the sequence reaction using the BigDye® Terminator V1.1 Cycle Sequencing Kit (Applied Biosystems, United Kingdom) and subsequently analyzed on an ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, United Kingdom). DNA sequence clustal alignments were done using the LaserGene software package (DNASTAR). Species determination was done on basis of highest sequence identity of PCR products from *Leptospira* reference strains (Gravekamp et al., 1993; Oliviera et al., 2003; Rossetti et al., 2005; Priya et al., 2007).

3. Results

3.1. Isolation

The culture with 0.1 mL blood inoculation became positive after two weeks. The isolate was named strain MAVJ 401. Under the darkfield microscope, strain MAVJ 401 showed typical *Leptospira* motility and morphology. The strain grew well in EMJH and Fletcher medium at 30 °C.

3.2. Serological characterization

When testing the strain against a panel of 38 rabbit anti-*Leptospira* sera to determine potential serogroups, highest agglutination titers were found against serogroup Sarmin serovar Weaveri and serogroup Javanica serovar Poi. Low cross-agglutinating titers were also produced with members of the serogroups Icterohaemorrhagiae and Celledoni. No agglutinations were found with reference sera from intermediate and saprophytic reference strains, suggesting a pathogenic status of the isolate.

Subsequent testing with the panel of mAbs against serovars of the Icterohaemorrhagiae and Sarmin groups only revealed a titer 1:320 against one of the 18 mAbs in the panel. No match was found with the agglutination pattern of any of the serovars in these two serogroups (results not shown). The agglutination pattern obtained with the mAbs against serovars of the Javanica group was most similar with that of serovar Javanica, strain Veldrat Batavia 46 (Table 1). No match was found with serovars of the closely related serogroup Celledoni and, again, serogroup Sarmin.

Cross-agglutinations and CAAT were performed to confirm the presumptive results obtained via mAbs typing.

Cross-agglutination experiments were executed between strain MAVJ 401 and antiserum against all serovars from the groups Javanica, Sarmin and Celledoni and vice versa. No significant cross-agglutinations (>10% compared to the homologous agglutination) were observed with sera from the serogroups Celledoni and Sarmin and vice versa, serum against MAVJ 401, virtually excluding that MAVJ 401 belongs to these serogroups. A significant cross-agglutination titer in both cross-agglutination experiments was only found against serogroup Javanica serovar Fluminense strain Aa3. Surpisingly only low cross-agglutination titers were found against serovar Javanica strain Veldrat Batavia 46.

CAAT was performed in duplicate and independently by two persons to assure reproducibility. The following reference strains were included in the test, Javanica group: serovar Fluminense strain Aa3, serovar Sofia strain Sofia 874, serovar Coxi strain Cox, serovar Javanica strain Veldrat Batavia 46, serovar Sorexjalna strain Sorex Jalná, serovar Zhengkang strain L 82 and serogroup Sarmin; serovar Machiguenga strain MMD 3, serovar Rio strain Rr 5 and serovar Weaveri strain CZ 390.

According to the definition of the International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Leptospira* (1984, 1987), strain MAVJ 401 was not serologically identical to any of these strains (Table 2) and therefore MAVJ 401 represents a new serovar, designated Arenal. Based on the initial serological reactions it is proposed that this serovar is placed within the pathogenic serogroup Javanica.

Table 1
Comparison of agglutination titers of strain MAVJ 401 and the reference serovar Javanica, strain Veldrat Batavia 46 with mAbs against serogroup Javanica

mAb	Reciprocal titers against strain MAVJ 401	Reciprocal titers against strain Veldrat Batavia 46
F12C3	–	–
F20C3	–	–
F20C4	320	320
F70C20	–	–
F98C4	–	–
F98C5	–	–
F98C8	5120	5120
F98C12	20480	5120
F98C17	–	–
F98C19	10240	10240
F98C20	–	≤80

(–) No agglutination.
Up to a 4-fold titer difference is acceptable in mAbs typing.

Table 2
Cross-agglutinations and CAAT between MAVJ 401 and reference strains

Serum	Strain	Cross agglutination (%)[a]	CAAT, residual titer (%)[b]
Aa3	MAVJ 401	50	50
MAVJ 401	Aa3	12.5	100
Sofia 874	MAVJ 401	12.5	50
MAVJ 401	Sofia 874	0.2	ND
Cox	MAVJ 401	6.25	50
MAVJ 401	Cox	0.4	ND
Veldrat Batavia 46	MAVJ 401	1.5	100
MAVJ 401	Veldrat Batavia 46	0.2	ND
Sorex Jalná	MAVJ 401	100	100
MAVJ 401	Sorex Jalná	0.2	ND
L 82	MAVJ 401	12.5	100
MAVJ 401	L 82	0.8	ND
MMD 3	MAVJ 401	50	100
MAVJ 401	MMD 3	6.25	ND
Rr 5	MAVJ 401	25	50
MAVJ 401	Rr5	6.25	ND
CZ 390	MAVJ 401	25	100
MAVJ 401	CZ 390	1.5	ND

[a] (Heterologous titer: homologous titer) × 100%; >10% is significant.
[b] (Homologous titer after absorption: homologous titer before absorption) × 100%; <10% indicates similarity of the serovars.

3.3. Species determination

Consistent with its pathogenic status, DNA from MAVJ 401 was amplified by primer pair G1/G2 (Gravekamp et al., 1993). To determine the species of MAVJ 401, the sequence of its G1/G2 amplicon was compared with 65 other sequences (Oliviera et al., 2003; Rossetti et al., 2005; Priya et al., 2007). The sequence of the amplicon showed highest percentage identity with a number of strains from *L. santarosai*, i.e. 97.1% with serogroup Sejroe; serovar Caribe strain TRVL 61866 and serovar Gorgas strain 1413 U, serogroup Mini; serovar Georgia strain LT 117 and Tabaquite strain TRVL 3214, serogroup Pyrogenes; serovar Princestown strain TRVL 112499, serogroup Javanica; serovar Vargonis strain 24, serogroup Sarmin; serovar Weaveri strain CZ 390 and 96.7% identity with serogroup Pomona, serovar Tropica strain CZ 299.

Percentages sequence identity outside *L. santarosai* ranged from 71.3% (*L. meyeri*, serovar Semaranga strain Veldrat Semarang 173) to 94.7% (*L. weilii* serovar Mengrun strain A 102 and *L. weilii*, serovar Coxi strain Cox). Taking the highest percentage of identity with eight strains of *L. santarosai*, we believe that MAVJ 401 belongs to this species.

Please cite this article in press as: Valverde, M.A. et al., Arenal, a new *Leptospira* serovar of serogroup Javanica, isolated from a patient in Costa Rica, Infect. Genet. Evol. (2008), doi:10.1016/j.meegid.2008.02.008

4. Discussion

We describe the isolation and characterization of a novel *Leptospira* serovar isolated from a Costa Rican patient. The patient was admitted to the hospital with signs and symptoms compatible with leptospirosis and standard antibiotic treatment with penicillin was effective. Leptospirosis was serologically confirmed. It likely concerns here an occupational disease as the patient worked on a fish farm where he obviously acquired the infection via fish ponds contaminated with urine of carrier animals.

The morphology and motility of the bacterium under darkfield microscopy is consistent for the genus *Leptospira*. Serologically, the isolate showed titers notably against members of the serogroups Javanica and Sarmin. Cross-agglutination titers were also found in the serogroups Icterohaemorrhagiae and Celledoni. This likely represents intra-serogroup cross-agglutinations because serogroups Javanica and Celledoni on one hand and Javanica, Sarmin and Icterohaemorrhagiae on the other hand form 'serogroup complexes' comprising antigenic related serovars (Hartskeerl et al., 2006). Because of this overlapping antigenic relationship between these groups and the fact that highest agglutinating titers were produced with serovars of serogroup Javanica we suggest to place MAVJ 401 into this serogroup.

We found contrasting data by mAbs typing and the CAAT. mAbs typing generated a pattern that was highly similar to that of the reference serovar Javanica strain Veldrat Batavia 46 of the Javanica group. However, cross-agglutination and CAAT revealed only little similarity with this serovar. Moreover, CAAT, which is the standard method to determine the serovar as basic taxon, revealed that this isolate is unique. The serovar status is mainly, if not exclusively, based on the composition and structure of the highly antigenic LPS (Faine et al., 1999). A likely explanation of the discrepancy in typing with monoclonal and polyclonal sera is that panels of agglutinating mAbs are directed to a limited number of epitopes while polyclonal hyperimmune sera cover the full spectrum of epitopes. Apparently, it is possible that a set of mAbs recognizes a limited number of common epitopes on furthermore different LPS in distinct serovars within a serogroup. As shown in this study, incorrect mAbs-based identification can be avoided by determining cross agglutination with polyclonal hyperimmune serum against the presumably corresponding reference strain.

We designated the isolate serovar Arenal after the volcano in the Costa Rica near the residence of the patient in the province Alajuela.

DNA sequence analysis indicated that serovar Arenal most likely belongs to species *L. santarosai*, which is distributed almost exclusively in Latin America (Chappel et al., 1998).

Serovar Arenal likely is not an exotic serovar and might be common in and around the Alajuela province of Costa Rica. Recently, two out of 21 isolates obtained from Costa Rica were identified as serovar Arenal implying that 13.6% (3/22) of the isolates consisted of Arenal. The two additional Arenal isolates, preliminary coded as isolate 7 and 11, were cultured from severely ill patients living in the Puntarenas province that flanks Alajuela. Molecular analysis of MAVJ 401/isolate 7 by Multilocus Sequence Typing showed that it formed a distinct branch that was positioned closely to, but apart from the clade of *L. santarosai* (Ahmed et al., 2006). This supports the unique character of this novel serovar, also on genotypical grounds.

The infection source of isolate 11 is unknown. Infection with isolate 7 was very likely acquired via contact with cattle. The environment of the fish farm of MAVJ 401 makes it possible that the ponds have been contaminated with urine of infected cattle. It is therefore tempting to speculate that cattle form the infection reservoir of this novel serovar. However, further research on potential infection sources in the region will be needed to confirm or refute this.

L. santarosai, serovar Arenal, type strain MAVJ 401 has been deposited under this designation in the culture collections of the National Reference Center for Leptospirosis, Costa Rican Institute for Research in Nutrition and Health, Tres Ríos, Costa Rica and the WHO/FAO/OIE and National Collaborating Centre for Reference & Research on Leptospirosis, Royal Tropical Institute, Amsterdam, Netherlands. The novel serovar designation of strain MAVJ 401 has been ratified by the International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of *Leptospiraceae*.

Acknowledgments

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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 200 年 4 月 10 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	Portsmouth woman's death under investigation dailypress.com, April 11, 2008	公表国 米国	
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
異型クロイツフェルト・ヤコブ病(vCJD)に関連すると疑われる脳変性疾患を呈した米国の女性の症例が報告された。しかし，感染症，脳内酸素欠乏，肝不全，腎不全，毒物暴露，代謝疾患，脳腫瘍，頭蓋内圧の上昇，栄養不足など多数の原因が，本症例の脳疾患に関連していると考えられており，原因究明には更なる調査が必要である。MRI 又は脳スキャンの結果が，アトランタの疾病対策センターに送付され，バージニア大学及び National Prion Disease Pathology Surveillance Center (NPDPSC) で更に調査されることになっているが，結果が出るまでには数ヶ月間を要すると考えられている。	BYL-2008-0316

報告企業の意見	今後の対応
弊社の血漿分画製剤は米国の血漿を使用しているが，現在までに報告されている米国での vCJD 3 例は，米国以外の国で暴露された患者に限定されている。また，弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されており，弊社製剤による vCJD 感染リスクは極めて低いと考えられる。	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。

(14)

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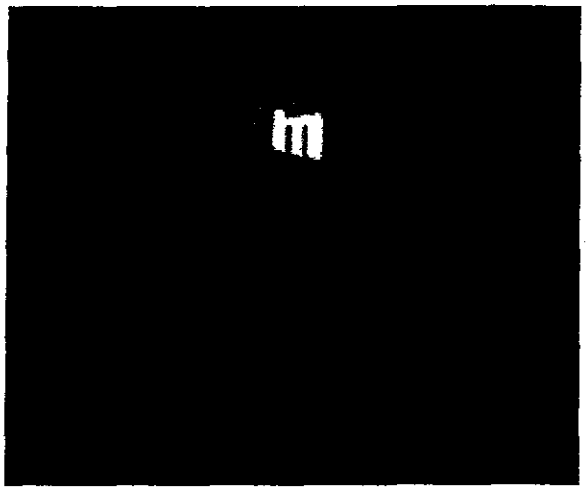
Portsmouth woman's death under investigation

By VERONICA GORLEY CHUFO

247-4741

April 11, 2008

RICHMOND



The illness and Wednesday death of a Portsmouth woman spurred a Virginia Department of Health investigation Thursday.

The woman suffered from encephalopathy, a degenerative brain disease. Her illness has been linked in news reports to variant Creutzfeldt-Jakob Disease — the human form of mad cow disease.

It's a very rare condition related to the consumption of beef infected with bovine spongiform encephalopathy. It's always fatal, the health department said in a news release.

The woman's name was not released by the health department but news reports have identified her as Aretha Vinson.

The illness could have been caused by a number of things, State Health Commissioner Karen Remley said in the release.

"Infections, lack of oxygen to the brain, liver failure, kidney failure, toxic exposures, metabolic diseases, brain tumors, increased intracranial pressure and poor nutrition are all related to encephalopathy," Remley said. "Further testing is the only way to know what caused this illness."

An MRI, or brain scan, was sent to the Centers for Disease Control and Prevention in Atlanta. Additional tests will be handled by the University of Virginia and the National Prion Disease Pathology Surveillance Center in Cleveland. Results are expected to take several months.

At least 200 cases of variant Creutzfeldt-Jakob Disease have been reported worldwide since 1996. Three cases have been reported in U.S. residents, and they were all exposed outside the country, Remley said. It's not spread casually from person to person.

For more information, visit cdc.gov, cjdfoundation.org or vdh.virginia.gov.

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3-2

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 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	Prion Filter for Donated Blood Medgadget LLC, April 9, 2008	公表国 カナダ	
販売名（企業名）						

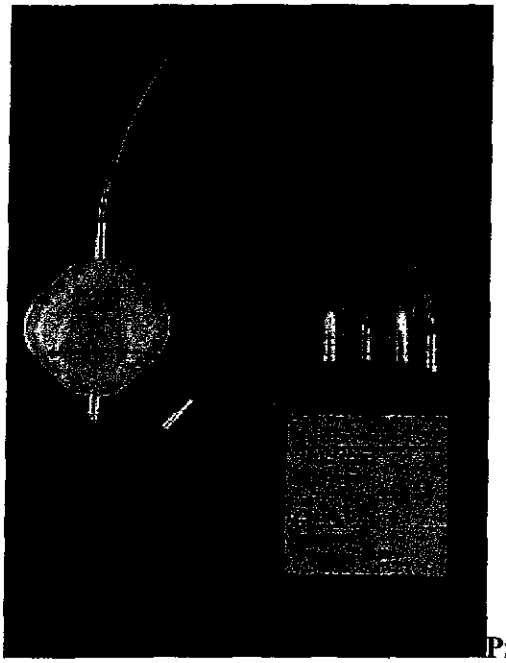
研究報告の概要	使用上の注意記載状況・その他参考事項等
カナダ，ケベック州の ProMetic Life Science 社が血液中の異型クロイツフェルト・ヤコブ病(vCJD)プリオンを除去するフィルターを開発した。 ProMetic Life Science 社の開発チームは，血液中のプリオンと親和性が高いペプチドをスクリーニングした後，市販のポリメタクリル酸樹脂にペプチドを固定し，これを膜状にしたものを幾層にも重ねて本フィルターを開発した。本使い捨てフィルターにより，1時間足らずで 1 ユニットの汚染血液からプリオンを除去することが可能であり，又，この工程によって血液自体の変性はおきない。さらに，プリオン感染ハムスターのフィルター処理した血液をプリオン非感染ハムスターに投与したところ，疾患は発現しなかった。	BYL-2008-0317

報告企業の意見	今後の対応
現在までに，血液中における vCJD 検査は可能となっていない。本フィルターの使用により，輸血による vCJD 感染に対する安全性は高まると考えられる。弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されている。	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。

(57)

Print

Prion Filter for Donated Blood



ProMetic Life Sciences, a company out of Mont-Royal, Quebec, has developed a blood filter touted to remove prions responsible for variant Creutzfeldt-Jakob disease (vCJD). Considering that currently there is no available test for vCJD in donated blood, filtering may soothe the nerves of potential transfusion recipients.

> The team took five years to create the hand-sized filter, screening millions of small peptides to find one that had the strongest affinity for the prions found in contaminated blood. They stuck the best peptide onto commercial polymethacrylate resins, and then sandwiched these in alternating layers with a membrane
>
> In tests, the disposable filter can clean the prions out of a single pack of contaminated blood in less than an hour. No prions remain in the cleaned blood, which is otherwise unchanged by the process. Tests with prion-infected hamsters showed that their filtered blood could be injected into disease-free hamsters with no ill effects.
>
> The team hope that the UK's National Blood Service could be using the device by the end of this year. Peter Edwardson, ProMetic's vice-president of medical technologies, says that Ireland's clinical trial, aiming to confirm that the filtered red blood cells are just as effective as untreated blood when transfused into humans, should be complete in a few months.

More at the *Royal Society of Chemistry*...

ProMetic Life Sciences...

Flashbacks: Leukotrap® Affinity Prion Reduction Filter

Posted on April 9, 2008 09:14 AM

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