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# 1 dies, 1 ill after receiving kidneys

The Boston Globe

## Donor infected with hard-to-find virus

**By Stephen Smith, Globe Staff | May 13, 2008**

A 70-year-old woman has died, and a 57-year-old man is critically ill in a Boston hospital after each received a kidney from a donor infected with a hard-to-detect virus, health authorities said yesterday.

The donor, a 49-year-old homeless man who suffered irreversible brain damage after cardiac arrest, carried a germ called lymphocytic choriomeningitis virus, or LCMV, the same infection that killed three transplant patients from Massachusetts and Rhode Island in 2005. The virus, most often transmitted by rodents, is usually unnoticed by healthy people who suffer no more than flulike symptoms.

Knowing that organs perish quickly, doctors test donors for what is easily analyzed, such as the AIDS virus, hepatitis, and a common herpes germ. But the lack of quick tests for less common conditions prevents screening for diseases such as the lymphocytic choriomeningitis virus.

Because the demand for organs always far exceeds the supply, recipients will accept organs even from high-risk donors such as the homeless. Waiting too long for a new kidney, liver, or heart can prove riskier.

"People are literally dying for organs," said Dr. Alfred DeMaria, top disease tracker at the Massachusetts Department of Public Health. "The list of potential things you can test for is enormous. But balancing that against the risk of not getting the organs, you have to make some decisions about what's feasible and what's not feasible to test for."

The homeless donor died in mid-March. After his family authorized the removal of viable organs, doctors took his kidneys. He had been tested for the AIDS virus, the liver diseases hepatitis B and C, and other diseases regularly checked by the New England Organ Bank, the region's organ procurement agency. There was no evidence of worrisome infections.

Still, his status as a man who had lived on the street, potentially exposed to a host of dangerous germs, led transplant surgeons to brand him as a high-risk donor.

Transplant surgeons at the hospitals with the two potential recipients - the woman was at Boston Medical Center, the man at Beth Israel Deaconess Medical Center - alerted the patients that the donor was regarded as high risk. The surgeons and patients decided to proceed.

"We all know that as much as we explain to the patients and inform them, they're relying on us and our medical judgment about whether this is a safe transplant," said Dr. Douglas W. Hanto, chief of the Division of Transplantation at Beth Israel Deaconess. "We feel a tremendous sense of responsibility to the patient and their family and feel terrible that this patient has had this infection and a bad outcome.

"But, on the other hand, we see patients who die every day on dialysis" awaiting a kidney transplant, he said.

The 57-year-old man transplanted at Hanto's hospital had lingered four years on the waiting list for a kidney. According to the United Network for Organ Sharing, an independent agency that sets organ procurement policies, 80,130 patients in the United States currently need a kidney.

It was the woman transplanted at Boston Medical who got sicker sooner after returning home. Like the donor and the other recipient, the woman was not identified by health authorities, who cited patient confidentiality laws.

The woman returned to Boston Medical about two weeks after her surgery, said Dr. Greg Grillone, the hospital's interim chief medical officer. She had a fever, diarrhea, "but oddly, symptoms not specific to the kidney," Grillone said.

Her condition kept deteriorating and, in mid-April, the woman died. Doctors at the hospital were stumped. There was no obvious cause of her precipitous demise.

But it turned out that one of the surgeons involved in the case, Dr. Amitabh Gautam, had been connected to

the 2005 Rhode Island and Massachusetts transplant cases.

He became suspicious that the Boston Medical patient had the same virus and alerted the federal Centers for Disease Control and Prevention. The virus has been known to have spread via transplant only two other times, in Wisconsin and Australia.

"Interestingly, what happened was this doctor had seen this before and thought, 'OK, this is a long shot, but I have seen it before and it can happen,' " Grillone said.

"If you take your car to the auto dealer with some very, very rare problem and you're lucky enough to get the mechanic who saw that same problem three years ago in the same make or model of the car, he might think: 'Oh, I saw this same problem three years ago. it might be the same problem," he said.

The man who had received his kidney at Beth Israel Deaconess returned with a fever 2 1/2 weeks after the surgery. On April 18, the doctors there got word that the Boston Medical patient had died. A transplant specialist at Beth Israel Deaconess also speculated that the virus might be at fault.

Samples from the deceased donor and the two patients were rushed to the CDC in Atlanta. All three tested positive for the virus, and investigators said all evidence points to the donor. The 57-year-old recipient remains in intensive care and is receiving the only drug known to possibly treat the virus.

"I don't believe this ever put the general public at risk," said Dr. Anita Barry, who leads the Boston Public Health Commission's investigation of the infections. "You have to be very, very unlucky to get LCMV from a transplant."

The virus is not transmitted casually from person-to-person; in addition to transplants, the only identified human transmissions have been from mother to fetus. Most people who are exposed catch it from the droppings of rodents, including wild animals and pets.

Because the virus causes few health problems in those who contract it, there has been little incentive to develop a rapid test.

The only tests currently available take time and are not widely available, said Dr. Eileen Farnon, a CDC medical epidemiologist.

"If you had a few days or a week for testing you could do that," Farnon said. "But in general that's not how the organ transplantation business works."

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医薬品　研究報告　調査報告書

<table>
<tr><td colspan="2">識別番号・報告回数</td><td></td><td>報告日</td><td>第一報入手日<br>2008年5月7日</td><td colspan="2">新医薬品等の区分<br>該当なし</td><td>総合機構処理欄</td></tr>
<tr><td colspan="2">一般的名称</td><td>別紙のとおり</td><td rowspan="2">研究報告の<br>公表状況</td><td rowspan="2">Infect Genet Evol. 2008 Mar 4. [Epub ahead of print]</td><td rowspan="2">公表国<br>コスタリカ</td><td rowspan="2"></td></tr>
<tr><td colspan="2">販売名（企業名）</td><td>別紙のとおり</td></tr>
<tr><td>研究報告の概要</td><td colspan="5">問題点：コスタリカにおいて、高病原性で新しい血清型に分類されるレプトスピラ株がヒトから分離された。<br><br>コスタリカにおいて、地域で流行しているレプトスピラの血清型を同定するため、通院している患者からレプトスピラを分離・解析した。レプトスピラ症の症状を呈して入院していた患者から分離された MAVJ401 株は、ウサギ抗血清パネルで Javanica 血清群型の血清型に対して著しく凝集価が上昇したが、標準的な Cross Agglutinin Absorption Test では血清学的にユニークであった。そのため MAVJ401 株は、Javanica 血清群型の新しい血清型（Arenal と命名）であると推測された。また、MAVJ401 株は、遺伝子学的解析によりラテンアメリカ諸国で多く発生している種である Leptospira santarosai に分類された。同じ地区の重症患者から分離された株も Arenal と同一の血清型であったことから、これが外来の血清型ではなく、この地域に流行する新規の高病原性の血清型であると考えられた。この新しい血清型に分類されるレプトスピラ株は、地域の公衆衛生と家畜衛生に脅威をもたらすおそれがある。</td><td>使用上の注意記載状況・<br>その他参考事項等<br><br>記載なし</td></tr>
<tr><td colspan="3">報告企業の意見</td><td colspan="4">今後の対応</td></tr>
<tr><td colspan="3">別紙のとおり</td><td colspan="4">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。</td></tr>
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別紙

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| 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人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑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献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500 注射用 |
| 報告企業の意見 | レプトスピラ症は、病原性レプトスピラ感染に起因する人獣共通の細菌(スピロヘータ)感染症である。レプトスピラは通常、長さ 6～20μm、直径 0.1μm のらせん状の細菌で、病原性レプトスピラと非病原性レプトスピラに大別される。病原性レプトスピラは、げっ歯類をはじめ多くの野生動物や家畜(ウシ、ウマ、ブタ、ヒツジなど)、ペット(イヌ、ネコなど)の腎臓に保菌され、尿中に排出される。ヒトは、保菌動物の尿で汚染された水や土壌から経皮的あるいは経口的に感染する。レプトスピラ症は急性熱性疾患であり、感冒様症状のみで軽快する軽症型から、黄疸、出血、腎障害を伴う重症型(ワイル病)まで多彩な症状を示す。レプトスピラは現在、13 の遺伝種からなり、さらに免疫学的性状により 250 以上の血清型に分類されている。日本におけるレプトスピラ症の患者数は近年激減したが、南西諸島・本土南部地域では他の地域に比べて多く散発している。また世界的に見ると、特に東南アジアや中南米などの亜熱帯、熱帯地域で患者発生が多い。レプトスピラは感染初期にヒトの血液や尿から直接観察される場合があることから、本剤の原料への混入を完全に否定できないと考え、本報告を行った。<br>仮に、製造原料にレプトスピラが混入していたとしても、弊所で製造している全ての血漿分画製剤の製造工程には、約 0.2μm の「無菌ろ過工程」および、レプトスピラよりも小さいウイルスの除去を目的とした平均孔径 19nm 以下の「ウイルス除去膜ろ過工程」が導入されており、これらの工程により除去されるものと考えられる。更に、これまでに当該製剤によるレプトスピラ感染の報告例は無い。<br>以上の点から、当該製剤はレプトスピラ感染に対する安全性を確保していると考える。しかし、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。 |

*現在製造を行っていない

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# Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica

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ABSTRACT

Leptospirosis is a worldwide distributed zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*. The basic taxon of *Leptospira* is the serovar. Currently, nearly 300 serovars have been identified. Leptospirosis is particularly prevalent in warm and humid tropical regions where conditions for transmission and survival of pathogenic leptospires in the environment are optimal. Leptospirosis probably constitutes a serious veterinary and public health problem in Central America but solid figures are missing. To determine distribution of leptospirosis in Costa Rica and to identify locally circulating pathogenic serovars, we performed a sentinel-based study, isolating and characterizing leptospires from patients attending hospitals. Strain MAVJ 401 was isolated from a hospitalized patient in the Alajuela province. The isolate produced agglutination titers notably with reference rabbit antisera against serovars of serogroup Javanica but appeared serologically unique in the standard Cross Agglutinin Absorption Test. Therefore, MAVJ 401 was considered to represent a new serovar, designated Arenal, of the serogroup Javanica. Genotypic analysis revealed that strain MAVJ 401 belongs to *Leptospira santarosai*, a species that almost exclusively occurs in Latin America. This is not a unique finding of an exotic serovar. Recent isolates from severely ill patients in the same region appeared to be identical to Arenal.

We have identified a novel highly virulent serovar from a patient in Costa Rica that is common in this area, thus posing a threat for the local public and veterinary health.



## 1. Introduction

Leptospirosis is a worldwide zoonosis, transmitted to humans through contaminated water or direct exposure to the urine of infected animals. Human infection may be acquired through occupational, recreational, or avocational exposures. Direct contact with infected animals accounts for most infections in farmers, veterinarians, abattoir workers, meat inspectors, rodent control workers and other occupations which require contact with animals. Indirect contact is important for sewer workers, miners, soldiers, septic tank cleaners, fish farmers, gamekeepers, canal workers, rice field workers, taro farmers, banana farmers and sugar cane cutters (Levett, 2001).

The clinical spectrum of the disease ranges from mild influenza-like to severe forms such as the Weil's syndrome, characterized by hepato-renal dysfunctions and a bleeding tendency and Acute Respiratory Distress Syndrome (ARDS) with mortality rates exceeding 50% (Levett, 2001; McBride et al., 2005).

Development of a subclinical infection or clinical disease might depend on both host and causative agent related factors such as immunological competence, age, physical condition and virulence and size of the inoculum, respectively. Animals with subclinical infections as well as those that recover from the clinical disease become a potential source of infection for other susceptible hosts, because they continue to excrete leptospires for a prolonged period of time (Faine, 1982; Faine et al., 1999).

The causative agents of leptospirosis belong to the genus *Leptospira*, which contains both saprophytic and pathogenic species (Levett, 2001). The isolation and identification of an infecting *Leptospira* strain is cumbersome and time consuming. Isolation is difficult due to the slow growth rate, notably when combined with a concomitant contamination with faster growing microorganisms, and stringent and fastidious in vitro culture requirements of these bacteria (Faine, 1994; Faine et al., 1999). The

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initial identification of a *Leptospira* is morphological, by dark field microscopy observation. Definitive identification of the isolates requires the use of serological and molecular techniques (Dikken and Kmety, 1978; Brenner et al., 1999; Levett, 2003). In the conventional classification system, all pathogenic leptospires belong to the species *Leptospira interrogans* sensu lato (Dikken and Kmety, 1978; Faine and Stallman, 1982). Based on serological criteria, strains of *Leptospira* are differentiated into serovars, which represent the basic taxon (ICSB Sub-committee on the taxonomy of Leptospira, 1987; Kmety and Dikken, 1993). Serovars that are antigenically related are placed into serogroups. Serogroups do not have an official taxonomic status, but are of clinical and epidemiological importance (Levett, 2003). The list is updated periodically and more than 250 pathogenic serovars arranged in 26 serogroups are currently known. The recent genotypical classification system is based on DNA homology. In this system, leptospires are placed into 17 *Leptospira* species of a pathogenic, saprophytic and doubtful nature (Yasuda et al., 1987; Perolat et al., 1998; Brenner et al., 1999; Levett et al., 2006). There is a poor correlation between the serological and genotypic classification systems (Brenner et al., 1999; Yasuda et al., 1987).

The species *Leptospira santarosai* contains 61 serovars of multiple serogroups (Brenner et al., 1999). The type strain of *L. santarosai*, serovar Shermani strain 1342 K was isolated from a spiny rat (*Proechymis semispinousus*) in the Panama Canal Zone (Yasuda et al., 1987). Several additional reports confirmed that *L. santarosai* is pathogenic for humans and domestic animals (Brenner et al., 1999; Hsieh and Pan, 2004; Rossetti et al., 2005).

In this paper, we describe a new leptospiral serovar belonging to the species *L. santarosai* isolated from the blood of a severely ill leptospirosis patient.

## 2. Materials and methods

### 2.1. Case description

A 45-year-old man was hospitalized in Ciudad Quesada San Carlos Hospital, Costa Rica, with a 3–4 day history of fever, headache and myalgia. The patient is a biologist employed by a Costa Rican fish farm. At the day of admission his temperature was 39°. He had tachycardia and his blood pressure was 120/60 mmHg. Clinical examination showed a conscious man, with bilateral headache, sore throat, provoked myalgia of the legs, hepatalgia, hepatomegaly, and conjunctivitis. There were no signs of rash, meningeal irritation and cervical rigidity. Laboratory tests revealed increased SGOT:79.8 U/L (normal range (nr) 12.0–46.0), 76.2 U/L (nr 3–50), creatine phosphokinase: 915 U/L (nr 24–195), direct bilirubin: 0.53 mg/dL (nr 0.0–0.2), total bilirubin: 1.49 mg/dL (nr 0.0–1.0), associated with hyperglycemia: 143 mg/dL, alkaline phosphatase: 202 U/L (value is within normal range, nl), albumin: 3.3 g/dL (nl), and protein levels: 5.92 g/dL (nl), ureic nitrogen: 8.62 mg/dL (nl), creatinine: 1.26 mg/dL (nl). The leukocyte count was 8, $2 \times 10^3/\mu L$ with 80% polymorph nuclear forms. Thrombocytopenia: $145 \times 10^3/\mu L$ (last control: $99 \times 10^3/\mu L$) was also observed. Results of urinalysis were normal. Malaria blood smears, blood cultures and serology for dengue were negative.

The patient received a 7-day treatment with penicillin, 2 million units 4 times a day, which resulted in a resolution of symptoms. Oral treatment with penicillin was continued for 6 more days.

Leptospirosis was confirmed by seroconversion in the Microscopic Agglutination Test (MAT) with a titer of 1:100 with serovar Canicola in the second sample. Also the rapid screening test Lepto dipstick (Gussenhoven et al., 1997) gave a positive outcome (data not shown).

### 2.2. Bacterial culture

Culturing was performed in Ellinghausen and McCullough modified Johnson and Harris (EMJH) culture medium (DifcoTM). Aliquots of 0.1 and 0.01 mL of heparin anticoagulanted whole blood were inoculated into 6 mL EMJH culture medium. Incubation was at 30 °C and cultures were inspected by darkfield microscopy for growth of leptospires at regular intervals. Isolates were subcultured and maintained in EMJH medium and in Fletcher medium supplemented with 5 fluoro-uracil (200 μg/mL) as a selective inhibitor for contaminating microorganisms (Faine and Stallman, 1982; Faine et al., 1999; Hartskeerl et al., 2006).

### 2.3. Microscopic agglutination test

The microscopic agglutination test (MAT) was performed as per standard procedure (Comisión Científica Permanente sobre Leptospirosis de la AAVL, 1994) starting with a serum dilution of 1:20 up to 1:20480. The highest dilution of serum showing 50% reduction in free-moving leptospires under dark field microscope was considered the end-titre. Rabbit anti-*Leptospira* sera were prepared following the standard procedure (ICSB Sub-committee on the taxonomy of *Leptospira*, 1984).

### 2.4. Serological typing: MAT with group sera and monoclonal antibodies

To identify the isolate up to serogroup level, MAT was performed following standard procedure using a panel of 38 anti-*Leptospira* rabbit antibodies (Dikken and Kmety, 1978; Hartskeerl et al., 2006). Isolates were further typed at the serovar level by performing MAT with panels of monoclonal antibodies (mAbs) that characteristically agglutinate serovars from the serogroups Icterohaemorrhagiae and Sarmin (F12C3, F20C3, F20C4, F52C1, F52C2, F70C4, F70C7, F70C13, F70C14, F70C20, F70C24, F70C26, F82C1, F82C2, F82C7, F82C8, F89C3, and F89C12) as described by Korver et al. (1988) and from serogroup Javanica (F12C3, F20C3, F20C4, F70C20, F98C4, F98C5, F98C8, F98C12, F98C17, F98C19 and F98C20) with cross-agglutinations of serovars of the closely related serogroups Sarmin and Celledoni (Alex et al., 1993).

### 2.5. Cross Agglutinin Absorption Test

The Cross Agglutinin Absorption Test (CAAT), the standard assay for serological classification of *Leptospira* serovars was carried out by staff of INCIENSA as described elsewhere (Dikken and Kmety, 1978; Kmety and Dikken, 1993; Hartskeerl et al., 2006, ICSB Sub-committee on the taxonomy of *Leptospira*, 1984). Staff of the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute, The Netherlands confirmed the CAAT results.

### 2.6. Genetic characterization

Strains and isolates were grown at 30 °C in EMJH medium and harvested by centrifugation during the late logarithmic phase. DNA was isolated as described by Boom et al. (1990). PCR was performed on the DNA extracts using the primer set G1/G2 that specifically amplifies a 285 bp fragment of the *secY* gene from all pathogenic species except *L. kirschneri* (Gravekamp et al., 1993; Oliviera et al., 2003). PCR conditions and controls were as previously described (Gravekamp et al., 1993; Bal et al., 1994). PCR products were analyzed by electrophoresis in 1.5% agarose gels, stained with ethidium bromide using standard procedures and subsequently judged by eye under UV illumination.

For sequencing, DNA concentration of PCR products was adjusted in the range of 10–20 ng per reaction and applied to

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