストラリアの研究者がブタインフルエンザに感染する人々のIgG2抗体レベル低いと重篤になる可能性があり、治療に免疫グロブリンが有用である可能性があることを示唆する報告である。 インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程にて十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えるものではないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人フィブリノゲンを濃縮・精製した製剤であり、ウイルス不活化を目的として、製造工程においてリン酸トリ-n-ブチル(TNBP)／ポリソルベート80処理、ウイルス除去膜によるろ過処理、凍結乾燥の後、60℃、72時間の加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

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Print Story: Low levels of key antibodies may lead to severe disease, study suggests on Yahoo! ... 1/3 ページ

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THE CANADIAN PRESS

Low levels of key antibodies may lead to severe disease, study suggests

Wed Sep 16, 6:09 PM

By Helen Branswell, Medical Reporter, The Canadian Press

TORONTO - Australian researchers may have uncovered a clue as to why some people who catch swine flu suffi life-threatening illness.

And if they are right, there is an existing weapon in the treatment arsenal that could help reduce the pandemic death toll. The group found that pregnant women who became severely ill with the new H1N1 virus had low leve of a particular antibody that is known to fight off viruses and help the body respond to vaccine.

Moderately ill women were much less likely to have significantly suppressed levels of the antibody, the researchers reported. "We all believe we may have stumbled onto something very interesting," said Dr. Lindsay Grayson, director of infectious disease at Austin Health, a network of three hospitals in Melbourne.

"To our knowledge it's the first time that a correlation or an association is being noted between severe influenza o any sort and a subtle but potentially important immune deficiency."

The team made the discovery when Grayson's colleague, Dr. Claire Gordon, ordered a test that looked at antibody levels - not just by class, but looking at individual subtypes within those classes. The call was made in the case of a very sick patient whose decline was particularly rapid, and the team was debating whether immune globulin - a blood product containing antibodies harvested from donated blood - might help.

The testing showed the patient had low levels of an antibody called IgG2, which Grayson admitted came as a surprise. They started ordering tests on all their swine flu patients in ICU.

"What we found was almost everyone, all the patients who needed ICU were IgG2 deficient," he said in an interview from San Francisco, where the data were presented at ICAAC, the annual meeting of the American Society for Microbiology. Severe cases had IgG2 levels that were about one-third of those detected in people who were moderately ill.

While the work was only done in pregnant women, Grayson and others said it would be useful to look to see if this deficiency might explain why a small subset of swine flu cases become gravely ill while most people only suffer through a bout of the flu.

It's known that between two and 20 per cent of people have some antibody deficiency, he said, though not all of those people would be IgG2 deficient.

Three of four critically ill patients treated with immune globulin survived, defying predictions of those caring for them.

Dr. Donald Low, chief microbiologist at Toronto's Mount Sinai, said the findings are exciting, if preliminary, and

http://ca.news.yahoo.com/s/capress/090916/national/flu_severity_clue?printer=1　134　2009/09/18

might explain why aboriginals seem to be at greater risk of developing severe disease if they contract swine flu. He suggested the hypothesis should be studied further.

"It would be a fishing expedition, but obviously worthwhile." "I think the bottom line is that this is obviously something that has to be looked into.

And it may have therapeutic implication. ... It could be a marker for women at higher risk if they get infected to get more severe disease."

But Dr. Anand Kumar, an intensive care specialist from Winnipeg who treated a lot of severely ill swine flu patients in the spring and early summer, was not as optimistic.

"The results are just what I'd expect in any group of critically ill," he said by email. Kumar, who is also an infectious diseases specialist, said it is not uncommon for all antibody levels to drop with critical illness and the more severe the sickness, the steeper the drop.

But he does think the notion of treating pandemic flu patients with antibodies harvested from other people makes sense, though he believes the immune globulin should be from people who've recovered from swine flu and have antibodies specific to the virus.

Grayson admitted they can't say at this point whether there is a cause-and-effect relationship at work here, meaning low IgG2 levels in the patients predisposed them to suffering from more severe disease once they caught the virus.

But he doesn't believe the reverse is at play, that the infection caused the low IgG2 levels.

"We don't think that influenza is causing this deficiency. We think that instead the influenza is picking out those people who have the deficiency," he said.

The numbers are admittedly small and will require further study, likely in the Northern Hemisphere. Swine flu rates are dropping in Melbourne, Grayson said.

Still, 16 of 19 severely ill patients had very low IgG2 levels, compared to three of 20 with moderate illness.

The team looked at healthy pregnant women and found that about 60 per cent of them were mildly deficient in IgG2 levels, which leads them to believe this may be one of the immune system changes that occurs to allow a pregnant woman to carry a foreign body - a fetus - without rejecting it. But Grayson said the group needs to follow women after they deliver to see if their IgG2 levels rise to normal levels.

Grayson said while the group's work hasn't proven their hypothesis, Northern Hemisphere doctors caring for the sickest of swine flu patients in the weeks and months to come should consider checking IgG2 levels and using immune globulin, which is often given to people seriously ill with some bacterial infections.

"In many ways, this is applying a general principle that we apply to bacteria diseases to now say well, 'Gee, we've made this interesting observation. This might work for influenza,"' he said.

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Follow Canadian Press Medical Writer Helen Branswell's flu updates on Twitter at CP-Branswell

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医薬品
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識別番号・報告回数			報告日	第一報入手日 2009年7月24日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①ポリエチレングリコール処理人免疫グロブリン ②人免疫グロブリン		研究報告の公表状況	CDC/MMWR 58(28);773-778/2009/07/24	公表国 アメリカ	
販売名（企業名）	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②グロブリン-Wf（ベネシス）					

研究報告の概要

てんかん発作、脳炎、脳症、ライ症候群と他の神経学的障害を含む神経学的合併症は、季節性インフルエンザAまたはBウイルスの気道感染に関連していることは以前に報告されているが、新型インフルエンザA（H1N1）ウイルスでは報告されていなかった。
2009年5月28日、保健社会福祉省（DCHHS）は新型インフルエンザA（H1N1）ウイルス感染症と関連した神経学的合併症を発症し5月18日～5月28日にかけてテキサス州ダラスの病院に入院した小児4人について CDC に報告した。
この報告は、それら4人の症例の臨床的特徴をまとめたものである。
患者は7歳、10歳、11歳、17歳でインフルエンザ様疾患（ILI）とてんかん発作の徴候、精神状態の変化が認められた。4人の患者のうち3人は、脳波図（EEG）の異常を示した。
4人の患者全てにおいて、鼻咽頭検体から新型インフルエンザA（H1N1）ウイルスRNAが検出されたが、脳脊髄液（CSF）では検出されなかった。
抗ウイルス薬療法は、オセルタミビル（4人の患者）とリマンタジン（3人の患者）であった。
4人全ての患者は、完全に回復し、退院後、神経学的後遺症は見られなかった。
これらの所見は、新型インフルエンザA（H1N1）ウイルスによる気道感染の後でも季節性インフルエンザと同様に神経学的合併症が発現することがあることを示している。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びろ過膜処理（ナノフィルトレーション）及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
新型インフルエンザA（H1N1）ウイルスについても、季節性インフルエンザと同様に神経学的合併症が発現し得るとの報告である。 インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程にて十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えるものではないと考えるので、特段の措置はとらない。

(21)

CDC MMWR Weekly
July 24, 2009 / 58(28);773-778

Neurologic Complications Associated with Novel Influenza A (H1N1) Virus Infection in Children -- Dallas, Texas, May 2009

Neurologic complications, including seizures, encephalitis, encephalopathy, Reye syndrome, and other neurologic disorders, have been described previously in association with respiratory tract infection with seasonal influenza A or B viruses (*1--2*), but not with novel influenza A (H1N1) virus. On May 28, 2009, the Dallas County Department of Health and Human Services (DCHHS) notified CDC of four children with neurologic complications associated with novel influenza A (H1N1) virus infection admitted to hospitals in Dallas County, Texas, during May 18--28. This report summarizes the clinical characteristics of those four cases. Patients were aged 7--17 years and were admitted with signs of influenza-like illness (ILI) and seizures or altered mental status. Three of the four patients had abnormal electroencephalograms (EEGs). In all four patients, novel influenza A (H1N1) viral RNA was detected in nasopharyngeal specimens but not in cerebrospinal fluid (CSF). Antiviral therapy included oseltamivir (four patients) and rimantadine (three patients). All four patients recovered fully and had no neurologic sequelae at discharge. These findings indicate that, as with seasonal influenza, neurologic complications can occur after respiratory tract infection with novel influenza A (H1N1) virus. For children who have ILI accompanied by unexplained seizures or mental status changes, clinicians should consider acute seasonal influenza or novel influenza A (H1N1) virus infection in the differential diagnosis, send respiratory specimens for appropriate diagnostic testing, and promptly initiate empirical antiviral treatment, especially in hospitalized patients.

Case Identification

Since April 22, DCHHS has requested all hospitals in Dallas County to report details concerning patients admitted with novel influenza A (H1N1) virus infection. As of July 20, DCHHS had identified 405 persons with laboratory-confirmed novel influenza A (H1N1) virus infection in the greater Dallas area, including 44 hospitalized patients. No deaths had been reported. Of confirmed novel influenza A (H1N1) virus infections, 83% were in patients aged <18 years. Among these pediatric cases, 145 children, including 26 who were hospitalized, were identified through the Children's Medical Center of Dallas (CMCD) laboratory-based surveillance program. Medical records from admission and discharge for all hospitalized H1N1 patients are routinely screened by DCHHS epidemiology staff. Characteristics of hospitalized patients are compiled on an ongoing basis, with further investigation of cases noted to have unusual features and severe illness.

A patient with acute neurologic complications associated with novel influenza A (H1N1) virus infection was defined as having laboratory-confirmed novel influenza A (H1N1) virus infection of the respiratory tract associated with seizures, encephalopathy, or encephalitis within 5 days of ILI symptom onset, without evidence of an alternative etiology. Encephalopathy was defined as

altered mental status lasting ≥24 hours. Encephalitis was defined as encephalopathy plus two or more of the following: fever ≥100.4°F (≥38.0°C), focal neurologic signs, CSF pleocytosis, EEG indicative of encephalitis, or abnormal neuroimaging indicative of infection or inflammation (*1--2*).

During April 22--July 20, seven possible cases of neurologic complications associated with novel A (H1N1) virus infection were identified. Three cases were excluded because the neurologic complications were determined to have alternative etiologies (e.g., hypocalcemia and apnea related to prematurity) or did not meet the case definition (e.g., altered mental status for <24 hours). Of the remaining four cases described in this report, one patient (patient A) was initially reported by a community hospital in Dallas on May 18. The three other cases were reported by CMCD to DCHHS during May 23--27. No additional cases had been reported in Dallas County through July 20.

Nasopharyngeal swab specimens collected from all three patients admitted to CMCD were tested for influenza A and B antigens by either Directigen EZ Flu A+B rapid enzyme immunoassay (EIA) (BD [Becton, Dickinson, and Company], Sparks, Maryland), QuickVue Influenza A+B test (EIA) (Quidel, San Diego, California), or D3 Ultra direct fluorescent assay (Diagnostic Hybrids, Athens, Ohio). All positive specimens were sent to DCHHS, and novel influenza A (H1N1) virus was identified by real-time reverse transcription--polymerase chain reaction (rRT-PCR) using CDC-approved primers and probe sets. All CSF samples were tested at CDC using rRT-PCR for influenza, enteroviruses, parechovirus, adenovirus, and human parainfluenza virus serotype 3. CSF for patients B and D were tested for additional viruses by a commercial laboratory (Viracor).*

Case Reports

Patient A. On May 17, a previously healthy black male aged 17 years visited a community hospital emergency department after 1 day of fever reaching 102.6°F (39.2°C), cough, headache, dizziness, and weakness. Influenza A was diagnosed by EIA, and the patient was discharged home with a prescription for oseltamivir. The patient was admitted the next day to another community hospital because of increased generalized weakness, disorientation to place, and markedly slow and intermittent responsiveness to questions. On physical examination, the patient was noted to be confused and unable to provide history of his own illness. He also was unable to lift his arms above his shoulders or stand. He had taken 1 dose of oseltamivir the morning of admission. A computed tomography (CT) head scan revealed pan-sinusitis, and CSF was normal (Table). The patient received ceftriaxone for 2 days, which was discontinued when CSF bacterial cultures indicated no growth. He received oseltamivir throughout his hospital admission. His mental status returned to normal on day three. He was discharged on day four with no apparent sequelae and completed a 5-day total course of oseltamivir.

Patient B. On May 23, a previously healthy Hispanic male aged 10 years was taken to a Dallas community hospital via emergency medical services after a 3-minute generalized tonic-clonic seizure and subsequent postictal mental state. The seizure occurred after 4 days of fever reaching 104.0°F (40.0°C), cough, decreased appetite, and fatigue. His family reported that the patient had contact with another child with ILI symptoms before the patient's illness onset. Upon initial evaluation in the emergency department, the patient was afebrile. A chest radiograph revealed a left lower lobe infiltrate, and a CT head scan was normal except for an incidentally noted single punctuate calcification in left frontal cortex. Influenza A was detected in a nasopharyngeal swab specimen by EIA. Three hours later, the patient had a second 3-minute generalized seizure. Intravenous (IV) lorazepam and ceftriaxone were administered, and the patient was transferred to a CMCD intensive-care unit.

On admission to CMCD, the patient was febrile, confused, and drowsy. He had difficulty

answering questions and made frequent inappropriate attempts to get out of bed. CSF analysis was normal. He was administered IV fosphenytoin to prevent additional seizures, vancomycin and ceftriaxone for empirical treatment of bacterial pneumonia, supplemental oxygen via bilevel positive airway pressure for oxygen saturations <92%, and anticonvulsants. Over the ensuing 2 days, he had intermittent fevers reaching 102.0°F (38.9°C). On hospital day four, he had a prolonged partial complex seizure with focal onset (eye deviation to the right) and secondary generalization, lasting 30--40 minutes, which eventually was controlled by 4 doses of IV lorazepam and a bolus of IV fosphenytoin. Oseltamivir and rimantadine were initiated. Brain magnetic resonance imaging (MRI) with magnetic resonance angiography was normal, and an EEG was consistent with encephalopathy (Table). His mental status returned slowly to baseline by hospital day seven, when he was discharged without apparent sequelae to continue levetiracetam, amoxicillin, and clindamycin, and complete a 5-day course of oseltamivir.

Patient C. On May 26, a white male aged 7 years with a history of a simple febrile seizure 1 year previously was taken to a Dallas community hospital via emergency medical services after a seizure and 2 days of cough, nasal congestion, and fatigue. On the day of admission, he had been found at home on the floor, with tonic movements of his upper and lower extremities lasting at least 2 minutes. On admission to the community hospital, he was noted to have postictal drowsiness and a temperature of 100.8°F (38.2°C). A diagnosis of influenza A was made by EIA. Blood tests, CSF, and a CT head scan were normal (Table).

The patient was transferred the same day to CMCD, where he exhibited normal mental status and no fever or seizures. A brain MRI showed nonspecific white matter abnormalities not characteristic of infection or inflammation. Localized cerebral dysfunction was evident on EEG (Table). Oseltamivir and rimantadine were started on hospital day one, and the patient was discharged on hospital day three without any neurologic sequelae, to complete a 5-day course of both antivirals and to continue levetiracetam until reassessment by neurologists in 3 months.

Patient D. On May 27, a black male aged 11 years with a history of asthma was taken to CMCD because of 1 day of fever and vomiting. A household contact, his grandmother, had an upper respiratory infection 3 days before his illness. One day before admission, he had a fever of 102.0° F (38.9°C), fatigue, headache, abdominal pain, and vomiting, and was given bismuth subsalicylate twice and one 81 mg aspirin. At CMCD, he was febrile. Neurologic examination revealed ataxia. Soon after admission, the patient had a seizure consisting of episodic eye rolling and tongue thrusting. An EIA test for influenza A was positive, and oseltamivir, rimantadine, cefotaxime, and acyclovir were initiated.

During the first 2 hospital days, the patient was disoriented, had visual hallucinations, had difficulty responding to questions and following commands, had slow speech, and required supplemental oxygen via facemask for mild hypoxia and hypopnea attributed to decreased respiratory drive associated with encephalopathy. Chest radiograph was normal. An EEG was consistent with encephalopathy, and a CT head scan was normal (Table). The patient's mental status returned to normal by hospital day four. He completed a 5-day course of oseltamivir.

Reported by: *AS Evans, MD, S Agadi, MD, JD Siegel, MD, Univ of Texas Southwestern Medical Center; WM Chung, MD, JT Carlo, MD, Dallas County Health and Human Svcs, Dallas, Texas. TM Uyeki, MD, J Sejvar, MD, S Lindstrom, PhD, D Erdman, DrPH, S Oberste, PhD, National Center for Immunization and Respiratory Diseases; SJ Olsen, PhD, Div of Emerging Infections and Surveillance Svcs, National Center for Preparedness, Detection, and Control of Infectious Diseases; F Dawood, MD, OW Morgan, PhD, EIS officers, CDC.*

Editorial Note: Infection with seasonal influenza virus can be associated with neurologic complications (*1–2*), but the frequency with which these occur with novel influenza A (H1N1) virus infection is unknown. This is the first report describing patients with neurologic

complications associated with novel influenza A (H1N1) virus infection. The severity of the neurologic disease in the four patients described in this report was less than the typical disease described in two studies of neurologic complications associated with seasonal influenza (*1--2*), which included reports of severe static encephalopathy and death. Only two of the four patients described in this report had seizures, and none died or had neurologic sequelae at discharge. Considering that clusters of influenza-associated encephalopathy in children have been reported during previous community outbreaks of seasonal influenza (*1--2*) and that children appear to be infected with novel influenza A (H1N1) virus more frequently than adults (*3*), additional neurologic complications in children are likely to be reported as the pandemic continues. Clinicians should consider influenza associated encephalopathy in the differential diagnosis of children with ILI and seizures or mental status changes, and remain aware of the potential for severe neurologic sequelae associated with seasonal or novel influenza A (H1N1) virus infection.

Neurologic complications in children associated with seasonal influenza have included acute cognitive and behavioral problems, focal neurologic deficits, and death from neurologic complications (*4*). Influenza-associated neurologic complications are estimated to account for up to 5% of cases of acute childhood encephalitis or encephalopathy (*4*) and were reported in 6% of influenza-associated deaths among children during one influenza season (2003--04) in the United States (*5*). The epidemiology of influenza-associated encephalopathy has been described extensively in Japan, where incidence has appeared to be higher than in other countries (*1*). In Japan, approximately 80% of influenza-associated encephalopathy cases occur in children aged <5 years (*1,6*), and neurologic signs typically develop within 1--2 days of influenza symptom onset (*1,6*). Manifestations have included seizures, altered consciousness, incoherence, irritability, and psychotic behaviors (*1,6*). Outcomes reported in one case-series from Japan ranged from complete resolution (in nearly 50% of cases), to mild (20%) or severe neurologic sequelae (10%), to death (20%) (*6*).

Neuroimaging results in influenza-associated encephalopathy might be normal, but in severe cases, abnormalities can include diffuse cerebral edema and bilateral thalamic lesions. EEG might show diffuse abnormalities (*1,2,4*). Only rarely is influenza virus detected in CSF, suggesting that neurologic manifestations might be an indirect effect of influenza respiratory tract infection (*2,7*).

For patients with respiratory illness and neurologic signs, diagnostic testing for possible etiologic pathogens associated with neurologic disease, including influenza viruses, is recommended (*8*). Health-care providers also should consider a diagnosis of Reye syndrome in patients with viral illness and altered mental status. Although one of the patients described in this report, patient D, received a salicylate-containing product and aspirin, no evidence of Reye syndrome was observed. Salicylates and salicylate-containing products should not be administered to children with influenza or other viral infections because of the increased risk for developing Reye syndrome (*9*).

Antiviral treatment should be initiated as soon as possible for any hospitalized patient with neurologic symptoms and suspected seasonal influenza or novel influenza A (H1N1) virus infection (2).† Although respiratory specimens should be obtained for appropriate diagnostic testing before administering antiviral agents, clinicians should not wait for the results before beginning treatment. Antiviral medications have been shown to decrease the risk for complications from influenza (*10*); however, the effectiveness of antiviral treatment to prevent influenza-associated encephalopathy sequelae is unknown. Clinicians also should send respiratory specimens for appropriate diagnostic testing. Although no vaccination against novel influenza A (H1N1) virus is available currently, CDC recommends that all children aged >6 months receive annual seasonal influenza vaccination to prevent illness and complications from infection with seasonal influenza virus strains.§

Acknowledgments

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* Viruses detected by the Luminex multiplex respiratory viral panel [xTAG] are influenza A and B; parainfluenza 1, 2, and 3; respiratory syncytial virus A and B; adenovirus; human metapneumovirus; and rhinovirus.

† CDC guidance on antiviral therapy available at http://www.cdc.gov/h1n1flu/recommendations.htm.

§ CDC recommendations for seasonal influenza vaccination available at http://www.cdc.gov/mmwr/pdf/rr/rr5707.pdf.

TABLE. Selected characteristics and laboratory, radiologic, and neurodiagnostic results for four patients with neurologic complications associated with novel influenza A (H1N1) virus infection* — Dallas, Texas, May 2009

Characteristic	Patient A	Patient B	Patient C	Patient D
Age (yrs)	17	10	7	11
Sex	Male	Male	Male	Male
Race/Ethnicity	Black, non-Hispanic	Hispanic	White, non-Hispanic	Black, non-Hispanic
Dates of hospitalization	May 18--21	May 23--29	May 26--28	May 27--30

Neurologic complication(s) diagnosed	Encephalopathy	Seizures, encephalopathy	Seizures	Encephalopathy
Interval from respiratory illness onset to neurologic symptoms (days)	1	4	2	1
Fever (maximum temperature)	102.6°F (39.2°C)	104.0°F (40.0°C)	100.8°F (38.2° C)	102.0°F (38.9°C)
Admission laboratory data				
Serum electrolytes, chemistry	Normal (except initial creatinine 1.3 mg/dL [normal range for age: 0.3--1.0 mg/dL])	Normal	Normal (except sodium 131 mmol/L [normal range: 134--146 mmol/L])	Normal
Liver function tests (U/L)	ND†	AST§ 28, ALT¶ 51, GGT** 29	AST 36, ALT 12, GGT 29	AST 41, ALT 27, GGT <10, ammonia 28 mmol/L (repeat testing normal)
Blood bacterial culture	ND	*S. epidermidis*, Micrococcus (contaminants), no growth x2	No growth	No growth
Urine bacterial culture	ND	ND	ND	No growth
Other	Creatine kinase 75 U/L (normal range: 22--269 U/L)	Urine toxicology screen positive for benzodiazepines only	---	Urine toxicology screen positive for caffeine, salicylate, and acetaminophen; serum salicylate level <1 mg/dL
Cerebrospinal fluid (CSF) analysis				
WBC†† (per mm3) (differential)	2 (differential ND)	2 (65%L 31%M)	4 (differential ND)	4 (95%L 5%M)
RBC§§ (per mm3)	18	0	2	1
Glucose (mg/dL) (normal range: 50--80 mg/dL)	39	63	58	65
Protein (mg/dL) (normal range: 10--45 mg/dL)	37	50	15	21
Bacterial culture	No growth	No growth	No growth	No growth
Neurodiagnostic testing				
Computed tomography	No intra-parenchymal abnormality; pan-sinusitis	Single punctuate calcification in left frontal cortex	No intracranial abnormality	No intracranial abnormality; sphenoid sinusitis
			Cortical nonspecific	

Magnetic resonance imaging	ND	No parenchymal abnormality	scattered T2 hyperintense foci within the cerebral white matter	No intracranial abnormality
Electroencephalogram	ND	Generalized continuous polymorphic delta slowing, without epileptogenic focus; consistent with mild/moderate encephalopathy	Midline parietal intermittent polymorphic delta slowing, without epileptogenic focus; consistent with localized cerebral dysfunction	Posterior background slowing, no epileptiform activity; consistent with mild encephalopathy
Viral testing and antiviral therapy				
Influenza EIA¶¶	Positive***	Positive	Positive	Positive
Influenza DFA†††	ND	ND	ND	Positive
CSF influenza rRT-PCR§§§	Negative	Negative	Negative	Negative
rRT-PCR	Enteroviruses: negative	Enteroviruses: negative	Enteroviruses: negative	Enteroviruses: negative
	Parechovirus: negative	Parechovirus: negative	Parechovirus: negative	Parechovirus: negative
	Adenovirus: negative	Adenovirus: negative	Adenovirus: negative	Adenovirus: negative
	HPIV-3¶¶¶: negative	HPIV-3: negative	HPIV-3: negative	HPIV-3: negative

TABLE. (Continued) Selected characteristics and laboratory, radiologic, and neurodiagnostic results for four patients with neurologic complications associated with novel influenza A (H1N1) virus infection — Dallas, Texas, May 2009

Characteristic	Patient A	Patient B	Patient C	Patient D
				HSV†††† rRT-PCR: negative
Other testing	ND	CSF respiratory viral panel (RVP)****	ND	Enterovirus rRT-PCR: negative
				CSF RVP: negative
Antiviral therapy	Oseltamivir	Oseltamivir and rimantadine	Oseltamivir and rimantadine	Oseltamivir and rimantadine

* A patient with acute neurologic complications associated with novel influenza A (H1N1) virus infection was defined as having laboratory-confirmed novel influenza A (H1N1) virus infection of the respiratory tract associated with seizures, encephalopathy, or encephalitis within 5 days of influenza-like illness symptom onset, without evidence of an alternative etiology. Encephalopathy was defined as altered mental status lasting ≥24 hours. Encephalitis was defined as encephalopathy plus two or more of the following: fever ≥100.4°F (≥38.0°C), focal

neurologic signs, cerebrospinal fluid pleocytosis, an electroencephalogram indicative of encephalitis, or abnormal neuroimaging indicative of infection or inflammation.

† Not done.

§ Aspartate transaminases (normal range: 10--45 U/L).

¶ Alanine aminotransferase (normal range: 10--50 U/L).

** Gamma glutamyltranspeptidase (normal range: 3--30 U/L).

†† White blood cell count.

§§ Red blood cell count.

¶¶ Enzyme immunoassay. All four patients had nasopharyngeal specimens obtained and tested for influenza A and B antigen by using Directigen EZ Flu A+B (EIA), QuickVue Influenza A+B test (EIA), or direct fluorescent assay using D3 Ultra.

*** All four patients' nasopharyngeal specimens were confirmed positive for novel influenza A (H1N1) virus by Dallas County Department of Health and Human Services, using CDC-approved primers and probes.

††† Direct fluorescent assay.

§§§ Real-time reverse--transcription polymerase chain reaction (performed at CDC).

¶¶¶ Human parainfluenza virus type 3.

**** CSF viral PCR testing was performed by Viracor, using the Luminex multiplex respiratory viral panel (xTAG), which tests for 10 different viruses (influenza A and B; parainfluenza 1, 2, and 3; respiratory synctial virus A and B; adenovirus; human metapneumovirus; and rhinovirus).

†††† Herpes simplex virus.

Morbidity and Mortality Weekly Report
Centers for Disease Control and Prevention
1600 Clifton Rd, MailStop E-90, Atlanta, GA
30333, U.S.A

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2009 年 4 月 14 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Swine Influenza - Advice for Veterinarians and Swine Producers. http://www.inspection.gc.ca/english/anima/disemala/swigri/swigrifse.shtml	公表国 カナダ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
カナダ食品検査庁（ＣＦＩＡ）はブタインフルエンザのヒトへの感染に関する報告を発表した。 カナダ食品カナダ食品検査庁（ＣＦＩＡ）はアメリカ南部およびメキシコでブタインフルエンザのヒトへの感染を発表すると共に要請に応じて支援や専門的知識の提供を行っている。 また，ヒトーヒト感染経路によるブタインフルエンザ感染が発生していた可能性があると指摘している。 これまでカナダにおけるブタの感染や死亡が増加している兆候は認められていないが，予防策としてＣＦＩＡは養豚業者，獣医および研究所にブタ疾患の監視や報告といった体制を強化するよう要請している。またブタインフルエンザ感染が疑われるブタが認められた場合は獣医，地域の保健局或いはＣＦＩＡに報告するよう要請している。同時に，カナダ公衆衛生局（ＰＨＡＣ）は重篤なインフルエンザ様症状が出現した場合には医療機関に連絡するよう勧告している。	BYL-2009-0374 New England Journal of Medicine 360 2605-2615 The Lancet Infectious Disease 9; 339-340, 2009 http://ec.europa.eu/food/animal/diseases/influenzaAH1N1/docs/Conclusions_AH1N1_090609.pdf http://www.who.int/mediacentre/news/statements/2009/h1n1_pandemic_phase6_20090611/en/index.html http://wwwn.cdc.gov/travel/content/outbreak-notice/novel-h1n1-flu-global-situation.aspx

報告企業の意見	今後の対応
本製品に使用されている原材料の原産国外でのウイルス感染発症の報告である。 ウイルス病原体はエンベロープウイルスであり，本製剤の製造工程におけるウイルス除去・不活化工程は，エンベロープウイルスに対しては効果的である。 したがって，本報告は本製剤の安全性に大きな影響を与えるものではないと考える。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

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Canadian Food Inspection Agency - Animal Health - Fact Sheet - Swine Influenza　1/2 ページ

BYL-2009-0374

Canada

Canadian Food Inspection Agency　Agence canadienne d'inspection des aliments

Animals > Animal Diseases > Swine Influenza

Swine Influenza - Advice for Veterinarians and Swine Producers

The Canadian Food Inspection Agency (CFIA) has been notified of cases of human swine influenza (swine flu) in the southern United States and Mexico. Information to date indicates that human-to-human transmission of the virus has occurred. The Public Health Agency of Canada (PHAC) is currently coordinating the Canadian response to this situation, and the CFIA is providing support and expertise as required. For more information, visit http://www.phac-aspc.gc.ca.

At this point, there are no signs of increased disease or death in Canadian swine. However, as a precaution, the CFIA is asking producers, veterinarians and labs to increase their vigilance in monitoring for and reporting swine disease. Suspected cases of illness in pigs should be reported to veterinarians, provincial authorities or the CFIA. Similarly, PHAC recommends that anyone who is experiencing severe flu-like symptoms contact their health care provider.

What is swine influenza?

Swine influenza is a contagious respiratory disease of pigs. The disease is commonly seen in North and South America, Asia and Europe. Illness is cause by type A Influenza viruses, which also affect a range of other animals, as well as humans.

Are humans affected by swine influenza?

Yes, but human cases of swine influenza are normally uncommon. Most often, cases involve people who have had close contact with pigs, such as farmers and veterinarians. Some cases of human-to-human transmission have been reported. Symptoms of human illness are similar to regular flu: cough, nausea, body aches, fatigue, runny nose and congestion.

Although the risk of human illness is low, anyone having contact with pigs or potentially contaminated equipment should thoroughly wash their hands and limit contact with possibly infected pigs.

Swine, avian and human influenza viruses can combine within pig cells to form new influenza viruses. Flu-like symptoms in swine or people that may have had contact with swine should be reported to animal or public health professionals. Doing so will allow health authorities to maintain a current understanding of the viruses circulating in the animal and human populations.

What are the symptoms in pigs?

Signs of swine influenza include the following:

- fever
- loss of appetite
- weight loss
- coughing
- sneezing
- nasal discharge
- difficulty breathing

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http://www.inspection.gc.ca/english/anima/disemala/swigri/swigrifse.shtml　2009/04/27

- reduced fertility or abortion

Swine influenza generally does not lead to death, and affected animals usually recover within five to seven days.

How do pigs become infected?

Normally, virus spreads when infected pigs cough or sneeze in close quarters with other pigs. Contaminated equipment or other objects may also play a role in transmitting virus. Influenza virus from birds and humans can also infect pigs.

How can pigs be protected?

The following actions can potentially prevent swine influenza:

- vaccinating animals
- ensuring farm working maintain good hygiene
- following strict biosecurity practices
- providing adequate ventilation in barns
- identifying and segregating sick animals as early as possible

What roles do veterinarians and producers play?

Veterinarians should work closely with clients to develop management strategies to limit the incidence and spread of swine influenza. As part of this approach, veterinarians suffering from the "flu" should limit contact with pigs, and farm workers should follow similar advice. Given the current situation, particular caution should be exercised with visitors to farms, especially those who may have recently returned from the southern United States or Mexico.

Does swine influenza affect food safety?

No, swine influenza is not a food safety concern.

For additional information: www.inspection.gc.ca

Date modified: 2009-04-26

別紙様式第2-1

No. 13

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		総合機構処理欄
				2009. 7. 21	該当なし		
一般的名称	人血清アルブミン		研究報告の公表状況	Shimada T, Gu Y, Kamiya H, Komiya N, Odaira F, Sunagawa T, Takahashi H, Toyokawa T, Tsuchihashi Y, Yasui Y, Tada Y, Okabe N. Euro Surveill. 2009 Jun 18;14(24). pii: 19244.	公表国		
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)				日本		使用上の注意記載状況・その他参考事項等 赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/50mL 血液を原料とすることに由来する感染症伝播等

研究報告の概要

○日本におけるインフルエンザA型(H1N1)ウイルス感染の疫学:2009年5月～6月

2009年5月9日～6月4日の期間中、日本の16の都道府県から、インフルエンザA型(H1N1)ウイルス確定症例が合計401例報告された。最も感染の多かった2地域は、高校でアウトブレイクが発生し休校に至った大阪府と神戸市であった。報告時(2009年6月18日)において、いずれの症例の症状も季節性インフルエンザ症状と同様であり、重症または死亡症例は報告されていない。

報告企業の意見

2009年5月9日～6月4日の期間中、日本における新型インフルエンザ(H1N1)確定症例が合計401例報告され、報告時(2009年6月18日)において重症または死亡症例はなかったとの報告である。

インフルエンザウイルスは脂質膜を持つRNAウイルスである。本剤によるインフルエンザウイルス感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているため、本剤の安全性は確保されていると考える。

今後の対応

日本赤十字社では、問診で発熱などの体調不良者を献血不適としている。更に、平成21年5月18日付薬食血発第0518001号「新型インフルエンザの国内発生に係る血液製剤の安全性確保について」に基づき、新型インフルエンザの患者又は罹患の疑いのある患者と7日以内に濃厚な接触があった人の献血を制限するほか、献血後に新型インフルエンザと診断された場合には当該血漿の使用を禁止している。新型インフルエンザが流行した場合、献血者減少につながることも予想されることから、今後も引き続き情報の収集に努める。

(23)

Rapid communications

Epidemiology of influenza A(H1N1)v virus infection in Japan, May - June 2009

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Between 9 May and 4 June 2009, a total of 401 laboratory-confirmed cases of influenza A(H1N1)v virus were reported in Japan, from 16 of the 47 Japanese prefectures. The two areas most affected were Osaka prefecture and Kobe city where outbreaks in high schools occurred leading to school closures. To date all cases have had symptoms consistent with seasonal influenza and no severe or fatal cases have been reported.

Following the emergence of a new influenza A(H1N1) virus (henceforth: influenza A(H1N1)v virus) and the relevant declarations by the World Health Organization (WHO) [1], the Ministry of Health, Labour and Welfare (MHLW) of Japan launched a case-based surveillance for influenza A(H1N1)v virus infection in addition to the existing sentinel surveillance system for seasonal influenza and imposed entry screening on travelers from affected areas (Canada, Mexico and the United States) starting from 28 April 2009 [2].

The following case definitions of suspected and confirmed cases have been used:

A **suspected case** of influenza A(H1N1)v virus infection is defined as a person with high fever (>38°C) OR at least two acute respiratory symptoms (nasal obstruction/rhinorrhea, sore throat, cough, fever/feverishness) AND who meets at least one of the following criteria:
a) within the last seven days returned from a country or region with an epidemic of influenza A(H1N1)v;
b) was in close contact (within two meters) with a confirmed case within the past seven days;
c) handled samples suspected of containing influenza A(H1N1)v virus in a laboratory or other setting within the past seven days;

A **confirmed case** of influenza A(H1N1)v virus infection is defined as a person with high fever (>38°C) OR at least two acute respiratory symptoms (nasal obstruction/rhinorrhea, sore throat, cough, fever/feverishness) AND influenza A(H1N1)v virus infection that has been laboratory confirmed by real-time PCR and/or viral isolation.

For all travellers from the affected areas who are febrile at the entry, a quarantine officer performs a rapid diagnostic test for influenza. If the result of rapid test is positive for influenza A, a PCR test for influenza A(H1N1)v is done. The Quarantine Law and the Pandemic Influenza Preparedness Action Plan of the Japanese Government request confirmed cases and close contacts of confirmed cases to be hospitalised/isolated for seven days considered to be the infectious period [3,4].

The primers for conventional and real-time RT-PCR for the detection of A(H1N1)v virus were developed by the National Institute of Infectious Diseases and became available on 29 April. All 75 prefectural and municipal public health institutes and quarantine stations in Japan became ready to perform conventional and real-time RT-PCR test by 4 May. Since the first laboratory-confirmed cases were reported on 9 May, the number of cases of influenza A(H1N1)v increased continuously, resulting in a total of 401 laboratory-confirmed cases as of 4 June 2009. This report summarises the epidemiological characteristics of the confirmed cases reported in Japan from May to June.

The first four laboratory-confirmed cases of influenza A(H1N1)v were reported at the Narita International Airport quarantine station on 9 May 2009. The patients were travellers who returned from Canada on 9 May. Although all of them showed mild symptoms, they were hospitalised in an isolation ward of a designated hospital for seven days, in accordance with the Quarantine Law and the Pandemic Influenza Preparedness Action Plan of the Japanese Government [3,4].

The first laboratory-confirmed cases without travel history were detected on 16 May as follows:

A high school in Ibaraki city, in Osaka prefecture near the border with Hyogo prefecture, noticed an increase in the number of absentees due to influenza-like symptoms in the middle of May 2009. On 16 May the school was closed in conformity with the School Health Law [5]. According to this law (enacted in 1958), influenza-like illness/seasonal influenza is one the infectious diseases that can trigger school closure. The number of absentees that leads to school closure is decided by the school authorities. In many cases, 5 to 10 absentees in a class may lead to closing the class; 2-3 closed classes may lead to school closure.

None of the sick high school pupils in Ibaraki had travel history to the countries affected by the new influenza. On 16 May, five teenagers were confirmed with influenza A(H1N1)v virus infection: one from the school in Ibaraki in Osaka prefecture, and four from Kobe City in the neighbouring Hyogo prefecture. Subsequently, outbreaks in three schools were reported during the next few days in these adjacent prefectures. The local governments of Kobe City and Osaka prefecture implemented extensive school closures, deciding to close not only schools with infected students but all schools in both districts, for one to two weeks from 16 May. As a result, over

4,200 schools with around 650,000 children/students were closed. By 19 May, the number of confirmed cases reported in the two districts reached 172. However, after school closures, the number of new confirmed cases decreased (Figure 1). By 4 June a total of 357 cases were reported from the two prefectures.

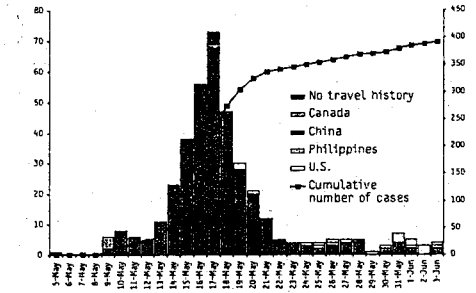
Outside these two prefectures only sporadic cases were reported, the majority of whom had a travel history abroad or an epidemiological link to a traveller from affected areas including Osaka (Figure 2). In all, confirmed cases were reported from 16 of the total of 47 Japanese prefectures.

Reflecting the outbreaks in high schools described above, confirmed cases in the age group of 15-19 years accounted for 64% (256) of all cases, followed by 10% (40) of cases in the age group of 10-14 years. Only four cases (1%) were over 60 years of age (Figure 3). Overall, the median age of cases was 16.0 (range 1-69 years). Male cases accounted for 63% (254) and female cases for 37% (147) of all cases. Large outbreaks observed in high schools may have contributed to the difference in gender (as more boys than girls attend the affected schools).

Information on clinical symptoms was available for 217 confirmed cases (Figure 4). The most frequent were fever (206, 95%), cough (128, 59%), and sore throat (85, 39%). Thirteen cases (6%) reported diarrhoea and five cases (2%) had nausea.

Figure 1

Confirmed cases of influenza A(H1N1)v virus infection in Japan, by date of onset and cumulative number as of 4 June 2009 (n=392*)



* Nine cases without the record of onset of illness were excluded

Figure 2

Geographical distribution of confirmed cases of influenza A(H1N1)v virus infection in Japan as of 4 June 2009 (n=401)

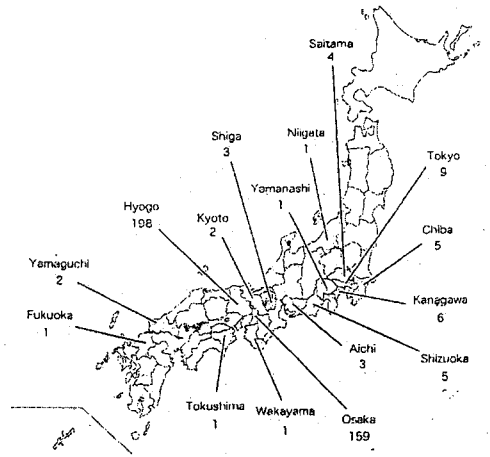


Figure 3

Age distribution of confirmed cases of influenza A(H1N1)v virus infection in Japan as of 4 June 2009 (n=401)

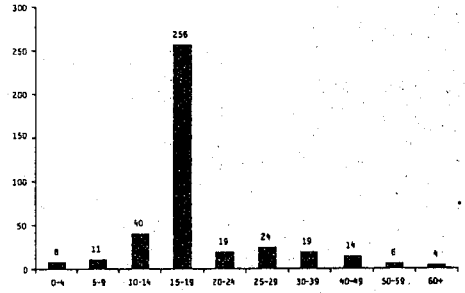
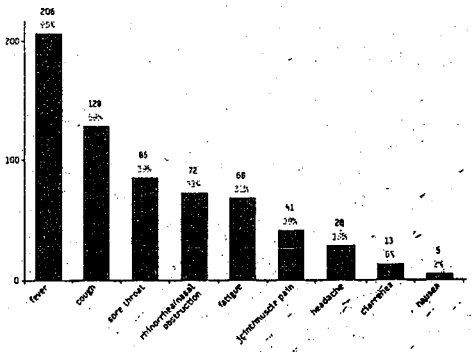


Figure 4

Clinical symptoms of confirmed cases of influenza A(H1N1)v virus infection in Japan as of 4 June 2009 (n=217)



Antiviral treatment of either oseltamivir or zanamivir was prescribed to about 90% of the 217 confirmed cases with known clinical symptoms.

No cases with pneumonia and/or respiratory failure, requiring ventilatory support, were reported. Other severe symptoms such as multiple organ failure were not reported either. Only three cases required hospitalisation due to underlying medical conditions, although a total of 135 cases were hospitalised for the purpose of isolation based on the Quarantine Law and the Pandemic Influenza Preparedness Action Plan of the Japanese Government [3,4].

Among the confirmed cases, six (including two cases aged over 60 years) had underlying diseases: asthma (3), asbestosis (1), epilepsia (1), myodystrophia (1); and one case was pregnant. As of 4 June 2009, no severe or fatal case had been reported.

The epidemiological characteristics of the patients with influenza A(H1N1)v virus infection have been reported by the investigation teams including members of IDSC/NIID and local government, who conclude that the severity of disease is similar to that of seasonal influenza [6,7].

The next steps include addressing the questions of how to improve the surveillance system to detect, monitor, and control the cases of influenza A (H1N1)v and how to prepare for the more severe cases as the epidemic is expected to expand in the winter season. We need to decide when the case-based surveillance for influenza A(H1N1)v should be ceased and integrated into the sentinel surveillance of seasonal influenza. To evaluate the pathogenicity, planned surveillance systems, such as severe pneumonia surveillance and ILI cluster surveillance, should be launched before the coming winter season. The Pandemic Influenza Preparedness Action Plan of the Japanese Government also needs to be amended so that medical resources would not be wasted by the patients with mild symptoms merely for the purpose of isolation.

Acknowledgement

We thank Dr Yamashita, Dr Morikane, Dr Shigematsu, Dr Taya, Dr Yahata, Ms Otake and Ms Maeda for their review and support.

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Citation style for this article: Shimada T, Gu Y, Kamiya H, Komiya N, Odaira F, Sunagawa T, Takahashi H, Toyokawa T, Tsuchihashi Y, Yasui Y, Tada Y, Okabe N. Epidemiology of influenza A(H1N1)v virus infection in Japan, May - June 2009. Euro Surveill. 2009;14(24):pii=19244. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19244

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-4

識別番号・報告回数		回	報告日 年 月 日	第一報入手日 2009 年 7 月 6 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称				Tamiful resistance, Denmark http://www.promedmail.org/pls/otn/f?p=2400:1001:52145918594326::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1004,78150	公表国	
販売名（企業名）		研究報告の公表状況			英国	
研究報告の概要	豚インフルエンザのパンデミックに対する主要薬剤である Tamiflu [oseltamivir]に耐性を示す初めての症例が報告された。Roche Holding AG 社は，デンマークで Tamiflu に耐性を示す新型インフルエンザ（H1N1）患者例を確認した。同社役員の David Reddy 氏によると，季節性インフルエンザでも同様の事例は生じ得るため予想外の事ではないと述べている。今回の症例は Tamiflu を服用していた豚インフルエンザ患者であった。同氏は，市中に Tamiflu 耐性の H1N1 株が蔓延している兆候ではないことを強調した。				使用上の注意記載状況・その他参考事項等	
					BYL-2009-0390	
					http://www.promedmail.org/pls/otn/f?p=2400:1001:737050559495972 5::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1010,78237	
報告企業の意見		今後の対応			http://www.promedmail.org/pls/otn/f?p=2400:1001:737050559495972 5::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1010,78236	
今回，初めて Oseltamivir 耐性の新型インフルエンザ(H1N1)の症例が発表された。この後，日本および中国においても同様の Oseltamivir 耐性インフルエンザが確認された。しかしながら，これらの耐性インフルエンザウイルスは散発性の発生にとどまっていると考えられる。新型インフルエンザ治療において は Oseltamivir が非常に重要な位置を占めているが，今後同様の耐性ウイルスのことを考慮し，Zanamivir の重要性も増し，同薬剤の備蓄に関しての対策も必要となってくると考えられる。		現時点で新たな安全対策上の措置を講じる必要はないと考える。今後も，ヒト感染症の急激な伝播拡大やそのような感染症に関する薬剤耐性の情報収集に努める。			

24

Archive Number 20090630.2359
Published Date 30-JUN-2009
Subject PRO/AH/EDR> Influenza A (H1N1) - worldwide (78): Tamiflu resistance, DK

INFLUENZA A (H1N1) - WORLDWIDE (78): TAMIFLU RESISTANCE, DENMARK
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Mon 29 Jun 2009
Source: BBC News [edited]
<http://news.bbc.co.uk/1/hi/health/8124987.stm>

Experts have reported the 1st case of swine flu that is resistant to Tamiflu [oseltamivir], the main drug being used to fight the pandemic. Roche Holding AG confirmed a patient with H1N1 influenza in Denmark showed resistance to the antiviral drug. David Reddy, company executive, said it was not unexpected given that common seasonal flu could do the same.

The news comes as a 9 year old girl has become the 3rd to die in the UK with swine flu. It is understood from her doctors at Birmingham Children's Hospital that she had underlying health conditions. It is not yet known whether swine flu contributed to her death.

Meanwhile, the Department of Health has announced a big jump in the number of patients in England confirmed with swine flu, up 1604 since Friday [26 Jun 2009], taking the UK total so far to 5937. A Health Protection Agency spokeswoman stated that: "Routine sampling in the UK has shown that there is currently no resistance to oseltamivir or zanamivir." Experts have been using Tamiflu, also known as oseltamivir, in a bid to stop the H1N1 spreading in communities. If taken early, it ensures that symptoms are mild and reduces the chance of a victim giving the illness to someone else.

This 1st reported case of resistance developed in a swine flu patient taking Tamiflu. Mr Reddy stressed that there were no signs of a Tamiflu-resistant strain of H1N1 circulating in the community. This is in contrast to seasonal H1N1 flu, where a Tamiflu resistant strain emerged last year [2009] and is now widely circulating. Experts fear if this were to happen, it could render Tamiflu ineffective [in treatment of the swine flu H1N1 virus infection].

Another antiviral drug, called zanamivir or Relenza, made by GlaxoSmithKline, is also effective against swine flu. The UK government has been stockpiling these antiviral drugs and currently has enough to treat half of the population, with a contract to bring that up to 80 per cent as soon as possible. Supplies of flu vaccine have also been ordered, and the 1st doses could be administered in the autumn [2009].

A spokeswoman for the Health Protection Agency said: "The Health Protection Agency continues to watch for antiviral resistance and will be carrying out regular sample testing throughout this outbreak. We have been monitoring antiviral drug resistance since the beginning of this outbreak. Routine sampling in the UK has shown that there is currently no resistance to oseltamivir or zanamivir." Virologist Professor John Oxford said: "I'm not

surprised about this finding. The question is whether it is going to spread. We will soon know the answer."

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communicated by:
ProMED-mail <promed@promedmail.org>

[According to the European Influenza Surveillance Scheme Weekly Electronic Bulletin of 26 Jun 2009 (<http://www.euroflu.org/>), all but one pandemic A(H1N1) viruses tested have been sensitive to oseltamivir and zanamivir but resistant to M2 inhibitors, although widespread (98 per cent) Tamiflu
resistance has been observed in seasonal A(H1N1) viruses. [see posting Influenza A (H1N1) - worldwide (83): antiviral resistance 20090705.2417] The emergence of Tamiflu-resistant 209 swine-origin A H1N1 influenza virus is not unexpected in view of the widespread and somewhat indiscriminate use of the drug in the treatment of what is still a relatively mild disease. It remains to be seen whether the Tamiflu-resistant virus will spread in Europe and beyond and appear
independently elsewhere. It is presumed that the Tamiflu-resistant virus isolated in Denmark remains sensitive to the alternate neuraminidase inhibiter Relenza. - Mod.CP]

[see also:
Influenza A (H1N1) - worldwide (77): case count 20090627.2338
Influenza A (H1N1) - worldwide (76): comments on 1918 virus (03) 20090625.2309
Influenza A (H1N1) - worldwide (74): susp. origin 20090624.2303
Influenza A (H1N1) - worldwide (73): case count, epidemiology 20090622.2288
Influenza A (H1N1) - worldwide (72): case count, epidemiology 20090619.2261
Influenza A (H1N1) - worldwide (70): risk factors 20090619.2260
Influenza A (H1N1) - worldwide (69): other viral infections 20090618.2254
Influenza A (H1N1) - worldwide (68): southern hemisphere 20090618.2253
Influenza A (H1N1) - worldwide (65): antivirals in pregnancy 20090616.2224
Influenza A (H1N1) - worldwide (64): case count, pandemic 20090616.2221
Influenza A (H1N1) - worldwide (62): Egypt, Lebanon 20090611.2150
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Influenza A (H1N1) - worldwide (60): Egypt (Cairo) 20090608.2117
Influenza A (H1N1) - worldwide (59): Worldwide 20060608.2117
Influenza A (H1N1) - worldwide (58): USA, Africa 20090607.2109
Influenza A (H1N1) - worldwide (57): Brazil, USA 20090605.2090
Influenza A (H1N1) - worldwide (55) 20090603.2056
Influenza A (H1N1) - worldwide (47): China, epidemiology 20090526.1962
Influenza A (H1N1) - worldwide (45) 20090525.1951
Influenza A (H1N1) - worldwide (42) 20090523.1929
Influenza A (H1N1) - worldwide (39) 20090521.1903
Influenza A (H1N1) - worldwide (37) 20090520.1893
Influenza A (H1N1) - worldwide (34) 20090518.1863
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Influenza A (H1N1) - worldwide (29) 20090515.1824
Influenza A (H1N1) - worldwide (26) 20090514.1798
Influenza A (H1N1) - worldwide (23) 20090511.1764
Influenza A (H1N1) - worldwide (21) 20090510.1749
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Influenza A (H1N1) - worldwide (17) 20090508.1722
Influenza (H1N1) - worldwide (15) 20090507.1709
Influenza A (H1N1) - worldwide (13) 20090506.1695
Influenza A (H1N1) - worldwide (11): coincident H3N2 variation 20090505.1679
Influenza A (H1N1) - worldwide (09) 20090504.1673
Influenza A (H1N1) - worldwide (07) 20090503.1658
Influenza A (H1N1) - worldwide (05) 20090503.1657
Influenza A (H1N1) - worldwide (03) 20090501.1646
Influenza A (H1N1) - worldwide (02): case counts 20090430.1638
Influenza A (H1N1) - worldwide 20090430.1636
Influenza A (H1N1) "swine flu": worldwide (07), update, pandemic 5 20090429.1622
Influenza A (H1N1) "swine flu": worldwide (06) 20090428.1614

Influenza A (H1N1) "swine flu": worldwide ../ 20090428.1601
Influenza A (H1N1) "swine flu": worldwide (03) 20090428.1600
Influenza A (H1N1) "swine flu": Worldwide (02) 20090427.1586
Influenza A (H1N1) "swine flu": Worldwide 20090427.1583
Influenza A (H1N1) virus, human: worldwide 20090426.1577
Influenza A (H1N1) virus, human - New Zealand, susp 20090426.1574
Influenza A (H1N1) virus, human - N America (04) 20090426.1569
Influenza A (H1N1) virus, human - N America (03) 20090426.1566
Influenza A (H1N1) virus, human - N America (02) 20090425.1557
Influenza A (H1N1) virus, human - N America 20090425.1552
Acute respiratory disease - Mexico, swine virus susp 20090424.1546
Influenza A (H1N1) virus, swine, human - USA (02): (CA, TX) 20090424.1541
Influenza A (H1N1) virus, swine, human - USA: (CA) 20090422.1516
Influenza A (H1N1) virus, swine, human - Spain 20090220.0715]

.....cp/msp/sh

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-5

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2009 年 7 月 6 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	World now at the start of 2009 influenza pandemic http://www.who.int/mediacentre/news/statements/2009/h1n1_pandemic_phase6_20090611/en/index.html	公表国 スイス	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
WHO 事務局長 Chan 氏は，今回のこのインフルエンザの感染の拡大は，現在までの知見や専門家等が評価した結果から，科学的な観点で，インフルエンザパンデミックの基準を満たしたことが判明し，この事実に基づいて感染のフェーズを５から６に引き上げる事としたと表明した。一方で，感染の広がりはフェーズ６ではあるが，重症度としては，中等度と位置づけている。各国に対しては，感染の第二波に備えるよう強く要望を出すとともに，このインフルエンザ感染への対応として，感染症例がまだ確認されていない或は少数確認されているにとどまっている国では監視の継続を求め，既に感染が拡大している国においては感染症患者への適切な管理に力を注ぐべきであることを求めている。また，ヒトや物の移動制限や国境閉鎖は推奨しないと表明している。 さらに，WHO はインフルエンザワクチン製造業者に対し，季節性インフルエンザワクチンの製造が間もなく完了する事から，その後はこの新型インフルエンザに対するワクチンを，全力を挙げて製造するよう要望している。	BYL-2009-0391

報告企業の意見	今後の対応
現在，伝播が拡大した新型インフルエンザ(H1N1)の流行に対し，最大の流行を示すフェーズ６と判定，宣言された。本インフルエンザは多くは重症化しない傾向があるが，感染に備えたワクチンの確保が要求される。また，インフルエンザ治療薬である Oseltamivir や Zanamivir の確保にも努める必要がある。	引き続き，新型インフルエンザ感染について，さらに健康を脅かす情報に注視し，情報の収集に努める。

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BYL-2009-0391

World Health Organization

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Statement to the press by WHO Director-General Dr Margaret Chan
11 June 2009

World now at the start of 2009 influenza pandemic

Dr Margaret Chan
Director-General of the World Health Organization

Ladies and gentlemen,

In late April, WHO announced the emergence of a novel influenza A virus.

This particular H1N1 strain has not circulated previously in humans. The virus is entirely new.

The virus is contagious, spreading easily from one person to another, and from one country to another.
As of today, nearly 30,000 confirmed cases have been reported in 74 countries.

This is only part of the picture. With few exceptions, countries with large numbers of cases are those with good surveillance and testing procedures in place.

Spread in several countries can no longer be traced to clearly-defined chains of human-to-human transmission. Further spread is considered inevitable.

I have conferred with leading influenza experts, virologists, and public health officials. In line with procedures set out in the International Health Regulations, I have sought guidance and advice from the Emergency Committee established for this purpose.

On the basis of available evidence, and these expert assessments of the evidence, the scientific criteria for an influenza pandemic have been met.

I have therefore decided to raise the level of influenza pandemic alert from phase 5 to phase 6.

The world is now at the start of the 2009 influenza pandemic.

We are in the earliest days of the pandemic. The virus is spreading under a close and careful watch.

No previous pandemic has been detected so early or watched so closely, in real-time, right at the very beginning. The world can now reap the benefits of investments, over the last five years, in pandemic preparedness.

We have a head start. This places us in a strong position. But it also creates a demand for advice and reassurance in the midst of limited data and considerable scientific uncertainty.

Thanks to close monitoring, thorough investigations, and frank reporting from countries, we have some early snapshots depicting spread of the virus and the range of illness it can cause.

We know, too, that this early, patchy picture can change very quickly. The virus writes the rules and one, like all influenza viruses, can change the rules, without rhyme or reason, at any time.

Globally, we have good reason to believe that this pandemic, at least in its early days, will be of moderate severity. As we know from experience, severity can vary, depending on many factors, from one country to another.

On present evidence, the overwhelming majority of patients experience mild symptoms and make a rapid and full recovery, often in the absence of any form of medical treatment.

Worldwide, the number of deaths is small. Each and every one of these deaths is tragic, and we have to brace ourselves to see more. However, we do not expect to see a sudden and dramatic jump in the number of severe or fatal infections.

We know that the novel H1N1 virus preferentially infects younger people. In nearly all areas with large and sustained outbreaks, the majority of cases have occurred in people under the age of 25 years.

In some of these countries, around 2% of cases have developed severe illness, often with very rapid progression to life-threatening pneumonia.

Most cases of severe and fatal infections have been in adults between the ages of 30 and 50 years.

This pattern is significantly different from that seen during epidemics of seasonal influenza, when most deaths occur in frail elderly people.

Many, though not all, severe cases have occurred in people with underlying chronic conditions. Based on limited, preliminary data, conditions most frequently seen include respiratory diseases, notably asthma, cardiovascular disease, diabetes, autoimmune disorders, and obesity.

At the same time, it is important to note that around one third to half of the severe and fatal infections are occurring in previously healthy young and middle-aged people.

Without question, pregnant women are at increased risk of complications. This heightened risk takes on added importance for a virus, like this one, that preferentially infects younger age groups.

Finally, and perhaps of greatest concern, we do not know how this virus will behave under conditions typically found in the developing world. To date, the vast majority of cases have been detected and investigated in comparatively well-off countries.

Let me underscore two of many reasons for this concern. First, more than 99% of maternal deaths, which are a marker of poor quality care during pregnancy and childbirth, occurs in the developing world.

Second, around 85% of the burden of chronic diseases is concentrated in low- and middle-income countries.

Although the pandemic appears to have moderate severity in comparatively well-off countries, it is prudent to anticipate a bleaker picture as the virus spreads to areas with limited resources, poor health care, and a high prevalence of underlying medical problems.

Ladies and gentlemen,

A characteristic feature of pandemics is their rapid spread to all parts of the world. In the previous century, this spread has typically taken around 6 to 9 months, even during times when most international travel was by ship or rail.

Countries should prepare to see cases, or the further spread of cases, in the near future. Countries where outbreaks appear to have peaked should prepare for a second wave of infection.

Guidance on specific protective and precautionary measures has been sent to ministries of health in all countries. Countries with no or only a few cases should remain vigilant.

Countries with widespread transmission should focus on the appropriate management of patients. The testing and investigation of patients should be limited, as such measures are resource intensive and can very quickly strain capacities.

WHO has been in close dialogue with influenza vaccine manufacturers. I understand that production of vaccines for seasonal influenza will be completed soon, and that full capacity will be available to ensure the largest possible supply of pandemic vaccine in the months to come.

Pending the availability of vaccines, several non-pharmaceutical interventions can confer some protection.

WHO continues to recommend no restrictions on travel and no border closures.

Influenza pandemics, whether moderate or severe, are remarkable events because of the almost universal susceptibility of the world's population to infection.

We are all in this together, and we will all get through this, together.

Thank you.

別紙様式第2－1

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 2009年10月22日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	新型インフルエンザに関する報道発表資料（農林水産省、2009年10月21日）	公表国 日本	
販売名（企業名）	別紙のとおり					

研究報告の概要	使用上の注意記載状況・その他参考事項等
問題点：新型インフルエンザに感染した豚が国内で初めて確認された。 1.経緯 大阪府の養豚農場で分離されたインフルエンザウイルスについて、(独)農研機構動物衛生研究所が、H 亜型検査（遺伝子解析）及びN亜型検査（遺伝子解析）を実施した結果、本ウイルスは、H1N1亜型であり、現在国内で流行している新型インフルエンザウイルスと同一であることが本日確認されました。 2.対応 大阪府において、当該農場に対して、臨床検査、遺伝子検査（PCR検査法）により異常がないことが確認されるまで、飼養豚の移動を自粛するよう要請しました。 なお、当該農場からと畜場へは、検査で陰性を確認した豚のみを出荷することとしています。	記載なし

報告企業の意見	今後の対応
別紙のとおり	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

MedDRA/J ver.12.0

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別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免疫グロブリン、⑤人免疫グロブリン、⑥人免疫グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥ペプシン処理人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン、⑬乾燥スルホ化人免疫グロブリン、⑭乾燥スルホ化人免疫グロブリン*、⑮乾燥濃縮人活性化プロテインC、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅷ因子、⑱乾燥濃縮人血液凝固第Ⅷ因子、⑲乾燥濃縮人血液凝固第Ⅷ因子、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥濃縮人血液凝固第Ⅸ因子、㉒乾燥濃縮人血液凝固第Ⅸ因子、㉓乾燥濃縮人血液凝固第Ⅸ因子、㉔乾燥抗破傷風人免疫グロブリン、㉕乾燥抗破傷風人免疫グロブリン、㉖抗HBs人免疫グロブリン、㉗抗HBs人免疫グロブリン、㉘トロンビン、㉙フィブリノゲン加第ⅩⅢ因子、㉚フィブリノゲン加第ⅩⅢ因子、㉛乾燥濃縮人アンチトロンビンⅢ、㉜乾燥濃縮人アンチトロンビンⅢ、㉝ヒスタミン加人免疫グロブリン製剤、㉞ヒスタミン加人免疫グロブリン製剤、㉟人血清アルブミン*、㊱人血清アルブミン*、㊲乾燥ペプシン処理人免疫グロブリン*、㊳乾燥人血液凝固第Ⅸ因子複合体*、㊴乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤ガンマーグロブリン筋注450mg/3mL「化血研」、⑥ガンマーグロブリン筋注1500mg/10mL「化血研」、⑦献血静注グロブリン“化血研”、⑧献血グロブリン注射用2500mg「化血研」、⑨献血ベニロンーⅠ、⑩献血ベニロンーⅠ静注用500mg、⑪献血ベニロンーⅠ静注用1000mg、⑫献血ベニロンーⅠ静注用2500mg、⑬献血ベニロンーⅠ静注用5000mg、⑭ベニロン*、⑮注射用アナクトC2,500単位、⑯コンファクトF、⑰コンファクトF注射用250、⑱コンファクトF注射用500、⑲コンファクトF注射用1000、⑳ノバクトM、㉑ノバクトM注射用250、㉒ノバクトM注射用500、㉓ノバクトM注射用1000、㉔テタノセーラ、㉕テタノセーラ筋注用250単位、㉖ヘパトセーラ、㉗ヘパトセーラ筋注200単位/mL、㉘トロンビン“化血研”、㉙ボルヒール、㉚ボルヒール組織接着用、㉛アンスロビンP、㉜アンスロビンP500注射用、㉝ヒスタグロビン、㉞ヒスタグロビン皮下注用、㉟アルブミン20%化血研*、㊱アルブミン5%化血研*、㊲静注グロブリン*、㊳ノバクトF*、㊴アンスロビンP1500注射用
報告企業の意見	インフルエンザウイルス粒子は70～120nmの球形または多形性で、8本の分節状マイナス一本鎖RNAを核酸として有する。エンベロープの表面に赤血球凝集素(HA)とノイラミダーゼ(NA)のスパイクを持ち、その抗原性により16種類のHA亜型および9種類のNA亜型に分類される。 今回の新型インフルエンザの原因ウイルスは、1930年代以降に発見された米国由来のブタインフルエンザウイルス、ヒトインフルエンザウイルス（H3N2）、鳥インフルエンザウイルスの3つのウイルスの遺伝子がブタインフルエンザとして再集合してできたウイルスに、さらにユーラシア大陸由来のブタインフルエンザウイルスの遺伝子の一部の分節が再集合して加わったものであると推察されている。新型インフルエンザは、これまでのところ限られた知見しか得られていないが、そのヒトからヒトへの感染伝播経路は従来の季節性インフルエンザに準ずると考えられている。すなわち、感染・発病者の咳やくしゃみとともに口から発せられる飛沫による飛沫感染が主な感染経路であり、患者との直接、間接の接触による接触感染も感染経路としての可能性がある。臨床症状であるが、これまでのところ、この新型インフルエンザのヒトへの病原性は、高病原性鳥インフルエンザウイルスA/H5N1のヒト感染例とは異なって、ヒトに対する病原性はそれほど高くはないと考えられている。 (http://idsc.nih.go.jp/idwr/douko/2009d/17douko.html) 弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に従い、ウシウイルス性下痢ウイルス（BVDV）、仮性狂犬病ウイルス（PRV）、ブタパルボウイルス（PPV）、A型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したインフルエンザウイルスは、エンベロープの有無、核酸の種類等からモデルウイルスとしてはBVDVが該当すると考えられるが、上記バリデーションの結果から、弊所の血漿分画製剤の製造工程がBVDVの除去・不活化効果を有することを確認している。また、これまでに当該製剤によるインフルエンザの報告例は無い。 以上の点から、当該製剤はインフルエンザウイルスに対する安全性を確保していると考える。

*現在製造を行っていない

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INF2009-005

農林水産省

プレスリリース

平成21年10月21日
農林水産省

大阪府における豚への新型インフルエンザの感染事例について

本日、大阪府の養豚農場の豚から分離されたウイルスは現在国内で流行している新型インフルエンザウイルスであることが確認されました。
当該農場に対して、臨床検査、遺伝子検査により異常がないことが確認されるまで、飼養豚の移動を自粛するよう要請しました。
なお、世界保健機関(WHO)等の国際機関によれば、適切に処理された豚肉を人が食べてインフルエンザに感染することはありません。

1.経緯

大阪府の養豚農場で分離されたインフルエンザウイルスについて、(独)農研機構動物衛生研究所が、H亜型検査(遺伝子解析)及びN亜型検査(遺伝子解析)を実施した結果、本ウイルスは、H1N1亜型であり、現在国内で流行している新型インフルエンザウイルスと同一であることが本日確認されました。

2.対応

大阪府において、当該農場に対して、臨床検査、遺伝子検査(PCR検査法)により異常がないことが確認されるまで、飼養豚の移動を自粛するよう要請しました。
なお、当該農場からと畜場へは、検査で陰性を確認した豚のみを出荷することとしています。

■ 報道機関へのお願い

1. 現場での取材は、本病の豚への感染を引き起こすおそれもあることから、厳に慎むようお願いします。
2. 今後とも、本病に関する情報提供に努めますので、生産者等の関係者や消費者が根拠のない噂などにより混乱することがないよう、ご協力をお願いします。

世界保健機関(WHO)等の国際機関によれば、適切に処理された豚肉を人が食べてインフルエンザに感染することはありません。

―― お問い合わせ先 ――
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農林水産省

別紙様式第2-1　　No. 17

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		総合機構処理欄
				2009. 7. 21	該当なし		
一般的名称	人血清アルブミン				公表国		使用上の注意記載状況・その他参考事項等
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)		研究報告の公表状況	CDC. Available from: http://www.cdc.gov/travel/content/outbreak-notice/chikungunya-fever.aspx	米国		赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/50mL 血液を原料とすることに由来する感染症伝播等

研究報告の概要

○米国疾病対策センター(CDC)によるアウトブレイク情報　アジアにおけるチクングニヤ熱

現状:2009年1月以降、チクングニヤ熱症例数の増加がタイ、マレーシア、インドを含むアジアの一部地域で報告されている。チクングニヤ熱は蚊の媒介でヒトに感染が広がるウイルス感染症である。症状には、急な発熱、関節痛(腫脹を伴うこともある)、悪寒、頭痛、吐き気、嘔吐、腰痛、紅斑などがある。流行地域は主にアフリカとアジアだが、2007年にはイタリアでの限定的な伝播が見られた。

タイでは、2009年7月22日時点で、プーケットなどの観光地を含むタイ南部で大規模なアウトブレイクが発生している。タイ保健省によると50の県で34,200例が記録されたが、死亡例はなかった。マレーシアでは、7月18日時点で2,900例以上のチクングニヤ熱症例が主に北部地域から報告された。インドでは、4月29日時点で2,700例以上の症例が主に南部地域から報告されたが、死亡例はなかった。報告数の増加に伴い、アジアの他の国々では監視を強化している。

渡航者向け勧告:流行地への渡航者は蚊に刺されないよう、朝晩に戸外に出る場合は虫除けを使用すること。罹患した場合は医師の診察を受けること。また、他人への感染拡大を防ぐため、蚊に刺されないよう注意すること。

チクングニヤ熱の潜伏期間は通常3〜7日である。症状は数日〜2週間持続するが、数週間にわたって疲労感を感じる患者もいる。ほとんどの患者が関節痛や関節炎を報告しており、数週間〜数ヶ月続くこともある。症状はデング熱によく似ているが、出血やショック症状は通常見られず、ほとんどは入院を必要としない。患者は自然治癒し、死亡に至ることは滅多にない。チクングニヤ熱の治療薬はないため、治療は対症療法が中心となる。

報告企業の意見	今後の対応
タイ、マレーシア、インドにおいてチクングニヤ熱のアウトブレイクが発生し、米国疾病対策センターが渡航者向けに蚊に刺されないよう注意喚起する情報を発表したとの報告である。 チクングニヤウイルスは脂質膜を持つRNAウイルスである。これまで、本製剤によるチクングニヤウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。	念のため今後も情報収集に努める。なお、日本赤十字社では帰国(入国)後4週間は献血不適とし、輸入感染症の防止に努めている。

(27)

Centers for Disease Control and Prevention
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Outbreak Notice
Chikungunya Fever in Asia
This information is current as of today, August 17, 2009 at 00:28 EDT

Updated: July 29, 2009

Situation Information

Since January 2009, a growing number of cases of chikungunya fever has been reported in parts of Asia, including Thailand, Malaysia, and India. Chikungunya fever is a disease caused by a virus that is spread to people through the bite of infected mosquitoes. Symptoms can include sudden fever, joint pain with or without swelling, chills, headache, nausea, vomiting, lower back pain, and a rash. Chikungunya mainly occurs in areas of Africa and Asia. In 2007, limited transmission of Chikungunya virus occurred in Italy (/travel/destinations/Italy.aspx).

Thailand

As of July 22, 2009, a large outbreak of chikungunya fever has affected the southern region of Thailand (/travel/destinations/Thailand.aspx) including some tourist destinations, such as Phuket. According to the Ministry of Public Health in Thailand, over 34,200 cases have been documented this year in 50 provinces, with no deaths reported. The most affected areas are the southern provinces of Songula, Narathiwat, Pattani, and Yala.

Recent reports show that Chikungunya virus has now from the southern provinces to all other regions of the country.

Malaysia

As of July 18, 2009, the Ministry of Health in Malaysia (/travel/destinations/Malaysia.aspx) has reported over 2,900 cases of chikungunya fever. The most affected areas are the northern provinces of Kedah, followed by Selangor, Kelantan, Perak and Sarawak.

India

As of April 29, 2009, the Directorate of National Vector Borne Disease Control Programme in India (/travel/destinations/India.aspx) has reported over 2,700 suspected cases of chikungunya fever, with no deaths reported. The most affected areas are the Karnataka, followed by Andhra, Goa, and Kerala states.

In response to the growing number of reports, other countries in Asia have increased surveillance for chikungunya fever.

Advice for Travelers

No medications or vaccines are available to prevent a person from getting sick with chikungunya fever. CDC recommends that people traveling to areas where chikungunya fever has been reported take the following steps to protect themselves from mosquito bites.

- The best way to avoid Chikungunya fever is to avoid mosquito bites. When outdoors during the day and at night, use insect repellent (http://www.cdc.gov/ncidod/dvbid/westnile/qa/insect_repellent.htm#proper) on exposed skin.
  - Look for a repellent that contains one of the following active ingredients: DEET, picaridin (KBR 3023), Oil of Lemon Eucalyptus/PMD, or IR3535. Always follow the instructions on the label when you use the repellent.
  - In general, repellents protect longer against mosquito bites when they have a higher concentration (%) of any of these active ingredients. However, concentrations above 50% do not offer a distinct increase in protection time. Products with less than 10% of an active ingredient may offer only limited protection, often from 1-2 hours.
  - The American Academy of Pediatrics (/travel/forward.aspx?t=aHR0cDovL3d3dy5hYXAub3JnL3B1YmxpY2VkL0JSX1JlcGVsbGVudHMuaHRt-QBZtvSqqfw%3d) approves the use of repellents with up to 30% DEET on children over 2 months of age.

If you get sick with a fever and think you may have chikungunya fever, you should seek medical care. Although there is no specific treatment for the disease, a doctor may be able to help treat your symptoms. Avoid getting any other mosquito bites, because you could transmit the disease to other people through mosquitoes.

For more travel health information, see the destinations (/travel/destinations/list.aspx) section and search for the country you are planning to visit.

More Information

The incubation period for chikungunya (time from infection to illness) can be 2-12 days, but is usually 3-7 days. Chikungunya fever typically lasts a few days to 2 weeks, but some patients feel fatigue lasting several weeks. Most patients have reported severe joint pain or arthritis, which may last for weeks or months. The symptoms are similar to those of dengue fever, but, unlike dengue, people who have chikungunya fever do not usually experience hemorrhage (bleeding) or go into shock. People with chikungunya fever generally get better on their own and rarely die from the disease.

There is no specific drug treatment for chikungunya fever, and medical care is usually focused on treating the symptoms of the disease. Bed rest, fluids, and mild pain medications such as ibuprofen, naproxen, or acetaminophen (paracetamol) may relieve symptoms of fever and aching, provided there are no medical contraindications for using these medications. Most people are not sick enough to need to stay in the hospital. All people who become sick with chikungunya fever should be protected against additional mosquito bites to reduce the risk of further transmission of the virus.

For more information, see—

- Chikungunya (http://www.cdc.gov/ncidod/dvbid/Chikungunya/CH_FactSheet.html) (CDC Fact Sheet)
- Traveling with Children: Resources (http://wwwn.cdc.gov/travel/contentChildTravel.aspx) (CDC Travelers' Health website)

Other Mosquito-Related Diseases

In many of the areas where chikungunya is present, there are other diseases spread by mosquito bites, such as dengue (/travel/yellowbook/2010/chapter-5/dengue-fever-dengue-hemorrhagic-fever.aspx), malaria (/travel/yellowbook/2010/chapter-2/malaria.aspx), Japanese encephalitis (/travel/yellowbook/2010/chapter-2/japanese-encephalitis.aspx), and yellow fever (/travel/yellowbook/2010/chapter-2/yellow-fever.aspx). If you are traveling to any tropical and subtropical areas of the world, you should take steps to avoid mosquito bites.

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  National Center for Preparedness, Detection, and Control of Infectious Diseases

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識別番号・報告回数			報告日	第一報入手日 2009年7月30日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①ポリエチレングリコール処理人免疫グロブリン ②人免疫グロブリン	研究報告の公表状況	Transfusion (Malden) 2009; 49(7): 1488-1492	公表国 アメリカ		
販売名（企業名）	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②グロブリン-Wf（ベネシス）					

研究報告の概要

米国における新しいパルボウイルスB19遺伝子型3の発見と分析

背景：パルボウイルスB19（B19V）は、核酸検出でヒト血漿においてしばしば同定される病原体である。3つのB19V遺伝子型は、全体のDNA配列で10%を超える相違のある分離株に基づいて定義された。B19V遺伝子型3は、主にガーナで、散発的にブラジルおよびフランスから報告されてきたが、過去に北アメリカでの報告はなかった。

研究設計と方法：B19Vを広い特異性で検出するためのPCR測定系が開発された。この測定法の性能は、81,000人以上のドナー由来の約440,000の臨床的サンプルを検査することによって評価された。B19Vタイター、DNA配列と抗体濃度の判定は、重要なサンプルで実施された。

結果：この評価の結果、DNA配列分析によってB19遺伝子型3に感染していると確認された米国の1人のドナーから28日の間に8回の血漿ドネーションを行っていることが確認された。この一連のドネーションのB19Vタイターは、ピークにおいて10^{11}IU/mLを超えるウイルス力価に達した。ウイルス力価は、免疫グロブリンM（IgM）抗体価の増加に合わせてその後数回のドネーションにおいて減少した。IgG濃度も増加したが、IgM濃度より約7日遅れた。

結論：これは、米国における血漿ドナーから検出されたB19V遺伝子型3の最初の報告である。我々のデータはこの遺伝子型の低い発生率を示唆している最近の報告と合致しているにも関わらず、それは血液及び血漿ドナーとの関連性が増していることを示している。

報告企業の意見	今後の対応
米国において血漿ドナーからB19V遺伝子型3が検出された最初の報告である。 献血ヴェノグロブリン-IHについては、万一本剤の原料血漿にB19が混入したとしても、CPVをモデルウイルスとしたウイルスバリデーション試験成績及びB19を用いた不活化・除去試験の結果から、本剤の製造工程において十分に不活化・除去されると考えている。 グロブリン-Wfについては、血漿分画製剤の製造工程での十分な不活化・除去が困難であり、本ウイルスの伝播リスクを完全に否定することはできないため、1996年11月より、使用上の注意にパルボウイルスB19についての記載を行い注意喚起を図っている。なお、一変承認後に製造されたロットについては、献血ヴェノグロブリン-IHと同様に、製造工程において不活化/除去されると考えている。	本報告は本剤の安全性に影響を与えるものではないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。

1. 慎重投与

(5) 溶血性・失血性貧血の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕

(6) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕

2. 重要な基本的注意

(1) 略

1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルスB19等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。

6. 妊婦、産婦、授乳婦等への投与

妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルスB19の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕

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TRANSFUSION COMPLICATIONS

Discovery and analysis of a novel parvovirus B19 Genotype 3 isolate in the United States

Lori A. Rinckel, Brett R. Buno, Todd M. Gierman, and Douglas C. Lee

BACKGROUND: Parvovirus B19 (B19V) is a pathogen frequently identified in human plasma donations through the detection of nucleic acids. Three B19V genotypes have been defined based on isolates having greater than 10% divergence in overall DNA sequence. B19V Genotype 3 is a rarely occurring genotype that has been detected primarily in Ghana with sporadic reports in Brazil and France but has not been previously reported in North America.

STUDY DESIGN AND METHODS: A polymerase chain reaction assay was developed with broad specificity for B19V detection. The performance of this assay was assessed by testing approximately 440,000 clinical samples representing more than 81,000 individual donors. Determinations of B19V titer, DNA sequence, and antibody concentrations were performed on samples of interest.

RESULTS: This assessment identified a series of eight plasma donations spanning 28 days from a single donor in the United States infected with B19V Genotype 3 as confirmed by DNA sequence analysis. The B19V titer of this series of donations showed virus titers that peaked at greater than 10^{11} IU/mL. The virus titer decreased significantly over the next several donations coinciding with an increase in immunoglobulin M (IgM) levels. The immunoglobulin G levels also increased but lagged approximately 7 days behind the IgM levels.

CONCLUSION: This is the first report of a B19V Genotype 3 detected from a plasma donor located in the United States. Although our data are consistent with recent reports suggesting low incidence for this genotype, they indicate its increasing relevance among blood and plasma donors.

Human parvovirus B19 (B19V) is a commo human pathogen that possesses a mutatio rate that is uncharacteristically high for DNA virus.[1,2] B19V DNA sequence homolog is used to classify B19V into three genotypes.[3] The gene types are defined as having approximately 10% divergenc in overall DNA sequence. Genotype 1 is the most prevale B19V currently circulating in North America and Europ and is represented by the prototype strain Au (GenBan Accession Number M13178).[4] Genotype 2 also circulate in North America and Europe but with lower frequenc than Genotype 1 and is represented by the prototyp strain A6 (GenBank Accession Number AY064475).[5] Genc type 3 is endemic to Ghana but has also been foun in Brazil and France.[6] Genotype 3 is represented by tw prototype strains, V9 and D91.1 (GenBank Accessio Numbers AX003421 and AY083234, respectively).[3] Numei ous genetic variants of B19V exist within each of thes three genotypes. By definition, these variants differ by les than 10% in their DNA sequence when compared to th genotypic prototype.

These three genotypes were shown to constitute on single serotype through various functional, structural, an immunologic studies;[7] current evidence suggests that a variants of B19V induce similar pathologies.[8] A primar interest and concern with the wide assortment of B19 genotypes and variants is diagnostic. Polymerase chai reaction (PCR) assays developed around specific referenc standards frequently fail to detect accurately specifi B19V genotypes and variants.[5,9-11] Therefore, developers o molecular diagnostics and the biologics industry have al

ABBREVIATION: B19V = parvovirus B19.

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increased interest in the prevalence of emerging B19V variants.

Here, we report a unique isolate of B19V. This isolate was discovered in a series of human plasma donations from a single donor located in the United States. Analysis indicated that this isolate exhibits strong DNA sequence homology to B19V Genotype 3. Analysis of the series of donations from this donor demonstrated the expected clinical pattern of antibody response to this B19V Genotype 3 infection. This is the first report of the discovery and characterization of B19V Genotype 3 among US plasma donors.

MATERIALS AND METHODS

A quantitative real-time PCR assay was developed with a target region within the NS1 coding sequence of the B19V genome utilizing oligonucleotide primers and probes purchased from Integrated DNA Technologies (IDT, Coralville, IA). The assay used two detection probes for the B19V target. One detection probe contained the DNA sequence of the B19V Au Genotype 1 prototype strain; the second probe contained a DNA sequence that is a consensus derived from the B19V A6 Genotype 2 prototype strain and the V9 and D91.1 Genotype 3 prototype strains. Both probes were labeled with the same fluorophore. The assay also incorporated a third detection probe for a competitive internal control that was labeled with a different fluorophore than that of the two B19V target probes. Test results indicating a PCR signal for the internal control, B19V target, or both were deemed valid; results indicating no PCR signal for both the internal control and the B19V target were deemed invalid. The quantitation standards used in the real-time PCR assay were dilutions of plasmid pYT104-C, which contains a B19V Genotype 1 strain (Au) genome.[12] A quantitative standard curve was used to assign values (copies/mL) to test samples. The results expressed as copies/mL were converted to IU/mL using a correlation factor of 2.9 copies/IU, determined by comparing the potency of the First WHO International Standard for B19V DNA nucleic acid test assays (99/800) to the potency of the dilution of pYT104-C used to create the quantitation standards.[13]

The performance of the B19V assay was assessed against a qualitative B19V assay that served as the test of record using a study sample of approximately 440,000 donor samples corresponding to roughly 81,000 individual donors. Both assays were designed to detect all three B19V genotypes; neither assay was designed to discriminate among the three genotypes. Donation samples were tested initially in pools of 384 or 480 samples to increase testing efficiency. Additional testing of B19V-reactive samples was performed using a B19V PCR assay (artus RealArt, Parvo B19 PCR assay, Qiagen, Hilden, Germany). Antibody detection was performed on test samples in duplicate using the B19V immunoglobulin M (IgM) and immunoglobulin G (IgG) enzyme immunoassay (EIA) kits (Biotrin, Dublin, Ireland).

DNA sequencing was performed on PCR amplicons generated using primers containing B19V consensus DNA sequences. The purified PCR amplicons were sequenced by primer walking performed at Lark Technologies (Houston, TX). The contiguous DNA sequences were assembled using sequence analysis cloning software (Vector NTI, Invitrogen Corp., Carlsbad, CA). DNA sequence alignments were performed with Vector NTI and with the GenBank database using BLAST.[14]

RESULTS

The performance of a new B19V assay was assessed using a study sample set consisting of approximately 440,000 donor samples, representing roughly 81,000 individual donors. The performance of the B19V assay was benchmarked against results obtained using an earlier version assay. During the course of the study, 1 in 2400 donor samples tested reactive for B19V. Review of results discordant between the two assays identified several samples for follow-up analysis. This investigation identified two reactive donations that were ultimately linked to a series originating from a single donor resident in the United States. Using a lookback process coupled with follow-up testing, a series comprising eight donations, designated P0 through P7, was identified and these units were pulled from the inventory for continued research. The two plasma samples with the highest titer from this series, P1 and P2, were used to characterize the B19V isolate. Additional testing using the Qiagen artus RealArt Parvo B19 PCR assay yielded negative results for neat and diluted samples (neat and 1:480).

DNA sequencing of B19V amplicons generated from P1 and P2 using our assay showed that both donations contained identical DNA target sequences. This preliminary sequence information also suggested that nucleic acid isolates from P1 and P2 have higher DNA sequence homology to B19V Genotype 3 than to Genotypes 1 and 2. The preliminary DNA sequence information was used to design and synthesize a new detection probe (P1 probe) containing 100% DNA sequence homology to the P1 and P2 isolates. This new detection probe was used to quantitate sample viral loads at 8×10^{11} IU/mL for P1 and 3×10^{10} IU/mL for P2 (Fig. 1).

The DNA sequence was determined for 4846 nucleotides of the P1 B19V genome (GenBank Accession Number FJ265736). Analysis of the DNA sequence from P1 (Fig. 2) shows that this B19V isolate has the highest DNA sequence homology to representative isolates of B19V Genotype 3. This sequence exhibited 97% homology to B19V strain V9 and 96% homology to B19V strain D91.1, suggesting that the P1 isolate belongs to Genotype 3

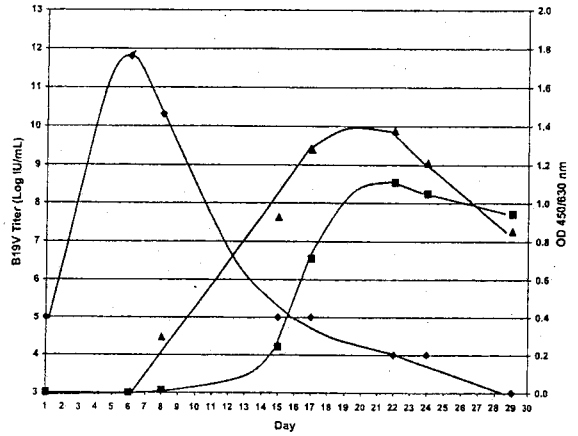
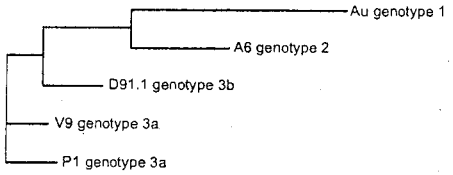


Fig. 1. B19V titer (◆) and IgM (▲) and IgG (■) levels for the series of Genotype 3 donations. The B19V titer is expressed in IU/mL.

A



B

	Genotype 1 Au	Genotype 2 A6	Genotype 3b D91.1	Genotype 3a V9	P1
Genotype 1 Au	100	91	90	89	89
Genotype 2 A6		100	93	93	92
Genotype 3b D91.1			100	98	96
Genotype 3a V9				100	97
P1					100

Fig. 2. Global DNA sequence alignment of B19V strain P1 with the prototype strains for B19V Genotype 1, Au; Genotype 2, A6; Genotype 3b, D91.1; and Genotype 3a, V9. (A) Phylogenetic tree. (B) Numerical comparison of DNA sequence homologies. This analysis shows that P1 is a member of B19V Genotype 3a.

Subtype B19/3a.[15] The P1 isolate is significantly less homologous to B19V Genotypes 1 and 2 with 89% homology to B19V Genotype 1 prototype strain Au and 92% homology to B19V Genotype 2 prototype strain A6.

When tested using the P1 probe, the B19V titers of this series of donations show the expected pattern for a B19V infection. B19V titers increased rapidly, peaking within several days after infection and then decreased gradually for several weeks (Fig. 1).

In addition, IgM and IgG B19V antibody levels were detected in this series of plasma donations using EIA methods (Fig. 1). For reference, the day of the P0 donation is referred to as Day 1. The IgM response was first detected on Day 8 (P3), peaked on Day 22 (P5), and decreased thereafter. The IgG antibody response was first detected on Day 15 (P3), peaked on Day 22 (P5), and gradually decreased through Day 29 (P7). The B19V antibody levels detected in these plasma donations displayed an increase in IgM level concurrent with the decrease in B19V titer (Fig. 1).

DISCUSSION

This is the first report of a B19V Genotype 3 detected in a blood or plasma donation in the United States. Previously, B19V Genotype 3 had been reported to occur primarily in the African country of Ghana, with less frequent reports in Brazil and France. The frequency of B19V Genotype 3 in Ghana was reported to be approximately 100% of the strains identified.[16] The frequency of B19V Genotype 3 in Brazil was approximately 50% and in France was approximately 11% of the strains identified.[3,6] Not only is the identification of a B19V Genotype 3 in the United States noteworthy, but also our characterization of this B19V Genotype 3 infection in this donation series has demonstrated that isolates of this genotype can achieve the high virus titers typically associated with acute B19V Genotype 1 infections. In contrast, previous reports concerning B19V Genotype 3 have suggested that high-titer infections involving this genotype occur infrequently.[3,16,17] The titers of the isolates described in these prior reports, however, may reflect late or persistent infections which would exhibit lower titers than an initial infection. The B19V titers of the series of donations in this report show the expected pattern for an acute infection where virus titers increase rapidly, peak within several days after infection, and then decrease gradually over a period of several weeks.

The B19V isolate described in this report, designated P1, was found to exhibit strong DNA sequence homology with B19V Genotype 3. Alignment to V9 and D91.1, both Genotype 3 isolates, demonstrated significantly higher DNA sequence homology (at least 94%) than to representative isolates for Genotypes 1 and 2. More specifically, P1

appears to belong to Genotype 3 Subtype B19/3a.[15] Subtype B19/3a was reported to be prevalent in Ghana whereas Subtype B19/3b appears to be more prevalent in Western Europe and Brazil.[15]

The B19V titers in these donations increased rapidly and peaked at a titer of approximately 8×10^{11} IU/mL. Our results also show that the decrease in B19V titer was concurrent with an increase in IgM antibodies. The increase in IgM antibodies was followed by an increase in the levels of IgG antibodies. These results concur with published works that suggest that the Genotype 1 antigens present in the Biotrin EIA kit are effective for the detection of Genotype 3 antibodies.[18] These results are also consistent with the suggestion that a single serotype may exist for the different B19V genotypes.[7]

Recent discussion concerning the incidence of the B19V Genotype 3 infection among blood and source plasma donors has suggested that the prevalence of this genotype in the United States is low or absent.[2] The comparison of the performance of two B19V assays in this limited, high-throughput sample set (approx. 440,000 donations) identified B19V at a frequency of 1:2400 donations. This detection frequency is typical for the time of year at which the study was conducted (based on data from nearly 8 years of high-throughput testing). When the study results were analyzed by donor, B19V-reactive donations were associated with 117 individual donors among 81,000 total donors (approx. 1:700). In contrast, the putative detection frequencies for samples and donors reactive for B19V Genotype 3 appear significantly lower. Samples containing high-titer B19V Genotype 3 (i.e., $>10^6$ IU/mL) were detected at the rate of 1:220,000 and were contributed by a single donor among the 81,000 donors comprising the sample set (1:81,000). Whether these frequencies accurately reflect the incidence of Genotype 3 within the source plasma donor population remains unclear, because the assays used in this study were not designed to differentiate among the three genotypes. Moreover, this study was designed to evaluate assay performance, rather than B19V epidemiology. Nevertheless, the fact that this study resulted in the identification and interdiction of 8 plasma units from a single donor, 2 of which contained sufficient B19V to exceed the prescribed limits for plasma fractionation pools, underscores the increasing relevance of assays that can detect B19V Genotype 3.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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別紙様式第2-1

No. 18

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	総合機構処理欄
				2009. 7. 21	該当なし	

一般的名称	人血清アルブミン	研究報告の公表状況	FDA, CBER. Available from: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm071592.htm.	公表国 米国
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)			

研究報告の概要

○血漿分画製剤によりヒトパルボウイルスB19が伝播する危険性を低減するための核酸検査(NAT)にかかる業界向けガイダンス
米国食品医薬品局(FDA)は、血漿分画製剤製造に使用される原料血漿および回収血漿の工程内検査として、ヒトパルボウイルスB19核酸増幅検査(NAT)の実施を製造業者に対し勧告するガイダンスを発表した。これは2008年7月付ガイダンス案の最終決定版である。
B19は脂質膜を持たない一本鎖DNAウイルスで、熱処理やS/D処理等の一般的な不活化法に抵抗性があり、小型であるため除去も困難である。輸血用血液製剤や血漿分画製剤の投与によりウイルス感染が起こり、妊婦や血液疾患、免疫不全の患者等、影響を受けやすい患者が感染すると死亡(または胎児水腫、流産、死産等)する可能性も否定できない。輸血用血液による感染はまれだが、B19急性感染期で無症候の献血者から採血した最高10^{13}IU/mL程度のウイルスを含む血液が血漿分画製剤の原料として用いられた場合には、多数の血漿をプールして製造することから感染リスクが高くなる。しかし、ウイルス血症期の献血者血液中には、長期にわたり低濃度のウイルスと中和抗体としてのB19抗体が共存しており、低濃度のウイルスを保有する献血者を排除することは、プール血漿中のB19抗体レベルを下げることになるため、望ましくない。
FDAは、血漿分画製剤の製造業者に対し、B19 DNAを検出するため、以下の手順を導入することを勧告する。
1. 全ての血漿分画製剤の製造に際し、製造工程中の品質管理としてB19 DNAのNATを実施し、製造原料となる血漿プール中のB19 DNA濃度が10^{4}IU/mLを超えないことを保証する。
2. 血漿分画製剤の製造原料となる血漿製剤に対し、B19のミニプールNATを実施する。B19のNATに用いるプライマーやプローブは、現在判明している全ての遺伝子型を検出できるものであること。
3. 血漿製剤がB19陽性であることが判明し、使用により血漿プール中のウイルス力価が10^{4}IU/mLを超える可能性がある場合には、その血漿を血漿分画製剤の製造原料として用いてはならない。
製造業者は、原料血漿及び回収血漿中のB19 DNA検出に用いるNATのバリデーションを行い、血漿プール中のB19 DNAが10^{4}IU/mLを超えないことを実証すること。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
米国食品医薬品局が、血漿分画製剤製造に使用される原料血漿および回収血漿の工程内検査として、ヒトパルボウイルスB19核酸増幅検査(NAT)の実施を製造業者に対し勧告するガイダンスの最終版を発表したとの報告である。 ヒトパルボウイルスB19(B19V)は脂質膜のないDNAウイルスである。これまで、本剤によるB19V感染の報告はない。B19は耐熱性とされていたが最近、液状加熱で容易に不活化できることが明らかにされた。本製剤の製造工程には、当該工程が含まれている。また最終製品についてB19-NAT陰性であることを確認していることから、本製剤の安全性は確保されている。	念のため今後も情報収集に努める。なお、日本赤十字社では、以前よりRHA法によるB19抗原検査を導入しウイルス量の多い血液を排除してきた。2008年からさらに感度の高い化学発光酵素免疫測定法(CLEIA)を導入し、10^{6}IU/mL以上のB19を含む血液を陽性と判定し排除するものであることから、現在は原料血漿プール中のウイルス濃度が10^{4}IU/mL以下となっている。今後も輸血用血液及び血漿分画製剤の安全性向上のために努力する。

(29)

JRC2009T-037

Guidance for Industry

Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Human Parvovirus B19 Transmission by Plasma-Derived Products

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD) (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
July 2009

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Guidance for Industry

Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Human Parvovirus B19 Transmission by Plasma-Derived Products

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

We, FDA, are issuing this guidance to provide you, manufacturers of plasma-derived products, with recommendations for performing nucleic acid testing (NAT) for human parvovirus B19 as an in-process test for Source Plasma and recovered plasma used in the further manufacturing of plasma-derived products. Such testing will identify and help to prevent the use of plasma units containing high levels of parvovirus B19. This guidance also recommends how to report to FDA implementation of parvovirus B19 NAT.

We recognize that in the current business practice for parvovirus B19 NAT in-process testing, several weeks can elapse between collection of the units of Source Plasma or recovered plasma and identification of B19 NAT-positive pools or units. We encourage manufacturers of plasma-derived products to employ practices that will reduce the time between product collection and in-process testing to allow for the meaningful notification of blood and plasma collection establishments of positive test results within the dating period of any blood components intended for use in transfusion.

This guidance finalizes the draft guidance of the same title, dated July 2008.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Human parvovirus B19 is a small, non-enveloped single stranded DNA virus. Virus clearance studies, using non-human parvoviruses as models for parvovirus B19, have indicated that this virus is highly resistant to all commonly used inactivation methods, including heat and

solvent/detergent (S/D) treatment, and is also difficult to remove by filtration because of its small size. More recent studies have demonstrated that human parvovirus B19 may be more readily cleared than certain model animal parvoviruses (Refs. 1, 2, 3 and 4). The parvovirus B19 can be transmitted by blood components and certain plasma derivatives, and may cause morbidity to susceptible recipients such as pregnant women (and their fetuses exposed in utero), persons with underlying hemolytic disorders, and immune compromised individuals (Refs. 5 and 6). The disease transmission by transfusion of blood components is rare. However, extremely high levels of parvovirus B19, up to 10^{12} IU/mL, in plasma of acutely infected but asymptomatic donors may present a greater risk in plasma derivatives due to pooling of large numbers of plasma units in the manufacture of these products. The virus can be detected by NAT in plasma pools when there are high levels of parvovirus B19 DNA in viremic donations. For example, the parvovirus B19 DNA can be detected in various plasma-derived products, particularly in coagulation factors (Refs. 7 and 8). There have been a few reports of parvovirus B19 infection associated with the administration of coagulation factors (Refs. 9 and 10) and S/D Treated Pooled Plasma (Refs. 5 and 11). Parvovirus B19 DNA is less frequently detected in albumin and immunoglobulin products and, when detected, the levels are usually low. There are no confirmed reports that albumin and immunoglobulin products have transmitted parvovirus B19 infection.

We have held or participated in several meetings to discuss the potential risk of parvovirus B19 infection by plasma-derived products, and the strategy for reducing such risk. The meetings included FDA-sponsored NAT workshops in 1999 and 2001 (Refs. 12 and 13), Blood Products Advisory Committee (BPAC) meetings in 1999 and 2002 (Refs. 14, 15, and 16), the National Heart, Lung, and Blood Institute-sponsored Parvovirus B19 workshop in 1999 (Ref. 5), and an ad hoc Public Health Service (PHS) panel in 2002 (discussed at the 2002 BPAC meeting (Ref. 16)). In these meetings, it was recognized that viral inactivation/removal steps that are routinely used in the manufacturing process of plasma-derived products do not alone appear to be sufficient to completely clear the virus if high viral load is present in the starting material. Therefore, in these meetings, a common recommendation for mitigating the risk of parvovirus B19 transmission by plasma derivatives has been to limit the virus load in the manufacturing plasma pool by testing the plasma donations for high titer parvovirus B19 DNA, using a minipool format. This viral load reduction strategy combined with the ability of the manufacturing process to clear the residual virus could greatly reduce the risk of parvovirus B19 infection by plasma-derived products.

The recommended limit in this guidance for viral load of parvovirus B19 DNA in the manufacturing plasma pool (i.e., not to exceed 10^4 IU/mL) was primarily derived from studies that were conducted on the transmission of parvovirus B19 associated with S/D Treated Pooled Plasma (Refs. 5, 11, and 14). In principle, testing in a minipool format to measure the viral load for parvovirus B19 DNA in a manufacturing plasma pool is acceptable in order to exclude only the high-titer plasma donations, thereby avoiding too great a loss of plasma for further manufacturing. Furthermore, during the viremic period for parvovirus B19 infected donors, which can be very lengthy, low levels of parvovirus B19 coexist with parvovirus B19 antibodies

(potentially complexing with and neutralizing the virus). Therefore, it is undesirable to remove plasma units with low levels of B19 DNA, because it would diminish the parvovirus B19 antibody levels in plasma pools and in some of the resulting plasma-derived products (Refs. 17 and 18).

III. RECOMMENDATIONS

We recommend that you implement the following procedures to detect the presence of parvovirus B19 DNA:

- For all plasma-derived products, you should perform parvovirus B19 NAT as an in-process test to ensure that the viral load of parvovirus B19 DNA in the manufacturing pools does not exceed 10^4 IU/mL.
- Use parvovirus B19 NAT on minipool samples to screen plasma units intended for further manufacturing into plasma-derived products. Primers and probes selected for parvovirus B19 NAT should detect all known genotypes of the virus (Ref. 19).
- When identified, you should not use individual plasma units, intended for further manufacturing into plasma-derived products, when such units are found to have a titer of parvovirus B19 DNA that might result in plasma manufacturing pools exceeding a parvovirus B19 DNA titer of 10^4 IU/mL.

You should assess validation data demonstrating the accuracy, sensitivity, specificity, reproducibility, and other performance characteristics of the parvovirus B19 NAT assay used for the detection of parvovirus B19 DNA in the Source Plasma and recovered plasma, and for demonstrating that the viral load of parvovirus B19 DNA in the manufacturing pool does not exceed 10^4 IU/mL.

If the above recommendations are implemented, you must inform FDA, as required under 21 CFR 601.12(a). You may submit these changes as a "Supplement-Changes Being Effected" supplement (CBE supplement), under 21 CFR 601.12(c)(5).

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別紙様式第2-1
番号16

医薬品
医薬部外品　研究報告　調査報告書
化粧品

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	厚生労働省処理欄
				2009年9月14日	該当なし	

一般的名称	トロンビン	研究報告の公表状況		公表国
販売名（企業名）	①献血トロンビン経口・外用5000単位「ベネシス」(ベネシス) ②献血トロンビン経口・外用1万単位「ベネシス」(ベネシス)		Clinical Infection Deiseases 2009; 49: 852-860	マレーシア

研究報告の概要

（背景）Plasmodium knowlesi（P. knowlesi）は東南アジアで次第にヒト・マラリアの原因と認められつつある。しかし、自然感染の詳細なプロスペクティブな臨床研究がない。

（測定法）急性P. knowlesi感染患者のpresentationと経過の系統的な研究において、臨床および検査データは2006年7月～2008年2月にKapit病院（サラワク、マレーシア）にPCRで確定された急性マラリアで入院した過去に治療経験のない非妊娠の成人から集められた。

（結果）152人の患者のうち、107人（70%）がP. knowlesiに、24人（16%）はP. falciparumに感染しており、そして、21人（14%）は三日熱マラリア原虫を持っていた。P. knowlesi感染者は、非特異的な発熱性の疾患を呈し、入院患者のベースライン中央値の寄生虫値は1387parasites/μL（四分位数間領域：6-222,570parasites/μL）を有し、そして全ての症例は入院又はその次の日に血小板減少を呈していた。
P. knowlesi感染患者のほとんど（93.5%）は、クロロキンとプリマキン治療に反応した合併症を伴わないマラリアであった。熱帯熱マラリアのWHO基準に基づくと、P. knowlesi感染の7人の患者（6.3%）は、入院時点で重症感染であった。最も頻度の高い合併症は呼吸困難であった。それは4人の患者では入院時にみられ、あとの3人の患者では入院後に発症した。入院時のP. knowlesi寄生虫血症は呼吸困難の独立した決定因子であり、入院時の血清クレアチニンレベル、血清ビリルビンそして血小板数と同様であった（各々P<0.002）。2人のP. knowlesiマラリア患者は死亡し、1.8%（95%信頼区間；0.2-6.6%）の致死率を示した。

（結論）Knowlesiマラリアは、広範囲の疾患を起こす。大部分の症例は合併症を伴わず、速やかに治療に反応する。しかし、約10人に1人の患者が潜在的に致命的な合併症を発病する。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、陽イオン交換体処理により人トロンビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程においてリン酸トリ-n-ブチル(TNBP)/ポリソルベート80処理、ウイルス除去膜によるろ過処理、凍結乾燥の後、60℃、72時間の加熱処理を施しているが、使用に際しては、次の点に十分注意すること。

(30)

報告企業の意見	今後の対応
東南アジアでPlasmodium knowlesiが原因のマラリアはだんだん増えており、患者の多くはクロロキンとプリマキン治療に反応した合併症を伴わないマラリアであったが、約10人に1人の患者が致命的な合併症を発病する可能性があることについての報告である。 Plasmodium knowlesiは長い間、サルに感染するマラリア原虫とみなされていたが、ヒトに感染する5番目のPlasmodium属原虫と認められるようになってきている。 血漿分画製剤からのマラリア伝播の事例は報告はされていない。FDAが2000年6月に発行した"Guidance for Industry: Recommendations for Donor Questioning Regarding Possible Exposure to Malaria"においては、赤血球成分または血小板用の供血についてはマラリアに関連した供血停止条件を規定しているものの、血漿成分用の供血や血漿分画製剤の原料用の供血については規定していない。感染患者におけるマラリア原虫はメロゾイドの形で存在すると考えられ、このものは2-3μmの卵型であるとされている（最新医学大辞典第2版、医歯薬出版、1996）。万一、原料血漿にマラリア原虫が混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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MAJOR ARTICLE

Clinical and Laboratory Features of Human *Plasmodium knowlesi* Infection

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Background. *Plasmodium knowlesi* is increasingly recognized as a cause of human malaria in Southeast Asia but there are no detailed prospective clinical studies of naturally acquired infections.

Methods. In a systematic study of the presentation and course of patients with acute *P. knowlesi* infection, clinical and laboratory data were collected from previously untreated, nonpregnant adults admitted to the hospital with polymerase chain reaction–confirmed acute malaria at Kapit Hospital (Sarawak, Malaysia) from July 2006 through February 2008.

Results. Of 152 patients recruited, 107 (70%) had *P. knowlesi* infection, 24 (16%) had *Plasmodium falciparum* infection, and 21 (14%) had *Plasmodium vivax*. Patients with *P. knowlesi* infection presented with a nonspecific febrile illness, had a baseline median parasitemia value at hospital admission of 1387 parasites/μL (interquartile range, 6–222,570 parasites/μL), and all were thrombocytopenic at hospital admission or on the following day. Most (93.5%) of the patients with *P. knowlesi* infection had uncomplicated malaria that responded to chloroquine and primaquine treatment. Based on World Health Organization criteria for falciparum malaria, 7 patients with *P. knowlesi* infection (6.5%) had severe infections at hospital admission. The most frequent complication was respiratory distress, which was present at hospital admission in 4 patients and developed after admission in an additional 3 patients. *P. knowlesi* parasitemia at hospital admission was an independent determinant of respiratory distress, as were serum creatinine level, serum bilirubin, and platelet count at admission ($P < .002$ for each). Two patients with knowlesi malaria died, representing a case fatality rate of 1.8% (95% confidence interval, 0.2%–6.6%).

Conclusions. Knowlesi malaria causes a wide spectrum of disease. Most cases are uncomplicated and respond promptly to treatment, but approximately 1 in 10 patients develop potentially fatal complications.

Five species of *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*) cause naturally acquired malaria in humans. The most recently identified species is *P. knowlesi*, which we previously reported to be the most common cause of hospitalization for malaria in the Kapit Division of Sarawak in Malaysian Borneo [1]. Further studies of blood samples from patients presenting with malaria in Sarawak, Sabah, and Peninsular states confirmed a much wider distribution within Malaysia [2]. There have also been reports of locally acquired *P. knowlesi* infections from Southern Thailand, the Myanmar-China border, the Philippines, and Singapore [3–7], indicating that transmission occurs in many Southeast Asian countries.

P. knowlesi is primarily a chronic infection of the long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaques [8]. It is easily confused with *Plasmodium malariae* on blood film microscopy in cases of human infection, because the morphologic appearances are almost identical [9, 10]. However, *P. knowlesi* is unique amongst the primate and human malarias in that it has a 24-h erythrocytic cycle [10], which is a characteristic that is likely to accelerate the development of complications [2]. Information on the characteristics of knowlesi malaria in humans, however, is restricted to single case reports [3, 5, 7]; our previous retrospective study of 94 patients with uncomplicated cases, in

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which we described available data relating to clinical features at presentation only [1]; and our report of 4 fatal cases [2]. We have, therefore, undertaken a detailed, systematic, prospective study of the presentation and clinical course of patients with a diagnosis of confirmed acute knowlesi malaria.

PATIENTS AND METHODS

Study site. This prospective study was conducted in the Kapit Division, which has a total population of 109,000 people of mostly Iban ethnicity [1]. A single World Health Organization (WHO) level 2 hospital serves the Division, together with 3 polyclinics and 22 rural health clinics. Health policy mandates that all patients with malaria are hospitalized until negative blood smear results are obtained on 2 consecutive days. Treatment for malaria is provided free of charge.

Subjects. Recruitment was consecutive and took place during 2 periods totalling 17 months from July 2006 through February 2008. All nonpregnant patients aged ≥15 years who were admitted to Kapit Hospital with a blood film result positive for any *Plasmodium* species were eligible, provided that there was no significant comorbid disease and that they had taken no antimalarial treatment within the previous 14 days. Subsequent confirmation of malaria species was determined by nested polymerase chain reaction assays [1]. All patients provided witnessed informed consent to the study procedures, which were approved by the Medical Research Ethics Subcommittee of the Malaysian Ministry of Health. In an initial 2-month pilot study, most cases of *P. vivax* and *P. falciparum* infection were among logging camp workers returning from long periods in Oceania or Equatorial Africa, respectively. Because the demographic characteristics and background immunity of these patients were significantly different from those of patients with knowlesi malaria, their clinical and laboratory data are presented but are not compared directly with data for patients with *P. knowlesi* infection.

Clinical procedures. Detailed demographic characteristics, history, and examination findings were recorded on a standard form. A baseline blood sample was obtained for routine biochemical and hematological testing, and regular monitoring of temperature, blood pressure, and pulse rate was started. Treatment was administered promptly according to the Malaysian Ministry of Health Guidelines. Because there are no current guidelines for *P. knowlesi* malaria, the guidelines for *P. malariae* were used. Patients with uncomplicated knowlesi malaria received oral chloroquine (25 mg base/kg over a 3-day period) followed by primaquine (15 mg daily for 2 days) given as a gametocidal agent. Oral and/or intravenous hydration was administered at the discretion of the treating physician. Patients presenting with or developing features of severe malaria were treated in accordance with WHO guidelines [11] except that the thresholds for hyperparasitemia and anemia were changed to >100,000 asexual forms/μL of whole blood and <7.1 g of hemoglobin/dL, respectively, to allow for the low immunity levels of the local population. If indicated clinically, patients were transferred to Sibu Hospital for intensive care.

All patients were assessed clinically and by microscopic examination of blood films on each inpatient day. Additional laboratory tests were performed as indicated by the clinical state of the patient. Parasite clearance time and fever clearance time were taken as the number of days to the first of at least 2 follow-up assessments at which the patient had negative blood film results and was afebrile, respectively. When the patient was afebrile and had negative blood film results for 2 consecutive days, additional blood samples were obtained for routine biochemical and hematological tests before discharge. Patients returned on the 28th day after hospital admission for clinical review and blood tests.

Laboratory procedures. All blood films were examined by 2 experienced microscopists. The parasite density was first determined at Kapit Hospital on the basis of the number of parasites per 500 white blood cells and the total white blood cell count for each patient. Microscopic examination was repeated in Kuching, with the second microscopist blinded to the initial result. The mean of the 2 parasite densities was used in data analysis. Parasite DNA was extracted from blood spots that had been collected on filter paper, and the *Plasmodium* species was determined by nested polymerase chain reaction for *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, as described elsewhere [1, 12].

Hematological profiles were determined on site using semiautomated methods (Sysmex model KX-21N). Serum sodium, potassium, glucose, creatinine, bilirubin, alanine aminotransferase (ALT), and albumin levels were either assayed on site (AVL 9180 and Hitachi 902; Roche/Hitachi, Roche Diagnostics) or serum samples were stored at −80°C before transfer on dry ice to the Biochemistry Department, Fremantle Hospital (Freemantle, Australia), for analysis (Cobas Integra 800; Roche Diagnostics). An additional uncuffed blood sample was collected into a chilled fluoride-oxalate tube, centrifuged immediately and separated plasma stored at −80°C before transfer on dry ice to Fremantle Hospital for plasma lactate assay (COBAS INTEGRA 800). Other laboratory investigations, including blood cultures, urine dipstick testing, microscopic examination, and chest radiography were performed as indicated clinically.

Statistical analysis. Data were analyzed using SPSS software, version 15.0 (SPSS). Normally distributed variables were compared using the Student's *t* test or analysis of variance and the Scheffé post hoc test. All other data were analyzed using nonparametric methods (the Wilcoxon rank-sum test or Friedman test). Proportions were compared with use of Fisher's exact test. Multiple logistic or linear regression analysis using forward conditional modeling was performed to determine baseline associates of complications or markers of severity, respectively. Plausible predictive variables with a statistically significant ($P < .05$) univariate association with the specific severity outcome were selected for inclusion in the model. These variables were log-transformed prior to model entry if they were nonnormally distributed and a stepwise forward selection procedure was then performed to identify the significant independent associates in each case.

RESULTS

Baseline characteristics. The number of patients who participated in the study in relation to all malaria admissions to Kapit Hospital during the recruitment period is shown in figure 1. Their baseline demographic and clinical features are summarized in table 1. *P. knowlesi* infections were acquired locally by both sexes and across all age groups, with 93 (87%) of patients reporting recent activities in the jungle or forest-fringe in the Kapit Division. All regions along the Rejang River and its associated tributaries were represented, and there was no significant clustering of cases. Confirming our pilot study findings, most of the cases of vivax and falciparum malaria (31 cases; 69%) were imported, and the numbers were relatively small.

The overall median duration of symptoms prior to hospitalization was 5 days (interquartile range, 3–5 days), but 2 patients were unwell for >10 days before hospitalization. Symptoms were typically nonspecific. Fever and chills were present in almost all cases, and other frequent symptoms included abdominal pain, breathlessness, and productive cough. Tachypnea, pyrexia, and tachycardia were common clinical signs (table 1).

The results of baseline laboratory investigations are summarized in table 2. The level of parasitemia at hospital admission was relatively low in the *P. knowlesi* group, but there was a wide range that included 3 patients (2.8%) with parasite densities >100,000 parasites/μL and 33 patients (30.8%) with densities <500 parasites/μL. The most common abnormal laboratory finding was thrombocytopenia (<150,000 platelets/μL), which was present in 104 patients (98%), with 31 (29%) of 107 patients having a platelet count <50,000 platelets/μL. The 3 patients who did not have thrombocytopenia (155,000, 152,000, and 167,000 platelets/μL) had low parasitemias (5, 126, and 170 asexual forms/μL, respectively), and all became thrombocytopenic within 24 h (with nadir values of 90,000, 131,000, and 112,00 platelets/μL, respectively). Lymphopenia was found in 7 (6.5%) of patients at presentation, but all patients had normal values by the time of hospital discharge. Anemia was uncommon at hospital admission. Only 5 (4.6%) of the patients had a hemoglobin concentration <10 g/dL, whereas none of the patients met the criteria for severe anemia. Mild hepatic dysfunction, usually comprising an elevated serum ALT level and a low serum albumin level, was relatively common. Mild-to-moderate hyponatremia (range, 122–135 mmol/L) was evident in 29% of cases, all of which responded to rehydration and antimalarial therapy.

On the basis of WHO criteria for severe falciparum malaria [11], 8 (7.5%) of the patients with *P. knowlesi* infection had severe infections at presentation (table 3). The most frequent clinical presentations of severe infection were respiratory distress (diagnosed in 4 patients on the basis of a respiratory rate >30 breaths/min, oxygen saturation <94% by pulse oximetry, auscultatory findings, and radiographic changes), hyperparasitemia (3 patients), and jaundice (serum total bilirubin >43 μmol/L in 3 patients). There were 3 cases of renal failure (serum

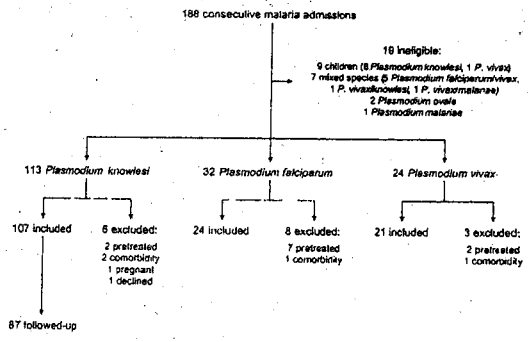

Figure 1. Flow chart showing patient recruitment, exclusion, and follow-up in a study of human *Plasmodium knowlesi* infection in Malaysia.

Table 1. Demographic and Clinical Characteristics of Patients Admitted to Kapit Hospital (Sarawak, Malaysia) with Untreated Malaria Categorized by *Plasmodium* Species

Variable	*Plasmodium knowlesi* (*n* = 107)	*Plasmodium falciparum* (*n* = 24)	*Plasmodium vivax* (*n* = 21)	*P*
Age, years				
Mean value (± SD)	44.9 ± 14.94[a]	38.7 ± 9.64	35.5 ± 10.61	.006
Range	16–79	15–53	15–51	
Male sex	56.1[b,c]	95.8	100	<.001
Iban ethnicity	91.6	95.8	76.2	.073
Occupation				<.001
Farmer	49.5	4.2	9.5	
Logging/plantation worker	27.1[b,c]	91.7	71.4	
Other	23.4	4.2	19	
Self-reported previous malaria	26.2[b,c]	75	57.1	<.001
Previous foreign travel	19.6[b,c]	91.7	71.4	<.001
Foreign travel within previous 4 weeks	0.9[b,c]	83.3	52.4	<.001
Duration of illness, median days (IQR)	5 (3–7)	2.5 (1–4.75)	3 (1–5)	<.001
Symptom				
Fever/chills	100	91.7	95.1	NA
Headache	94.4	87.5	52.4	NA
Rigors	89.7	79.2	85.7	NA
Malaise	89.7	91.7	66.7	NA
Anorexia	83.2	70.8	52.4	NA
Myalgia	87.9	79.2	90.2	NA
Cough	56.1	54.7	47.6	NA
Nausea	56.1	87.5	28.5	NA
Vomiting	33.6	41.7	19.0	NA
Abdominal pain	52.3	37.5	23.8	NA
Diarrhea	29.0	47.5	33.3	NA
Clinical findings				
Axillary temperature, median °C (IQR)	37.6 (37.0–38.5)	37.8 (37.0–38.5)	37.0 (36.8)	NA
Respiratory rate, median breaths/min (IQR)	26 (22–31)	25.5 (22.3–28.5)	27 (24.5–29.0)	NA
Pulse rate, mean beats/min (± SD)	95 ± 16	99 ± 17	97 ± 18	NA
Arterial blood pressure, mean mmHg (± SD)	89 ± 11	85 ± 9	89 ± 9	NA
Capillary refill time, median secs (IQR)	2 (2–3)	2 (2–3)	2 (2–3)	NA
Palpable liver	24.3	29.2	16.7	NA
Palpable spleen	15.0	20.8	23.8	NA

NOTE. Data are percentage of patients, unless otherwise indicated. IQR, interquartile range; NA, not assessed; SD, standard deviation.

[a] $P < .05$ vs *P. vivax*.
[b] $P < .01$ vs *P. falciparum*.
[c] $P < .01$ vs *P. vivax*.

creatinine level ≥265 μmol/L despite fluid resuscitation), 2 cases of hypotension (systolic blood pressure ≤80 mmHg despite fluid resuscitation), and 1 case of hypoglycemia (venous plasma glucose level <2.2 mmol/L). There were no cases of unrousable coma. A combination of features was present at hospital admission in 3 patients.

Clinical course. Clinical and parasitological outcomes together with changes in key hematological and biochemical variables during hospitalization and at day 28 for patients with knowlesi malaria are summarized in tables 4 and 5. There was no clinical, laboratory, or radiological evidence of other infections or conditions at study entry, during hospitalization, or at follow-up that would have influenced outcome. When patients with knowlesi malaria were discharged from the hospital, platelet counts had increased, and all patients had values that were within the normal range by day 28. Most of the remaining hematological and biochemical parameters had improved by hospital discharge. Abnormal laboratory values had resolved in all 87 patients with knowlesi malaria who attended for day 28 review.

Three patients, including 2 patients without complications at hospital admission, developed respiratory distress (table 3). A total of 7 (6.5%) of the 107 patients in the knowlesi group, all of whom were female, presented with or developed respiratory distress. Of those patients with evidence of severe knowlesi malaria either at presentation or during treatment, 2 died (table 3). Patient 1 had parasitemia at presentation (parasite density, 222,570 parasites/μL), evidence of multiorgan failure,

Table 2. Laboratory Results for Patients Admitted to Kapit Hospital with Untreated Malaria Categorized by *Plasmodium* Species

Variable	Normal range	*Plasmodium knowlesi* (*n* = 107)	*Plasmodium falciparum* (*n* = 24)	*Plasmodium vivax* (*n* = 21)
Parasite count, parasites/μL	NA	1387 (6–222,570)	26,781 (1840–271,760)	4258 (324–32,132)
Hemoglobin level, g/dL	11.3–15.7	13.3 (12.0–14.3)	12.9 (12.3–13.6)	13.5 (12.6–13.8)
White blood cell count, × 10^3 cells/μL	3.1–10.3	5.6 (4.7–7.0)	6.3 (5.3–8.6)	6.1 (4.9–7.8)
Neutrophil count, mean neutrophils × 10^3/μL (± SD)	.2–5.3	3.7 ± 1.8	4.6 ± 2.4	4.6 ± 2.2
Lymphocyte count, × 10^3 cells/μL	0.8–2.7	1.5 (1.1–2.0)	1.0 (0.8–1.4)	1.0 (0.6–1.7)
Platelet count, mean value × 10^3 platelets/μL (± SD)	150–450	71 ± 35	108 ± 59	118 ± 51
Prothrombin time, secs	NA	13 (12–15)	15 (13–16)	12 (12–14)
Blood group O, % of patients	NA	28.0	12.5	9.5
Serum creatinine level, μmol/L	<133	86 (73–100)	89 (80–97)	89 (76–98)
Serum sodium level, mmol/L	136–152	137 (135–140)	138 (135–140)	138 (135.5–141)
Serum total bilirubin, μmol/L	<21	13 (9–18)	17 (12–22)	16 (10–21)
Serum alanine aminotransferase level, IU/L	<40	36 (25–54)	26 (20–40)	27 (13–55)
Serum albumin level, g/dL	>36	36 (33–39)	38 (35–41)	41 (39–46)
Serum glucose level, mmol/L	4–8	6.2 (5.3–6.7)	6.4 (5.7–7.2)	6.2 (5.5–7.0)
Plasma lactate level, mmol/L	<2	1.6 (1.2–2.0)	1.5 (1.2–2.0)	1.5 (1.1–2.0)

NOTE. Unless otherwise indicated, data are median value (interquartile range). NA, not applicable.

hypoglycemia, and lactic acidosis. This patient died within 6 h after hospital admission despite intensive treatment with intravenous quinine, broad spectrum antibiotics, and ionotropic and ventilatory support. Patient 8 presented with symptoms and signs of a right hemiparesis and sensory inattention and had a history of uncontrolled hypertension. The patient's parasite density at hospital admission was 214,000 parasites/μL. She was treated with intravenous quinine but developed respiratory distress that required mechanical ventilation. After showing signs of improvement, she experienced neurological deterioration on the seventh day of hospitalization and died 24 h later. No neuroimaging studies were possible.

Baseline* P. knowlesi *parasitemia, complications, and markers of severity. Patients reporting breathlessness or vomiting had greater geometric mean parasite counts than did those who did not report these symptoms ($P = .025$ and $P = .038$, respectively). In a logistic regression model, presentation with or development of respiratory distress was positively and independently associated with the admission ln(parasitemia) and inversely associated with the admission hemoglobin level ($P = .004$ and $P = .015$, respectively). In multiple linear regression, (1) ln(parasitemia) and age were independent positive associates of ln(admission serum creatinine) ($P < .001$ and $P = .007$, respectively), (2) ln(parasitemia) and ln(plasma glucose) were independent associates of ln(admission serum total serum bilirubin) ($P = .003$ and $P = .008$, respectively), and (3) ln(parasitemia) was an independent associate of the ln(admission platelet count) and absolute differences between day 28 and hospital admission platelet counts ($P = .002$ and $P = .004$, respectively). In other multivariate models, ln(parasitemia) was not an independent associate of the admission hemoglobin level ($P = .49$) or serum ALT level ($P = .70$). In receiver operating characteristic curve analysis, parasitemia was a good predictor of complications after excluding hyperparasitemia (area under the receiver operating characteristic curve, 0.90 [95% confidence interval, 0.82–0.98]; $P < .001$). The prespecified 100,000/μL threshold was highly specific (specificity, 100%) but had a sensitivity of 30%.

DISCUSSION

The present study provides the first detailed, prospective evaluation of *P. knowlesi* infection in an area of Malaysian Borneo in which it is the most common locally acquired human malaria. Although there were demographic differences between the 3 groups of patients with malaria, there were no presenting symptoms or signs that distinguished knowlesi malaria from either falciparum or vivax malaria. Consistent with available—albeit, incomplete—retrospective data [1, 2], most cases of knowlesi malaria were uncomplicated and responded promptly to treatment with chloroquine and primaquine, but complications developed in nearly 1 in 10 patients. Because the number of cases of severe knowlesi malaria was small, an accurate case fatality rate is difficult to ascertain, but the case fatality rate was 1.8% (95% confidence interval, 0.2%–6.6%) in our sample. Malaria may have been a contributory factor rather than the sole cause in our patient who presented with a stroke. Nevertheless, *P. knowlesi* infections occur in older as well as younger adult patients in the Kapit Division, and the vital organ dysfunction caused by this parasite may unmask underlying significant comorbidities.

Despite the significantly lower peripheral blood parasitemia, the patients with knowlesi malaria had clinical and laboratory profiles that were largely similar to those for patients with *P.*

Table 3. Details of Knowlesi Patients Presenting with (Patients 1–8) or Developing (Patients 9 and 10) Severe Malaria

Patient	Age, years	Sex	Hyperparasitemia	Hypotension	Acute renal impairment	Jaundice	Hypoglycemia	Lactic acidosis	Severe anemia	Acute pulmonary edema or respiratory distress syndrome	Outcome
1	68	F	Yes (parasite count, 222,570 parasites/μL)	Yes (systolic blood pressure, 80 mm Hg)	Yes (serum creatinine level, 320 μmol/L)	Yes (total serum bilirubin, 45 μmol/L)	Yes (plasma glucose level, <1.1 mmol/L)	Yes (plasma lactate level, 17.4 mmol/L)	No	Yes	Died
2	36	M	Yes (parasite count, 178,000 parasites/μL)	No	Yes (serum creatinine level, 385 μmol/L)	No	No	No	No	No	Discharged
3	50	F	No	No	No	Yes (total serum bilirubin, 87 μmol/L)	No	No	No	Yes	Discharged
4	71	M	No	Yes (systolic blood pressure, 79 mm Hg)	No	No	No	No	No	No	Discharged
5	66	M	No	No	No	Yes (total serum bilirubin, 66 μmol/L)	No	No	No	No	Discharged
6	61	F	No	No	No	No	No	No	No	Yes	Discharged
7	69	F	No	No	Yes (serum creatinine level, 418 μmol/L)	No	No	No	No	Yes	Discharged
8	36	F	Yes (parasite count, 214,000 parasites/μL)	No	No	Yes (total serum bilirubin, 178 μmol/L)	No	No	No	Yes	Died
9	73	F	No	No	No	No	No	No	No	Yes	Discharged
10	54	F	No	No	No	No	No	No	No	Yes	Discharged

NOTE. Severe malaria was defined on the basis of World Health Organization criteria for severe falciparum malaria [11]: hyperparasitemia was defined as >100,000 parasites/μL. Severe anemia was defined as hemoglobin concentration <7.1 g/dL. Hypotension was defined as systolic blood pressure ≤80 mm Hg; Acute renal impairment was defined as a serum creatinine level >265 μmol/L despite rehydration. Jaundice was defined as serum bilirubin level >43 μmol/L. Hypoglycemia was defined as a serum glucose level <2.2 mmol/L. Hyperlactatemia was defined as a lactate level >6.0 mmol/L. Acute pulmonary edema or respiratory distress was defined as a respiratory rate >30 breaths/min plus oxygen saturation <94% on room air and/or pulmonary infiltrates visible on a chest radiograph.

Table 4. Measures of Outcome in Patients Categorized by *Plasmodium* Species

Variable	*Plasmodium knowlesi* (n = 107)	*Plasmodium falciparum* (n = 24)	*Plasmodium vivax* (n = 21)
Fever clearance time, h	20 (12–31)	20 (11–37)	16 (4–28)
Parasite clearance time, days	1 (1–2)	3 (2–3.75)	3 (2–3)
Duration of hospitalization, days[a]	3 (3–4)	4 (4–5)	4 (3–4)

NOTE. Data are median value (interquartile range).

[a] Excludes 2 patients who died and 2 patients with hospital admission and day 1 data only.

falciparum and *P. vivax* infection, with a wide spectrum of illness. The most frequent complication in our cohort was respiratory distress, which affected 1 in 15 patients. It is also a relatively common sequelum of severe falciparum malaria [13]. Respiratory distress can reflect pulmonary edema, acute respiratory distress syndrome, or metabolic acidosis. In our group, a pulmonary, rather than metabolic, etiology was the main cause, because we measured blood lactate concentrations and had access to chest radiographs and pulse oximetry. The strong association between parasitemia at hospital admission and the development of respiratory distress in our patients suggests that parasite-specific effects that increase pulmonary capillary permeability rather than iatrogenic fluid overload or the syndrome of inappropriate anti-diuretic hormone secretion are responsible, as in falciparum malaria [14]. Patients with falciparum malaria who develop respiratory distress have a relatively poor prognosis [13], and both of our patients who died developed this complication. Respiratory distress has also been reported as a rare complication of vivax [15–17] and ovale [18, 19] malaria. We cannot explain the disproportionate number of female patients with this complication in the *P. knowlesi* group.

Although the women in our cohort, compared with the men, had lower serum albumin concentrations at presentation (34.5 g/L vs 38.0 g/L; $P < .001$), sex association has not been reported in the case of the other human malarias and is likely to be attributable to the play of chance in the present study.

The *P. knowlesi* parasitemia at hospital admission was also strongly and independently associated with renal dysfunction, and 3 patients developed renal failure despite resuscitation and rehydration. As with respiratory distress, this is another complication of falciparum malaria that could be mediated by the parasite [20], although the microvascular sequestration that may contribute to *P. falciparum*–associated renal dysfunction [21] is not known to occur in *P. knowlesi* infection. The presence of *P. knowlesi* parasitemia at hospital admission was also independently associated with the total serum bilirubin but not serum ALT level. This could reflect relatively brisk hemolysis associated with the short (24-h) erythrocytic cycle rather than abnormal liver function, but the median parasitemia was low, and there was no inverse association with hemoglobin level at hospital admission. It is still possible that hepatic dysfunction is a relatively late vital organ complication of *P. knowlesi* malaria

Table 5. Changes in Laboratory Test Results between Hospital Admission and Discharge and Hospital Admission and Day 28 in Patients with *Plasmodium knowlesi* Infections

Variable	Change from hospital admission to discharge (n = 103)	Change from hospital admission to day 28 (n = 87)
Hemoglobin level, g/dL	−1.3 ± 1.0	0 ± 1.3[a]
White blood cell count, ×10³ cells/μL)	0.1 ± 1.8	1.2 ± 2.1[a]
Neutrophil count, median value × 10³ cells/μL (IQR)	−0.6 (−1.5 to 0.6)[a]	0.5 (−0.5 to 1.45)
Lymphocyte count, median value × 10³ cells/μL (IQR)	0.7 (0.40–1.3)[a]	0.9 (0.4–1.5)[a]
Platelet count, median value × 10³ platelets/μL (IQR)	65 (31–113)[a]	184 (144–222)[a]
Serum creatinine level, median μmol/L (IQR)	−8.5 (−19 to 1)[a]	−12 (−21 to 0)[b]
Serum sodium level, median mmol/L (IQR)	2 (0.1–5)[a]	3 (0–8)[a]
Serum total bilirubin, median μmol/L (IQR)	−6 (−15 to −3)[a]	−7 (−12.9 to −4.3)[a]
Serum alanine aminotransferase level, median IU/L (IQR)	−1 (−10 to 11)	−18 (−32.9 to −4)[a]
Serum albumin level, g/dL	−1.0 ± 2.8	4.8 ± 4.1[a]
Serum glucose, median mmol/L (IQR) (n = 56)	−0.4 (−1.1 to 0.8)	−0.6 (−1.21 to 0.37)[b]
Plasma lactate level, median mmol/L (IQR) (n = 56)	0.1 (−0.5 to 0.4)	−0.1 (−0.5 to 0.5)

NOTE. Unless otherwise indicated, data are mean value ± standard deviation. IQR, interquartile range.

[a] $P < .01$.

[b] $P \leq .05$.

but—as evidenced by patient 1, who presented with jaundice, hypoglycemia, and lactic acidosis—it is one with potentially devastating metabolic consequences.

Consistent with the nonsequestering nature of *P. knowlesi*, we did not observe significant neurologic sequelae except in patient 8, who had evidence of a stroke in the context of preexisting cerebrovascular risk. In addition, in contrast with the group of patients with *P. falciparum* infection, the group of patients with *P. knowlesi* included no patients with severe anemia. Both severe anemia and neurologic disturbance have been reported recently as common manifestations of severe vivax malaria [22, 23], but these complications were observed in patients who were younger than those in the present study and in areas of much greater malaria transmission of multiple *Plasmodium* species.

Despite the very high prevalence of thrombocytopenia among our patients with *P. knowlesi* infection (100%, compared with <80% in other human malarias [24–26]), none had a clinically evident coagulopathy. This is consistent with the relative infrequency of bleeding episodes complicating severe falciparum malaria [11], but it is possible that a low platelet count (52,000 platelets/μL) and prolonged prothrombin time (17 sec) contributed to an intracerebral hemorrhage in the patient with knowlesi malaria who died of a probable stroke. The almost invariable presence of thrombocytopenia could facilitate diagnosis of knowlesi malaria. In addition, the significant association between platelet count and *P. knowlesi* parasite density and, in turn, the relationship between parasitemia and markers of severity, could imply that very low platelet counts are of prognostic significance. Such a relationship has been found among African children with falciparum malaria [27].

Although our study included relatively few patients with severe knowlesi malaria, we provide preliminary data relating to the incidence of severe disease. A larger study on the main complications and pathophysiology of knowlesi malaria is in progress, with the aim of establishing specific criteria for severity. It is likely that those for severe falciparum malaria, including neurologic sequelae, severe anemia, and hyperparasitemia [11], may not adequately address the unique biologic properties of *P. knowlesi*. In the case of falciparum malaria, $\geq$250,000 parasites/μL (or 5% parasitized erythrocytes) is conventionally used [11], but thresholds as low as 100,000/μL have been associated with increased mortality and have been used for nonimmune patients [28, 29]. It is therefore important to determine knowlesi-specific markers of disease severity, especially an accurate risk-associated threshold parasitemia.

Our study shows that knowlesi malaria is a significant cause of morbidity in the Kapit Division, extends available data to characterize the spectrum of illness and its clinical course, and confirms our previous observation that life-threatening complications can supervene [2]. Knowlesi malaria is widely distributed in Southeast Asia; it affects mainly people who enter forests or the forest fringe, but the transmission ecology of this potentially serious disease may be changing [30]. Recently, European travellers to Malaysia have received a diagnosis of knowlesi malaria following their return home [31, 32]. The increase in tourism in Southeast Asia may mean that more cases are detected in the future, including in Western countries. Clinicians assessing a patient who has visited an area with known or possible *P. knowlesi* transmission should be aware of the diagnosis, its clinical manifestations, and its course.

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No. 28

医薬品 研究報告 調査報告書

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研究報告の概要

○英国の匿名扁桃検体中の疾患関連プリオンタンパク質の陽性率:横断的便乗検査

目的:英国の一般集団における疾患関連プリオンタンパク質(PrP(CJD))のより正確な陽性率を確認し、健康への脅威となる変異型クロイツフェルトヤコブ病(vCJD)伝播を低減するための適切な公衆衛生対策に役立てること。

デザイン、調査対象:イングランドおよびスコットランド全域で選択的扁桃摘出術で摘出された匿名扁桃腺検体を対象に、横断的便乗調査を実施した。

主要評価項目:分析原理の異なる2つの酵素免疫法を用いて調べたPrP(CJD)の有無(いずれかの方法で陽性となった場合は、免疫組織化学法または免疫ブロット法による更なる検査を行う)。

結果:2008年9月末までに63,007検体の検査が終了した。このうち12,753例は、vCJDのほとんどが発生した1961～85年生まれの集団であり、19,908例は感染牛肉および加工品によりBSEに曝露した可能性がある1986～95年生まれであった。検査検体のうち、両方の酵素免疫法で明らかな陽性となったものはなかった。1つの検査法で陽性となり、もう一方があいまい(擬陽性)となったのは2検体のみで、残り9検体は両方とも擬陽性結果となった。276検体は、どちらか一方の検査法で初回陽性であった。繰り返し陽性率は15%以下であり、検査法とカットオフの定義により左右された。免疫組織化学法または免疫ブロット法を実施した検体(初回陽性となった276検体すべてを含む)のうちPrP(CJD)陽性となった検体はなかった。

結論:観察された扁桃検体中のPrP(CJD)有病率は、1961～95年生まれの集団では0/32,661(95%信頼区間0～113/100万)であった。1961～85年生まれの有病率は0(95%信頼区間0～289/100万)で、過去の虫垂組織の調査(292/100万、95%信頼区間60～853/100万)より低かったが統計的に矛盾はなかった。引き続き扁桃検体を集めて検索することで、特に年長の集団、あるいは他の補完的な大規模な匿名の組織調査(特に剖検組織)の検索により、PrP(CJD)の陽性率の算出精度は更に高まるであろう。

報告企業の意見	今後の対応
英国の匿名扁桃検体中の疾患関連プリオンタンパク質の陽性率調査において、PrP(CJD)陽性が確認された検体はなかったとの報告である。	これまでの疫学研究等では、血液製剤を介して古典的CJD(弧発性、遺伝性および医原性CJD)が伝播するという証拠はない。またCJDの病原因子とされる異常プリオンがアルブミン製剤の製造工程で効果的に除去されるとの報告もあるが、輸血あるいは第Ⅷ因子製剤によりvCJDに感染する可能性が示唆されたことから、今後も引き続き情報の収集に努める。なお、日本赤十字社は、CJD、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)、CJDの既往歴(本人、血縁者)、hGH製剤投与の有無を確認し、該当するドナーを無期限に献血延期としている。

使用上の注意記載状況・その他参考事項等
赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/50mL 血液を原料とすることに由来する感染症伝播等

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RESEARCH

Prevalence of disease related prion protein in anonymous tonsil specimens in Britain: cross sectional opportunistic survey

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ABSTRACT

Objective To establish with improved accuracy the prevalence of disease related prion protein (PrP^{dcp}) in the population of Britain and thereby guide a proportionate public health response to limit the threat of healthcare associated transmission of variant Creutzfeldt-Jakob disease (vCJD).

Design Cross sectional opportunistic survey.

Study samples Anonymised tonsil pairs removed at elective tonsillectomy throughout England and Scotland.

Setting National anonymous tissue archive for England and Scotland.

Main outcome measure Presence of PrP^{dcp} determined by using two enzyme immunoassays based on different analytical principles, with further investigation by immunohistochemistry or immunoblotting of any samples reactive in either assay.

Results Testing of 63 007 samples was completed by the end of September 2008. Of these, 12 753 were from the birth cohort in which most vCJD cases have arisen (1961-85) and 19 908 were from the 1986-95 cohort that would have been also exposed to bovine spongiform encephalopathy through infected meat or meat products. None of the samples tested was unequivocally reactive in both enzyme immunoassays. Only two samples were reactive in one or other enzyme immunoassay and equivocal in the other, and nine samples were equivocally reactive in both enzyme immunoassays. Two hundred and seventy six samples were initially reactive in one or other enzyme immunoassay; the repeat reactivity rate was 15% or less, depending on the enzyme immunoassay and cut-off definition. None of the samples (including all the 276 initially reactive in enzyme immunoassay) that were investigated by immunohistochemistry or immunoblotting was positive for the presence of PrP^{dcp}.

Conclusions The observed prevalence of PrP^{dcp} in tonsils from the 1961-95 combined birth cohort was 0/32 661 with a 95% confidence interval of 0 to 113 per million. In the 1961-85 cohort, the prevalence of zero with a 95% confidence interval of 0 to 289 per million was lower than, but still consistent with, a previous survey of appendix tissue that showed a prevalence of 292 per million with 95% confidence interval of 60 to 853 per million. Continuing to archive and test tonsil specimens, especially in older birth cohorts, and other complementary large scale anonymous tissue surveys, particularly of post-mortem tissues, will further refine the calculated prevalence of PrP^{dcp}.

INTRODUCTION

Although the risk to the population of Britain of dietar exposure to the bovine spongiform encephalopath agent that causes variant Creutzfeldt-Jakob disease (vCJD) has been virtually eliminated, the occurrenc to date of four cases of vCJD infection resulting fror blood transfusion has made real the threat of a second ary epidemic through healthcare associated human t human transmission.[1-4] These cases from blood trans fusion have also established the existence of an infec tive asymptomatic stage in human vCJD. Estimatin the prevalence of this asymptomatic infective stage although technically challenging, is essential to guid a proportionate public health response to reduce th risk of healthcare associated transmission.

Measurement of prevalence in the 1961-85 birt cohort is a priority, given that 138 of the 167 cases c vCJD to date in Britain have been in this group (with 3 cases in the 1961-9 and 99 in the 1970-85 birt cohorts). Data are available from previous analyses c appendix and tonsil specimens for the presence of dis ease related prion protein (designated PrP^{dcp}) b immunohistochemistry and immunoblotting.[5 6] Th first study screened 11 947 appendix specimens an 1427 tonsil specimens by immunohistochemistry an found three positives in the appendixes from the 1961 85 birth cohort, giving a prevalence of 292 (95% cor fidence interval 60 to 853) per million.[5] A second stud found no positives in 2000 tonsil specimens screene by both immunohistochemistry and immunoblotting half of these tonsils were from patients aged ove

9 years and hence in the birth cohort likely to have had dietary exposure to bovine spongiform encephalopathy. Uncertainty about the true prevalence was increased when back calculation using plausible assumptions from the observed clinical vCJD cases suggested a much lower prevalence of sub-clinical vCJD infection than would be predicted from the finding of PrP^{CJD} in three appendixes.[5,7]

The absence of a suitable blood test for PrP^{CJD}, and doubt about the clinical interpretation for a patient of a positive test result from testing any tissue, created major organisational and technical challenges for our large scale prevalence survey of PrP^{CJD}. To facilitate semi-automated enzyme immunoassay screening, we chose anonymised surgically removed tonsil pairs collected prospectively for the study reported here, rather than appendix tissue already archived in paraffin blocks that would have needed more labour intensive and slower immunohistochemical screening. PrP^{CJD} is known to accumulate to relatively high levels in the tonsils of people with vCJD, although, because of the difficulty of identifying such cases, it has not yet been shown to be present pre-clinically.[8,9]

Commercially available enzyme immunoassay kits are routinely used for testing for bovine spongiform encephalopathy, scrapie, and other animal prion diseases; however, when our survey began no validated kits were available for testing human samples for PrP^{CJD}. We therefore issued a formal tender calling for manufacturers to take part in an enzyme immunoassay selection study and to supply suitable kits. The companies that responded were each sent two blinded panels of samples. Two assays, from Microsens and Bio-Rad, were able to detect brain from vCJD cases diluted 10^{-3} and spleen diluted 10^{-2} into tonsil homogenate (Jillian Cooper, personal communication), and we selected these for use in this study. We now report the results of testing of the first 63 007 specimens from the intended collection of 100 000 in a national anonymous tissue archive.

METHODS

Test validation

We obtained unfixed palatine tonsil samples from 32 sheep with scrapie and 10 that were uninfected, as well as aliquots of unfixed frozen tonsil tissue taken at autopsy from six patients who died of vCJD. We prepared 12% homogenates from these and tested them by both enzyme immunoassays after making a dilution series from 10^{-1} to 10^{-5} with negative human tonsil homogenate. We used a panel of 250 human tonsils that had been previously tested and found to be negative by immunoblotting and immunohistochemistry as examples of "true" negative controls.[6]

Survey tissue samples

Paired tonsil samples from people of all ages, and from operations done between January 2004 and September 2008, were collected from hospitals throughout England and Scotland. One tonsil of the pair was collected as fresh tissue chilled to 4C, and the other tonsil was collected in formalin. Tonsils arrived at the study centre an average of 65 (mode 50, median 113) hours after operation. Once transferred to suitable containers, samples were stored either at −80C (fresh tissue) or at room temperature (fixed tissue).

Patients or their carers were given a leaflet explaining the aims of the study and that any result from testing their tonsil could not be traced back to them. An explicit paragraph and tick box to exercise a right to opt out of inclusion in the survey was included in the pre-tonsillectomy consent forms.

Investigatory algorithm

We homogenised a specimen of each tonsil pair and screened it with both enzyme immunoassays. We defined samples as "reactive," "high negative," or "negative" by a calculation based on the optical density readings from enzyme immunoassay for each microtitre plate. A reactive sample was within three standard deviations of the cut-off, and a high negative was within four standard deviations. We further investigated all samples that were initially reactive in either enzyme immunoassay or gave a high negative result in both enzyme immunoassays by immunoblotting and immunohistochemistry. We re-tested any sample that was high negative in one or other enzyme immunoassay by both enzyme immunoassays, and if it gave a reactive or high negative result in either we investigated it further by immunoblotting and immunohistochemistry. On occasion, we repeated immunoblotting tests with the same and with alternative antibodies.

Definition of a positive result

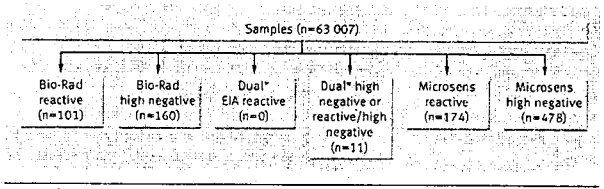
We defined a tonsil positive for PrP^{CJD} as one identified by enzyme immunoassay that was immunohistochemistry positive, had the expected specific protein band pattern in immunoblotting, or both.

RESULTS

Test performance

At a dilution of 10^{-3}, 31 of 32 scrapie sheep samples were reactive in both enzyme immunoassays, and at a 10^{-4} dilution 21were reactive in the Microsens enzyme immunoassay and 16 were reactive in the Bio-Rad enzyme immunoassay. One positive sample was detectable only at a dilution of 10^{-1}. Dilutions of 10^{-2} and 10^{-3} could be detected by immunoblotting.

The six tonsil aliquots from human vCJD cases varied in the amount of lymphoid germinal centre tissue that was present, as judged by visual inspection. Depending on the quality of the tissue, PrP^{CJD} was detectable down to a dilution of 10^{-3} in the Microsens enzyme immunoassay and 10^{-2} in the Bio-Rad enzyme immunoassay (table 1). The amount of PrP^{CJD} detected varied, as judged by the optical density values. This variation may have been due to biological differences in some cases, but an important contributory factor will have been the quality of the available tissue. Immunoblotting of aliquots of the vCJD samples showed that the expected specific band patterns of PrP^{CJD} were



Enzyme immunoassay screening of human tonsil tissue homogenates for PrP^{CJD} *Dual enzyme immunoassay (EIA) reactive samples gave optical density readings above the cut-off classified as "reactive" in both Bio-Rad and Microsens tests; dual high negative or reactive/high negative samples gave optical density readings above the cut-off classified as "high negative" in both Bio-Rad and Microsens tests or was reactive in one and high negative in the other. All EIA reactive samples and most high negative samples were subject to both immunoblotting and immunohistochemistry testing (see text)

detectable. The sensitivities of the enzyme immunoassays were comparable to the immunoblotting results.

Survey specimens collected

Between January 2004 and October 2008, a total of 67 696 tonsil pairs had been archived after collection from 134 hospital trusts throughout England and Scotland. We received forms without tonsil tissue for 1426 patients who objected and 762 in whom clinical pathology examination had been requested. All regions of England contributed samples, and 5651 came from Scotland between January 2006 and September 2008.

We also tested another 2015 anonymous specimens, from tonsillectomies done in the southeast of England between July 2000 and August 2002, of which half were from patients aged over 9 years at operation, and that were untested as part of an earlier survey.[6]

Table 1| Enzyme immunoassay results on available tonsil tissue from six variant Creutzfeldt-Jakob disease (vCJD) cases (including sample of brain from one case)*: highest dilutions for reported result

	Bio-Rad		Microsens	
Dilution†	Optical density	Interpretation	Optical density	Interpretation
Specimen 1:				
Tonsil 10^{-2}	0.06	High negative	0.12	Reactive
Brain 10^{-3}	0.39	Reactive	0.08	Reactive
Specimen 2:				
Tonsil 10^{-2}	0.04	Negative	0.11	Reactive
Specimen 3:				
Tonsil 10^{-2}	0.06	High negative	0.20	Reactive
Specimen 4:				
Tonsil 10^{-1}	0.04	Negative	0.10	Reactive
Specimen 5:				
Tonsil 10^{-3}	0.04	Negative	0.09	Reactive
Specimen 6:				
Tonsil 10^{-1}	0.13	Reactive	0.21	Reactive

*Three specimens supplied by National CJD Surveillance Unit (including paired tonsil and brain) and three by MRC Prion Unit.
†Dilution from 12% homogenate (10^0); 10^{-1} dilution is therefore equivalent to 0.012 g/ml vCJD tonsil tissue homogenate; as dilution is in negative homogenate, total tissue concentration was 0.12 g/ml for all samples tested.

Enzyme immunoassay screening results

By the end of September 2008, we had screened 63 00 samples with both enzyme immunoassays and, wher indicated, completed investigatory testing (figure).

In one or other of the enzyme immunoassays, 27 samples gave an optical density defined as reactive an 638 were classed as high negative (figure). To defin the repeat reactivity rate by enzyme immunoassay we retested 487 reactive and high negative sample by enzyme immunoassay at the beginning of the pro ject, before immunohistochemistry and immunoblot ting confirmatory testing. The repeat reactivity rat was 15% (7/48) for the initially reactive samples an 3.5% (4/116) for the initially high negative samples ir the Bio-Rad enzyme immunoassay. The equivalent fig ures for the Microsens enzyme immunoassay were 12% (7/60) and 10% (26/263). All initially reactive samples and any initially high negative samples tha gave a repeat reactive or high negative result enzyme immunoassay were subject to immunohisto chemistry and immunoblotting confirmatory testing Any samples that were initially reactive or high nega tive but which were not repeat tested by enzyme immu noassay went directly for immunohistochemistry and immunoblotting (figure).

No samples were clearly reactive in both enzyme immunoassays. One was reactive by Microsens and high negative by Bio-Rad, and another was reactive by Bio-Rad and high negative by Microsens. Nine were high negative by both the Microsens and Bio-Rad enzyme immunoassays. Seven of these 11 samples were methionine homozygote at codon 129 of the prion protein gene (*PRNP*) and four were heterozygote; only four (three homozygote and one heterozygote) were from people born before 1996 and therefore likely to have had dietary exposure to bovine spongiform encephalopathy.

Immunoblotting results

We demonstrated satisfactory immunoblotting perfor mance, using two different protocols in two separate laboratories, by testing the tonsil tissue taken at autopsy from vCJD patients, as well as by spiking experiments using scrapie sheep tonsil tissue, scrapie infected hamster brain, and human vCJD brain tissue.

None of the survey sub-sample investigated by immunoblotting gave a protein banding pattern consistent with the presence of PrP^{CJD}. Some samples that showed a single band, which was not consistent with any expected pattern, were re-tested by immunoblotting either with the same antibody or with different antibodies, including 3F4 and a secondary antibody designed to reveal non-specific antibody interactions. Only one sample still showed a single immunoblotting band; it was methionine homozygote at codon 129 and from a patient in the 1986-90 birth cohort, and it was negative by immunohistochemistry.

Immunohistochemistry results

More than 800 tonsils, selected on the basis of the enzyme immunoassay results, have been investigated

by immunohistochemistry in one or other of two experienced laboratories, and none was scored positive for PrP^{CJD}.

Prevalence estimates

Overall, 32 661 (52%) of the 63 007 samples tested came from people born in 1995 or earlier who were alive at the time when bovine spongiform encephalopathy contaminated meat was being consumed (table 2). The observed prevalence of PrP^{CJD} in this group was zero (95% confidence interval 0 to 113 per million). Combining the 1986-90 and 1991-5 cohorts gave a prevalence of zero with an upper 95% confidence limit of 185 per million. The prevalence in the combined 1996-2000 and 2001-7 unexposed cohorts was also zero with an upper 95% confidence limit of 122 per million.

Although the zero per million prevalence seen in the 1961-85 cohort (upper 95% confidence limit 289 per million) was different from the 292 per million (95% confidence interval 60 to 853 per million) found in the earlier survey of appendix tissue,[5] the 95% confidence intervals for both surveys overlapped (a formal comparison of the prevalence estimates gives a P value of 0.09).

DISCUSSION

Initial results from testing the tonsil specimens in a national anonymous tissue archive have shown the prevalence of PrP^{CJD} to be zero in 63 007 overall and zero in 12 753 in the birth cohort in Britain in which most cases of vCJD have occurred. Interpretation of this finding, and of the difference between it and the earlier survey of appendix tissue, depends critically on three factors: the sensitivity of the test system chosen to screen the tonsil specimens, the representativeness of the sample specimens of the people most vulnerable to vCJD disease, and the natural history of the infectivity of bovine spongiform encephalopathy in individual patients, particularly the time when PrP^{CJD} first appears pre-clinically in tonsil compared with appendix tissue and how long it persists.

Table 2 | Prevalence of disease related prion protein (PrP^{CJD}) in Britain by birth cohort (positive/total; rate per million with 95% confidence intervals*)

Birth cohort	Current (2004-September 2008) national tissue survey: tonsils	Earlier† (1995-9) national tissue survey	
		Appendices	Tonsils
1940 and before	NA	NA	0/225
1941-60	NA	0/573	0/266
1961-85	0/12 753; 0 (0 to 289)	3/10 278; 292 (60 to 853)	0/694
1986-90	0/9 564; 0 (0 to 386)	0/396	0/119
1991-5	0/10 344; 0 (0 to 357)	NA	0/106
1996-2000	0/15 708; 0 (0 to 253)	NA	0/17
2001-7	0/14 638; 0 (0 to 252)	NA	NA
Total	0/63 007; 0 (0 to 59)	3/11 247; 267 (55 to 779)	0/1 427; 0 (0 to 2 582)

NA=not available.
*95% confidence interval calculated only when denominator exceeds 1000.
†Data from separate tissue survey of 2000 tonsils (July 2000-August 2002) in southeast England (including London)[6] not included.

Test sensitivity

Three experiments investigated the sensitivity of the enzyme immunoassays. The first was the enzyme immunoassay selection study, the second was the interrogation of the enzyme immunoassays with tonsil tissue from sheep with scrapie, and the third was the use of tonsil tissue from patients who died from vCJD. Overall, these indicated that the Microsens enzyme immunoassay was more sensitive than the Bio-Rad enzyme immunoassay for detection of PrP^{CJD} in lymphatic tissue. The most sensitive detection was by the Microsens enzyme immunoassay with a sample containing 12 µg vCJD tonsil tissue; the equivalent for the Bio-Rad enzyme immunoassay was 480 µg vCJD tonsil tissue (table 1). When used for screening, 12 000 µg tonsil tissue was applied to the Microsens enzyme immunoassay and 48 000 µg to the Bio-Rad enzyme immunoassay. Therefore, the two enzyme immunoassays should have been sufficiently sensitive to detect PrP^{CJD} in tonsils from asymptomatic people incubating vCJD if levels of PrP^{CJD} were a 10th to a 1000th of those in patients with symptoms.

The dual enzyme immunoassay tonsil screening protocol may be at least as sensitive as any other large scale testing for abnormal prion protein that could have been used. The enzyme immunoassays use different test principles and antibodies, perhaps reinforcing the sensitivity of each. Reading of the results was automated, and we used a range of controls on each 96 well plate of tests. We deemed the use of a single enzyme immunoassay cut-off value as commonly applied to screen a population with many positives to be inappropriate, as this particular set of samples was expected (and found) to be overwhelmingly negative. Therefore, we calculated the cut-off value for each plate individually, and this method almost doubled the number of specimens that were selected for further investigation by immunoblotting and immunohistochemistry.

Several reasons exist why a specimen could have given a false high (reactive or high negative) optical density reading in either or both enzyme immunoassays: inadequate proteinase K digestion of PrP^{C} (the normal cellular form of PrP) for the Bio-Rad enzyme immunoassay, inadequate removal of PrP^{C} bound to the capture polyanion for the Microsens enzyme immunoassay, non-specific antibody interactions owing to the high antibody concentration in tonsil tissue, and poor sample quality or technical failures. Therefore, applying more specific immunoblotting and immunohistochemistry tests to confirm whether PrP^{CJD} was present was essential.

In comparison with immunohistochemistry, the volume of tonsil tissue screened by enzyme immunoassay was relatively large. Immunohistochemistry on appendix tissue may also be less specific than immunoblotting, so that prevalence estimated by immunohistochemistry screening may tend to overestimate the true situation.[9] However, to tackle the lingering uncertainty that screening immunohistochemistry might be more sensitive than dual enzyme immunoassay screening, a further study to re-test 10 000 of the archived tonsils by immunohistochemistry has been commissioned. These 10 000 samples comprise those from patients in the 1961-85 birth cohort, as well as any samples that gave optical density readings above the cut-offs in either of the two enzyme immunoassays. The results from this major undertaking should be available some time during 2009.

Two of the three positive samples in the retrospective immunohistochemistry study of appendix tissue were valine homozygous at codon 129 of *PRNP*.[5 10] Therefore, we can be confident that the antibodies used in our immunohistochemistry analysis would have showed PrP^{CJD} in a valine homozygote if it was present. The antibodies used in the enzyme immunoassay and immunoblotting would similarly be likely to detect PrP^{CJD} in a valine homozygote and, by extension, PrP^{CJD} in a heterozygote. Although the immunoblotting profiles of valine homozygote and heterozygote vCJD are unknown, they may be expected to consist of three or four glycoforms.[11] The immunoblotting profile of the spleen in a case of asymptomatic vCJD infection in a heterozygote patient showed similarities to that in clinical vCJD spleen samples in methionine homozygote patients, with a predominance of the diglycosylated band.[2] We did not observe by immunoblotting any pattern similar to any recognised profiles in sporadic CJD or vCJD.[12-16] The only repeatedly anomalous immunoblotting pattern seen was of a single immunoblotting band in an immunohistochemistry negative sample, which was methionine homozygote at codon 129 of *PRNP*.

Representativeness of sample

The age and sex characteristics of the samples in our study reflected the current age and sex distribution of people having tonsillectomy: 72% of those born in 1995 or earlier in our survey were female, compared with 48% of those born since 1995. Although only 44% of vCJD cases to date have been in women, we do not think that the predominance of females in our older sample of tonsils could have biased our findings with respect to prevalence of PrP^{CJD}.

Given the very strong association between PrP^{CJD} and people who are homozygous for methionine at *PRNP* codon 129,[5] it is important to note that our sample was likely to have been representative of this genetic susceptibility: an analysis of 466 of the tonsils in our survey showed 47% to be methionine homozygotes at codon 129, consistent with what was expected.[10 17-20] Therefore, of the 32 661 tonsils tested from people born before 1996, approximately 15 351 (47%) would have been from methionine homozygotes.

Several differences must be considered when comparing results between surveys. First and foremost is that previously appendix tissues were screened by immunohistochemistry, whereas we screened tonsil tissue by enzyme immunoassay. Secondly, an average of 10 years elapsed between when the previous large sample from the 1961-85 birth cohort had their appendixes removed (during 1995-9) until our sample had their tonsils removed (mostly in 2006-7)—10 years during which abnormal prion protein levels might be expected to have increased rather than diminished. Within this birth cohort, however, the average age of appendicectomy was estimated to be four years older than the average age of tonsillectomy, so the average duration of the opportunity for PrP^{CJD} to increase between the appendicectomy samples and the tonsillectomy samples would have been about six years. On the other hand, the relatively older appendix sample that was collected earlier may conceivably have contained a wave of infectivity in the 1961-85 cohort of the British population that was not present in the younger tonsil group that was sampled later.

Detailed information on previous operative history was sought on every vCJD case diagnosed in Britain. Seventeen of 167 patients were reported to have had tonsillectomy; 14 of these were in the 1961-85 birth cohort, and the remaining three were in the pre-1961 birth cohort. None was likely to have had specimens included in this or the earlier tonsil survey (Hester Ward, personal communication).[6]

Natural history

While PrP^{CJD} has been found consistently by immunoblotting and immunohistochemistry in tonsil tissue from patients with vCJD,[8 9 21-24] PrP^{CJD} in a tonsil from an asymptomatic person has yet to be reported. Given, however, that tonsillar tissue has been shown to accumulate PrP^{Sc} before the onset of clinical disease in non-human primates and well before the onset of clinical disease in sheep experimentally infected orally with bovine spongiform encephalopathy,[25 26] we considered tonsil tissue to be a reliable substrate for a survey of prevalence in humans. Also, the use of fresh tonsil tissue allowed more comprehensive laboratory testing, if necessary, after the initial screening assays.

PrP^{CJD} has been observed to accumulate in appendix tissue in vCJD (19/20 positive/tested)[9 27 28] and, in two cases, before symptoms developed.[29 30] However, data on the timing of the appearance of PrP^{CJD} in different peripheral lymphoreticular tissues during the prolonged incubation period of vCJD are sparse. The rate of accumulation of PrP^{CJD} in tonsil and appendix tissue could differ such that the findings of surveys of appendix and tonsil tissues would also differ. The positive samples found in the appendix survey presumably came from people who were infected a relatively short time earlier, during the peak of the bovine spongiform encephalopathy epidemic.[5] Moreover, should the incubation period for prion disease be considerably longer in people with different genotypes, uncertainty about the timing of the appearance of detectable PrP^{CJD} in these will increase, with concomitant implications for the interpretation of results of PrP^{CJD} prevalence surveys.

Animal experiments have shown that high infectivity, and indeed disease, can be present in the absence of detectable proteinase K resistant PrP^{Sc}.[31] The extent to

WHAT IS ALREADY KNOWN ON THIS TOPIC

Statistical back calculation based on cases of vCJD to 2004 has given estimates of between 10 and 190 further clinical cases over the next few decades

A study of archived appendix and tonsil tissues found a prevalence of lymphoreticular accumulation of pathogenic prion protein consistent with the existence of between 520 and 13 000 sub-clinical cases

Therefore, a discrepancy exists between estimates, which needs to be resolved to ensure that proportionate public health measures are implemented

WHAT THIS STUDY ADDS

Testing of tissue from more than 63 000 tonsils, of which 12 763 were from the 1961-85 birth cohort, has not shown evidence for the presence of the pathogenic form of the prion protein

The prevalence of sub-clinical vCJD infection in Britain may be lower than that given by previous estimates, with an upper limit of 289 per million in the 1961-85 birth cohort

which this observation can be generalised is, however, unclear, as PrP^{CJD} has been shown to be present in the lymphoid tissues of all vCJD patients tested.[9 27] If other, more reliable, indicators of vCJD become available, screening the existing samples with tests for these markers, and thereby determining whether any vCJD positives have been missed by looking only for PrP^{CJD}, may be possible.

Data from animal experiments also show "clearance" of abnormal prion protein after inoculation.[31 32] Therefore, the abnormal prion protein found in the earlier survey of appendix tissue may conceivably have been transient and eventually cleared without leading to disease, so that the appendix survey result would not have been replicated by the later tonsil survey.

Conclusion

We tested more than 32 000 tonsils from people in the age range most exposed to meat contaminated with bovine spongiform encephalopathy, and believed to be asymptomatic when sampled, for disease related prion protein. Using two sensitive enzyme immunoassays, with selective application of specific immunoblotting and immunohistochemistry techniques, we found no samples positive for PrP^{CJD}, a prevalence of 0 per million (with an upper 95% confidence limit of 113 per million). For the 1961-85 birth cohort, the prevalence of zero with a 95% confidence interval of 0 to 289 per million was lower than, but still consistent with, the earlier study of appendix tissue (60 to 853 per million). A P value of 0.09 applies to the comparison of the two prevalence estimates. These two surveys may not, however, be directly comparable owing to differences in testing methods, tissues sampled, and the time the tissues were removed (typically about 10 years earlier in the previous study). More data are needed through continuing the testing of tonsils from people born before 1996, despite the low frequency of tonsillectomy in older birth cohorts. In addition, creation and testing of other anonymous tissue archives, such as one based on coronial autopsies, or a repeat of the appendix survey on an even larger scale, should provide a larger sample set of the people most exposed to the bovine spongiform encephalopathy agent.[33]

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This study was originally proposed by a Medical Research Council and Department of Health committee. It was overseen by an Expert Advisory Group on the Laboratory Testing Strategy for Large Scale Abnormal Prion Prevalence Studies with a membership of N Andrews, D W G Brown, J P Clewley, J Cooper, R Eglin, E Gadd, O N-Gill, M Head, D A Hilton, J W Ironside, G Jackson, C M Kelly, G Mallinson, D Matthews, P Minor (chair), P P Mortimer, N Raven, J R Stephenson, and J D F Wadsworth. Data from this anonymous tonsil survey were discussed in public at meetings of the Spongiform Encephalopathy Advisory Committee (www.seac.gov.uk) in December 2007 and April 2008; and see a statement issued in August 2008 (www.seac.gov.uk/statements/state-cjd-infections.pdf).
Contributors: JPC designed and analysed the laboratory studies and wrote the paper with ONG, who initiated the study and did clinical and epidemiological analyses. CMK recruited hospitals to the study and did epidemiological analyses. NA did statistical and epidemiological analyses. KV organised the National Anonymous Tissue Archive laboratory, tonsil processing, and enzyme immunoassay testing. GM, MK, and RD did the immunoblotting. DAH, PE, JWI, LMcC, and DLR did the immunohistochemistry. JWI provided some of the vCJD clinical tissue used in the work. HEA did the codon 129 genotyping. JPC and ONG are the guarantors.
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