Table. Infection parameters of 7 persons exposed to TBEV by eating nonpasteurized goat cheese, Austria, 2008*

Sex/ age, y	Incubation, d	Symptoms/signs	Diagnosis	Hospitalized, d	Virologic parameters: Material	TBEV ELISA: IgM	TBEV ELISA: IgG	TBEV NT	TBEV infection confirmed
M/43	11	Fever, cephalea, meningism, aseptic urethritis; CSF: pleocytosis	ME	18	Serum	Pos	Pos	Pos	Yes
					CSF	Bor	Pos		
M/65	10	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	30	Serum	Pos	Pos	Pos	Yes
					CSF	Bor	Bor		
F/60	14	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	25	Serum	Pos	Pos	Pos	Yes
					CSF	Pos	Pos		
M/44	9	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	9	Serum	Pos	Pos	Pos	Yes
					CSF	Pos	Bor		
F/37	NA	None	NA	0	Serum	Pos	Pos	Pos	Yes
F/7	NA	None	NA	0	Serum	Pos	Pos	Pos	Yes
F/45	NA	None	NA	0	Serum	Neg	Neg	Neg	No

*TBEV, tick-borne encephalitis virus; NT, neutralization test; CSF, cerebrospinal fluid; Ig, immunoglobulin; ME, meningioencephalitis; pos, positive; bor, borderline; NA, not applicable; neg, negative.

The 4 domestic pigs kept at the alpine pasture and fed with the whey and goat milk, however, were seropositive (TBEV HI- and neutralizing antibodies detected), which indicated TBEV infection, but no clinical signs were observed. Infection with TBEV has been reported in wild boars (*4*,*5*). Serum samples from 105 goats from pastures in the neighborhood also were investigated for TBEV-specific antibodies; all goats were seronegative.

Conclusions

Our analyses showed that the 6 humans and the 4 pigs were infected through the milk of 1 goat, which had been transported by car from a TBE–nonendemic valley to the alp 12 days before production of the TBEV-contaminated cheese. Experiments have demonstrated that infected domestic animals (i.e., goats, sheep, and cows) can excrete TBEV into milk for ≈3–7 days, beginning as early as the second or third day postinfection (*6*–*9*). In addition, although cheese was produced once or twice each week, only this ≈1-kg batch of cheese transmitted TBEV. Therefore, all the evidence indicates that the goat was infected at the alpine pasture at an altitude of 1,564 m. Indeed, some ticks were collected from cows that had stayed at this altitude during the entire summer. Analyses of these ticks for TBEV by PCR, however, yielded only negative results.

Our findings provide further evidence for the expansion of TBEV-endemic regions to higher altitudes in central Europe. For example, longitudinal studies in the Czech Republic, a country with similar climatic and ecologic conditions to those of Austria, showed a shift in *Ixodes ricinus* ticks and TBEV, from 700 m in 1981–1983 to 1,100 m altitude in 2001–2005 (*10*,*11*). Likewise, Zeman and Beneš demonstrated that the maximum altitude at which TBEV is found in the Czech Republic gradually moved upward during 1970–2000, corresponding to the rise in temperature during the same period (*12*). In Scandinavia, a northward extension of the geographic range of *I. ricinus* ticks and TBEV since the mid-1980s has also been recognized (*1*,*13*–*15*). Climatic changes most likely are the major driving forces for the geographic changes in the distribution of TBEV and its main vector, *I. ricinus*, in Europe.

This report also emphasizes the efficiency of oral transmission of TBEV to humans and to pigs. Six of the 7 persons who ate the cheese and all 4 pigs fed residual milk or whey from the same cheese became infected. Given the excellent effectiveness of the TBE vaccine (*2*), vaccination probably could have prevented all 6 human cases.

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References

1. Lindquist L, Vapalahti O. Tick-borne encephalitis. Lancet. 2008;371:1861–71. DOI: 10.1016/S0140-6736(08)60800-4
2. Heinz FX, Holzmann H, Essl A, Kundi M. Field effectiveness of vaccination against tick-borne encephalitis. Vaccine. 2007;25:7559–67. DOI: 10.1016/j.vaccine.2007.08.024
3. Süss J. Epidemiology and ecology of TBE relevant to the production of effective vaccines. Vaccine. 2003;21(Suppl 1):S19–35. DOI: 10.1016/S0264-410X(02)00812-5
4. Borcić B, Raos B, Kranzelić D, Abu Eldan J, Filipović V. The role of large wildlife in the maintenance of natural foci of tick-borne meningoencephalitis in northern Croatia. Acta Med Iugosl. 1990;44:399–406.

5. Zeman P, Januska J. Epizootiologic background of dissimilar distribution of human cases of Lyme borreliosis and tick-borne encephalitis in a joint endemic area. Comp Immunol Microbiol Infect Dis. 1999;22:247–60. DOI: 10.1016/S0147-9571(99)00015-6
6. Van Tongeren HA. Encephalitis in Austria. IV. Excretion of virus by milk of the experimentally infected goat. Arch Gesamte Virusforsch. 1955;6:158–62. DOI: 10.1007/BF01247065
7. Gresiková M. Excretion of tick-borne encephalitis virus in the milk of subcutaneously infected cows. Acta Virol. 1958;2:188–92.
8. Gresiková M. Recovery of the tick-borne encephalitis virus from the blood and milk of subcutaneously infected sheep. Acta Virol. 1958;2:113–9.
9. Gresiková M, Rehacek J. Isolation of the tick encephalitis virus from the blood and milk of domestic animals (sheep and cow) after infection by ticks of the family *Ixodes ricinus* L. Arch Gesamte Virusforsch. 1959;9:360–4. DOI: 10.1007/BF01248828
10. Daniel M, Danielová V, Kriz B, Kott I. An attempt to elucidate the increased incidence of tick-borne encephalitis and its spread to higher altitudes in the Czech Republic. Int J Med Microbiol. 2004;293(Suppl 37):55–62.
11. Danielová V, Kliegrová S, Daniel M, Benes C. Influence of climate warming on tickborne encephalitis expansion to higher altitudes over the last decade (1997–2006) in the Highland Region (Czech Republic). Cent Eur J Public Health. 2008;16:4–11.
12. Zeman P, Beneš C. A tick-borne encephalitis ceiling in central Europe has moved upwards during the last 30 years: possible impact of global warming? Int J Med Microbiol. 2004;293(Suppl 37):48–54.
13. Lindgren E, Gustafson R. Tick-borne encephalitis in Sweden and climate change. Lancet. 2001;358:16–8. DOI: 10.1016/S0140-6736-(00)05250-8
14. Skarpaas T, Ljøstad U, Sundøy A. First human cases of tickborne encephalitis, Norway. Emerg Infect Dis. 2004;10:2241–3.
15. Stjernberg L, Holmkvist K, Berglund J. A newly detected tick-borne encephalitis (TBE) focus in south-east Sweden: a follow-up study of TBE virus (TBEV) seroprevalence. Scand J Infect Dis. 2008;40:4–10. DOI: 10.1080/00365540701522934

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No. 27

医薬品 研究報告 調査報告書

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研究報告の概要

○インドで分離されたバガザウイルス(BAGV)の遺伝子学的特性と脳炎患者から採取した血清中抗BAGV抗体のエビデンス
1996年のインドのケララ州における脳炎アウトブレイクの調査時、コガタアカイエカのプールからアルボウイルスが分離された。補体結合検査により、日本脳炎とウエストナイルウイルスに交差反応を起こす可能性のあるアルボウイルスの特徴が明らかとなった。プラークを精製したアルボウイルス分離株に対する過免疫血清を使用し、プラーク減少／中和検査を行った。血清は日本脳炎ウイルスで陽性を示さず、ウエストナイルウイルスで弱陽性であった。全ORF配列解析で、当該アルボウイルスはバガザウイルス(BAGV)の特徴を示した(アフリカのBAGV DakAr B209株とのヌクレオチド相同性94.80%)。疾患急性期の脳炎患者から採取した血清は、15%(8/53)がBAGV中和抗体陽性を示した。これは、インドで分離されたBAGVの初の報告である。抗BAGV中和抗体の存在は、人間集団がBAGVに暴露されていたことを示唆する。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

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

⑤

報告企業の意見	今後の対応
1996年のインドのケララ州における脳炎アウトブレイク時に患者がバガザウイルスに感染していたことが判明したとの報告である。	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。

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Short Communication

Genetic characterization of Bagaza virus (BAGV) isolated in India and evidence of anti-BAGV antibodies in sera collected from encephalitis patients

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During investigations into the outbreak of encephalitis in 1996 in the Kerala state in India, an arbovirus was isolated from a *Culex tritaeniorhynchus* mosquito pool. It was characterized as a Japanese encephalitis and West Nile virus cross-reactive arbovirus by complement fixation test, A plaque reduction–neutralization test was performed using hyperimmune sera raised against the plaque-purified arbovirus isolate. The sera did not show reactivity with Japanese encephalitis virus and were weakly reactive with West Nile virus. Complete open reading frame sequence analysis characterized the arbovirus as Bagaza virus (BAGV), with 94.80 % nucleotide identity with African BAGV strain DakAr B209. Sera collected from the encephalitic patients during the acute phase of illness showed 15 % (8/53) positivity for anti-BAGV neutralizing antibodies. This is the first report of the isolation of BAGV from India. The presence of anti-BAGV neutralizing antibodies suggests that the human population has been exposed to BAGV.

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An outbreak of Japanese encephalitis (JE) was reported from the Allapuzza, Thiruvanthapuram and Kottayam districts of Kerala state, India, during 1996. Only 33 % (50/150) of the sera collected from hospitalized cases were confirmed as JE by immunoglobulin M (IgM) ELISA. Other clinical specimens were not available for further investigations. Entomological investigations during the outbreak were carried out and 184 mosquito pools collected from the affected area were processed for isolation in 2-day-old Swiss mice by the intra-cranial route (Rodrigues *et al.*, 1980; George *et al.*, 1984). One pool from *Culex tritaeniorhynchus* showed sickness in inoculated mice. Brains from sick mice were harvested and suspended in 10 % bovalbumin phosphate saline. The suspensions were stored at −70 °C and designated as the arbovirus isolate (96363). The isolate showed cross-reactivity with anti-JE virus (JEV) and anti-West Nile virus (WNV) immune sera in a complement fixation (CF) test (Pavri & Ghosh, 1969; Rodrigues *et al.*, 1980; Damle *et al.*, 1998).

The isolate did not react with immune sera raised against other circulating arboviruses, including Chandipura (*Rhabdoviridae*), Sindbis (*Togaviridae*), Chikungunya (*Togaviridae*), Kyasanur forest disease (*Flaviviridae*), Batai (*Bunyaviridae*) and Dengue (*Flaviviridae*) viruses (Paul *et al.*, 1970; Rodrigues *et al.*, 1980; George *et al.*, 1984).

In this study, we present the genetic characterization of the arbovirus isolate and serological analysis of available sera collected from encephalitis patients during 1996. The Institutional Animal Ethical Committee approved this work and ethical guidelines were strictly followed according to their recommendations. The arbovirus isolate was plaque-purified to rule out the possibility of isolation of both JEV and WNV from the mosquito pool. The mouse brain stock of the arbovirus isolate was passaged twice in porcine stable kidney (PS) cells to amplify the virus. A single plaque was selected from the first PS cell passage and then subjected to two sequential rounds of plaque purification (total of three plaque-to-plaque transfers), followed by amplification in PS cells. The cell culture supernatant from PS cells was clarified by centrifugation at 3220 *g* for 10 min at 4 °C, supplemented with 20 % fetal bovine serum (FBS) and the aliquots were stored at −80 °C and designated as the arbovirus stocks. Generation of the arbovirus virus-specific polyclonal hyperimmune sera,

The GenBank/EMBL/DDBJ accession number of the Indian Bagaza virus isolate sequenced in this paper is EU684972.

A supplementary figure showing the phylogenetic analysis of BAGV based on nucleocapsid, membrane, non-structural (NS) 1, NS2, NS3, NS4 and NS5 gene sequences is available with the online version of this paper.

plaque reduction neutralization test (PRNT) and genetic characterization studies were performed using the PS-amplified arbovirus stocks. Since the CF test characterized the isolate as a JEV and WNV cross-reactive arbovirus, PRNTs were performed to determine the antigenic relationship among these viruses. An *in vitro* neutralization test was carried out using PS-adapted JEV (strain 733913), WNV (strain 804994) and the arbovirus isolate (strain 96363), as described previously (Bondre *et al.*, 2007). The threefold-diluted hyperimmune sera were mixed with 100 p.f.u. of each virus and the infectivity was determined in PS cells. The serum dilution showing 80 % plaque reduction (ND_{80}) was considered as a neutralizing end point. As shown in Table 1, the highest neutralizing activity was observed with homologous sera. In heterologous neutralization between the arbovirus isolate and WNV, both viruses showed cross-reactivity with each other, although this was weaker than the homologous neutralization. The JEV-specific hyperimmune sera did not neutralize the arbovirus isolate, even at a dilution of 1 : 5.

As the CF test characterized the 96363 isolate as a JEV- and WNV-reactive arbovirus and the heterologous neutralization showed that it had weak reactivity with WNV, we genetically characterized the isolate. A 1050 nt fragment from the NS5 region of the sample was amplified by RT-PCR using flavivirus-specific universal primers that amplify the partial NS5 fragment from a number of flaviviruses (Kuno, 1998). The genomic RNA of plaque-purified arbovirus grown in PS cells was isolated using QIAamp viral RNA kit (Qiagen) according to the manufacturer's protocol. The RT-PCR amplification was carried out as described by Kuno *et al.* (1998) and the amplified product was sequenced as described previously (Bondre *et al.*, 2007). BLAST analysis showed 99.90 % nucleotide identity (PNI) with African Bagaza virus (BAGV) strain DakAr B209, followed by 95 PNI with Israel turkey meningoencephalitis virus (ITMV). RT-PCR amplification and complete genome sequencing of BAGV-India was achieved by using overlapping primers designed by aligning available flavivirus sequences from GenBank with CLUSTAL_X 1.83 software (Thompson *et al.*, 1997). RT-PCR amplification of overlapping genomic fragments was carried out as described previously (Bondre *et al.*, 2007). PCR products were column-purified (QIAquick PCR purification kit; Qiagen) and both strands were sequenced by using a Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems) and an automated Sequencer (ABI Prism 310 Genetic Analyzer). A 10281 nt genomic sequence of BAGV-India (GenBank accession no. EU684972) coding a 3426 aa complete open reading frame (ORF) was obtained. Multiple alignments of nucleotide sequences were carried out by using CLUSTAL_X 1.83. The phylogenetic analysis of the complete genome sequence of BAGV-India was assessed by using MEGA (Tamura *et al.*, 2007). For analysis in MEGA, Jukes–Cantor and nucleotide maximum composite likelihood models were utilized, employing the neighbour-joining algorithm. The topologies generated in the neighbour-joining algorithm were confirmed by using the maximum-likelihood method, as implemented in the software Treefinder 2008, with the gamma-distributed rate variation with four rate categories (HKY+γ4) model of nucleotide substitution (Jobb *et al.*, 2004). The reliability of different phylogenetic groupings was evaluated by using the bootstrap test (1000 bootstrap replications). The genetic distance between different viruses was obtained by using the *P*-distance model in MEGA. Phylogenetic trees were constructed by using the complete genomic sequence of the Indian BAGV isolate (this study) and complete genomic sequences (from GenBank) of representative strains from different genomic groups in the *Flaviviridae*. Similarly, phylogenetic analysis of genomic fragments encoding different proteins – nucleocapsid, pre-membrane and membrane, envelope and non-structural (NS) proteins 1–5 – was carried out to understand the relationship between African and Indian BAGV isolates and other flaviviruses.

Comparative analysis of both the Indian and African (AY632545) BAGV complete ORF coding nucleotide sequences showed 94.8 (PNI). The difference of 515 nt (5.2 %) resulted in 77 aa (2.24 %) differences throughout the ORF of Indian and African (DakAr B209) BAGV isolates (Kuno & Chang, 2007). A difference of 20 aa was documented in the structural protein coding region (14 nt in the nucleocapsid with 2 aa differences, 40 nt in the membrane with 13 aa and 73 nt in the envelope with 5 aa), while a difference of 57 aa was documented in the NS protein coding region (71 nt in the NS1 region with 8 aa differences, 50 nt in the NS2 region with 7 aa, 95 nt in the NS3 region with 9 aa, 48 nt in the NS4 region with 19 aa and 119 nt in the NS5 region with 14 aa). Additionally, compared with BAGV-DakAr B209, one deletion (at nt 7424) and four additions (nt 7438–7439, 7444 and 7463) were documented in the NS4B region of BAGV-India.

Phylogenetic analysis using the complete sequence of the Indian BAGV ORF showed that this sequence had a close genetic relationship with the African BAGV-DakAr B209 strain and clustered together with the *Culex* mosquito-transmitted clade on the phylogram (Fig. 1). Similar tree topologies were obtained with both models (Jukes–Cantor and maximum composite likelihood) that were used to construct the complete ORF sequence based on the phylogenetic tree obtained by using the neighbour-joining algorithm. The phylogenetic analysis of individual gene sequences coding for nucleocapsid, membrane, NS1, NS2, NS3 and NS4 showed similar tree topologies, which were comparable with complete genome sequence-based analysis (Supplementary Fig. S1, available in JGV Online). The PNI using nucleocapsid and membrane coding gene sequences of Indian and African BAGV isolates was 96.00±1.25 and 92.30±1.20, respectively. Analysis of the NS proteins NS1, NS2, NS3 and NS4 of both the BAGV isolates showed 94.40±0.70, 95.10±0.60, 95.20±0.50 and 95.80±0.60 PNI, respectively. A number of previous phylogenetic studies on flaviviruses mostly attempted to use envelope coding sequences. We also determined the genetic relationship of BAGV-India using the additional envelope sequences of representative members from different *Flaviviridae* groups. In envelope sequence-based analysis, BAGV-India grouped together with the African DakAr B209 strain (95.90±0.80 PNI) along with other members of the Ntaya virus group of the *Flaviviridae* (Fig. 2). Envelope sequence analysis of the African BAGV strain (AF372407; Gaunt *et al.*, 2001) showed that it had a closer relationship (99.00±0.40 PNI) with DakAr B209 strain than BAGV-India (94.80±1.70 PNI). Among other members of the Ntaya virus group, ITMV showed a close relationship (93.40–95.50 PNI) with all three BAGV strains, followed by a more distant relationship with Ntaya virus (76.00–77.00 PNI) and Tembusu virus (74.00–75.00 PNI). As partial NS5 sequences from additional

Table 1. Homologous and heterologous cross-neutralization test using hyperimmune sera against JEV, WNV and arbovirus (BAGV) isolates

Serum giving 80 % plaque reduction was considered to be at the neutralizing end point. ND_{80} values are given.

Virus strain	Hyperimmune sera against:		
	JEV (733913)	WNV (804994)	BAGV (96363)
JEV	501	5	<5
WNV	<5	239	31
BAGV	<5	21	67

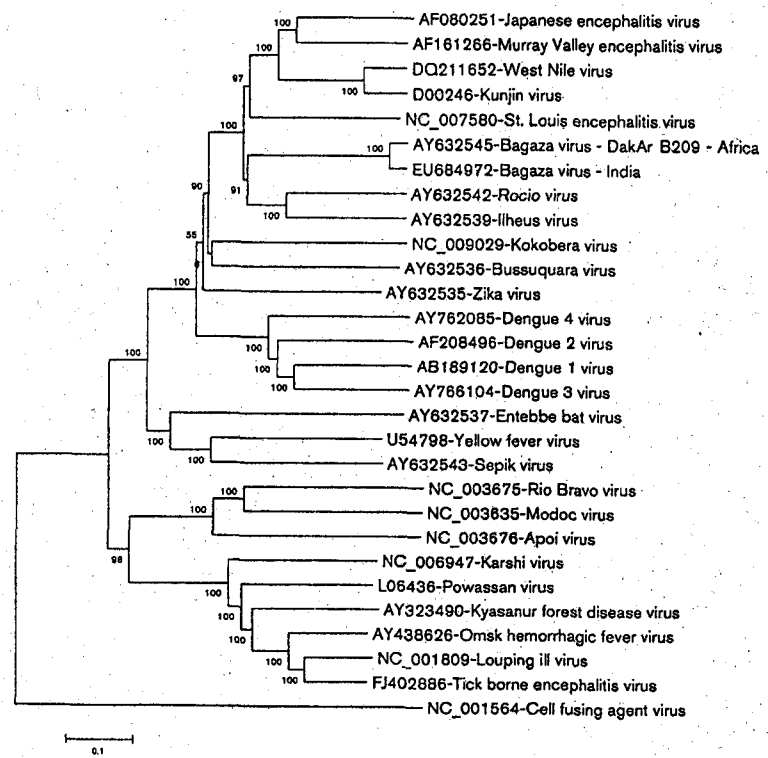

Fig. 1. Phylogenetic analysis of the BAGV complete ORF sequence using the nucleotide maximum composite likelihood model of the neighbour-joining algorithm. Cell fusing agent virus was used as an outgroup in phylogenetic analysis. GenBank accession numbers are given on the figure. Numbers at the nodes indicate bootstrap support for each node. Bar, nt substitutions per site.

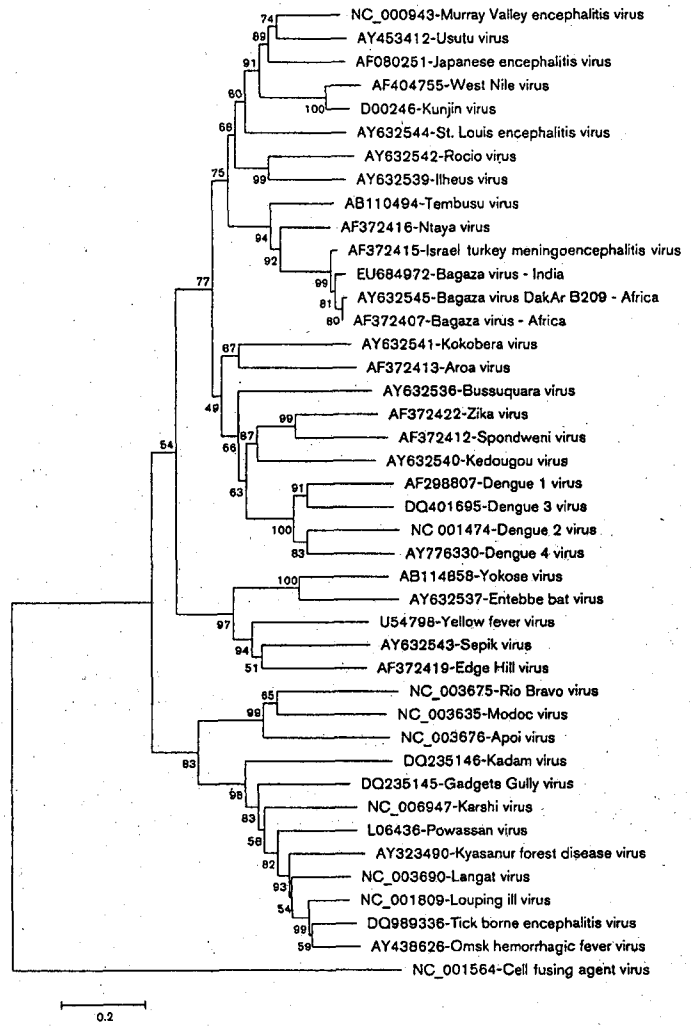

Fig. 2. Phylogenetic analysis of BAGV based on partial envelope sequences. The tree was constructed by using MEGA, by the neighbour-joining with nucleotide maximum composite likelihood model. Bootstrap confidence level (1000 replicates) and a confidence probability value based on the standard error test were calculated using MEGA and are indicated at the nodes. Partial envelope sequences of additional viruses (where complete genome sequences were not available) were used in the phylogenetic analysis. Cell fusing agent virus was used as an outgroup in phylogenetic analysis. GenBank accession numbers are given on the figure. Numbers at the nodes indicate bootstrap support for each node. Bar, nt substitutions per site.

members of the *Flaviviridae* were available in GenBank, we performed separate analysis to determine the genetic relationship of BAGV-India with these viruses (data not shown). With NS5 analysis, both the BAGV sequences grouped together, with 99.90±0.10 PNI, in the Ntaya virus group. However, in NS5 sequence analysis, the nucleotide identities of BAGV and other members of the Ntaya virus group were comparable with envelope sequence analysis. BAGV DakAr B209 and Indian strains showed 95.20–95.30 PNI with ITMV, 76.50–76.60 PNI with Ntaya virus and 75.10–75.30 PNI with Tembusu virus.

We documented one nucleotide insertion and four nucleotide deletions in the complete ORF sequence of Indian and African BAGV strains. The envelope sequence analysis of an additional BAGV strain from Africa indicates a closer genetic relationship with BAGV DakAr B209 than the Indian BAGV strain. These data indicate independent circulation of both the African and Indian isolates in different geographical areas. Although the time and mode of introduction of BAGV in India is unknown, we hypothesize that it may represent a genetic variant of the BAGV strain which originated in the African continent and was dispersed and established in areas with similar climatic conditions and favouring vector multiplication. Dispersal of the flaviviruses from the Old World to the New World and the co-existence of related viruses sharing antigenic, host and vector similarities have been supported by molecular phylogenetic analyses (Sabin, 1959; Gaunt *et al.*, 2001; Chevalier *et al.*, 2004; Mackenzie *et al.*, 2004; Petersen & Marfin, 2005; Gould *et al.*, 2006). However, to determine the precise genetic relationship, geographical origin and epidemiology, full genome sequence data of more strains will be helpful.

We isolated BAGV from a mosquito pool collected during a JE outbreak and studied its genetic relationship with other *Flaviviridae*. Since it was characterized as a JEV and WNV cross-reactive arbovirus (CF test), we determined the antigenic relationship with JEV and WNV by PRNT. Although the heterologous neutralization differentiated these as three distinct arboviruses, we documented weak cross-reactivity between WNV and BAGV (Table 1). The genetic relatedness of BAGV and WNV in several genomic regions might be the reason for antigenic cross-reactivity between these viruses (Kuno & Chang, 2007). We determined the previous exposure of hospitalized encephalitis patients with BAGV by analysing the sera stored at −80 °C for anti-BAGV neutralizing antibodies. The neutralization assay was performed with PS cell-adapted BAGV pools, as described previously (Bondre, *et al.*, 2007; Sapkal *et al.*, 2007). Only 15 % (8/53) of available sera showed reactivity with BAGV, while 24.14 % (14/53) were reactive with JEV (733913). Both the anti-JEV and anti-BAGV neutralizing antibody titres (ND_{80}) were in the range of 50–1250. All of the BAGV reactive sera were negative for JEV by IgM ELISA.

Recently, BAGV has been identified as one of the emerging and re-emerging human pathogens that causes febrile illness in humans (Woolhouse *et al.*, 2006). It belongs to the Ntaya group of *Flaviviridae* and has been isolated in the Central African Republic, Cameroon and Senegal, where it circulates between ornithophagic mosquitoes and birds (Digoutte, 1978; Traore-Lamizana *et al.*, 1994; Diallo *et al.*, 2005). It is genetically related to ITMV, which is a serious avian pathogen in the Middle East and southern Africa (Digoutte, 1978; Kuno *et al.*, 1998). The phylogenetic studies using envelope and NS5 sequences clearly suggest that there is a close genetic relationship between ITMV and BAGV. Other members of the Ntaya virus group are genetically distinct from BAGV and ITMV. Our preliminary findings on sera collected during the acute phase of illness from hospitalized patients indicates the presence of anti-BAGV neutralizing antibodies. This suggests that BAGV might be circulating in the area between ornithophagic mosquitoes and birds and incidentally the human population might be exposed to it. These observations need to be strengthened by investigating additional human clinical specimens from the region. However, our preliminary observations need to be confirmed by systematic study of the human population from the Allapuzza, Thiruvanthapuram and Kottayam districts of Kerala to understand the association of BAGV with human infections.

In conclusion, this study indicates the necessity of serious efforts to investigate the likely involvement of BAGV in sporadic human infections and outbreaks in other vertebrates occurring in the region. This can be achieved by developing BAGV-specific serological and molecular diagnostics for testing of human clinical specimens collected from the region. Additional studies addressing the potential of various mosquito species as vectors and birds as amplifying hosts, and sero-surveillance in domestic animals and the human population will add to our understanding of the epidemiology of arboviral diseases.

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References

Bondre, V. P., Jadi, R. S., Mishra, A. C., Yergolkar, P. N. & Arankalle, V. A. (2007). West Nile virus isolates from India: evidence for a distinct genetic lineage. *J Gen Virol* **88**, 875–884.

Chevalier, V., de la Rocque, S., Baldet, T., Vial, L. & Roger, F. (2004). Epidemiological processes involved in the emergence of vector-borne diseases: West Nile fever, Rift Valley fever, Japanese encephalitis and Crimean–Congo haemorrhagic fever. *Rev Sci Tech* **23**, 535–555.

Damle, R. G., Yeolekar, L. R. & Rao, B. L. (1998). Strain analysis and epitope mapping of West Nile virus using monoclonal antibodies. *Acta Virol* **42**, 389–395.

Diallo, M., Nabeth, P., Ba, K., Sall, A. A., Ba, Y., Mondo, M., Girault, L., Abdalahi, M. O. & Mathiot, C. (2005). Mosquito vectors of the 1998–

1999 outbreak of Rift Valley Fever and other arboviruses (Bagaza, Sanar, Wesselsbron and West Nile) in Mauritania and Senegal. *Med Vet Entomol* 19, 119–126.

Digoutte, J. P. (1978). *Bagaza (BAG) strain: Dak Ar B 209. Am J Trop Med Hyg* 27, 376–377.

Gaunt, M. W., Sall, A. A., de Lamballerie, X., Falconar, A. K., Dzhivanian, T. I. & Gould, E. A. (2001). Phylogenetic relationships of flaviviruses correlate with their epidemiology, disease association and biogeography. *J Gen Virol* 82, 1867–1876.

George, S., Gourie-Devi, M., Rao, J. A., Prasad, S. R. & Pavri, K. M. (1984). Isolation of West Nile virus from the brains of children who had died of encephalitis. *Bull World Health Organ* 62, 879–882.

Gould, E. A., Higgs, S., Buckley, A. & Gritsun, T. S. (2006). Potential arbovirus emergence and implications for the United Kingdom. *Emerg Infect Dis* 12, 549–555.

Jobb, G., von Haeseler, A. & Strimmer, K. (2004). TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evol Biol* 4, 18.

Kuno, G. (1998). Universal diagnostic RT-PCR protocol for arboviruses. *J Virol Methods* 72, 27–41.

Kuno, G. & Chang, G. J. (2007). Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol* 152, 687–696.

Kuno, G., Chang, G.-J. J., Tsuchiya, K. R., Karabatsos, N. & Cropp, B. C. (1998). Phylogeny of the genus *Flavivirus*. *J Virol* 72, 73–83.

Mackenzie, J. S., Gubler, D. J. & Petersen, L. R. (2004). Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med* 10, S98–S109.

Paul, S. D., Narasimha Murthy, D. P. & Das, M. (1970). Isolation of West Nile virus from a human case of febrile illness. *Indian J Med Res* 58, 1177–1179.

Pavri, K. M. & Ghosh, S. N. (1969). Complement-fixation tests for simultaneous isolation and identification of Dengue viruses, using tissue cultures. *Bull World Health Organ* 40, 984–986.

Petersen, L. R. & Marfin, A. A. (2005). Shifting epidemiology of *Flaviviridae*. *J Travel Med* 12, S3–S11.

Rodrigues, F. M., Bright Singh, P., Dandawate, C. N., Soman, R. S., Guttikar, S. N. & Kaul, H. N. (1980). Isolation of Japanese encephalitis and West Nile viruses from mosquitoes collected in Andhra Pradesh. *Indian J Parasitol* 4, 149–153.

Sabin, A. B. (1959). Survey of knowledge and problems in field of arthropod-borne virus infections. *Arch Gesamte Virusforsch* 9, 1–10.

Sapkal, G. N., Wairagkar, N. S., Ayachit, V. M., Bondre, V. P. & Gore, M. M. (2007). Detection and isolation of Japanese encephalitis virus from blood clots collected during the acute phase of infection. *Am J Trop Med Hyg* 77, 1139–1145.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24, 1596–1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25, 4876–4882.

Traore-Lamizana, M., Zeller, H. G., Mondo, M., Hervy, J. P. & Digoutte, A. J. (1994). Isolations of West Nile and Bagaza viruses from mosquitoes (*Diptera: Culicidae*) in central Senegal (Ferlo). *J Med Entomol* 31, 934–938.

Woolhouse, M. E. J., Gowtage-Sequeria, S. & Evans, B. (2006). Ti6: quantitative analysis of the characteristics of emerging and re-emerging human pathogens. Available at: www.foresight.gov.uk. Accessed 19 November 2007.

別紙様式第2-1

No. 22

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. 10. 7	該当なし	
一般的名称	人赤血球濃厚液		研究報告の公表状況	Planitzer CB, Modrof J, Yu MY, Krejl TR. Emerg Infect Dis. 2009 Oct. Available from http://www.cdc.gov/EID/content/15/10/1668.htm	公表国 米国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)					

研究報告の概要

○血液および血漿供血者の血漿中のウエストナイルウイルス感染(アメリカ合衆国)

2003～2008年に供給された米国の血漿由来静注用免疫グロブリン製剤(IGIV)中のウエストナイルウイルス(WNV)中和抗体価と最近の感染との関連を検討した。抗体価は供血者のWNV既感染率と密接に相関し、2008年ロットから既感染率は1%と推定された。

血漿由来IGIV製剤と、NATによる感染確定後の供血者由来血漿検体の中和抗体価を、古典的マイクロ中和試験により測定した。供血血液のWNVスクリーニング結果から1999年から2008年の各年における平均WNV感染数を算出した。米国疾病対策センターに報告された神経侵襲性症例数から推定し、その年の累積感染率を求めた。

IGIVのWNV中和抗体価は2003年から急速に増加し始めた。WNVスクリーニング結果から、2003年までに米国の人口の0.5%がWNVに感染したと推定された。米国の人口における既感染者の推定数は、IGIVの抗体価と平行して増加していた。2008年に出荷されたロットでは、中和抗体価は2.8～69.8、平均±SEMは21±1(n=256)であった。NATでWNV感染が確定した人から得られた血漿ではさらに抗体価が高く、検査した30名で平均±SEMは208±40となった。また、これらの結果から、米国の人口の1%が既にWNVに感染したと推定された。

米国の血漿由来IGIV製剤中の中和抗体価は上昇しており、特にWNV既感染供血者の抗体価が高いことから、WNVの予防や治療を目的としたIGIV製剤製造の可能性が示唆される。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2003～2008年に供給された米国の血漿由来静注用免疫グロブリン製剤中のウエストナイルウイルス(WNV)中和抗体価と最近の感染との関連を検討したところ、抗体価は供血者のWNV既感染率と密接に相関し、2008年ロットから既感染率は1%と推定されたとの報告である。	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、ウエストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき緊急対応の準備を進めているほか、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して対応について検討している。今後も引き続き情報の収集に努める。

(7)

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West Nile Virus Infection in Plasma of Blood and Plasma Donors, United States

Christina B. Planitzer, Jens Modrof, Mei-ying W. Yu, and Thomas R. Kreil

This study investigated the association of ongoing West Nile virus (WNV) infections with neutralizing antibody titers in US plasma-derived intravenous immune globulin released during 2003–2008. Titers correlated closely with the prevalence of past WNV infection in blood donors, with 2008 lots indicating a prevalence of 1%.

West Nile virus (WNV) is a flavivirus endemic to the United States; typically, hundreds of clinical cases of infection occur each year. The observed number of clinical WNV infections as collated by ArboNET (www.cdc.gov) and the incidence of asymptomatic WNV infections as shown by nucleic acid testing (NAT) of the US blood supply (*1*) indicate that ≈3 million WNV infections occurred in humans during 1999–2008.

Because the immune system elicits WNV neutralizing antibodies in response to WNV infection, detectable levels of WNV neutralizing antibodies in the blood of persons with previous WNV infection is expected. Consequently, lots of immune globulin-intravenous (human) (IGIV) manufactured from plasma collected in the United States contain WNV neutralizing antibodies (*2*). Those IGIV lots, each prepared from several thousand plasma donations to ensure a broad spectrum of antibodies, can be used as an epidemiologic tool that enables the surveillance of thousands of persons in a community through analysis of comparatively few samples. In this study, we demonstrated the increasing trend of WNV-neutralizing antibody titers in lots of IGIV.

Comparing these titers with those of persons with confirmed past WNV infection provides an independent measure of the percentage of the US population previously infected with WNV. Several WNV vaccine trials are ongoing or imminent, so information about the prevalence of past WNV infection in the United States is valuable for planning the demonstration of vaccine efficacy. Low incidence and lack of highly WNV-endemic areas in the United States preclude classic vaccine field trials because of study size requirements and cost-logistics difficulties.

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The Study

The WNV neutralization titers of several US plasma–derived IGIV products (Gammagard Liquid/KIOVIG; Gammagard S/D/ Polygam S/D; Iveegam EN [Baxter Healthcare Corporation, Westlake Village, CA, USA]) and plasma samples obtained from US blood donors after a NAT-confirmed WNV infection were determined by an infectivity assay as earlier described (*2*), adapted to a classical microneutralization format (*3*). WNV neutralization titers (i.e., the reciprocal dilution of a 1:2 series resulting in 50% neutralization [NT_{50}; detection limits <0.8 for undiluted IGIVs and <7.7 for 1:10 prediluted serum]) are reported as the mean ± SEM. An unpaired *t* test was used to evaluate whether titer differences between 2 groups were statistically significant.

Using an extrapolation derived from screening the US blood supply for WNV (*1*), we calculated the average annual number of WNV infections in the United States for 1999–2008. The total number of neuroinvasive cases reported for those years to the US Centers for Disease Control and Prevention (CDC) through ArboNET was multiplied by 256 (i.e., the factor between all WNV infections and neuroinvasive cases). The cumulative infection rate for each year during 1999–2008 was then calculated by dividing the infections occurring up to a specific year by the US population for that year (determined by US Census Bureau estimates [www.census.gov/popest/states/NST-ann-est.html]).

Although WNV was first introduced into the United States in 1999, only in 2003 did the mean WNV neutralization titers of IGIV lots released to the market start to increase markedly (Figure 1). According to extrapolations from the WNV screening of the US blood supply (*1*), by 2003, an estimated 0.5% of the US population had been infected with WNV, although most infections were asymptomatic.

A delay of ≈1 year occurs between the collection of plasma and the release of IGIV lots to the market; thus, the WNV-positive IGIV lots in 2003 reflect the larger number of WNV infections occurring in 2002. Using the same extrapolations from the US blood supply (*1*), we found that the ≈0.1% annual increments in the proportion of the US population with past WNV infection follow a straight line ($r^2 = 0.9996$), generally paralleled by the mean WNV neutralization titers of IGIV lots. During 2005–2008, when large numbers of lots of a single IGIV product (Gammagard Liquid) could be analyzed, the WNV neutralization titer increased by 3.6 per year ($r^2 = 0.9793$).

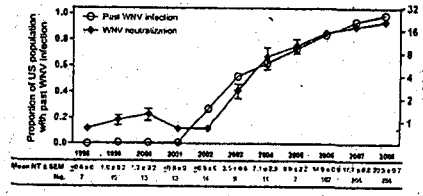


Figure 1. West Nile virus (WNV) neutralization titers of US plasma-derived immune globulin intravenous (human) (IGIV) lots by year of production and estimated percentage of the US population with past WNV infection by year. WNV neutralization titers were determined either for retention or lot release samples of 3 IGIV products produced during 1998–2005 or for a considerable proportion of Gammagard Liquid/KIOVIG lots produced during 2006–2008. Results are shown as mean ± SEM (limit of detection <0.8) by year of product release. For 5% of IGIV samples, titers were multiplied by 2 for comparison with the 10% IGIV samples at equivalent immunoglobulin concentrations. The percentage of the US donor population with past WNV infection was calculated from the number of neuroinvasive cases reported per year and the estimated ratio of neuroinvasive cases to total cases of WNV infection.

US plasma-derived IGIV lots released during 2008 showed variable WNV neutralization titers ranging from 2.8 to 69.8; mean ± SEM titer was 21 ± 1 (n = 256) (Figure 2). Compared with titers shown to be protective in an animal model of WNV infection (equivalent to >21 by the current assay) (*2*), ≈40% of the 2008 IGIV lots had higher titers.

Plasma obtained from persons with NAT-confirmed WNV infection had even higher titers; mean ± SEM titer was 208 ± 40 for 30 persons available for testing. When results were corrected for the immunoglobulin (Ig) G concentration in plasma (≈1%), compared with the 10% IGIV preparations, the mean neutralization titer of the plasma samples was ≈100× higher than that of the IGIV lots tested (2,080 vs. 21).

Conclusions

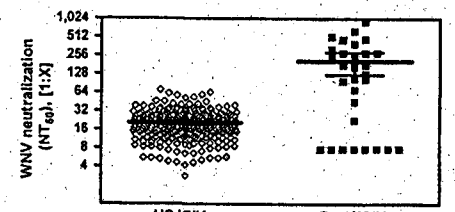
The most comprehensive collation of information about the incidence of WNV infection in the United States is available from ArboNET. When that information is combined with information obtained from the nationwide screening of the blood supply for WNV RNA by NAT (*1,4,5*), the current prevalence of past WNV in the US population is estimated to be ≈1%.

Busch et al. has noted that large-scale, community-based serologic surveys are hardly feasible because of their expense and because WNV ELISA assays are possibly biased by cross-reactions with other flaviviruses (*1*). Nevertheless, 7 seroepidemiologic studies have been performed (*6–12*). Cumulatively, 5,503 persons were tested for WNV infection by ELISA, and the results have shown highly divergent seroprevalence rates ranging between 1.9% (*6*) and 14.0% (*10*).

The use of IGIV lots, each representing the serostatus of several thousand donors in 1 sample, makes seroepidemiology practical (*13*) because it allows a large donor population to be surveyed by analyzing comparably few samples. The use of a more complex yet functional virus neutralization assay minimizes concerns about cross-reactivity with flaviviruses of other serocomplexes (e.g., dengue virus) that occasionally circulate in the US population. Also, epidemiologic considerations render interference by St. Louis encephalitis virus, a flavivirus within the same serocomplex, highly unlikely (*2*). The specificity of the neutralization assay was confirmed by testing IGIV lots manufactured from European-derived plasma against tick-borne encephalitis virus, a flavivirus closely related to WNV and circulating in Europe. Although these lots contained high neutralization titers against tick-borne encephalitis virus, only 1 of 20 had a detectable neutralization titer of 5 against WNV (unpub. data).

In this study, we determined that the mean titer of samples obtained during 2003–2008 from persons with a confirmed diagnosis of WNV infection was 100× higher than the mean titers of IGIV lots produced in 2008. This determination provides an independent experimental measure of the frequency of past WNV infection in the general US population, as reflected by the plasma/blood donor community, and the results correlate well with results of previously published theoretical extrapolations (*1*), which estimated that ≈1% of the population has already been infected with WNV.

The increasing levels of WNV neutralizing antibodies in IGIV lots from US plasma and the particularly high



	US IGIV	Past WNV
Mean ± SEM	21 ± 1	208 ± 40
No.	256	30

Figure 2. West Nile virus (WNV) neutralization by US plasma-derived immune globulin intravenous (human) (IGIV) released in 2008 and plasma from donors with past WNV infection (past WNV), confirmed by nucleic acid testing. WNV neutralization titers are shown as the mean ± SEM (limit of detection <0.8 for undiluted IGIVs and <7.7 for prediluted sera). NT_{50}, 50% neutralization titer.

titers in donors who have had a WNV infection suggest the possibility of preparing IGIV products with sufficiently high titers to be useful for WNV prophylaxis or treatment. Several ongoing or imminent WNV vaccine clinical trials stress the practical value of an independent confirmation of extrapolations that estimate the percentage of the US population with past WNV infection. Knowing the percentage of preexisting WNV seroprevalence as well as estimates of the mostly asymptomatic incidence rates (*14*) can be of vital importance in designing vaccine trials.

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Ms Planitzer is writing her PhD thesis on virus antibodies in immune globulins at the Global Pathogen Safety Group of Baxter BioScience in Vienna, Austria, in collaboration with the Medical University of Vienna, Austria. Her research focuses on determining functional antiviral properties of immunoglobulin preparations.

References

1. Busch MP, Wright DJ, Custer B, Tobler LH, Stramer SL, Kleinman SH, et al. West Nile virus infections projected from blood donor screening data, United States, 2003. Emerg Infect Dis. 2006;12:395–402.
2. Planitzer CB, Modrof J, Kreil TR. West Nile virus neutralization by US plasma-derived immunoglobulin products. J Infect Dis. 2007;196:435–40. DOI: 10.1086/519392
3. Ehrlich HJ, Muller M, Oh HM, Tambyah PA, Joukhadar C, Montomoli E, et al. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. N Engl J Med. 2008;358:2573–84. DOI: 10.1056/NEJMoa073121
4. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, et al. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med. 2005;353:460–7. DOI: 10.1056/NEJMoa044029
5. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med. 2005;353:451–9. DOI: 10.1056/NEJMoa044333
6. Mandalakas AM, Kippes C, Sedransk J, Kile JR, Garg A, McLeod J, et al. West Nile virus epidemic, northeast Ohio, 2002. Emerg Infect Dis. 2005;11:1774–7.
7. Meyer TE, Bull LM, Cain HK, Pascua RF, Travassos da Rosa A, Gutierrez CR, et al. West Nile virus infection among the homeless, Houston, Texas. Emerg Infect Dis. 2007;13:1500–3. PMID: 18257995
8. Schweitzer BK, Kramer WL, Sambol AR, Meza JL, Hinrichs SH, Iwen PC. Geographic factors contributing to a high seroprevalence of West Nile virus–specific antibodies in humans following an epidemic. Clin Vaccine Immunol. 2006;13:314–8. DOI: 10.1128/CVI.13.3.314-318.2006
9. Schellenberg TL, Anderson ME, Drebot MA, Vooght MT, Findlater AR, Curry PS, et al. Seroprevalence of West Nile virus in Saskatchewan's Five Hills Health Region, 2003. Can J Public Health. 2006;97:369–73.
10. Murphy TD, Grandpre J, Novick SL, Seys SA, Harris RW, Musgrave K. West Nile virus infection among health-fair participants, Wyoming 2003: assessment of symptoms and risk factors. Vector Borne Zoonotic Dis. 2005;5:246–51. DOI: 10.1089/vbz.2005.5.246
11. Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet. 2001;358:261–4. DOI: 10.1016/S0140-6736(01)05480-0
12. Michaels SR, Balsama GA, Kukreja M, Anderson C, Straif-Bourgeois S, Talati G, et al. Surveillance for West Nile virus cases in Louisiana 2001–2004. J La State Med Soc. 2005;157:269–72.
13. Audet S, Virata-Theimer ML, Beeler JA, Scott DE, Frazier DJ, Mikolajczyk MG, et al. Measles-virus-neutralizing antibodies in intravenous immunoglobulins. J Infect Dis. 2006;194:781–9. DOI: 10.1086/506363
14. Samuel MA, Diamond MS. Pathogenesis of West Nile virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J Virol. 2006;80:9349–60. DOI: 10.1128/JVI.01122-06

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別紙様式第 2-1
番号 14

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日		第一報入手日 2009年11月17日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人ハプトグロビン						
販売名（企業名）	ハプトグロビン静注 2000 単位 「ベネシス」（ベネシス）			研究報告の公表状況	・ FDA／Vaccines, Blood & Biologics/2009/11/6	公表国 アメリカ	

研究報告の概要

このガイダンスは、輸血によるウエストナイルウイルス(WNV)伝播の可能性を最小化するために、血液採取施設が講ずる措置についての推奨である。示された全血及び血液成分のドネーションのための推奨内容は以下の通りである。

A. 検査、ユニットの管理及びドナー管理

1. 輸注を意図して採取された全血および血液成分のドナーサンプルについて、WNV のスクリーニングを認可された NAT で 1 年を通じて行うことを推奨する。
2. 血液採取・取扱施設がミニプール NAT(MP-NAT)を用いてスクリーニングを行っているのならば、その施設は、陰性であったミニプールを構成している各試験サンプルのもとのユニット全てを、それらが WNV 以外の点について出荷可とすることが適切であるのならば、出荷することができる。FDA は、血液採取・取扱施設が NAT で陽性を示したミニプールを、それを構成する各検体に戻って個別 NAT(ID-NAT)を用いて検査し、そのミニプールが陽性となる原因となったユニット(単数または複数)を同定することを推奨する。

B. MP-NAT から ID-NAT への切替え

1. 当該施設が採取を行う地域内で「WNV の活動性が高い」ということを定義する判断基準を設定し、バリデートすること。
2. 当該施設が採取を行う地域で「WNV の活動性が高い」際に MP-NAT から ID-NAT へとスイッチするため、およびその地域での「WNV の活動性が高い」状態が収まったときに MP-NAT へと戻すための閾値を定めること。
3. MP-NAT から ID-NAT へのスイッチは可能な限り早期に行うべきだが、定めた閾値に達してから 48 時間以内に行うこと。
4. このような決定のプロセスについて SOP(標準作業手順書)を制定し、それに従うこと。

C. 検査実施報告書

1. 血液採取・取扱施設が認可を得ている施設であって、かつ、血液製剤の感染症検査を行うことがすでに FDA によって承認された施設では、認可を得ている WNV NAT 検査を製造者の使用説明書に従って当該施設で用いることができ、その場合には 21 CFR 601.12(d)に従って、検査法の変更について、その施設の FDA への年次報告中に記載して FDA に知らせなければならない。
2. 血液採取・取扱施設が認可を得ている施設であって WNV の NAT 検査を行うために新たな契約ラボを利用する場合であって、かつ、そのラボがすでに血液製剤の感染症検査を行っている場合には、その血液採取・取扱施設はその変更について FDA に報告しなければならず、またそのことは 21 CFR 601.12(c)(1)および(5)に従って"Supplement–Changes Being Effected"の申請を行うことによって報告しても良い。

D. 輸血を目的とした全血及び血液成分の表示

21 CFR 606.122(h)は、輸注を意図した血液製剤用の使用説明書("Circular of Information")としても知られている)には、安全でかつ有効な使用のために必要であれば、実施した検査名と結果を全て含めることを求めている。この 21 CFR 606.122(h)に準拠するために、WNV の NAT として認可を受けた検査を実施するに際しては、認可を受けた血液採取・取扱施設、認可を受けていない施設のどちらでも、そのような使用説明書を改訂して、WNV についての NAT 検査が陰性であったとの結果を含めるようにしなければならない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意

(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び 5 過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。