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 2010年3月3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	Infect Genet Evol 9:1240-1247	公表国 フランス	
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：フランスの家禽と殺場従業員に発生した非定型肺炎に関する調査から、クラミジアの新株の存在が示唆された。 フランスの家禽と殺場従業員に発生した非定型肺炎に関する調査から、クラミジアの新たな株の存在が示唆された。と殺場に家禽を供給した10農場における25群から得れらた検体を用いてPCR検査を行ったところ、同25群の内14群にクラミジア関連因子が認められた。同14群の内の1群の因子はChlamydophila psittaciと同定されたものの、他の群の因子はこれまでに分類されていないものであった。未分類因子が認められた群の中の異なる6群の検体を用いた感染実験の結果、それらの16S rRNAの遺伝子は非常に近い配列を有し、Chlamydophila属に属することは明らかであるものの、同属の新たな株である可能性が示唆された。今のところ、これらの因子が人畜共通感染症の感染因子であるかは不明である。	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.12.1

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人免役グロブリン、⑤人免役グロブリン、⑥人免役グロブリン、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役グロブリン*、㊳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アンスロビンP1500 注射用
報告企業の意見	クラミジア（Chlamydia）は 300nm 程度の大きさで、細胞内でのみ増殖する偏性細胞内寄生微生物であり、DNA と RNA を有し、2 分裂で増殖する。今回の報告は家禽と殺場従業員に発生した非定型肺炎に関する調査を機に、クラミジアの Chlamydophila 属における新株の可能性が示唆されたものであるが、それらが人畜共通感染症の感染因子であるかは不明である。 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、クラミジアよりも小さいウイルスの除去を目的としたウイルス除去膜ろ過工程が導入されているので、仮に製造原料にクラミジアが混入していたとしても、これらの工程により除去されるものと考えられる。更に、これまでに上記製剤によるクラミジア感染の報告例は無い。 以上の点から、上記製剤はクラミジア感染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

Infection, Genetics and Evolution 9 (2009) 1240–1247

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France

Karine Laroucau [a,*], Fabien Vorimore [a], Rachid Aaziz [a], Angela Berndt [b], Evelyn Schubert [b], Konrad Sachse [b]

[a] Bacterial Zoonoses Unit, French Food Safety Agency (AFSSA), 23 avenue du Général de Gaulle, 94706 Maisons-Alfort, France
[b] Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Jena, Germany

ARTICLE INFO

Article history:
Received 20 March 2009
Received in revised form 18 August 2009
Accepted 19 August 2009
Available online 26 August 2009

Keywords:
Chlamydophila spp.
New agent
Domestic poultry
Real-time PCR
Genotyping
DNA sequence analysis

ABSTRACT

Three cases of atypical pneumonia in individuals working at a poultry slaughterhouse prompted an epidemiological survey in 10 poultry farms that had supplied birds. Using a *Chlamydiaceae*-specific real-time PCR assay, chlamydial agents were detected in 14 of 25 investigated flocks. Rather unexpectedly, *Chlamydophila psittaci* was identified only in one of the positive flocks, whereas ArrayTube DNA micrparray testing indicated the presence of a new, so far unclassified member of the genus *Chlamydophila*.

For further characterization of the agent involved, positive cloacal swabs were used to inoculate embryonated chicken eggs and isolates were obtained from 6 different flocks. Sequencing of 16S rRNA genes revealed nearly identical sequences of all samples. Alignment with representative sequences of *Chlamydiaceae* showed the separate position of the present strains outside the currently recognized species of *Chlamydophila*, but clearly within this genus. In contrast, partial *ompA* gene sequences displayed considerable diversity among the isolates, which had already been observed in restriction enzyme analysis of *ompA* PCR products. These data suggest that each farm had been infected with a different strain of this new chlamydial agent, the zoonotic potential and the exact taxonomic status of which have yet to be defined.

1. Introduction

Chlamydial infections leading to outbreaks of avian chlamydiosis in domestic, companion and wild birds are regularly reported from all parts of the world. As their general importance is based on two aspects, i.e. economic losses to the bird owners and potential zoonotic transmission to humans, control measures are obligatory in a number of European countries, where specific state legislation is in force. The most prominent chlamydial agent in *Aves* is *Chlamydophila (C.) psittaci*, which was shown to occur in as many as 465 bird species (Kaleta and Taday, 2003). Following the recent revision of chlamydial taxonomy (Everett et al., 1999), this obligate intracellular bacterium now predominantly comprises avian serovars. The family *Chlamydiaceae* with its two genera *Chlamydia* and *Chlamydophila* currently combines a total of nine species, i.e. *Chlamydia trachomatis*, *Chlamydia suis* and *Chlamydia muridarum*, as well as *C. pneumoniae*, *C. abortus*, *C. caviae*, *C. felis*, *C. pecorum*, and *C. psittaci*, respectively.

The importance of *C. psittaci* as the causative agent of psittacosis or avian chlamydiosis in psittacine birds and domestic fowl has been known for decades. A number of recent reports have confirmed that its zoonotic potential remains significant in the face of regularly occurring outbreaks of disease in domestic fowl (Vanrompay et al., 1995; Gaede et al., 2008; Laroucau et al., 2009). In addition, infections can take a subclinical and/or chronic course (Harkinezhad et al., 2009). However, occasional detections of *C. abortus* (Herrmann et al., 2000; Pantchev et al., 2008) and so far non-classified chlamydial agents (Gaede et al., 2008), as well as genetic evidence on intermediate strains between *C. psittaci* and *C. abortus* (Van Loock et al., 2003) suggest that the spectrum of *Chlamydiaceae* spp. encountered in birds is not confined to a single species.

In this context, it should be noted that laboratory diagnosis of infections involving chlamydiae has undergone a remarkable methodological change in the past two decades (Sachse et al., 2009). While only a few specialized laboratories are still conducting routine isolation of chlamydiae using cell culture or embryonated eggs, DNA-based detection methods have become widely accepted. This implies that specific PCR tests for individual chlamydial species and/or pan-*Chlamydiaceae* assays are conducted, which are capable of detecting even small amounts of a known agent within a working day. However, new and hitherto

Abbreviations: MOMP, major outer membrane protein; RFLP, restriction fragment length polymorphism; rtPCR, real-time PCR.

* Corresponding author. Tel.: +33 1 49 77 13 00; fax: +33 1 49 77 13 44.
E-mail address: klaroucau@afssa.fr (K. Laroucau).

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doi:10.1016/j.meegid.2009.08.005

non-classified taxa will often be overlooked by this approach, so that the inclusion of a highly parallel screening assay, e.g. a DNA microarray test, is advisable.

Following the occurrence of symptoms of atypical pneumonia in a group of poultry slaughterhouse workers in France early in 2008, a diagnostic investigation in the slaughterhouse and the flocks of the suppliers was undertaken. Initial tests suggested involvement of chlamydiae, so that diagnostic examinations focused on *Chlamydiaceae* spp. In the present paper, we report the results of this study, which led to the identification of so far non-classified avian chlamydial agents.

2. Materials and methods

2.1. History of human infections

In the period from April 25 to May 30 in 2008, three individuals presented to their physicians with atypical pneumonia. Case 1, a 49-year-old woman, had fever and flu-like symptoms and was administered Naxy. Case 2 was a 25-year-old woman suffering from thoracic pain and fever. She had to be hospitalized and was treated with Clamoxyl and Rulide. Case 3, a man aged 62, had fever and complained about weariness. He was administered Zeclar. No diagnostic data from microbiology and serology are available.

2.2. Animal samples

Cloacal swabs were collected from poultry flocks received for slaughter and from birds belonging to the slaughterhouse owner. In each sampled flock, 10 animals were examined. One panel of swabs was stored in 1 ml of conservation buffer SPG (Spencer and Johnson, 1983) at −80 °C until inoculated into chicken eggs, whereas the other panel was stored dry at −80 °C until subjected to DNA extraction. Data on age, breed and flock of the examined birds are contained in Table 1.

2.3. DNA extraction

Dry cloacal swabs were subjected to DNA extraction using the QIAamp DNA Mini Kit (QIAGEN, Courtaboeuf, France) following the buccal swab protocol. For DNA extraction from aliquots of chlamydial cell culture and vitellus membranes, the tissue protocol of the same kit was used. Finally, DNA was eluted with 150 μl of AE buffer and stored at −20 °C until examination.

2.4. Direct detection of chlamydiae by real-time PCR

A *Chlamydiaceae*-specific real-time PCR targeting the 23S rRNA gene (23S-rtPCR) was used in this study (Ehricht et al., 2006). The protocol includes primers Ch23S-F (5′-CTGAAACCAGTAGCTTA-TAAGCGGT-3′), Ch23S-R (5′-ACCTCGCCGTTTAACTTAACTCC-3′), and probe Ch23S-p (FAM-5′-CTCATCATGCAAAAGGCACGCCG-3′-TAMRA). Each reaction mix contained 2 μl sample DNA template, 10 μl of Universal Mastermix 2× (Applied Biosystems, Courtaboeuf, France), 0.5 μl of each primer (25 μM), 2 μl of the probe (1 μM), and 5 μl deionized water. The temperature–time profile was 95 °C for 10 min, 45 cycles of 95 °C for 15 s and 60 °C for 60 s.

The *ompA*-based real-time PCR assay specific for *C. psittaci* was conducted as recently described (Pantchev et al., 2008).

2.5. Culture on embryonated chicken eggs

For culture, suspensions of cloacal swabs stored in conservation buffer at −80 °C were thawed, transferred into sterile Eppendorf

Table 1
Characteristics of investigated poultry flocks and results of diagnostic testing.

Flock ID	Breeder no.	Flock effective	Breed	Age at time of sampling (weeks)	23S-rtPCR *Chlamydiaceae*		*ompA*-rtPCR *C. psittaci*	Cell culture isolation
					No. of +ve samples	Mean Ct	No. of +ve samples	
Slaughterhouse								
08-1274/2	Slaughterhouse	500	Chicken	4	1/10 (10%)	38.6	0/1	
08-1274/1	breeder	500	Chicken	8	0/10	–	–	
08-1274/5		500	Chicken	12	0/10	–	–	
08-1274/4		500	Chicken	16	4/10 (40%)	33.0	0/4	
08-1274/3		500	Chicken	20	10/10 (100%)	28.8	0/10	+
08-1274/6		100	Guinea fowl	7	0/10	–	–	
08-1274/8		120	Guinea fowl	13	0/10	–	–	
08-1274/7		120	Guinea fowl	16	0/10	–	–	
Customers								
08-1274/9	Breeder 1	320	Chicken	20	3/10 (30%)	34.6	0/3	
08-1274/10		–	Guinea fowl	20	0/10	–	–	
08-1274/17		–	Duck	–	1/5 (20%)	35.1	1/1	+
08-1274/11	Breeder 2	600	Chicken	15	0/10	–	–	
08-1274/12		–	Guinea fowl	15	1/8 (12.5%)	40.1	0/1	
08-1274/13	Breeder 3[a]	600	Chicken	–	9/9 (100%)	26.3	0/9	+
08-1274/23		600	Chicken	12	10/10 (100%)	27.2	0/10	+
08-1274/24		600	Chicken	4	3/10 (30%)	39.0	0/3	
08-1274/14	Breeder 4	–	Chicken	–	0/10	–	–	
08-1274/15		–	Guinea fowl	–	0/8	–	–	
08-1274/16	Breeder 5[a]	600	Chicken	8	1/10 (10%)	38.8	0/1	
08-1274/19		600	Chicken	18	10/10 (100%)	27.0	0/10	+
08-1274/18	Breeder 6[a]	600	Chicken	18	0/10	–	–	
08-1274/20	Breeder 7[a]	600	Chicken	12	1/10 (10%)	38.9	0/1	
08-1274/21		600	Chicken	17	10/10 (100%)	26.6	0/10	+
08-1274/22	Breeder 8[a]	600	Chicken	16	9/10 (90%)	31.8	0/9	+
08-1274/25	Reproducer	407	Chicken	43	0/10	–	–	

[a] Breeders linked to the same supplier of 1-day-old chickens, i.e. 08-1274/25.

Table 2
Primers used for sequencing.

Targeted sequence	Primer name	Sequence[a] (5'-3')	PCR product size	References
16S rRNA	16S1 rp2	CGGATCCTGAGAATTTGATC CTACCTTGTTACGACTTCAT	1200 bp	Pudjatmoko et al. (1997) Thomas et al. (2006)*
ompA	191CHOMP CHOMP371	GCIYTITGGGARTGYGGITGYGCIAC TTAGAAICKGAATTGICCRTTIAYGTGIGCIGC	600 bp	Sachse and Hotzel (2003)

[a] Degenerate nucleotides: K=G, T; M=A, C; W=A, T; Y=C, T; I=Inosine.

tubes and centrifuged at 10,000 rpm for 5 min. Supernatant was transferred into a new sterile tube. Antibiotic solution containing 0.1 mg of vancomycin, 0.1 mg of streptomycin, 0.1 mg of kanamycin and 100 U of nystatin was added to the supernatant and the pellet suspensions, which were then incubated at 37 °C for 2 h before inoculation.

Yolk sacs of 7-day-old embryonated eggs were inoculated with 0.2 ml per egg, and five eggs per sample. For each set of inoculation, three eggs were inoculated with *C. psittaci* strain Loth as positive control, and three other eggs were kept separately as non-infected controls. Eggs were incubated at 38 °C and observed daily.

Vitellus membranes were collected and analyzed using a MIF test, i.e. a direct immunofluorescence assay (Chlamydia direct IF, BioMérieux, Marcy l'Etoile, France). One of the two monoclonal antibodies contained in this kit allowed the identification of *Chlamydiaceae* spp.

2.6. Cell culture

Chlamydial isolates were grown in Buffalo Green Monkey (BGM) kidney cells as previously described (Sachse et al., 2003). Three days after inoculation, coverslips were fixed with methanol, and the monolayer was stained using the IMAGEN™ Chlamydia kit (DAKO Ltd., Cambridgeshire, UK). For confocal laser scanning microscopy using a TCS-SP2 confocal microscope (Leica, Bensheim, Germany), the coverslips were stained with Evans Blue, FITC-labeled anti-Chlamydia antibody (Oxoid, Cambridgeshire, UK) and DAPI (Sigma, Taufkirchen, Germany).

2.7. DNA-based characterization

2.7.1. DNA microarray testing

The ArrayTube™ (AT) assay was used as described (Borel et al., 2008) to check samples for the presence of DNA from any of the currently accepted nine species of *Chlamydia* and *Chlamydophila*, as well as *Waddlia chondrophila* and *Simkania negevensis*. Briefly, DNA template was amplified and biotin labeled in 40 cycles of 94 °C/30 s, 55 °C/30 s, and 72 °C/30 s, using primers U23F-19 (5'-ATTGAMAGGCGAWGAAGGA-3') and 23R-22 (5'-biotin-GCYTAC-TAAGATGTTTCAGTTC-3'). Hybridization was conducted in the AT vessel at 58 °C for 1 h. Subsequently, the array was washed, and hybridization patterns were visualized using streptavidin-conjugated peroxidase-catalyzed precipitation and processed using the ATR-01 reader (Clondiag Chip Technologies, Jena, Germany) and the Iconoclust 2.3 program (Clondiag).

2.7.2. Multiple locus VNTR analysis

Samples were examined using the recently published MLVA procedure (Laroucau et al., 2008a).

2.7.3. Amplifications for DNA sequencing

Primers used for the partial amplification of *ompA* and 16S rRNA genes are listed in Table 2. PCR was performed in a total volume of 50 µl containing 4 µl of DNA template, 5 µl of 10× PCR reaction buffer, 2 U of Hot start *Taq* DNA polymerase (Qiagen), 400 µM of each deoxynucleotide triphosphate, and 0.5 µM of each flanking primer. The temperature–time profile for amplification of the 16S rRNA gene was 95 °C for 10 min, 4 cycles of 95 °C for 1 min, 40 °C for 1 min, 72 °C for 2 min and 36 cycles of 95 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min. The final extension step was at 72 °C for 10 min.

2.7.4. Genotyping using restriction fragment length polymorphism (RFLP) of ompA amplicons

Aliquots of 10 µl of PCR product (primers 191CHOMP/CHOMP371, from Sachse and Hotzel, 2003) were subjected to overnight restriction enzyme digestion using 1 U of *AluI* (Euromedex, Souffelweyersheim, France) in a total volume of 15 µl at 37 °C. The digestion products were then loaded onto a 4% gel (50% Nusieve–50% Metaphore) containing ethidium bromide and analyzed using a UV transilluminator.

2.7.5. DNA sequencing

PCR-amplified segments of *ompA* and 16S rRNA genes were purified using the QIAquick PCR Purification Kit (Qiagen). DNA sequencing was done at MWG Biotech France (Roissy, France). Nucleotide sequences have been deposited under GenBank no. GQ398026 to GQ398031 for 16S rRNA sequences and under GenBank no. GQ398032 to GQ398038 for *ompA* sequences.

2.7.6. Sequence analysis

All 16S rRNA and *ompA* gene sequences determined in this study were combined in an alignment with previously published sequences that were relevant for phylogenetic and epidemiological considerations. Accession numbers of these sequences are listed in Table 3. Sequence data were analyzed using the Bionumerics software package version 4.6 (Applied-Maths, Saint-Martens-Latem, Belgium) as a character dataset. Cluster analysis was conducted using the categorical parameter and the UPGMA coefficient.

3. Results

3.1. Examination of samples from infected flocks

In preliminary examinations following diagnosis of atypical pneumonia in humans, two working-surface area samples from the slaughterhouse where those workers were employed, 20 swabs collected from chickens and guinea fowl belonging to the flock of the slaughterhouse owner and two fecal samples from the same flock were tested by real-time PCR. The results given in Table 4 revealed the presence of *Chlamydiaceae* in the birds as well as in the slaughterhouse itself. Quite surprisingly, none of the samples was positive for *C. psittaci*.

Subsequently, samples were collected from 16 poultry flocks, all of which were regular suppliers to this slaughterhouse. The prevailing bird species were chickens of the "Barbezieux" and "Coucou" breeds, but guinea fowl (three flocks) and ducks (one flock) were also included. In addition, samples were taken from 8 flocks belonging to the slaughterhouse owner and the flock of the egg supplier of five breeders raising "Barbezieux" chickens (Breeders no. 3, 5, 6, 7 and 8). Essential data and testing results are given in Table 1. In real-time PCR examinations, *Chlamydiaceae* were detected in 14 flocks. Comparison of Ct values indicated different

Table 3
BLAST analysis of partial *ompA* sequences (about 480 nt) of samples from flocks 08-1274/3, 08-1274/9, 08-1274/13, 08-1274/19, 08-1274/21 and 08-1274/22.

Origin of strain	*ompA* partial fragment size (nt)	GenBank acc. no. of partial *ompA* sequence	Highest similarity to (GenBank acc. no.)	Total BLAST score	Query coverage	*E* value	Max identity
08-1274/3	480 bp	GQ398033	*Chlamydophila* sp. 6617-T5 (EU019096)	479	60%	6.00E−132	96%
			Chlamydophila sp. 6620-T4 (EU019095)	479	60%	6.00E−132	96%
			Chlamydophila sp. 6688-T2 (EU019094)	440	62%	3.00E−120	93%
08-1274/9 (swab)	489 bp	GQ398034	*Chlamydophila* sp. 6617-T5 (EU019096)	695	77%	0	99%
			Chlamydophila sp. 6620-T4 (EU019095)	686	76%	0	99%
			Chlamydophila sp. 6688-T2 (EU019094)	455	60%	1.00E−124	94%
			Chlamydophila sp. PEENT (U82955)	324	59%	3.00E−85	86%
08-1274/13 & 08-1274/23	480 bp	GQ398035, GQ398032	*Chlamydophila* sp. 6617-T5 (EU019096)	484	60%	1.00E−133	96%
			Chlamydophila sp. 6620-T4 (EU019095)	484	60%	1.00E−133	96%
			Chlamydophila sp. 6688-T2 (EU019094)	457	62%	3.00E−125	94%
08-1274/19	486 bp	GQ398036	*Chlamydophila felis* MOMP gene for major outer membrane protein (X61096)	182	30%	2.00E−42	89%
08-1274/21	489 bp	GQ398037	*Chlamydophila* sp. 6617-T5 (EU019096)	363	76%	6.00E−97	84%
			Chlamydophila sp. 6620-T4 (EU019095)	359	76%	8.00E−96	84%
			Chlamydophila sp. PEENT (U82955)	353	61%	4.00E−94	88%
			Chlamydophila sp. 6688-T2 (EU019094)	348	75%	2.00E−92	84%
08-1274/22	483 bp	GQ398038	*Chlamydophila* sp. 6688-T2 (EU019094)	320	74%	4.00E−84	82%
			Chlamydophila felis MOMP gene for major outer membrane protein (X61096)	204	72%	4.00E−49	77%

Table 4
Summary of preliminary diagnostic testing.

1st investigation	No. of analyzed samples	23S-rtPCR *Chlamydiaceae* No. of positive samples	*ompA*-rtPCR *C. psittaci* no. of positive samples
In the slaughterhouse	2	1/2	0/1
In the personal flock of the owner (chickens and guineafowl)			
Cloacal swabs	20	5/20	0/5
Fecal samples	2	1/2	0/1

levels of chlamydial excretion, with birds from flocks no. 3, 13, 19, 21, 22, and 23 being identified as high excretors and with almost 100% of animals testing positive. Notably, flock 08-1274/25, which was linked with flocks 08-1274/13, 08-1274/16, 08-1274/18, 08-1274/19, 08-1274/20, 08-1274/21, 08-1274/22, 08-1274/23 and 08-1274/24 for being their exclusive supplier of 1-day-old chicks, proved negative when tested.

Again, *C. psittaci* was not the predominant chlamydial agent, since only a single sample (from flock no. 08-1274/17, the only investigated duck flock) was positive in *C. psittaci*-specific real-time PCR and, subsequently, also in MLVA. The *C. psittaci*-specific MLVA genotype of the duck sample was 2:3:2:0:6:3:0:4 using primers ChlaPsi_280, ChlaPsi_480, ChlaPsi_605, ChlaPsi_810, ChlaPsi_222, ChlaPsi_281, ChlaPsi_929 and ChlaPsi_1778, respectively (data not shown).

3.2. Direct genotyping of clinical samples

While PCR using the classical *ompA* primers CTU/CTL (Denamur et al., 1991) failed to produce amplicons from positive non-*C. psittaci* samples (data not shown), the use of degenerate primers 191CHOMP/CHOMP371 enabled further characterization by RFLP. DNA extracts from real-time PCR-positive (Ct < 34) cloacal swabs were subjected to PCR and digested with *AluI*. As shown in Fig. 1, all restriction patterns within a flock were identical, but clearly differed among flocks 08-1274/3, 08-1274/9, 08-1274/13, 08-1274/21, 08-1274/22, and 08-1274/19. As the only exception, patterns from flocks 08-1274/3 and 08-1274/4 were identical. These 2 flocks belonged to the same breeder (i.e. the owner of the slaughterhouse, see Table 1). The same was observed for flocks 08-1274/13 and 08-1274/23, which also belonged to the same breeder (Breeder no. 3).

3.3. DNA-based characterization of isolated strains

In cell culture trials, duplicates of the PCR-positive dry swabs, which had been stored in SPG medium, were inoculated into embryonated chicken eggs. Isolates were successfully cultured from flocks 08-1274/3, 08-1274/13, 08-1274/19, 08-1274/21, 08-1274/22 and 08-1274/23 (Table 1). The same strains also grew well

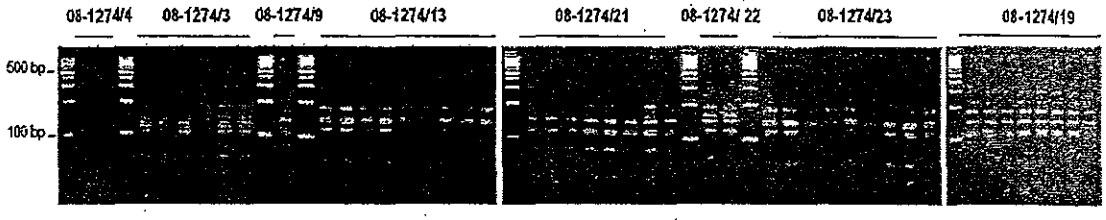

Fig. 1. Genotyping of cloacal swab samples by RFLP. *AluI* enzyme restriction profiles of partial *ompA* PCR products from clinical samples of 8 flocks. The following samples from the respective flocks were examined: 08-1274/4 (2 birds), 08-1274/3 (6 birds), 08-1274/9 (1 bird), 08-1274/13 (9 birds), 08-1274/21 (8 birds), 08-1274/22 (2 birds), 08-1274/23 (9 birds) and 08-1274/19 (8 birds). DNA size marker (GeneRuler™ 100 bp, Euromedex, France) was loaded between each flock. Fragment sizes (in bp) are given on the left-hand margin.

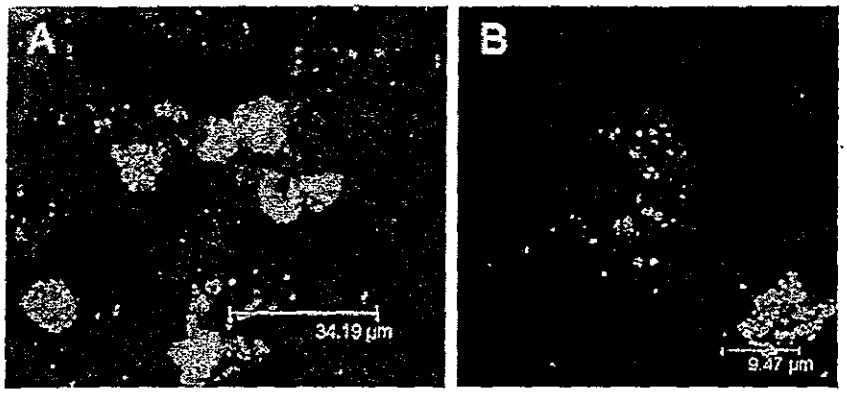

Fig. 2. Confocal laser scanning microscopic images of cell culture of isolate 08-1274/3. The chlamydial agent was grown in BGM cells, fixed with methanol on coverslips, and the monolayer was stained using Evans Blue for BGM cells (red color), FITC-labeled anti-Chlamydia antibody for chlamydial bodies (green, A) and DAPI for BGM cell nuclei (blue, A). Yellow: co-localization of chlamydial inclusions and cellular Evans Blue (A), FITC-labeled anti-Chlamydia antibody for chlamydial bodies (B). Scale bars are given on each image. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

in cell culture of BGM cells, where intracellular inclusions reminiscent of other chlamydial agents were observed (Fig. 2). DNA extracts from these cultures were examined by DNA microarray analysis using the AT test. Fig. 3 shows a typical hybridization pattern, where only the genus-specific probes generated specific positive signals, whereas species-specific signals were absent. These results indicated that DNA of any of the 9 established species of *Chlamydiaceae* was not present in the samples.

To establish the identity of the strains encountered in the poultry flocks, partial sequencing of the ompA gene was conducted for 6 isolates (from flocks 08-1274/3, 08-1274/13, 08-1274/19, 08-1274/21 08-1274/22 and 08-1274/23) and one swab sample from flock 08-1274/9. BLAST analysis shown in Table 3 revealed that *ompA* sequences of samples from flocks 08-1274/3, 08-1274/9 and 08-1274/13 exhibited the highest degree of similarity to a group of sequences from an outbreak of psittacosis in Germany (EU019094–EU019096), which had been tentatively classified as *Chlamydophila* spp. because they could not be assigned to any of the currently defined species (Gaede et al., 2008). Furthermore, *ompA* sequences of isolates from flocks 08-1274/9 and 08-1274/21 were found to have moderate similarity to another non-classified strain of *Chlamydophila* spp. (U82955) isolated from a peacock. The isolates from flocks 08-1274/19 and 08-1274/22 displayed moderate similarity to the *C. felis ompA* sequence and to one of the group of sequences from an outbreak of psittacosis in Germany, respectively. Sequences from isolates 08-1274/13 and 08-1274/23 (same breeder) were identical.

To explore the genetic relatedness of the strains from the present study, all sequences mentioned in Table 3 were aligned

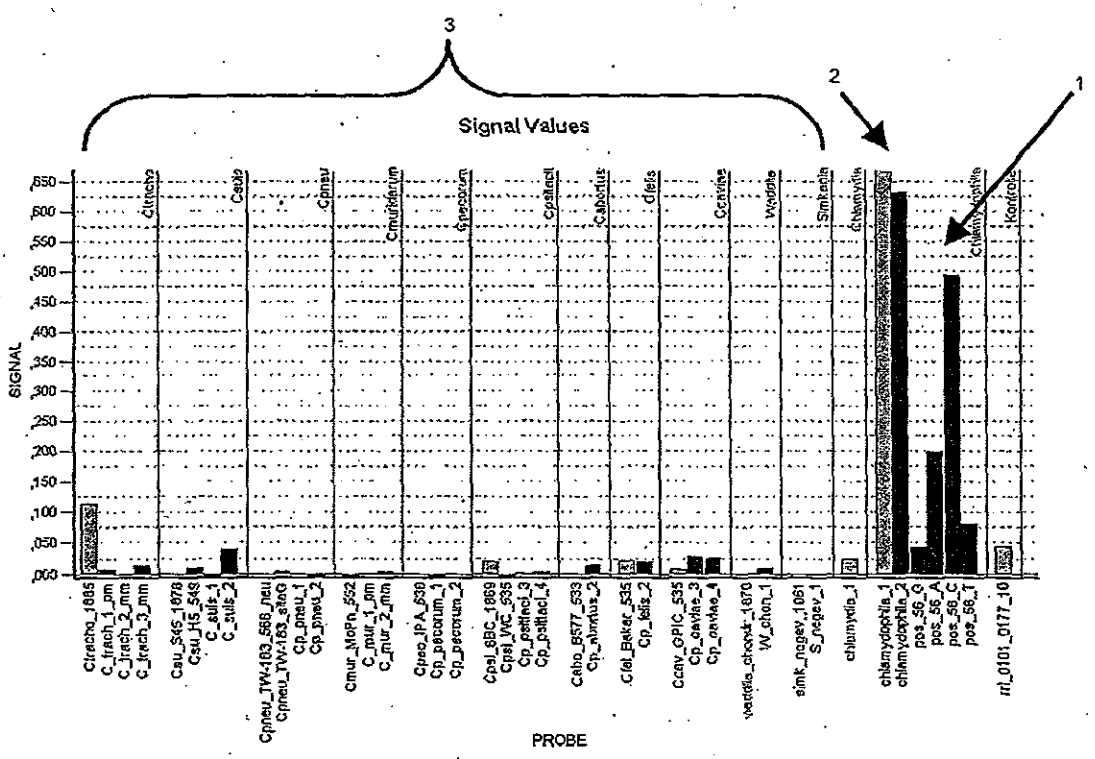

Fig. 3. Hybridization pattern obtained from examination of isolate 08-1274/3 using the ArrayTube DNA microarray assay. Barplot of hybridization signals; 1 consensus probe (family *Chlamydiaceae*), 2 genus-specific probes (*Chlamydophila*), 3 probes specific for the currently defined nine species of *Chlamydiaceae*.

with *ompA* sequences of strains representing the established species of *Chlamydia* and *Chlamydophila*. The dendrogram shown in Supplement 1 indicates that the strains described here form a separate cluster situated at the margin of the genus *Chlamydophila*.

As the *ompA* gene generally is distinguished by high intra-species diversity among chlamydiae, analysis of the more conserved 16S rRNA gene sequences was conducted to obtain alternative information on the identity and characterize the

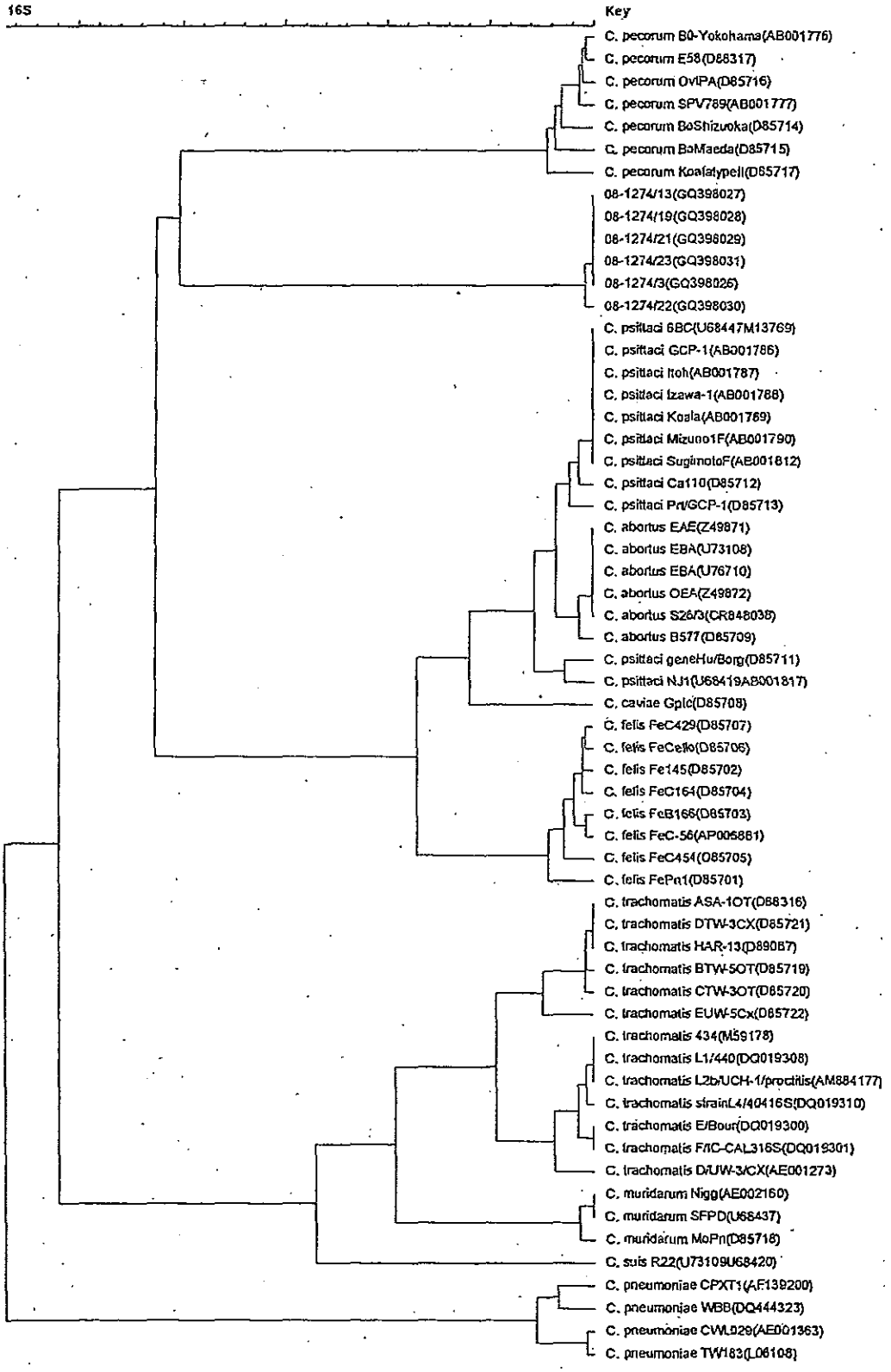

Fig. 4. Dendrogram for *Chlamydiaceae* based on a partial sequence analysis 16S rRNA genes. The tree was constructed by the neighbour-joining method from phylogenetic distances calculated by UPGMA method. Horizontal distances correspond to genetic distances, vertical distances are arbitrary.

taxonomic position of the present isolates. Segments of 1436 nt representing nearly the entire gene were sequenced from samples from the five strains and one clinical sample mentioned in Table 4. All sequences obtained were identical except for a single point mutation in 08-1274/22. Alignment with representative sequences of *Chlamydiaceae* confirmed the separate position of the present strains outside the existing species of *Chlamydophila*, but clearly within the genus. The corresponding dendrogram is shown in Fig. 4. Sequence similarity values given in Supplement 2 also clearly show the distinct genetic position of the present strains.

4. Discussion

So far, very few studies on avian chlamydiosis in chickens have been conducted in France and elsewhere. Veterinary investigations are usually undertaken when transmission to humans is suspected. To obtain more information, an epidemiological study on psittacosis involving 15 French administrative units is currently underway (http://www.invs.sante.fr/surveillance/psittacose/default.htm), whose aim is the determination of the incidence of hospitalized human cases, as well as the frequency of grouped cases, and risk assessment for exposed individuals. Additionally, the analysis of strains isolated from humans and animals and the description of breeding characteristics and working conditions should improve the knowledge on risk factors for animal-to-human transmission. Up to now, most of the recently confirmed cases of *C. psittaci* infection have been associated with ducks or exotic birds (Laroucau et al., 2008b, and unpublished data).

The present survey was prompted by the occurrence of hitherto unexplained atypical pneumonia in three French slaughterhouse workers in 2008. This poultry slaughterhouse had originally not been included in the national study mentioned above, but a veterinary survey was started as symptoms of the workers were reminiscent of psittacosis, without any microbiological confirmation.

Although no clinical signs were seen in the birds, diagnostic testing revealed the presence of *Chlamydiaceae* in most of the poultry flocks investigated, and some of the flocks were identified as high excretors. The levels of excretion were similar to those previously observed in *C. psittaci*-infected duck flocks, some of them associated with human infections (Laroucau et al., 2009). Rather unexpectedly, only one out of 73 *Chlamydiaceae*-positive samples of the present panel proved positive in *C. psittaci*-specific real-time PCR. When genotyping of the chlamydial strains involved was attempted, *C. psittaci*-specific VNTR primers failed to generate patterns characteristic for this species (except for the positive sample). Subsequently, the AT test revealed aberrant hybridization patterns, i.e. signals of the genus-specific probes for *Chlamydophila*, but the absence of grouped species-specific signals (Fig. 3). This combined evidence suggested that we were probably dealing with a novel chlamydial agent.

Analysis by RFLP of *ompA* gene segments directly amplified from the most high-titer real-time PCR-positive samples indicated the presence of a single strain within each investigated flock, but also revealed that the strains were different from each other. Thus, 6 isolates were obtained from 6 different flocks raising "barbezieux" or "cou nu" breeds. Two of them, which were isolated at the same farm, proved identical based on their partial *ompA* gene sequences (flocks 08-1274/13 and 08-1274/23). Partial sequencing of the 16S rRNA and *ompA* genes revealed that, while 16S rRNA gene sequences were highly similar among the isolates, the *ompA* sequences were distinguished by high inter-strain heterogeneity. This confirms observations by Everett et al. (1999), who pointed out that rRNA genes were subjected to evolutionary pressure to a far lesser extent than genes encoding outer membrane proteins, such as *ompA*. The same authors recommended that, in order to be classified as a member of *Chlamydiaceae*, a taxon should have less than 10% 16S rRNA gene diversity to any other member of the family. This condition is fulfilled for the isolates described in this study. Furthermore, comparison of the 16S rRNA sequences with those of the established species of *Chlamydiaceae* showed that the present avian strains formed a separate cluster within the genus *Chlamydophila* (Fig. 4). While the evidence gathered so far indicates that the 6 isolates belong to a new species, the authors are aware that more DNA sequence data, as well as morphological and other phenotypic data, are required to justify the definition of a new taxon. To address the epidemiological importance, we will further pursue the question whether these new microorganisms are occurring in other regions and countries. In any case, the present idea of taxonomic classification is still preliminary.

The present investigations were conducted in a limited geographical area, which involved, among others, a small poultry production unit dedicated to the barbezieux chicken breed. This chain involved a unique breeder (parental) flock which supplied 5 other breeders (Breeders no. 3, 5, 6, 7 and 8) dealing with fattening. The birds were all slaughtered in the slaughterhouse concerned. The first surprise was that one of the five breeders (Breeder no. 6) was not affected by these new *Chlamydophila* bacteria (Table 1). Six months later, follow-up sampling was done in another flock of this breeder, and, again, no *Chlamydiaceae* were detected in 10 animals randomly selected and examined 5 times in 16 weeks (at 3, 6, 8, 12 and 16 weeks of age, data not shown). Another surprise was the finding that the four farms having infected flocks harbored different strains, as shown by partial *ompA* gene sequencing, and that the strains were apparently associated to an individual farm. Indeed, sampling another flock of Breeder no. 5 confirmed the presence of one unique strain per farm based on partial *ompA* gene sequences (data not shown). In these circumstances, the source of infection remains unclear, but vertical transmission can be ruled out. It should be noted that the new *Chlamydophila* strains have also been detected in the "cou nu" breed of chicken (flocks 08-1274/3 and 08-1274/9). Interestingly, no *Chlamydiaceae* were found in guinea fowl ($n = 60$), although these birds were mixed with proven positive chickens (flocks 08-1274/2, 08-1274/3 and 08-1274/4).

Notably, no clinical signs were observed in animals of the respective flocks. Autopsy conducted on five birds in flocks 08-1274/21 and 08-1274/23, did not reveal any macroscopic lesions, despite the fact that PCR examination of spleen, liver, lung, and intestinal tissue samples demonstrated an intensive and dominant colonization of intestinal tissue with the new *Chlamydiaceae* spp. in comparison to the other organs that tested weakly positive (data not shown). This is in agreement with the observation of Gaede et al. (2008) that genetically related non-classified *Chlamydophila* spp. were found in symptomless chickens during an outbreak of clinical psittacosis.

The etiological importance of these new chlamydial isolates for human pneumonia has yet to be defined. So far, there has been no hard evidence of the strains being responsible for the three reported human cases of atypical pneumonia. Although clinical signs were reminiscent of psittacosis, i.e. high fever and pulmonary invasion, they cannot be regarded as specific. The three affected individuals were successfully treated with macrolide antibiotics (see Section 2). A possible approach to the assessment of the pathogenicity of the present strains could include experimental challenge trials in mice based on the protocol of Rodolakis et al. (1989).

Finally, it should be emphasized that detection of the presumed new members of *Chlamydiaceae* became possible because of the use of advanced yet complementary DNA-based diagnostic methods, i.e. real-time PCR in conjunction with the AT test. This combination, which was already suggested as a reference standard

(Sachse et al., 2009), can be further recommended for the laboratory diagnosis of animal and human chlamydial infections.

Conflict of interest statement

None of the authors (K. Laroucau, F. Vorimore, R. Aaziz, A. Berndt, E. Schubert, K. Sachse) has a financial or personal relationship with other people or organisations that could inappropriately influence or bias this paper.

Acknowledgments

We are grateful to Christine Grajetzki, Simone Bettermann, and Sabine Scharf who provided excellent technical assistance. We also thank Muriel Enique, Jean-Marie Gobin, Jean-Marie Arsicaud, Jérome Berthelot, David Leservoisier, Gilbert Marchand, Jacky Renaudin and the regional veterinary service for their precious help in flocks investigations and to Dr Loic Masson for his valuable help.

Part of the work conducted at Friedrich-Loeffler-Institut, Jena was funded by grant 01 KI 0720 "Zoonotic chlamydiae" from the Federal Ministry of Education and Research of Germany.

The study was an integral part of the European COST Action 855 "Animal chlamydioses and the zoonotic implications".

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2009.08.005.

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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. 12. 20	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		公表国
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)		Satake M, Mitani T, Oikawa S, Nagumo H, Sugiura S, Tateyama H, Awakihara S, Mitsutomi Y, Muraoka M, Tadokoro K. Transfusion. 2009 Oct;49(10):2152-7.	日本

研究報告の概要

○日本における初流血除去導入前後の血小板濃厚液の細菌汚染頻度

背景:血小板濃厚液(PC)の細菌汚染は、輸血医学における重大な感染リスクとなっている。細菌汚染を低減させる新しい戦略実施の必要性を評価するために、PCの正確な汚染頻度を明らかにすることが必要である。

試験デザインおよび方法:日本赤十字社が供給するPCの細菌汚染頻度を、初流血除去実施前後の有効期限切れPCを用いて調べた。偽陰性結果が可能な限り最少となる培養法をデザインした:保存から4日以上後に血小板検体をサンプリングし、好気性ボトルと嫌気性ボトルの双方に10mLの量を接種した。

結果:初流血除去実施前では、培養したPC21,786検体のうち36(0.17%)に細菌汚染があったことが確認された。実施後の汚染PC数は、21,783中11(0.05%)に減少し(減少率71%)、臨床的に重大な細菌の汚染の件数は、*Propionibacterium acnes*陽性PCを除き4件(0.018%)であった。献血者の血液に由来すると推定される細菌の汚染頻度は減少しなかった。

結論:初流血除去は細菌汚染頻度に確かな影響を及ぼす。西欧諸国と同等の細菌汚染頻度であり、培養スクリーニング検査が実施されていないにもかかわらず、日本でPC輸血後の敗血症反応発現率が低いことは、PCの保存期間が短い(72時間)ことが重要であると示唆される。

報告企業の意見	今後の対応
日本における献血血液の初流血除去導入についての評価報告である。血小板濃厚液の細菌汚染頻度は、初流血除去導入前後で0.17%から0.05%に減少し、臨床的に重大な細菌の汚染の件数は4件(0.018%)であった。培養スクリーニング検査が実施されていないにもかかわらず、輸血後の敗血症発現率が低いことは、PCの保存期間が短いことが重要であると示唆されたとの日本赤十字社の安全対策に関する報告である。	日本赤十字社では、輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に保存前白血球除去及び初流血除去を導入している。さらに、輸血情報リーフレット等により細菌感染やウイルス感染について医療機関へ情報提供し注意を喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

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

二

Frequency of bacterial contamination of platelet concentrates before and after introduction of diversion method in Japan

Masahiro Satake, Takako Mitani, Shinji Oikawa, Hideto Nagumo, Sayoko Sugiura, Hidemi Tateyama, Syuji Awakihara, Yoshiro Mitsutomi, Masato Muraoka, and Kenji Tadokoro

BACKGROUND: Bacterial contamination of platelet concentrates (PCs) is the major infectious risk in transfusion medicine. To evaluate the necessity of implementing novel strategies for the reduction of bacterial contamination, it is necessary to establish a precise contamination frequency in PCs.

STUDY DESIGN AND METHODS: The frequency of bacterial contamination in PCs issued by the Japanese Red Cross was determined using expired PCs before and after the implementation of the diversion method. The culture method was designed such that it yields the least possibility of false-negative results: platelet specimens were sampled after at least 4 days of storage and the inoculum volume was 10 mL for both aerobic and anaerobic bottle cultures.

RESULTS: Of the 21,786 PCs cultured, 36 (0.17%) were confirmed to be bacterially contaminated before the implementation of the diversion method. After its implementation, the number of contaminated PCs decreased to 11 of 21,783 (0.05%) with a reduction rate of 71% and the number of contaminations of clinical importance was 4 (0.018%) excluding PCs positive for *Propionibacterium acnes*. The frequency of contamination by bacteria presumed to originate from donors' blood did not decrease.

CONCLUSION: The effect of the diversion method on the frequency of bacterial contamination is robust. The low incidence of septic reactions after PC transfusion in Japan in spite of the contamination frequency being comparable to those in Western countries and the non-institution of culture screening suggests the importance of a short shelf life (72 hr) for PCs introduced in Japan.

Bacterial contamination of blood components is the major residual infectious risk in modern transfusion medicine in developed countries. The transfusion of blood components with clinically relevant bacterial species at certain concentrations can lead to sepsis or a fatal outcome in transfusion recipients. This is particularly true for platelet concentrate (PC) products that are stored at 20 to 24°C. It is generally accepted that the frequency of bacterial contamination in PCs is approximately 1 in 3000.[1,2] To prevent transfusion-mediated septic reactions, several preventive measures have been proposed or implemented in each step from blood drawing to bedside practice of transfusion, namely, the improvement of the skin disinfection procedure,[3-5] use of a diversion pouch,[6-8] screening by culture for bacteria,[9-14] pH or glucose measurement, screening by amplification of a bacterial genome sequence,[15-17] and use of pathogen reduction/inactivation technologies.[18]

In Japan, all PC products are obtained using the apheresis system and the expiry time for PCs has been limited

ABBREVIATIONS: JRC = Japanese Red Cross; PC(s) = platelet concentrate(s).

From the Japanese Red Cross Tokyo Metropolitan West Blood Center and the Japanese Red Cross Tokyo Metropolitan Blood Center, Tokyo; the Japanese Red Cross Hokkaido Blood Center, Hokkaido; the Japanese Red Cross Miyagi Blood Center, Miyagi; the Japanese Red Cross Aichi Blood Center, Aichi; the Japanese Red Cross Osaka Blood Center, Osaka; the Japanese Red Cross Okayama Blood Center, Okayama; the Japanese Red Cross Kyushu Blood Center, Fukuoka; and the Japanese Red Cross Blood Service Headquarters, Tokyo, Japan.

Address correspondence to: Masahiro Satake, Japanese Red Cross Tokyo Metropolitan West Blood Center, Midori-cho 3256, Tachikawa, Tokyo 190-0014, Japan; e-mail: ma-satake@tokyo.bc.jrc.or.jp.

Received for publication January 22, 2009; revision received March 18, 2009, and accepted March 31, 2009.

doi: 10.1111/j.1537-2995.2009.02243.x

TRANSFUSION 2009;49:2152-2157.

to 72 hours, which has undoubtedly contributed to the relatively infrequent occurrence of sepsis after PC transfusion. Over the past 8 years, however, two cases of septic reactions, including one fatal case, after PC transfusion have been confirmed by the Japanese Red Cross (JRC) blood center, and the necessity for the implementation of novel strategies for reducing bacterial contamination has been discussed. To this end, it is essential to establish a precise frequency of bacterial contamination in PCs processed under current regulations for blood procurement and processing. JRC systematically cultured more than 20,000 expired PCs using the culture conditions that were expected to provide the lowest possibility of false-negative results. JRC implemented the diversion method in October 2006 in the blood drawing process for PCs and reevaluated thereafter the bacterial contamination frequency in 20,000 expired PCs. In this article, we report the bacterial contamination frequency in PCs before and after the implementation of the diversion method and discuss the possible origin of bacteria detected in PCs obtained from otherwise healthy blood donors in Japan.

MATERIALS AND METHODS

Blood collection

In Japan, six types of PC categorized in accordance with the number of platelets (PLTs) contained have been approved, namely, Units 1, 2, 5, 10, 15, and 20. Unit 5 contains 1.0×10^{11} to 2.0×10^{11} PLTs and the number of PLTs contained in other units is in proportion to the unit number. Units 1 and 2 that are specifically used for infants or neonates, representing only 0.2% of all PCs, have been obtained from whole blood using the buffy coat method. All other units have been procured using the apheresis systems of CCS (Haemonetics, Inc., Tokyo, Japan), Terusys (Terumo, Inc., Tokyo, Japan), and Trima Accel (BCT Japan, Inc., Tokyo, Japan). In 2006, splitting of a larger unit was introduced for processing Units 1 and 2, and all PCs in Japan are now produced using the apheresis system. Unit 10 is the most frequently used, which contains 2.0×10^{11} to 3.0×10^{11} PLTs, representing approximately 80% of all PCs used in Japan.

For skin preparation for venipuncture, the donor's cubital fossa is cleansed by two courses of scrub with an isopropyl alcohol–containing cotton swab (One Shot plus, Hakujuji, Inc., Tokyo, Japan). Povidone-iodine alcohol (Isodine field solution, Meiji, Inc., Tokyo, Japan) is next applied on the area using a cotton-tipped applicator (Sterile Cotton Buds, Kawamoto, Inc., Osaka, Japan) in concentric circles away from the puncture site. After a minimum of 30 seconds of air drying, the donor's vein is punctured and blood is drawn. The diversion method was implemented in PC collection in October 2006 and in whole blood collection early in 2007. Twenty-five milliliters of initial flow of whole blood is collected in the integrated diversion pouch and the diverted blood is used for testing and blood archive registry.

Culture of expired PCs

There are 39 JRC blood centers all over Japan that process and distribute PCs. Among them, seven leading blood centers have laboratories with an automatic blood culture system, BacT/ALERT (Sysmex-bioMérieux, Tokyo, Japan). All PCs expiring in local blood centers after 72 hours of storage were further stored until Day 4 or 5 at 15 to 25°C and then sent to one of the leading laboratories. The PCs were maintained at 2 to 6°C during the transportation. The laboratories started PC culture between Day 4 and Day 9 but mostly did so on Day 5, 6, or 7. Common procedures were determined among the laboratories for sampling, inoculation, culturing, and retesting and were strictly followed by trained staff members; a PLT solution of more than 20 mL was drawn from the PC bag, of which 10 mL was inoculated into an anaerobic culture bottle and the remaining 10 mL into an aerobic bottle (BPN and BPA bottles, respectively, Sysmex-bioMérieux). All the procedures were conducted under aseptic conditions in a laminar air flow hood. The inoculated bottles were kept at 35°C in the BacT/ALERT system, and culture was continued until a positive signal was flagged or continued for 7 days in the absence of a positive signal. When a positive signal was flagged, culture was repeated using the original PC and frozen plasma obtained from the same donation. To confirm the bacterial species, bacteria-positive culture bottles were sent to the central laboratory of Tokyo Red Cross Blood Center and Tokyo Metropolitan Institute of Public Health.

Statistical analysis

The chi-square test was used to compare the bacterial contamination frequency of PCs procured before and after the implementation of the diversion method.

RESULTS

Frequency of bacterial contamination in PCs

During the period from May 2005 through April 2006 before the implementation of the diversion method, 21,786 expired PCs were cultured for bacterial examination. Culture started on Day 4, 5, 6, or 7 for 0.4, 20, 19, and 21% of PCs studied, respectively. There were 57 initial positive cultures, 10 of which were determined to have been caused by an inappropriate positive signal by the culture machine (Table 1). Of the remaining 47, 11 were defined as false-positive cultures on the basis of the negative result of the reculture of the aliquot from the original PC (Table 2). The number of confirmatory positive results

TABLE 1. Frequency of bacterial contamination in PCs collected with or without diversion method*

Variable	Without diversion	With diversion	Reduction (%) by diversion
Number of cultures	21,786	21,783	
Initially positive	57 (0.26)	23 (0.11)	
Machine failure	10	1	
False positive	11	11	
Confirmatory positive	36† (0.17)	11 (0.050)	71 (p = 0.0003)
Anaerobic only‡ (*P. acnes*)	24 (0.11)	7 (0.032)	71 (p = 0.004)
Aerobic and anaerobic§	13 (0.060)	4 (0.018)	70 (p = 0.052)

* Data are reported as number (%) unless otherwise specified.
† One culture was doubly contaminated by *P. acnes* and *Staphylococcus* sp.
‡ All the cultures that were anaerobic bottle positive and aerobic bottle negative were identified to be contaminated by *P. acnes*.
§ All the cultures positive for bacteria using aerobic culture bottle were identified to be also positive using anaerobic culture bottle.

TABLE 2. Bacterial species determined as false positive by repeat negative culture

Without diversion (*n* = 11)	With diversion (*n* = 11)
P. acnes (7)*	*P. acnes* (8)†
Bacillus sp. (2)	*Bacillus* sp. (2)
Brevibacillus choshinensis (1)	*Bacillus circulans* (1)
Staphylococcus saccharolyticus (1)	

* The number of cultures initially positive for *P. acnes* was 31.
† The number of cultures initially positive for *P. acnes* was 15.

was 36, representing 0.17% of all PCs cultured. Of these 36, 24 (0.11%) were identified only by anaerobic bottle culture and the bacterial species were all determined to be *Propionibacterium acnes* (Table 1). Thirteen (0.060%) were identified both by aerobic and anaerobic bottle culture. One PC was cocontaminated by *Staphylococcus* sp. and *P. acnes*. Eight non-*P. acnes* bacterial species were considered to be derived from the donors' skin and three from the donors' peripheral blood (Table 3). The remaining two PCs were contaminated by *Staphylococcus aureus* and could be derived either from transient skin flora or the donors' blood stream. *P. acnes* was initially detected in 31 PCs but was not detected in 7 PCs by repeated culture (Tables 2 and 3).

Two months after the introduction of the diversion method, when the staff in every donation site became proficient with the procedure, the culture study of expired PCs restarted in December 2006 and ended with the culture of 21,783 PCs in March 2008. Culture started on Day 4, 5, 6, or 7 for 0, 19, 21, and 24% of PCs studied, respectively. There was no significant difference in the date of start of culture between before and after diversion method. Table 1 shows the results of the study. Twenty-three were initially positive, of which one was caused by machine failure as described previously, 11 were false positive, and 11 were determined as confirmatory positive, representing 0.050% of all cultured PCs. Seven (0.032%) of the 11 PCs were found to be contaminated by *P. acnes* only by anaerobic bottle culture. The remaining four PCs were found to be contaminated by other species both by aerobic and by anaerobic bottle culture, indicating that PCs currently released from JRC blood centers after implementation of the diversion method have a 0.018% frequency of contamination by bacteria other than *P. acnes*. One of the four PCs was positive for *Staphylococcus epidermidis* that could be derived from the donors' skin, two were positive for *Streptococcus dysgalactiae* subsp. *equisimilis* and *Escherichia coli* that could be derived from the donors' peripheral blood, and one was positive for *S. aureus* that could be derived from either origin (Table 3). Our culture study of more than 40,000 expired PCs confirmed that the diversion of initial blood flow into the integrated pouch decreased the contamination rate for all bacterial species by 71% (p = 0.0003 by chi-square test, Table 1).

DISCUSSION

The frequency of bacterial contamination in PCs has been recently studied to evaluate the residual risks in the context of the implementation of culture screening. Most of them report the results obtained from routine culture screening conducted as a release test. There are, however, some limitations in those studies regarding the sensitivity of the culture method used, that is, the limited incubation time before sampling and the limited sample volume inoculated into culture bottles. In this study, the culture procedures employed were designed such that the possibility of false-negative results could be as low as possible: the storage period of PCs at 15 to 25°C before sampling was 4 days at the minimum; PCs were stored at a low temperature after 5 days of storage to prevent autolysis of fully grown bacteria; culture was conducted using anaerobic as well as aerobic bottles; the inoculation volume was 10 mL, which is the maximum volume for each culture bottle; and culture was continued for 7 days. Accordingly, the frequencies of bacterial contamination described in this article would be the highest values obtainable using available techniques for bacterial detection with minimal possibility of false-negative results, although possibility remains that false-negative results occur if bacteria die in PCs with storage.

TABLE 3. Confirmed bacterial species detected in PCs other than *P. acnes*

Estimated origin of bacteria	Without diversion	With diversion
Skin flora	*S. epidermidis* (4)*	*S. epidermidis* (1)
	Staphylococcus sp. (1)	
	CNS† (1)	
	S. saccharolylicus (1)	
	Gram(+) bacillus, nonspore (1)	
Transient skin flora or blood	*S. aureus* (2)	*S. aureus* (1)
Peripheral blood	*S. constellatus* (1)	*Streptococcus dysgalactiae* subsp. *equisimilis* (1)
	Salmonella serovar Choleraesuis (1)	*E. coli* (1)
	E. corrodens (1)	

* Number in parentheses is number of cases.
† CNS = coagulase-negative *Staphylococcus*.

Overall, the implementation of the diversion method significantly reduced the frequency of bacterial contamination in PCs by 71% for all bacterial species (Table 1). If the results obtainable using only aerobic culture bottles are considered, the contamination rates without or with the diversion method was 0.06 and 0.018%, respectively, both of which are the values comparable to those reported in Western countries.[1,9,10,13]

In Japan, approximately 700,000 PCs are released and transfused to patients every year. From the calculation based on the data shown in Table 1, it is estimated that as many as 770 PCs contaminated by *P. acnes* had been released every year from blood centers before the diversion method was implemented. Through the JRC hemovigilance, however, there has been no report of adverse reactions after PC transfusion that implicated the involvement of *P. acnes* contamination. In fact, *P. acnes* has been rarely reported to be of clinical significance in the literature.[18] Possible reasons for these are 1) the clinical virulence of *P. acnes* is usually considered to be very low, 2) PC bags currently used serve the suboptimal culture conditions for *P. acnes* in terms of oxygen delivery, and 3) the growing speed of *P. acnes* in PC bags is very low.[20] In this study, the time required for obtaining the positive signal using BacT/ALERT was 3.14 to 6.83 days (mean, 4.7 days) from the start of culture, indicating that it took a minimum of 7 days from PC donation, far exceeding the shelf life of PCs in Japan. The frequency of contamination with clinical relevance could, therefore, be expressed excluding *P. acnes*-contaminated PCs, namely, 0.018% or 126 products per year after the use of the diversion pouch. The significance of using anaerobic culture bottles here would be that the sample volume is doubled and that it often shows better sensitivity than using aerobic culture bottles.[21]

Jacobs and colleagues[22] calculated the rate of septic reactions after the transfusion of bacterially contaminated components as 41% and the rate of fatality among the septic reactions as 11% on the basis of their elaborate prospective study. With these figures and the contamination rate obtained from this study, the total number of septic reactions and the fatality in Japan are estimated as 52 and 5.7 per year, respectively. However, most of the PCs that Jacobs and colleagues described as contaminated are considered to have had a high bacterial load because the sensitivity of the culture method they used was relatively low (10 colony-forming units/mL) and some PCs that were contaminated were identified while they evaluated only PCs stored for 4 days or more. Moreover, most of the contaminated PCs were transfused 4 and 5 days after donation. Therefore, the rates of septic reactions and fatality described in their study are considered to be the results of the transfusion of PCs, most of which were heavily contaminated. The contaminated PCs in our study could include those that had a low bacterial load if they were transfused within the 3-day shelf life. The estimated frequencies of septic reactions and fatality in Japan that were described previously could, in this context, be overestimated.

After the implementation of the diversion method, the number of contaminated PCs possibly originating from the donors' skin flora except for *P. acnes* decreased markedly from eight to one ($p = 0.046$, Table 3). On the other hand, the contamination possibly caused by the bacteria from the donors' peripheral blood or transient skin flora remains to be a serious problem, showing a decrease in the number of contaminated PCs from five to three. These observations substantiate the theoretical mechanism of the effect of the method of initial flow diversion.

Through the extensive culture of more than 40,000 expired PCs, we identified several bacterial species that could have caused a serious clinical outcome if PCs contaminated at clinically relevant concentrations were transfused. Both *Streptococcus constellatus* and *Eikenella corrodens* sometimes cause periodontitis, local abscess, sepsis, or meningitis and are frequently found in the oral cavity or upper respiratory tract. JRC experienced a fatal case of sepsis caused by the transfusion of a PC contaminated by *Streptococcus pneumoniae*,[23] which must have been derived from the donor's upper respiratory tract or oral cavity. These observations suggest that the transient bacteremia caused by bacterial invasion into the blood

stream from the oral cavity, periodontal space, or upper respiratory tract is not a rare event but that people with such bacteremia represent a considerable proportion of otherwise healthy blood donors.

Three PCs contaminated by *S. aureus* were identified during the culture study. Through donor interview, it was verified that two of the three had atopic dermatitis on their cubital fossa or face. *S. aureus* has been implicated in bacteremia or sepsis in patients with atopic dermatitis[24,25] and it is possible that the organism invaded the donors' peripheral blood from their skin lesion and eventually contaminated PCs. Skin lesions such as atopic dermatitis may serve as a risk factor for bacteremia in blood donors regardless of whether the lesion is on the venipuncture site or not.

In spite of the considerably high rate of bacterial contamination of PCs, only two septic cases including one fatality have been confirmed over the past 8 years from 2000 through 2007. These figures are smaller than those reported by Eder and coworkers[9] for rate of fatality (1/500,000) and septic reactions (1/75,000) after the implementation of universal culture screening of PCs. The most likely reason for it is that the shelf life of PCs has been limited to 72 hours in Japan. We believe that administration of PCs as soon as possible after processing will remain as the best strategy of preventing transfusion-related sepsis whatever new strategies for the reduction of bacterial contamination in PCs are implemented in the near future, although we detected contamination with *E. coli* and *S. aureus*, two organisms that can grow rapidly in PLT products and lead to life-threatening reactions within 3 days of collection. Another reason may be that all PC products in Japan are obtained using the apheresis system, which would decrease the contamination rate in theory, although some recent articles argue for the similarity of contamination rate for apheresis PLTs and pooled PLTs.[3,26] It is also possible that low sensitivity of the current hemovigilance system contributes to the low rate of septic reactions in Japan. It is, however, highly likely that at least the fatality rate obtained represents a real occurrence, as suggested by Jacobs and coworkers,[22] because it is mandatory in Japan for clinicians to report any serious transfusion-related adverse effect to the Ministry of Health, Labour and Welfare, and every such event is also evaluated by the JRC headquarters.

The introduction of culture screening is unlikely in JRC blood centers because it will oblige us to extend the shelf life to 5 days, which would trade off the real merit of the currently short shelf life of PCs. To determine whether the implementation of a novel strategy such as pathogen inactivation/reduction[27,28] or point-of-issue testing of PCs using a rapid assay[29] is indispensable, it is essential to establish the data for patients to be rescued with the new strategy and the cost required for the institution and maintenance of the new strategy.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

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医薬品　研究報告　調査報告書

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<tr><td>販売名（企業名）</td><td colspan="2">別紙のとおり</td></tr>
<tr><td>研究報告の概要</td><td colspan="5">問題点：2009年から2010年初頭にかけてインドネシア、タイ、マレーシアにおいてチクングニヤウイルス熱のアウトブレイクが発生している。

2006年以降、アジア及びインド洋領域でチクングニヤ熱の活動が報告されているが、2009年にインドネシアでは12の県に渡って43,206例（死亡例は無し）が、タイではPhuketを含む南部を中心に49,069例超が、マレーシアではSarawak Kedah県北部を中心に4,430例超（死亡例は無し）の当該症例が報告された。また、インドネシアではSumatra島のlampung県南部において2009年12月後半から2010年1月初頭に6,700例のチクングニヤ感染例が、マレーシアでは2010年最初の5週間で325例のチクングニヤ熱症例が報告されている。</td><td>使用上の注意記載状況・
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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人免役グロブリン、⑤人免役グロブリン、⑥人免役グロブリン、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役グロブリン*、㊳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アンスロビンP1500注射用
報告企業の意見	チクングニヤウイルス（Chikungunya virus）は、トガウイルス科（Togaviridae）のアルファウイルス属（Alphavirus）に分類される1本鎖のRNAを核酸として持つ直径70nmのエンベロープを有する球状粒子であり、これまでに国内での発生、流行は報告されていないが、2010年12月までに海外からの輸入症例として15例の報告がある。チクングニヤウイルスは蚊によって媒介されるが、感染後ウイルス血症を起こすことから、血液を介してウイルス感染する可能性を完全に否定できないため本報告を行った。 上記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・不活化工程が存在しているので、仮にウイルスが原料血漿に混入していたとしても、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。今回報告したチクングニヤ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、BVDVの除去・不活化効果を有することを確認している。また、これまでに上記製剤によるチクングニヤウイルス感染の報告例は無い。 以上の点から、上記製剤はチクングニヤ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

INF2009－011

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Outbreak Notice
Chikungunya Fever in Asia and the Indian Ocean

This information is current as of today, April 07, 2010 at 21:25 EDT

Updated: February 18, 2010

Situation Information

Since 2006, parts of Asia and the Indian Ocean region have reported chikungunya fever activity. Several countries have increased surveillance for this disease, and cases continue to be reported throughout this region.

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.

The following examples highlight some recent chikungunya activity in Asia and the Indian Ocean region:

Indonesia

A chikungunya outbreak has been reported in the southern province of Lampung on the island of Sumatra. From the second half of December 2009 through the beginning of January 2010, 6,700 chikungunya cases were reported. In 2009, no deaths due to chikungunya fever were reported, although a total of 43,206 cases were reported across the country from 12 provinces.

Thailand

In 2009, a large outbreak of chikungunya fever affected the country, particularly the southern region, including some tourist destinations, such as Phuket. According to the Ministry of Public Health in Thailand, over 49,069 cases were documented in more than 50 provinces. Reports from Thailand show that chikungunya virus continues to circulate throughout the country.

Malaysia

In 2009, the Ministry of Health in Malaysia reported over 4,430 cases of chikungunya fever. No deaths were reported. The most affected areas are the northern provinces of Sarawak Kedah, followed by Kelantan, Selangor, and Perak. Chikungunya activity has continued in 2010, with an additional 325 cases reported in the first 5 weeks. The cases occurred predominately in Serawak.

Advice for Clinicians

Clinicians should be aware of the ongoing global chikungunya activity. Chikungunya may present in a similar fashion to malaria and dengue, with fever, chills, and generalized myalgias. However, after the acute illness, patients with chikungunya may have a prolonged course of arthralgias or arthritis, which may lead health-care providers to consider and begin testing for rheumatic diseases. These signs and symptoms can persist for several months.

For more information, please see Chikungunya Fever section of *CDC Health information for International Travel 2010.*

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.

- When outdoors during the day and at night, use insect repellent 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 only 1–2 hours.
  - The American Academy of Pediatrics 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 chikungnnya 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 if you are sick and a mosquito bites you, it can spread the disease to other people.

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

More Information

The incubation period for chikungunya (time from infection to illness) is usually 3–7 days, but it can range from 2–12 days. Chikungunya fever typically lasts a few days to 2 weeks, but some

patients feel fatigue lasting several weeks. Most patients report severe joint pain or arthritis, which may last for weeks or months. The symptoms are similar to those of dengue fever, but, unlike some types of dengue, people who have chikungunya fever do not experience hemorrhage (bleeding) or go into shock. People with chikungunya fever generally get better on their own and rarely die from the disease.

Medical care for chikungunya fever 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 (CDC Fact Sheet)
- Traveling with Children: Resources (CDC Travelers' Health website)

Other Mosquito-Related Diseases

In many of the areas where chikungunya is present, mosquito bites spread other diseases, such as dengue, malaria, Japanese encephalitis, and yellow fever. If you are traveling to any tropical and subtropical areas of the world, you should take steps to avoid mosquito bites.

- Page last reviewed: November 19, 2009
- Page last updated: February 18, 2010
- Page created: August 21, 2008
- Content source:
  Division of Global Migration and Quarantine
  National Center for Preparedness, Detection, and Control of Infectious Diseases

Centers for Disease Control and Prevention 1600 Clifton Rd. Atlanta, GA 30333, USA
800-CDC-INFO (800-232-4636) TTY: (888) 232-6348, 24 Hours/Every Day - cdcinfo@cdc.gov

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. 11. 19	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	Stramer S L, Linnen J M, Carrick J M, Krysztof D, McMilin K D, De Vera A, Hunsperger E A, Muñor J L, Dodd R Y. AABB Annual Meeting and TXPO; 2009 Oct. 24-27; New Orleans.	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)			米国

研究報告の概要 73

○2007年のデング熱アウトブレイク時におけるプエルトリコからの供血のデング熱ウイルス血症

背景:デング熱ウイルスは世界で最も重要なアルボウイルスであり、流行範囲を拡大させている。WNV同様、デング熱は蚊によって自然感染するが、輸血によっても伝播する。デング熱流行地域であるプエルトリコの2005年流行期後半のウイルス血症発現率は1:1300を示した。2007年に非常に大規模なデング熱アウトブレイクが発現し、流行期間中の供血者検体が保管された。

方法:ウイルス血症検査のために検体を2セットに分類した(アメリカ本土/プエルトリコで輸血された血液)。研究用transcription mediated amplification assay (TMA)により個別に検体を検査した。初回陽性(IR)検体に再検査を行い、反復陽性(RR)検体は確定とみなされた。プエルトリコのCDCデング熱部門にて、血清型およびウイルス量を明らかにするためPCR、蚊細胞培養、IgM検査などを実施した。RR血液供給先の病院に連絡を取り、受血者の調査を行った。

結果:合計15,350検体を検査し、28がIR、25がRRとなった。有病率は1:614であった。陽性血液のうち12(1:533)が米国本土に輸出され、13がプエルトリコに残った。特異性は99.98%であった。1:16希釈で14/25(56%)のRR供血が検出された。CDCの追加検査では、プエルトリコで循環している血清型1、2、3が示され、11/25(44%)の検体は、RNA力価10^5-10^9copies/mLであり、11検体すべてが細胞培養で感染性があった。9/11(82%)のPCR陽性検体が1:16希釈で検出された。IgM検査を行った6/22(27%)検体のうち2検体のみウイルスの定量が可能(10^6、10^8)であり、うち1つは1:16の希釈で検出された。残り4つのIgM陽性検体では1つのみが1:16希釈で陽性であり、合計2つのIgM陽性検体が希釈時に陽性であった。米国本土とプエルトリコで受血者調査を実施中である。

結論:流行期間中のウイルス血症頻度が高いことが示された。RNA陽性血液の半数近くがIgM陰性で高力価ウイルス血症があり、細胞培養で感染性が確認された。デング熱流行時には供血者のスクリーニングを検討すべきである。

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
プエルトリコにおけるデング熱流行期間中の供血者のウイルス血症頻度が高いことが示され、RNA陽性血液の半数近くがIgM陰性で高力価ウイルス血症があり、細胞培養で感染性が確認されたとの報告である。 デングウイルスは脂質膜を持つ中型RNAウイルスである。これまで、本製剤によるデング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。	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。

S64-030F

HOD RBCs Stored For 14 Days Are Significantly More Immunogenic Than Fresh HOD RBCs

J E Hendrickson[1] (jeanne.hendrickson@choa.org), E A Hod[2], S L Spitalnik[2], C D Hillyer[3], J C Zimring[3]. *[1]Department of Pediatrics, Emory University, AFLAC Cancer Center and Blood Disorders Service, Atlanta, GA, USA; [2]Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY, USA; [3]Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, USA*

Background: Within FDA limits, red blood cells (RBCs) are generally transfused without regard to length of in vitro storage. However, recent studies have raised concerns that transfusion of older stored (i.e. aged) RBCs may lead to adverse events in certain patients. We hypothesized that aged RBC transfusions would lead to higher rates of RBC alloimmunization, and developed a murine model to test this hypothesis. **Materials and Methods:** RBCs from HOD donors (expressing transgenic RBC specific hen egg lysozyme (HEL) fused to human Fyb) were collected in 12.3% CPDA, leukoreduced (LR) with a Pall neonatal LR filter, volume reduced to a Hct of 75%, and stored at 4° C for 14 days. C57BL/6 recipients were transfused intravenously with 500 µL of a 20% solution of fresh or aged (stored 14 days) LR or non-LR RBCs. Flow cytometric testing of HEL and Fyb expression on pre and post-transfusion RBCs was done, with 24 hour post-transfusion survival determined by extrapolation to time 0. Blood cultures were performed on representative samples prior to transfusion. Alloimmunization was tested 2 weeks post-transfusion by anti-HEL IgG ELISA using titrated sera. **Results:** In 5 of 6 independent experiments (n = 62 mice), transfused aged RBCs were 10-100 fold more immunogenic than fresh RBCs as determined by HEL specific ELISA ($p < 0.05$ by 2 way ANOVA with Bonferroni posttest). This increase in immunogenicity was also seen with LR RBCs; in 3 of 4 experiments (n = 42 mice), aged LR RBCs were more immunogenic than fresh LR RBCs ($p < 0.001$). In 2 of 2 experiments (n = 20 mice), aged RBCs washed 3 times in saline led to similar levels of alloimmunization as did unwashed aged RBCs. Gram's stain and culture of 7 of 9 representative units was negative. The calculated 24 hour post-transfusion survival for fresh, aged, and aged LR blood was 100%, 38.7% (95% CI 31.8-45.6), and 43.9% (95% CI 35.9-51.9). In 4 of 5 experiments, HEL and Fyb expression on aged RBCs was identical to that of fresh RBCs. **Conclusions:** Transfusion of LR and non-LR transgenic HOD RBCs, stored for 14 days in conditions similar to those used in human blood banking, induce higher levels of alloimmunization than freshly collected and transfused RBCs. This cannot be explained solely by the presence of contaminating WBCs or bacteria. In addition, because washed RBCs are as immunogenic as unwashed RBCs, the RBCs themselves may be responsible for the increased immunogenicity. Although the 24 hour post-transfusion survival is below the average for human RBCs, this study is a proof of principle testing of the effect of aging on RBC alloimmunization. The reproducibility of these findings in other RBC antigen systems, as well as the potential translational applicability, remains to be determined.

Disclosure of Commercial Conflict of Interest

J. E. Hendrickson: Nothing to disclose; C. D. Hillyer: Nothing to disclose; E. A. Hod: Nothing to disclose; S. L. Spitalnik: Nothing to disclose; J. C. Zimring: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. E. Hendrickson: Nothing to disclose; C. D. Hillyer: Nothing to disclose; E. A. Hod: Nothing to disclose; S. L. Spitalnik: Nothing to disclose; J. C. Zimring: Immucor Inc., Grants or Research Support

S65-030F

Crossmatch Incompatible RBCs Have an Intrinsic Range of Susceptibility to Hemolysis

J S Liepkalns[1] (jsliepk@emory.edu), J C Zimring[1]. *[1]Center for Transfusion and Cellular Therapies, Emory University School of Medicine, Atlanta, GA, USA*

Background: During crossmatch incompatible transfusions, clinically significant antibodies can lead to brisk hemolysis. However, for some blood group antigens, antibodies are hemolytic in certain patients but not in others. The reason for this variability is poorly understood. Using a mouse model of crossmatch incompatible transfusion involving human glycophorin A (hGPA) as an RBC antigen, we have previously observed that some hGPA RBCs clear but others continue to circulate despite being coated with IgG. We have also reported that the mechanism of resistance was neither antibody depletion nor saturation of the reticuloendothelial system. To further characterize hemolysis resistance, we tested whether resistance is an acquired or intrinsic property of the RBC. **Methods:** Incompatible hGPA RBCs and compatible wild-type RBCs were labeled with fluorescent dyes DiI and DiO, respectively. Mixtures of the labeled RBCs were transfused into wild-type recipients (transfusion 1) that had been passively immunized with a monoclonal antibody against hGPA (6A7). Two days post transfusion, RBCs were collected and were mixed with freshly isolated hGPA RBCs labeled in a third color (DiD). This mixture was then transfused into naive mice (transfusion 2), which were likewise passively immunized with 6A7. In all cases, RBC survival was determined through enumerating each population by flow cytometry. Clearance in transfusion 1 was determined by calculating survival of hGPA RBCs as a function of compatible wild-type RBCs. Hemolysis resistance was defined during transfusion 2 as decreased clearance of hGPA RBCs from transfusion 1 compared to clearance of fresh hGPA RBCs. Titrations of 6A7 were performed in transfusions 1 and 2. **Results:** In transfusion 1, hGPA RBCs showed initial rapid clearance proportional to the amount of 6A7 injected. In all cases, the surviving hGPA RBCs were 90-100% resistant to clearance in transfusion 2 when exposed to the same concentration of 6A7 as in transfusion 1. However, if an increased concentration of 6A7 was used in transfusion 2, then resistance to clearance was less (range 20-60%). **Conclusion:** The observation that hGPA RBCs are resistant to clearance by the same concentration of 6A7 in transfusion 2 as in transfusion 1, but are less resistant to increased amounts of 6A7 in transfusion 2, suggest that RBCs have a range of susceptibility to clearance as a function of antibody concentration. The mechanism of differential susceptibility to clearance is uncertain, but may include RBC age or antigen density.

Disclosure of Commercial Conflict of Interest

J. S. Liepkalns: Nothing to disclose; J. C. Zimring: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. S. Liepkalns: Nothing to disclose; J. C. Zimring: Immucor Inc., Grants or Research Support

Transfusion-Transmitted Diseases: Arboviruses

S66-030G

Dengue Viremia in Donations from Puerto Rico During the 2007 Dengue Outbreak

S L Stramer[1] (stramers@usa.redcross.org), J M Linnen[2], J M Carrick[2], D Krysztof[1], K D McMillin[3], A De Vera[4], E A Hunsperger[5], J L Muñoz[6], R Y Dodd[6]. *[1]Scientific Support Office, American Red Cross, Gaithersburg, MD, USA; [2]Gen-Probe, Inc., San Diego, CA, USA; [3]Biomedical Services – Alabama, American Red Cross, Birmingham, AL, USA; [4]Biomedical Services – Puerto Rico, American Red Cross, San Juan, PR, USA; [5]Centers for Disease Control (CDC), San Juan, PR, USA; [6]Holland Laboratory, American Red Cross, Rockville, MD, USA*

Background: Dengue virus is the most important arbovirus in the world; its range is expanding. Like WNV, dengue is transmitted naturally by the bite of an infected mosquito but also is transfusion transmitted. Data from 2005 in Puerto Rico (PR), a dengue-endemic area, demonstrated a rate of donor viremia of 1:1300 during the latter half of the 2005 epidemic season. In 2007, a much larger dengue outbreak occurred in PR from which samples from donors during the epidemic period were retained for testing to further confirm donor viremia rates and for recipient tracing of components from positive donations. **Methods:** Samples were retained in a repository and split into two sets for viremia studies: those units exported and transfused in the continental US and those transfused in PR. Samples were tested individually by a research transcription mediated amplification assay (TMA, Gen-Probe). Initially reactive (IR) samples were retested by the original TMA and an alternate TMA (alt TMA used for the units transfused in PR only) without dilution and at a 1:16 dilution to model pooling. All TMA-repeat reactive (RR) samples were considered confirmed. Additional virologic/infectivity and serologic testing was performed at the CDC dengue branch in PR including PCR to define the dengue serotype and viral load, mosquito cell culture and IgM testing. Hospitals receiving components from RR donations were contacted to initiate recipient tracing including a detailed questionnaire about symptoms and risk factors. The study was IRB approved. **Results:** A total of 15,350 samples were tested with 28 IR and 25 RR samples considered confirmed positive (pos) for a prevalence of 1:614 consisting of 12 dengue-pos donations exported from PR into the continental US (1:533) and 13 pos donations that remained in PR (1:689). Specificity was 99.98%. A 1:16 dilution detected 14/25 (56%) RR donations. Further supplemental testing (CDC) demonstrated dengue virus serotypes 1, 2 and 3 (corresponding to those circulating in PR); 11/25 (44%) samples had RNA titers of 10^5-10^9 copies/mL of which all 11 also infected C636 mosquito cell cultures. 9/11 (82%) PCR-pos

samples were detected at a 1:16 dilution. 6/22 (27%) samples tested for IgM were pos, only 2 of which had quantifiable virus (10^6 and 10^8) with 1 detected at a 1:16 dilution. Of the 4 remaining IgM pos samples, only 1 was pos at a 1:16 dilution (low level pos) for a total of 2 IgM-pos samples detected when diluted. Recipient tracing in the continental US and PR is underway. **Conclusions:** Like the prior study identifying dengue viremic donations in PR, this study demonstrates a high frequency of viremia during dengue-epidemic periods with nearly half of the RNA-pos donations lacking IgM, having high-titer viremia and infectious in cell culture. Screening of donors should be considered during dengue-epidemic periods.

Disclosure of Commercial Conflict of Interest

J. M. Carrick: Gen-Probe Incorporated, Ownership or Partnership; A. De Vera: Nothing to disclose; R. Y. Dodd: Nothing to disclose; E. A. Hunsperger: Nothing to disclose; D. Krysztof: Nothing to disclose; J. M. Linnen: Gen-Probe Incorporated, Stocks or Bonds; K. D. McMilin: Gen-Probe Incorporated, Other; J. L. Muñor: Nothing to disclose; S. L. Stramer: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. M. Carrick: Nothing to disclose; A. De Vera: Nothing to disclose; R. Y. Dodd: Nothing to disclose; E. A. Hunsperger: Nothing to disclose; D. Krysztof: Nothing to disclose; J. M. Linnen: Nothing to disclose; K. D. McMilin: Nothing to disclose; J. L. Muñor: Nothing to disclose; S. L. Stramer: Nothing to disclose

S67-030G

Highly Sensitive and Equivalent Detection of Dengue Virus Serotypes 1, 2, 3, and 4 with an Enhanced Transcription-Mediated Amplification Assay

J M Carrick[1] (jamesca@gen-probe.com), J Knight[1], C Lontoc-Bugay[1], C Motta[1], J B Wellbaum[1], C Fleischer[1], J L Muñor[2], S L Stramer[2], J M Linnen[1]. *[1]Gen-Probe Incorporated, San Diego, CA, USA; [2]American Red Cross, Gaithersburg, MD, USA; [3]Centers for Disease Control and Prevention, San Juan, PR, USA*

Background: Based on WHO estimates, the incidence of dengue has grown dramatically around the world in recent decades and is now considered to be a major international public health concern. To investigate the risk of dengue virus (DENV) transfusion transmission, we developed a prototype nucleic acid test (NAT) based on Transcription-Mediated Amplication (TMA) that was used to show the feasibility of detecting DENV RNA in asymptomatic blood donors from Honduras, Brazil, and Puerto Rico and in clinically ill patients from Puerto Rico. Our previous results demonstrated the importance of detecting all 4 DENV serotypes at low copy levels with equivalent sensitivity. Recently, we developed an improved TMA Assay with increased sensitivity for each of the 4 serotypes. **Methods:** The enhanced TMA assay uses the same technology as other PROCLEIX® assays, consisting of lysis and target capture of viral RNA followed by TMA and chemiluminescent detection by Hybridization Protection Assay (HPA). Analytical sensitivity for serotypes 1, 2, 3, and 4 were determined by probit analysis of results from testing serially diluted live DENV and DENV RNA transcripts. Live DENV was obtained from the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO. Assay specificity was determined by testing 988 US blood donor specimens and 8,680 donor specimens from Puerto Rico that were screened previously with the earlier version of the TMA assay. Previous screening of these specimens yielded 14 positive results. Samples were tested on the fully automated PROCLEIX® TIGRIS® System. **Results:** The enhanced dengue assay showed 95% detection at 14.9, 18.3, 13.0, and 16.4 copies/mL of DENV 1, DENV 2, DENV 3, and DENV 4, respectively. Analytical sensitivities for each of the four serotypes were determined to be not statistically different. There were no reactive samples among the US donations. The improved assay was able to detect all 14 positive donations identified by the original assay in the Puerto Rican donations; an additional 7 reactive samples were identified with the improved assay, of which 4 were repeat reactive. The overall assay specificity from testing the US and Puerto Rican donations was 99.97% (95% CI: 99.91-99.99). **Conclusions:** Using the improved dengue TMA assay we demonstrated reliable detection of all 4 serotypes of DENV below 20 copies/mL while maintaing high clinical specificity. The analytical and clinical sensitivity results from this study indicate that the improved dengue assay has the potential to identify a larger number of low viral load DENV infections in both blood screening and diagnostic applications.

Disclosure of Commercial Conflict of Interest

J. M. Carrick: Gen-Probe Incorporated, Ownership or Partnership; C. Fleischer: Nothing to disclose; J. Knight: Nothing to disclose; J. M. Linnen: Gen-Probe Incorporated, Stocks or Bonds; C. Lontoc-Bugay: No Answer; C. Motta: No Answer; J. L. Muñor: Nothing to disclose; S. L. Stramer: Nothing to disclose; J. B. Wellbaum: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. M. Carrick: Nothing to disclose; C. Fleischer: Nothing to disclose; J. Knight: Nothing to disclose; J. M. Linnen: Nothing to disclose; C. Lontoc-Bugay: No Answer; C. Motta: No Answer; J. L. Muñor: Nothing to disclose; S. L. Stramer: Nothing to disclose; J. B. Wellbaum: Nothing to disclose

S68-030G

Correlation between Yield of WNV NAT Screening of North Dakota Donors Over 6 Epidemic Seasons with WNV Seroprevalence at the End of 2008

M P Busch[1] (mbusch@bloodsystems.org), V Winkelman[2], J Dunn Williams[2], H E Prince[3], C Yeh[3], B Custer[1], L R Petersen[4]. *[1]Blood Systems Research Institute; & Univ California, San Francisco, San Francisco, CA, USA; [2]Blood Systems Laboratory, Tempe, AZ, USA; [3]Focus Diagnostics, Cypress, CA, USA; [4]DVBID, Centers for Disease Control & Prevention, Ft Collins, CO, USA*

Background: MP-NAT for WNV was implemented in 2003, with progressive enhancement in screening sensitivity over the next 6 years by using targeted ID-NAT in epidemic areas with increasingly stringent trigger criteria. In our system, North Dakota (ND) has had the highest overall rate of WNV+ donations. Seasonal yield has fluctuated, but remained below the 2003 peak yield. This lower yield may be partly attributable to prior WNV infections in the population, leading to population immunity. This cross-sectional study determined WNV antibody seroprevalence after the 2008 transmission season, and correlated this seroprevalence with annual NAT yield rates in the state. **Methods:** 5000 samples from ND blood donations were archived from late Oct-Dec 2008, >1 month after the last NAT yield donation and last WNV case report in ND. Samples from donors resident in ND were selected and tested for WNV IgG; IgG-positive donations were further tested for WNV IgM to identify recent infections (Focus Diagnostics). NAT yield cases (confirmed by replicate NAT/serology on index donation and/or follow-up samples) from ND donors were compiled by year, and further sorted into those detectable by MP-NAT (based on MP-NAT detection, or reactivity at 1:16 dilution if detected by ID-NAT) vs those detectable only by ID-NAT. Annual incidence was projected based on annual MP-NAT yield and a 6.9-day MP-NAT yield window period (Busch et al, EID, 2005). **Results:** Of 3594 donations by ND donors from Oct-Dec 2008 tested for IgG, 296 (8.2%; 95%CI 7.3-9.1) were positive for WNV IgG; of these 26 (8.8%) confirmed positive for WNV IgM. The yield of WNV MP-detectable (MP-NAT+) and ID-only detectable (ID-NAT+) donations, and the projected WNV incidence/year, are shown in the table. **Conclusions:** The proportion of ND residents previously exposed to WNV, based on donor IgG seropositivity in late 2008, is currently 8.2%. Thus the general decline in WNV NAT yield in the past 6 years is not attributable to human population immunity, but rather likely due to ecological factors influencing WNV transmission to humans. The 8.8% rate of IgM detection among IgG+ donations is consistent with the proportionate yield of infections in 2008 (7/124, 5.6%), with some contribution of persistent IgM from 2007 infections. Cumulative annual incidence projected from annual MP-NAT yield cases correlated reasonably well with observed IgG seroprevalence, suggesting that cumulative MP-NAT yield data from other areas can be used to project WNV infection rates throughout the US.

Disclosure of Commercial Conflict of Interest

M. P. Busch: Nothing to disclose; B. Custer: Nothing to disclose; J. Dunn Williams: Nothing to disclose; L. R. Petersen: Nothing to disclose; H. E. Prince: Nothing to disclose; V. Winkelman: Nothing to disclose; C. Yeh: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. P. Busch: Gen-Probe, Inc., Grants or Research Support, Travel Support or Honorarium, Chiron / Novartis, Grants or Research Support, Travel Support or Honorarium, Ortho, Travel Support or Honorarium, Abbott, Travel Support or Honorarium; B. Custer: Nothing to disclose; J. Dunn Williams: Nothing to disclose; L. R. Petersen: Nothing to disclose; H. E. Prince: Nothing to disclose; V. Winkelman: Nothing to disclose; C. Yeh: Nothing to disclose

Year	Donations	Total NAT+	MP-NAT+	ID-NAT+	Incidence
2003	66,109	62	42	20	3.3%
2004	67,117	1	1	0	0.1%
2005	68,150	10	5	5	0.4%
2006	68,652	16	6	10	0.5%
2007	73,640	28	12	16	0.9%
2008	76,306	7	5	2	0.4%

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. 11. 24	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		公表国
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)		ABC Newsletter #41. 2009 Nov 13; 4-6.	米国

研究報告の概要

○バベシア症拡大を示す研究を受け、米国赤十字は7州での供血検査の実施を提案

Transfusion誌で近く発表される最近の3つの研究で、供血血液におけるバベシア症の原因となる寄生虫の陽性率増加および輸血伝播バベシア症(TTB)の増加が認められた。1つはコネチカット州とマサチューセッツ州において供血血液のどの程度の範囲に拡大しているかを示した。2つ目の研究ではロードアイランド州において、輸血を介した伝播範囲を確認した。3つ目では、米国赤十字のヘモビジランス・プログラムを通して報告された症例を用いて、感染した供血者と受血者の特徴を調べている。これらの研究から、バベシア症とTTBの危険が増大しているという懸念が明らかになった。

これらのデータに基づき、米国赤十字は疾患が流行する北東部および中西部北部の7州で供血血液のIFA検査を開始する2つの計画を作成した。

バベシア症は、Ixodes属のマダニによって伝播し、米国ではほとんどが*Babesia microti*(マラリアと類似した赤血球を汚染する寄生虫)に起因する。大部分の感染者は無症候か軽症であるが、特定の健康要因を有する人では、重症、致命的となる場合もある。無症候感染は何ヶ月も続く可能性がある。現在、米国食品医薬品局(FDA)が認可したバベシア症の検査はなく、血液センターは供血者にバベシア症の既往を尋ねるのみである。しかし、大半の罹患者は自分の感染を知らないため、問診の効果には疑問がある。寄生虫保有者が供血した場合、受血者に輸血を介して感染させる可能性がある。これまでに赤血球と血小板で輸血による伝播が報告されている。合併症と死亡者数の急増により、TTBに対する懸念が浮上した。1997～2004年にFDAに報告されたTTB関連死亡例は1例のみだったが、2005年11月～2008年9月では9例以上となった。FDAは2008年9月にワークショップを開催し、AABBは70以上の症例の報告を受けて2009年8月に公報を発行した。

報告企業の意見	今後の対応
米国東部と中西部におけるバベシア症拡大を示す研究を受け、米国赤十字が7州での供血検査の実施を提案したとの報告である。	今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

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Red Cross Lobbying (continued from page 3)

In 2008, Congress appropriated $100 million in emergency funding to the American Red Cross to replenish its disaster relief reserves, which were depleted when the charity provided shelter, food and other services during a string of hurricanes earlier in the year. The Red Cross appropriation was set out in two sections 10502-03 of the Homeland Security bill, HR 2638 (Consolidated Security, Disaster Assistance, and Continuing Appropriations Act, 2009) and is explicitly for disaster relief purposes.

So far this year, the organization reports spending $154,890 on lobbying. Lobbyists for 2009 are listed as Cherae Bishop, Marc Decourcey, Neal Denton, Dawn Latham and Marin Reynes. (Sources: Senate Lobbying Disclosure Database; Implu Corp., an online business intelligence database) ♦

With Studies Showing Spread of Babesiosis, ARC Proposing to Test Donated Blood in Seven States

Three recent studies have discovered increases in the incidence of the parasite that causes babesiosis in donated blood and of transfusion-transmitted babesiosis (TTB). On the strength of that data, the American Red Cross (ARC) has developed two proposals to begin testing donated blood in states in the Northeast and the upper Midwest where the disease is endemic.

The picture emerging from the studies – each of which is forthcoming in *Transfusion* – shows babesiosis to be a growing threat. Each focuses on a different aspect of the problem. One study shows how widespread it is among blood donations in Connecticut and Massachusetts, another identifies the extent of its transmission through transfusions in Rhode Island, and the third determines the characteristics of infected donors and recipients, using cases reported through ARC's Hemovigilance Program.

> **Individually and collectively, the studies emphasize that concerns over the dangers of babesiosis and TTB are increasing. The ARC proposals involve setting up testing in affected areas, starting with Connecticut and potentially expanding to seven states – 16 percent of the nation's population.**

Babesiosis is carried by *Ixodes* ticks; in the US, it is mostly caused by *Babesia microti*, a parasite that is similar to malaria and that infects red blood cells. Most people infected with it do not experience any symptoms or experience only mild symptoms that can be mistaken for the flu; however, the disease can be severe and even fatal, particularly for people with certain complicating health factors. Asymptomatic infection may last for months. Currently, there is no Food and Drug Administration-approved test for the disease, and blood centers merely ask potential donors whether they have a history of babesiosis. But the fact that most people with the disease do not know they have it casts doubt on the effectiveness of the question.

If a person who carries the parasites donates blood, the disease can be transmitted through transfusion to a susceptible recipient. To date, transmission has been reported only with red blood cells (both fresh and frozen) and platelets.

Concerns about TTB have risen as the number of complications and deaths related to it has jumped. The Food and Drug Administration received only one report of a TTB-related death from 1997 to 2004; however, from November 2005 to September 2008, it received at least nine (see *ABC Newsletter*, 12/5/08). In September 2008, FDA held a workshop on TTB in the US. In August 2009, AABB issued a bulletin on it, prompted by reports of more than 70 cases of it (see *ABC Newsletter*, 8/14/09).

(continued on page 5)

Babesiosis (continued from page 4)

The three forthcoming studies aim at shedding light on the epidemiology of the babesiosis. In one study, led by Stephanie T. Johnson, MT (ASCP), MPH, who is with the ARC branch in Farmington, Conn., scientists tested blood donated at selected drives in Connecticut and Massachusetts from 2000 to 2007 for the presence of immunoglobulin (Ig)G antibodies to *Babesia microti*. Using an immunofluorescence assay (IFA), they found the antibodies in blood donated in all eight counties in Connecticut and three counties in Massachusetts. They also found it in blood donated not just during the season peak for the tick that causes the virus – from July through September – but also during the rest of the year.

Although the results of this study helped them identify particular areas and times of the year when the likelihood of *Babesia microti* in blood is highest, they also made clear that the threat extended beyond certain areas and months, which led the scientists to conclude that year-round, regional testing may be necessary to fully safeguard the blood supply from the transmission of the disease.

Scientists in Rhode Island reached a similar conclusion when they carried out a retrospective study in which they analyzed babesiosis cases that were reported to the Department of Health in that state from 1999 to 2007. Led by Leonard Mennel, DO, an infectious disease specialist and the director of infection control for the Rhode Island Hospital, this team identified 21 cases of TTB in the nine years they studied.

Their analysis of information about where donors lived and when they donated reinforced the finding in Johnson's study that some people with babesiosis lived in areas without high tick populations and had merely traveled to an area where babesiosis is more common. Drawing also on other studies that show that the virus can survive for extended periods in blood bank conditions, including refrigeration up to 35 days, these researchers conclude that TTB is possible any time of year and in any location. Their study also revealed a troubling rise in cases of TTB: from 1999 to 2007, 326,081 units of red blood cells were transfused, according to the Rhode Island Blood Center. The 21 cases of TTB during that period give an incidence rate for TTB of just more than 1 in 15,000 transfusions. However, by the last three years studied, that rate had risen to 1 in 9,000 units transfused.

To determine the characteristics of infected donors and recipients, the third team of researchers – led by Laura Tonnetti, PhD, a scientist with the ARC's Transmissible Diseases Department, Jerome H. Holland Laboratory, in Rockville, Md. – analyzed cases of suspected TTB that were reported to ARC's Hemovigilance Program from 2005 to 2007.

They carried out follow-up testing of previously collected blood donations, by IFA, Western blot, and/or real-time polymerase chain reaction (PCR) analysis. They found 18 definite or probable *Babesia microti* infections among transfusion recipients. Five of those recipients died. Of the 18 cases, two recipients had sickle cell disease and four were asplenic; 13 were between the ages of 61 and 84 and two were 2 years old or younger. The researchers concluded that TTB "can be a significant cause of transfusion-related morbidity and mortality," particularly when transfusion recipients were elderly, very young, or asplenic. Like the researchers in Rhode Island, these scientists also found that TTB stemmed both from donors who lived in areas where the disease is endemic as well as those who had merely traveled to those areas. They also found that IFA testing was more effective than PCR analysis: the former identified all 18 donors, while the latter identified only one.

What Should Be Done? The conclusions of these studies – that babesiosis can occur anywhere at any time, that the number of TTB cases is rising, and that TTB can lead to serious complications from transfusions, including death – gave new data to support ARC proposals for testing donated blood for evidence of infection, which Dr. Tonnetti discussed in a presentation at the recent AABB Meeting.

(continued on page 6)

Babesiosis (continued from page 5)

The first proposal is to establish testing donated blood in Connecticut by IFA. ARC's recommendations include year-round IFA testing under investigational new drug regulations. Only whole-blood donations would be tested. Donors associated with positive results would be deferred, and their donations would be discarded. Testing could be done throughout the state or only in highly endemic areas. The latter approach would be less expensive, but it may only identify one-third of at-risk donors, so ARC favors testing across the state.

Depending on the results of that project, said Dr. Tonnetti, ARC would like to expand the area to include Rhode Island, Massachusetts, New York, New Jersey, Minnesota, and Wisconsin. Connecticut was chosen as the starting point, she explained in a phone call, because earlier studies had found a number of endemic areas in the state. But she emphasized that expanding the testing to other states would be important, given that babesiosis and TTB can spread so easily. No timeline has been set for testing under either proposal.

Citations. Asad S, *et al.* Transfusion-transmitted babesiosis in Rhode Island. Transfusion. 2009 Sep 16 [epub ahead of print]; Johnson ST, *et al.* Seroprevalence of *Babesia microti* in blood donors from *Babesia*-endemic areas of the northeastern United States: 2000 through 2007. Transfusion 2009 Oct. 10 [epub ahead of print]; Tonnetti L, *et al.* Transfusion-transmitted *Babesia microti* identified through hemovigilance. Transfusion. 2009 Jul 16 [epub ahead of print] ♦

FDA Finalizes Guidance on Testing Donated Blood for West Nile Virus

The Food and Drug Administration has finalized its guidance for blood centers on how they should test donations of whole blood and blood products for West Nile Virus (WNV). This guidance replaces the draft guidance dated April 28, 2008, and it takes into account a number of the comments FDA received from America's Blood Centers (ABC) and other sources.

While the draft guidance included recommendations for screening cells, tissues, and cellular-based products, the final guidance covers only donations of whole blood and blood products. Key recommendations are that blood centers should test whole blood and blood products for WNV year-round; that they may use minipool tests when there is not high WNV activity in their area; that each center may establish its own criteria for high WNV activity; that centers switch to individual testing as soon as possible, but not later than 48 hours, after high WNV activity is found in their area; and that if a minipool tests as reactive for WNV, each unit in that minipool should be tested with an individual test. It also recommended that, for individual units that test positive, additional testing "may be of value in donor counseling."

Background. It has been known since 2002 that donors who were infected with WNV could be viremic but not have any symptoms; it has also been known that the virus could be transmitted through blood transfusions and organ transplantation. FDA began studies the following year aimed at evaluating nucleic acid tests (NAT) for detecting WNV, and it has approved biologics license applications for two NAT since 2005. Both tests are used for individual donor samples, and for minipools of samples taken from either 6 or 16 donations.

Studies have found that the individual test (ID-NAT) has greater sensitivity than the minipool test (MP-NAT), and that, in fact, up to 25 percent of viremic units were not detected by the MP-NAT. However, it is not feasible or practical to test every unit individually, because of limited availability of the tests and personnel and logistical issues. This guidance, then, is meant to clarify when blood centers should use ID-NAT and when they may use MP-NAT.

(continued on page 7)