別紙様式第２－１

医薬品　研究報告　調査報告書

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一般的名称	ﾃｸﾈﾁｳﾑ大凝集人血清ｱﾙﾌﾞﾐﾝ（^{99m}Tc）		研究報告の公表状況	ProMED-mail／20070423.1325(2007 年 4 月 23 日) 1:Herald Sun online, 2: Mailman School of Public Health, Columbia University, press release	公表国 オーストラリア	
販売名（企業名）	テクネ MAA キット（富士フイルムＲＩファーマ株式会社）					

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
オーストラリア（ヴィクトリア）において、一人のドナーから臓器提供を受けた 3 人（腎臓を移植された 63 歳女性、肝臓を移植された 64 歳の男性、腎臓を移植された 3 例目）の死因について、コロンビア大学とメルボルンの科学者らによって新たなウイルス（リンパ球性脈絡髄炎ウイルスに関連するアレナウイルス科）が発見された。しかしこのウイルスが単に付着しているだけか、組織拒絶と死の原因となるかどうかは未解決である。	特になし

報告企業の意見	今後の対応
臓器移植後死亡した 3 例の死因についての研究報告であり、これまで知られていない新ウイルス（リンパ球性脈絡髄炎ウイルスに関連するアレナウイルス科）が発見され、同科には出血熱性のラッサウイルスやマチュポウイルス等あることより、重大な感染症である可能性が考えられる。 本報告は、新たに判明した感染症に関するものであり、かつ、重大な感染症に関するものと判断する。	本報告はヒト血液を原料とする血漿分画製剤等とは直接関連するものではなく、現時点で特に当該生物由来製品に関し、措置等を行う必要はないと判断する。しかしこのウイルスが単に臓器に付着しているだけか、組織拒絶と死の原因となるかどうかは未解決であると報告されているため、今後も情報収集が必要であると考える。

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ARENAVIRUS, ORGAN TRANSPLANTS - AUSTRALIA (VICTORIA)
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[1]
Date: Sun 22 Apr 2007
Source: Herald Sun online [edited]
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Australia: Novel virus responsible for deaths of organ donation recipi
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A virus unknown to medical science was behind the deaths of 3 Victorians who received organs from the one donor. The unnamed bug has been linked to Ebola virus, [a virus] responsible for the deaths of thousands in central Africa since the 1970s. [This is an incorrect statement. The organ transplant-associated virus is not related to Ebola virus; see part [2] below. - Mod.CP]. After baffling local scientists, experts from New York's Columbia University were called in to help solve the mystery of the multiple transplant deaths being investigated by the coroner.

Initial investigations and tests had been unable to determine any common link between the donor and the 3 recipients. The presence of the virus in the recipients is thought to be a world first. One of the New York team said: "The discovery of this virus is of national and international significance."

The Sunday Herald Sun revealed the deaths in February 2007. A 63-year-old woman died after receiving a kidney transplant at Austin Hospital. A 64-year-old man died after receiving a liver transplant there. The 3rd victim received a kidney at Royal Melbourne Hospital.

The male donor whose organs carried the suspected killer bug had died in Dandenong Hospital of a brain hemorrhage in December 2006 after returning from overseas; it is believed most of his trip was spent in Europe.

The virus is part of the rodent-borne arenavirus family and can cause "old-world" diseases such as Yellow Fever, Ebola and Lymphocytic choriomeningitis. [This statement is incorrect: yellow fever is caused by a flavivirus and Ebola hemorrhagic fever is caused by a filovirus; only lymphocytic choriomeningitis (LCM) is caused by an old-world arenavirus. - Mod.CP]. Victoria's acting Chief Health Officer, Dr John Carnie, confirmed the virus [LCM virus?] had been detected in multiple samples from all 3 transplant patients. But there was no evidence the virus represented a public health risk, he said.

Health authorities are examining whether future donated organs can be screened for [LCM?] virus. A spokesman for the Victoria Coroner's office said families of the victims were told yesterday [21 Apr 2007]. There would be a formal inquest.

Experts from Columbia's Greene Infectious Diseases Laboratory helped

solve the mystery. Initial investigations and tests were unable to determine any common link between the donor and the 3 recipients. Dr Carnie said the risk to the public was minimal because "these viruses [?] affect immunocompromised people, and it is rarely fatal in those with normal immune systems. We have not had any indication of any unexplained illnesses among families of the donor or recipients," he said. "This would be the case if it was transmissible person to person. Our supposition is it was transmitted by organ transplantation."

Cutting edge techniques were used for the 1st time by the Greene lab -- in collaboration with Victorian Infectious Diseases Reference Laboratory -- to gene sequence the virus. "Our gene technology enables unbiased sequencing of all agents present," Columbia's Prof. Ian Lipkin said. "We found a handful (of combinations) that were related to Lassa virus or LCM virus [both old world arenaviruses - Mod.CP]. Using these clues we can confidently say this is a new virus, present in the original organs and so different than anything seen before."

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Communicated by:
ProMED-mail Rapporteur Brent Barrett

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[2]
Date: Sat 21 Apr 2007
Source: Mailman School of Public Health, Columbia University, press release [edited]
<http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/stor

Scientists Discover New Virus Responsible for Deaths of 3 Transplant Recipients From Single Donor in Victoria, Australia
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Knowledge of genetic sequence of virus will enable improvements in screening to enhance transplantation safety. Scientists in the Greene Infectious Disease Laboratory of Columbia University Mailman School of Public Health and colleagues in the Victoria Infectious Diseases Reference Laboratory in Melbourne, Australia and 454 Life Sciences have discovered a new virus that was responsible for the deaths of 3 transplant recipients who received organs from a single donor in Victoria, Australia.

The previously unknown virus, which is related to lymphocytic choriomeningitis virus (LCMV), was found using rapid sequencing technology established by 454 Life Sciences and bioinformatics algorithms developed in the Greene Laboratory with support from the National Institute of Allergy and Infectious Diseases. Known strains of LCMV have been implicated in a small number of cases of disease transmission by organ transplantation [see references below], however, the newly discovered virus is sufficiently different that it could not be detected using existing screening methods.

Over 30 000 organ transplants are performed in the U.S. each year. Knowledge of the genetic sequence of this virus will enable improvements in screening that will enhance the safety of transplantation.

Ian Lipkin, MD, director of the Greene Laboratory and Principal Investigator of the Northeast Biodefense Center, emphasized the importance of academic, public health, and industrial partnership in this work. "This was a team effort. Drs. Mike Catton and Julian Druce at the Victorian Infectious Disease Reference Laboratory reached out to us after a comprehensive state-of-the-art investigation failed to turn up leads," stated Dr. Lipkin. "We succeeded in identifying the virus responsible for the deaths by building on their work and utilizing new tools for pathogen surveillance and discovery developed in the Greene Laboratory and 454 Life Sciences."

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

[Lymphocytic choriomeningitis virtu (LCMV) is the type species of the genus _Arenavirus_ of the _Areanviridae_ family of bipartitie genome

RNA viruses. The reservoir hosts of almost all arenaviruses are rodents. LCMV is found in wild and laboratory mice, and other related "old world" arenaviruses are found in African species of rodents. Human LCMV infection may occur in rural and urban areas with high densities of rodents. Laboratory-acquired infections occur sporadically, and, previously, there have been a small number of cases of LCMV transmission by organ transplantation as mentioned by Professor Lipkin above. The virus detected by Professor Lipkin's group appears to be an LCMV-like agent but distinct from previously isolated strains of LCMV. It is unresolved, however, whether these organ-transplanted viruses are merely passengers or are responsible also for tissue-rejection illness and death. - Mod.CP]

[see also:
2005

LCMV, transplant recipients, fatal - USA (02) 20050526.1459
LCMV, transplant recipients, fatal - USA 20050524.1426
1995

LCMV & birth defects - USA 19951119.1095]
.....mpp/cp/msp/lm

A New Arenavirus in a Cluster of Fatal Transplant-Associated Diseases
致死性臓器移植関連疾患の新しいアレナウイルスクラスター

Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, Conlan S, Quan PL, Hui J, Marshall J, Simons JF, Egholm M, Paddock CD, Shieh WJ, Goldsmith CS, Zaki SR, Catton M, Lipkin WI.

N Engl J Med. 2008 Feb 6 [Epub ahead of print]

抄録
背景：同じドナーから同じ日に臓器移植を受けた 3 名の患者が、発熱性疾患により 4～6 週間後に死亡した。広範囲の感染性因子を対象として、培養、PCR、血清学的検査、オリゴヌクレオチドマイクロアレイ分析を行ったが、病原体を特定することはできなかった。
方法：2 名の患者に移植された肝臓及び腎臓から得られた RNA を評価した。他の方法で検出できなかった微生物塩基配列を特定するため、無作為ハイスループットシークエンスを行った。新規病原体のシークエンスの特異性は、培養と PCR、免疫組織化学的分析、血清学的分析によって確認された。
結果：ハイスループットシークエンスでは 103,632 塩基のシークエンスが得られ、このうち 14 塩基は、ある旧世界アレナウイルスを示していた。追加のシークエンス分析によって、この新しいアレナウイルスがリンパ性脈絡髄膜炎ウイルスと関連していることが明らかになった。特定のシークエンスに基づく特異的 PCR アッセイによって、移植を受けた患者の腎臓、肝臓、血液、脳脊髄液中にウイルスの存在が確認された。免疫組織化学的分析では、患者に移植された肝臓及び腎臓からアレナウイルス抗原が検出された。IgM 抗体と IgG 抗体がドナーの血清から検出された。同じ患者から時間を置いて 2 度採取した血清検体では、明らかなセロコンバージョンが見られた。
結論：無作為ハイスループットシークエンスは、病原体発見のための強力なツールである。疾患のアウトブレイク中にこの方法を使用することで、新しいアレナウイルスが固形臓器移植によって伝播したことを容易に確認することができた。

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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A New Arenavirus in a Cluster of Fatal Transplant-Associated Diseases

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ABSTRACT

Background Three patients who received visceral-organ transplants from a single donor on the same day died of a febrile illness 4 to 6 weeks after transplantation. Culture, polymerase-chain-reaction (PCR) and serologic assays, and oligonucleotide microarray analysis for a wide range of infectious agents were not informative.

Methods We evaluated RNA obtained from the liver and kidney transplants in two recipients. Unbiased high-throughput sequencing was used to identify microbial sequences not found by means of other methods. The specificity of sequences for a new candidate pathogen was confirmed by means of culture and by means of PCR, immunohistochemical, and serologic analyses.

Results High-throughput sequencing yielded 103,632 sequences, of which 14 represented an Old World arenavirus. Additional sequence analysis showed that this new arenavirus was related to lymphocytic choriomeningitis viruses. Specific PCR assays based on a unique sequence confirmed the presence of the virus in the kidneys, liver, blood, and cerebrospinal fluid of the recipients. Immunohistochemical analysis revealed arenavirus antigen in the liver and kidney transplants in the recipients. IgM and IgG antiviral antibodies were detected in the serum of the donor. Seroconversion was evident in serum specimens obtained from one recipient at two time points.

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Conclusions Unbiased high-throughput sequencing is a powerful tool for the discovery of pathogens. The use of this method during an outbreak of disease facilitated the identification of a new arenavirus transmitted through solid-organ transplantation.

Methods of cloning nucleic acids of microbial agents directly from clinical specimens offer new opportunities for the surveillance and discovery of pathogens. Molecular techniques have been used successfully in the identification of infectious agents such as the Borna disease virus, hepatitis C virus, Sin Nombre virus, human herpesviruses 6 and 8, *Bartonella henselae, Tropheryma whipplei,* West Nile virus, and the coronavirus associated with severe acute respiratory syndrome.[1]

The arenaviruses are enveloped, negative-strand RNA viruses in rodents; these viruses are most frequently transmitted to humans through exposure to infected urine. Infection with the prototype virus, lymphocytic choriomeningitis virus (LCMV), is typically asymptomatic or associated with mild, transient illness; however, LCMV has also been implicated in aseptic meningitis.[2] Human-to-human transmission of LCMV during pregnancy has been reported, and infection during the gestational period can result in fetal death, neurologic sequelae, and chorioretinopathy.[3] Fatal outbreaks of disease associated with human-to-human transmission of LCMV in recipients of solid-organ transplants have also been described.[4] We report the use of unbiased DNA sequencing in the discovery of a new LCMV-related arenavirus that caused fatal disease in three recipients of organs from a single donor.

Methods

Patients and Clinical Course

Three women in Australia who were 63 years of age (Recipient 1), 64 years of age (Recipient 2), and 44 years of age (Recipient 3) received a liver transplant (Recipient 2) or kidney transplants (Recipients 1 and 3) from one male donor who was 57 years of age. The donor died of cerebral hemorrhage 10 days after returning to Australia from a 3-month visit to the former Yugoslavia, where he had traveled in rural areas. The immediate post-transplantation course in the three transplant recipients was unremarkable; however, febrile illnesses with varying degrees of encephalopathy developed in all three, and they died 4 to 6 weeks after transplantation (Table 1). Bacterial and viral cultures; polymerase-chain-reaction (PCR) assays for herpesviruses 1 through 8, lyssavirus, influenza A and B viruses, respiratory syncytial virus, picornavirus, adenovirus, human parainfluenza virus, flavivirus, alphavirus, hantavirus, polyomavirus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, toxoplasma, *Mycobacterium tuberculosis,* and *Mycoplasma pneumoniae;* and viral and panmicrobial oligonucleotide microarray analysis[4] revealed no candidate pathogens.

View this table: **Table 1.** Characteristics of the Organ-Transplant Recipients.
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Unbiased High-Throughput Sequencing

RNA was extracted from the brain, cerebrospinal fluid, serum, kidney, and liver of Recipient 1, who had received a kidney transplant, and from the cerebrospinal fluid and serum of Recipient 2, who had received a liver transplant. As shown in Figure 1, after digestion with DNase I to eliminate human chromosomal DNA, RNA preparations were amplified by means of reverse-transcriptase PCR (RT-PCR) with the use of random primers.[5,6] Amplification products were pooled and sequenced with the use of the GSL FLX platform (454 Life Sciences), but DNA fragmentation was omitted.[7] After trimming to remove sequences derived from the amplification primer and after filtration to eliminate highly repetitive sequences, the data set was analyzed by subtracting fragments that matched human sequences, clustering nonredundant sequences,[8] and assembling them into contiguous sequences[9] for direct comparison with the GenBank databases of nucleic acids and proteins with the use of BLASTN and BLASTX software.[10] We analyzed the resulting alignments and assigned them to nodes in the National Center for Biotechnology Information taxonomy database, using a custom software application written in Perl (BioPerl version 5.8.5).

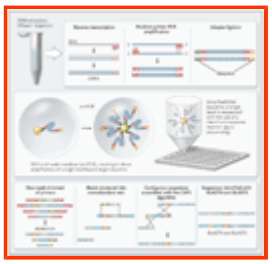

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Figure 1. High-Throughput Sequencing Method.

PCR denotes polymerase chain reaction.

PCR Quantitation of the Arenavirus Burden

RNA obtained from tissues, plasma, serum, and cerebrospinal fluid was reverse transcribed with the use of random hexamers. PCR was performed with the use of a SYBR Green assay (Applied Biosystems). The following cycling conditions were used: 50° C for 2 minutes and 95° C for 10 minutes, followed by 45 cycles at 95° C for 15 seconds and 60° C for 1 minute. Real-time PCR assays were performed with the following primer set:
5'AGTGCYTGCACAACATCGTTT3' (forward) and 5'CAATGCCCAGCYTGACAAT3' (reverse).
Thermal cycling was performed with the use of an ABI 7500 real-time PCR system (Applied Biosystems).

Viral Isolation and Analyses

Kidney tissue from Recipient 1 was homogenized in phosphate-buffered saline, centrifuged to pellet cellular debris, filtered, and used to inoculate Vero E6 cells. The cells were monitored daily by means of light microscopy for cytopathic effect and by means of RT-PCR for the presence of arenavirus nucleic acid in supernatant. Monolayers of cells showing cytopathic effects that were also positive for arenavirus nucleic acid were fixed with buffered 4% paraformaldehyde for indirect immunofluorescence and immunohistochemical microscopy and with buffered 2.5% glutaraldehyde for thin-section electron microscopy. Rabbit polyclonal antiserum against Old World arenaviruses, including LCMV, was used as the source of primary antibodies for immunohistochemical analysis. Secondary antibodies were alkaline phosphatase–conjugated goat antibodies against rabbit IgG.[4] Immunohistochemical assays were also performed with the use of formalin-fixed, paraffin-embedded tissue sections obtained from the liver and kidney of Recipient 1.

Virus-infected and noninfected (control) Vero E6 cells were fixed with methanol. Serum specimens from the donor, from the recipients, and from 100 randomly chosen control recipients of solid-organ transplants were applied to the fixed cells followed by fluorescein-labeled antihuman IgG or IgM secondary antibodies.

Complete Genome Sequencing and Phylogenetic Analyses

RNA extracted from the liver in Recipient 1 was used as a template to clone and sequence the L and S segments of the virus. The gene fragments obtained by means of pyrosequencing were used to design specific PCR primers; thereafter, consensus primers were designed on the basis of alignments of other arenavirus sequences with the use of the SCPrimer program.[11]

The L and S segments were assembled and sequenced as a series of overlapping genetic fragments. Evolutionary distances between the assembled segments were computed with the use of the Poisson correction method and expressed in units of amino acid substitutions per site in relationship to arenavirus L, glycoprotein precursor, and nucleoprotein amino acid segments in the GenBank database with the use of the MEGA program.[12] The percentage of replicate trees in which taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (see Figure 1a, 1b, and 1c of the Supplementary Appendix, available with the full text of this article at www.nejm.org). The nucleotide and amino acid homologies of each of the arenavirus genes (Z, L, GPC, and NP) to LCMV (the closest completely sequenced relative) are shown in Table 2. The sequences are deposited in GenBank (accession numbers EU136038 and EU136039).

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Table 2. Nucleotide and Amino Acid Homologies of the New Arenavirus to Other Arenaviruses.

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Results

RNA from tissue from Recipient 2, who had received a liver, and Recipient 1, who had received a kidney, was pooled and amplified for unbiased high-throughput sequencing,[7] yielding 103,632 sequence fragments. The sequences recovered ranged in size from 45 to 337 nucleotides, with a mean length of 162. Sequences derived from the amplification primer and highly repetitive sequences were eliminated, yielding a net of 94,043 sequences. These sequences were processed with the use of algorithms that subtract vertebrate sequences, assemble contiguous sequences, and compare the residual nucleotide and deduced amino acid sequences in all six potential open reading frames with motifs represented in databases of microbes.

At the nucleotide level, sequence data were uninformative; however, BLASTX analysis of the deduced protein sequence revealed 14 fragments that were consistent with Old World arenaviruses (12 S-segment and 2 L-segment fragments) sharing the closest relationship to LCMV.

Primers were designed for RT-PCR experiments to detect viral RNA in clinical specimens, assess the similarity of viral sequences among individual organs and recipients, and extend the viral sequence needed to facilitate characterization. Viral RNA was present in a total of 22 of 30 specimens of tissue, blood, or cerebrospinal fluid from all three transplant recipients (Table 3). The sequence was identical in all specimens, a finding that was consistent with the introduction of a single virus into all the recipients.

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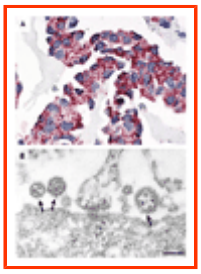
Table 3. Viral RNA and Antibody Titers in the Donor and Recipients.

Fresh-frozen kidney tissue from Recipient 1 was homogenized and used to inoculate cultures of Vero E6 cells. A cytopathic effect was observed only in the first passages; thereafter, morphologic characteristics did not differ between infected and control cells. Indirect immunofluorescence assays with the use of polyclonal antibodies against arenaviruses and LCMV showed cytoplasmic distribution of viral antigen. Immunostaining of viral antigens was also seen in infected cells by means of an indirect immunoalkaline phosphatase technique (Figure 2A). Quantitative RT-PCR assays showed increasing concentrations of viral nucleic acid with serial passage. Examination of infected Vero E6 cells by means of thin-section electron microscopy revealed extracellular particles with morphologic features that are characteristic of arenaviruses (Figure 2B).

Figure 2. Propagation of the New Arenavirus in Tissue Culture.

Panel A shows immunostaining of viral antigens in infected cells by means of an indirect immunoalkaline phosphatase technique. Panel B shows an electron micrograph of extracellular arenavirus-like virions. Particles



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(arrows) are round, vary in size, and have surface projections on the perimeter. Cellular ribosomes are visible within the virions. The length of the bar corresponds to 100 nm.

Immunofluorescence assays for serum antibodies that are reactive with infected Vero E6 cells revealed virus-specific IgM and IgG antibodies in the donor that were consistent with acute infection. Plasma and serum specimens from Recipient 2 that had been collected at two time points 19 days apart (11 days and 30 days after transplantation) were available for analysis. Virus-specific IgG and IgM antibodies were detectable only at the second time point, consistent with seroconversion.

Immunohistochemical analysis of specimens of the liver (Figure 3A) and kidney (Figure 3B) obtained from Recipient 1 showed focal immunostaining of arenavirus antigens. PCR surveys of 100 archived serum or plasma specimens from solid-organ transplant recipients who were not linked to the cluster and who had undergone transplantation in the same city and during the same time period revealed no evidence of infection with this pathogen.

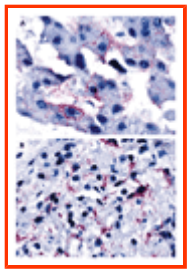

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Figure 3. Predominantly Membranous Distribution of Arenavirus Antigen.

The distribution of the arenavirus antigen is shown in the liver (Panel A) and kidney (Panel B) of Recipient 1. Formalin-fixed, paraffin-embedded tissue sections were incubated with polyclonal rabbit antiserum against lymphocytic choriomeningitis virus followed by alkaline phosphatase–conjugated secondary antibodies against rabbit IgG.

The 3301-nucleotide S-segment and 7215-nucleotide L-segment sequences were cloned from the kidney of Recipient 1 by means of PCR and sequenced. Phylogenetic characterization was limited by the paucity of available sequences deposited in public databases; nonetheless, L- and S-segment analyses were consistent with the presence of a new arenavirus. Whereas sequences

in the nucleoprotein and glycoprotein genes on the S segment were closest to the LCMV strain LE[13] and M1 and M2 isolates[14] (Table 2, and Figure 1b and 1c of the Supplementary Appendix), the L-segment sequence indicated a closer relationship to Kodoko virus. Strain LE was isolated in France from an infected fetus. M1 and M2 were isolated in Japan from wild mice. Kodoko virus was recently isolated in Africa from wild mice (Figure 1a of the Supplementary Appendix).[15] Reassortment is well described in arenaviruses and could account for differences in phylogenetic relationships based on L- and S-segment sequences. However, reassortment cannot be implicated without a complete genomic sequence for the viruses used in these phylogenetic analyses.

Discussion

Two clusters of transmission of arenavirus through solid-organ transplantation have been reported.[4] In each cluster, recipients linked to a single donor died of an unexplained infectious disease 9 to 76 days after transplantation. In neither cluster did the donor have a history of acute infectious disease or evidence of infection by PCR or serologic analysis; however, in one cluster, a pet hamster that had recently been introduced into the donor's household was found to be infected with the same virus that was detected in the recipients. LCMV was implicated after the results of viral culture and electron microscopy triggered specific immunohistochemical and molecular tests for arenaviruses.

In our cluster, a new arenavirus was first detected through unbiased high-throughput sequencing. Thereafter, the infection was confirmed by means of culture, electron microscopy, and specific immunohistochemical and serologic tests. As in the other two reported clusters of transplant-associated transmission, we detected no viral nucleic acids in the donor and found no history of acute infectious disease; however, the presence of IgG and IgM antibodies confirmed recent infection. We were also unable to obtain any information indicating that the donor had been exposed to rodents; however, his history of recent travel suggests that he may have been infected before returning to Australia from southern Europe, where such exposure may have occurred in a rural area.

Although we have not fulfilled Koch's postulates, evidence implicating this new virus in the outbreak of infection among patients who received transplants is compelling. All three recipients received organs from the same donor and died within days of one another after febrile illness. Identical viral sequences were obtained from all the recipients. The virus is new and was not detected in 100 organ recipients who were not linked to this cluster. The results of serologic analysis of specimens obtained from the donor were consistent with recent infection, and seroconversion was observed in one recipient.

Unbiased high-throughput sequencing has been used to characterize complex mixtures of microflora in environmental contexts[16]; we have shown that this strategy can be used to address a suspected outbreak of infectious disease. Its use in the context of investigating a cluster of cases of acute disease associated with organ transplantation facilitated the rapid implication of a new arenavirus not detected by other methods. This technique may prove useful as a new tool in the identification and surveillance of pathogens in chronic as well as acute disease.

Supported by grants from the National Institutes of Health (U54AI57158, to the Northeast Biodefense Center–Lipkin; U01AI070411; AI062705; and HL083850).

Drs. Du, Simons, and Egholm report being employees of 454 Life Sciences. Dr. Lipkin reports being a member of the scientific advisory board of 454 Life Sciences during a portion of the time the work reported here was pursued. Drs. Du, Simons, Egholm, and Lipkin report holding stock options in 454 Life Sciences before it was purchased by Roche Diagnostics in May 2007. No other potential conflict of interest relevant to this article was reported.

We thank David Riches for skillful technical assistance.

Source Information

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Characteristics of the Organ-Transplant Recipients

Table 1. Characteristics of the Organ-Transplant Recipients.

Recipient No.	Age	Diagnosis	Organ Transplanted	Clinical Course	Interval between Transplantation and Death
	yr				*days*
1	63	End-stage renal failure due to polycystic kidney disease	Kidney	Fever, sepsis, encephalopathy, acute tubular necrosis, graft rejection, radiographic evidence of chest infiltrates	36
2	64	Decompensated cirrhosis and hepatocellular cancer due to hepatitis C infection	Liver	Fever, confusion, encephalopathy with myoclonus, chest infiltrates	30
3	44	End-stage renal failure due to polycystic kidney disease	Kidney	Fever, graft rejection, intraabdominal hematomas and effusion, transplant nephrectomy, encephalopathic illness	29

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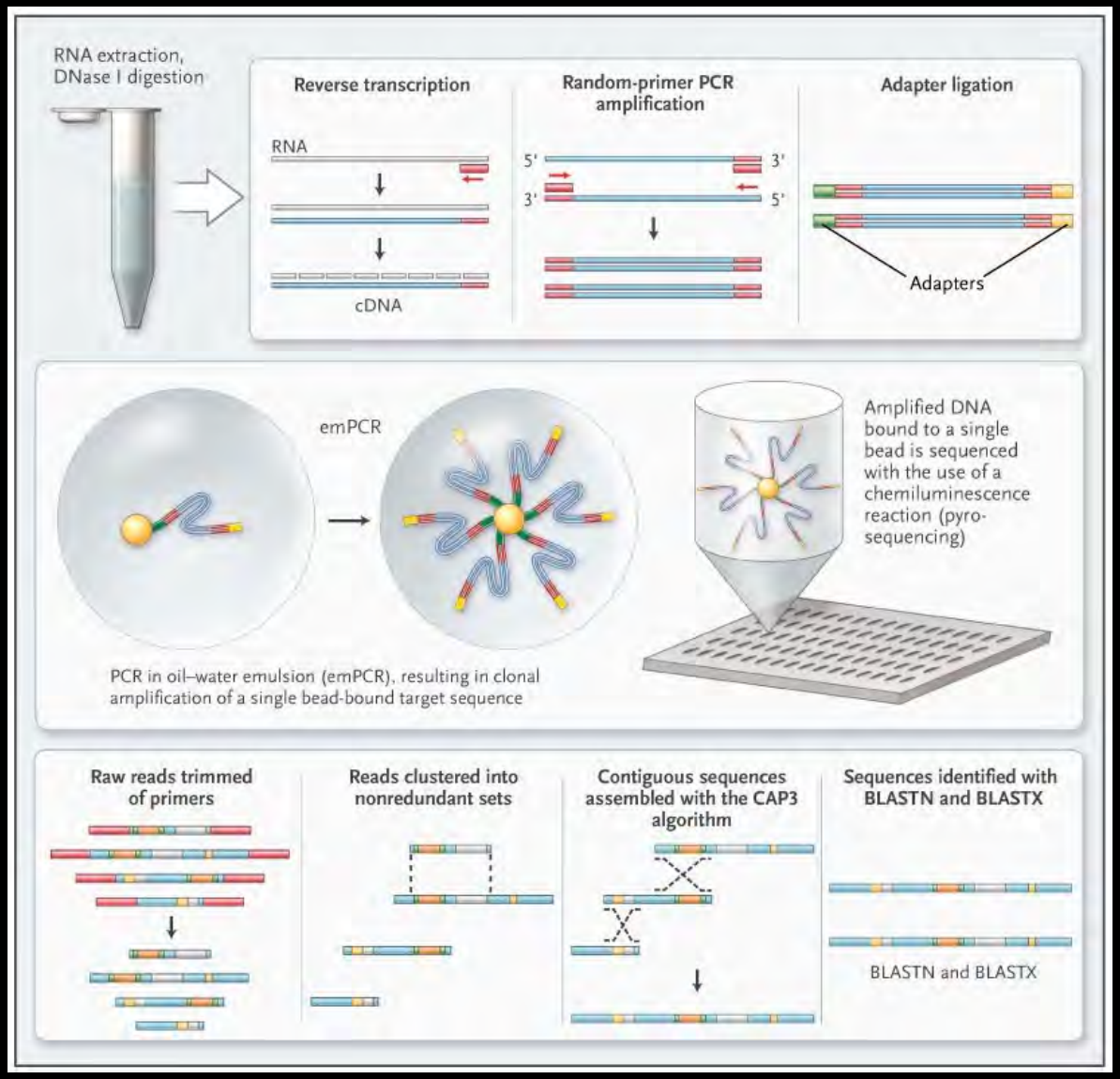
High-Throughput Sequencing Method



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Nucleotide and Amino Acid Homologies of the New Arenavirus to Other Arenaviruses

Table 2. Nucleotide and Amino Acid Homologies of the New Arenavirus to Other Arenaviruses.*

Gene	Accession No.	LCMV Strain	Homology	
			Amino Acid	Nucleotide
			percent	
GPC	AB261990	M2	94	86
NP	AB261990	M2	97	87
L	DQ286932	Marseille 12	82	79
Z	DQ286932	Marseille 12	79	72

* LCMV denotes lymphocytic choriomeningitis virus.

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Viral RNA and Antibody Titers in the Donor and Recipients

Table 3. Viral RNA and Antibody Titers in the Donor and Recipients.*

Specimen	Interval between Transplantation and Collection of Specimens	Viral RNA	Antibody Titer
	days	*copies/ml of RNA extract*	
Donor			
Serum	0	ND	1:80 IgG, 1:20 IgM
Spleen	0	ND	NA
Pancreas	0	ND	NA
Recipient 1 (kidney transplant)			
Plasma	0	ND	<1:10 IgG, <1:10 IgM
Plasma	27	889,200	NP
Plasma	33	614,900	NP
Cerebrospinal fluid	33	5,500	NP
Plasma	35†	1,000,000	NP
Urine	35†	88,000,000	NA
Heart	35†	33,200	NA
Spleen	35†	52,600	NA
Liver	35†	2,362,800	NA
Lung	35†	498,600	NA
Cerebrospinal fluid	35†	63,700	NP
Serum	35†	1,440,400	<1:10 IgG, <1:10 IgM
Brain	35†	16,600	NA
Rectal swab	35†	623,200	NA
Nasal swab	35†	55,400	NA
Axillary swab	35†	ND	NA
Kidney	35†	85,900	NA
Recipient 2 (liver transplant)			
Plasma	12	121,900	<1:10 IgG, <1:10 IgM
Mouth swab	24	457,000	NA
Bronchoalveolar lavage	19	1,163,400	NA
Cerebrospinal fluid	24	ND	NP
Plasma	24	346,200	NP
Serum	31†	347,600	1:40 IgG, 1:20 IgM
Recipient 3 (kidney transplant)			
Serum	−235	ND	<1:10 IgG, <1:10 IgM
Serum	0	ND	NP
Serum	24	415,500	NP
Serum	28	565,100	<1:10 IgG, <1:10 IgM

* NA denotes not applicable, ND not detected, and NP not performed.
† Specimens were obtained after death.

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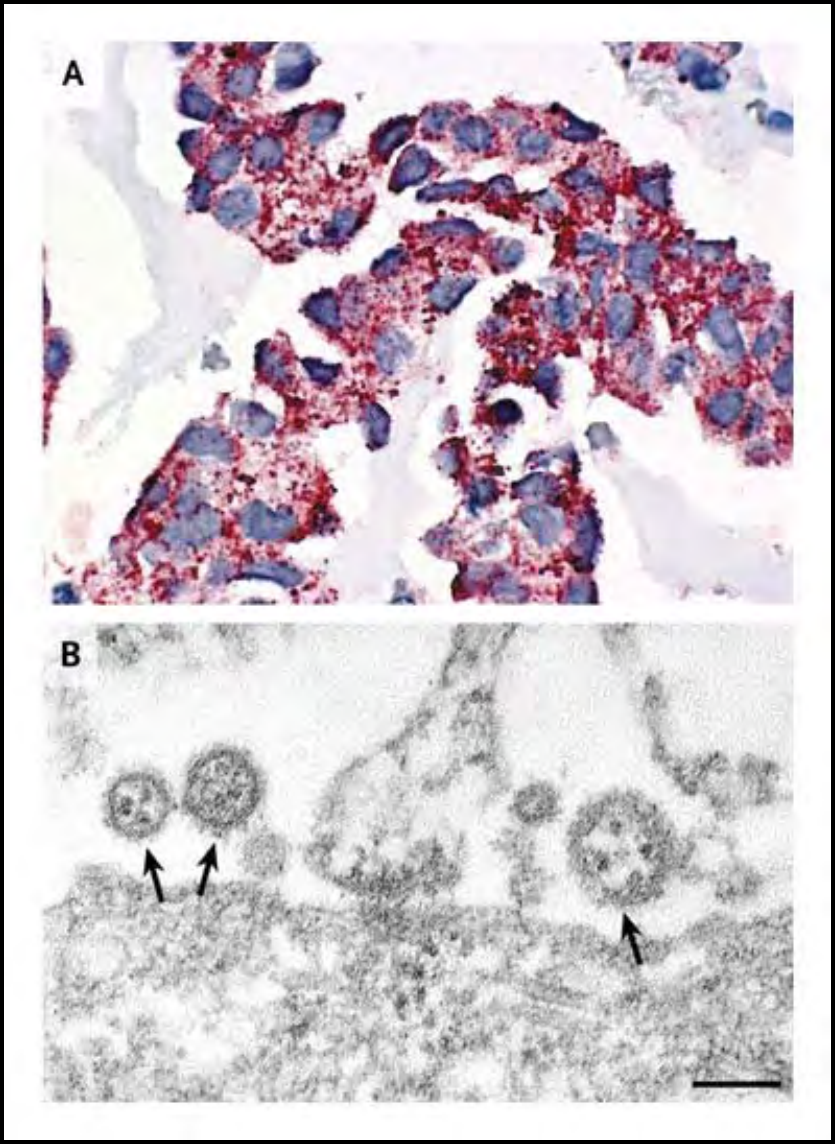
Propagation of the New Arenavirus in Tissue Culture



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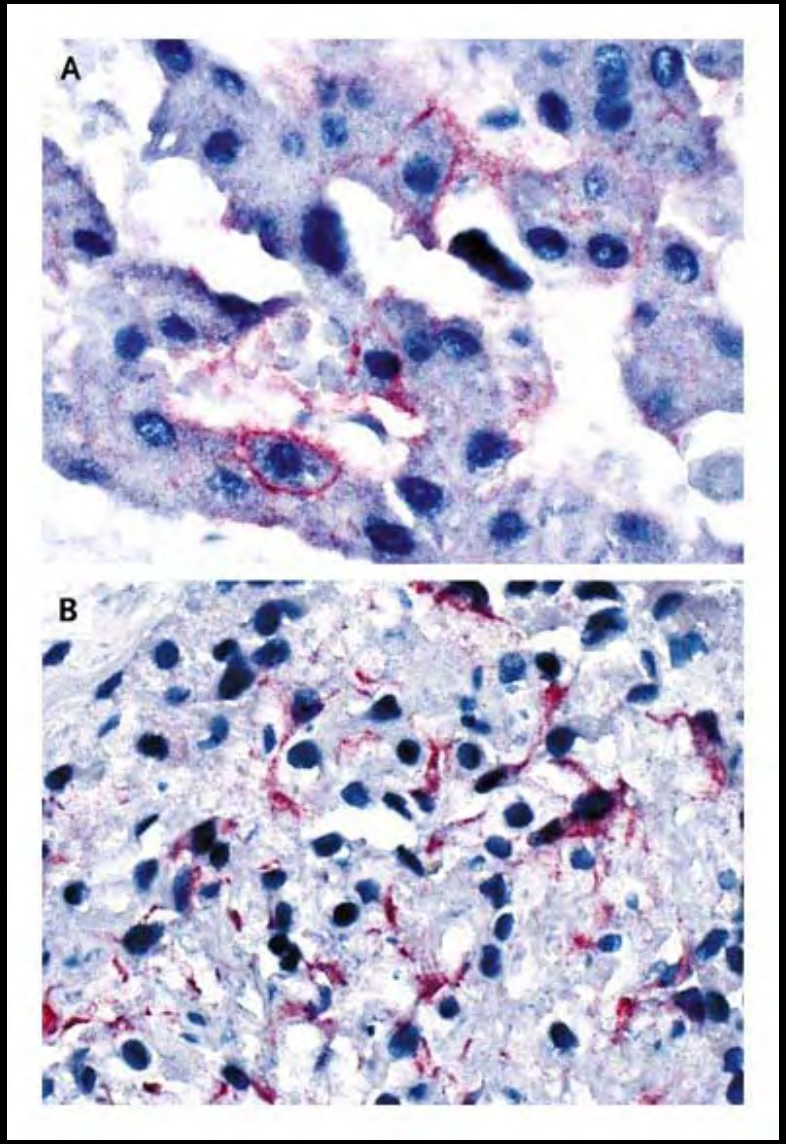
Predominantly Membranous Distribution of Arenavirus Antigen



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