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在日ラテンアメリカ人の慢性シャーガス病キャリアーと2次感染予防

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我が国の在日ラテンアメリカ人は既に40万人に達する勢いで増加している。そのうちブラジルからの滞在者が80%を占めており、その8万人が既に定住永住権を取得している。こうした中で、南米特有の風土病シャーガス病患者も散見されるようになった。近年各地医療機関から依頼のあった心疾患患者41名についてシャーガス病病原体*Trypanosoma cruzi (T.cruzi)* 血清抗体検査を行った。その結果15名 (36.58%) が明らかに陽性と判定され、シャーガス病が示唆された。更に、抗体陽性者について血液を材料にしたPCRを行った結果4名に*T.cruzi*－DNA産物を検出した。病原体の血液内生残が強く示唆されたので、更に血液培養を試みた結果2名 (抗体陽性者の13.3%) から*T.cruzi*虫体を分離することが出来た。即ち慢性の病原体キャリアーが日本に現存することが明らかとなった。ECGでは不整脈、心エコーで拡張型心筋症を示した。ブラジル、ボリビアの生活歴がある者に関しては、我が国では臨床経験の少ないシャーガス病感染を検討すべきである。

一方、消化器系の症状を訴える患者の検査依頼は皆無であったが、心室拡張症で通院している同一患者は消化器症状 (飲み込み困難、排便困難) をも訴えているものの、検査を受けていない。

本疾患の特徴は感染者の70%は病型が定まらない慢性感染で、一見健常者とみえることである。本人、家族もその感染を認知するものは少ない。

媒介昆虫の存在しない日本国内で感染が起こるとすれば、それは輸血感染、臓器移植による2次感染であると思われる。肝要な点は、事前の抗体チェックでこのような2次感染が防げることである。ラテンアメリカ人の多くを抱える地方自治体は健康保健支援環境を整備し、シャーガス病の2次感染を阻止すべく啓蒙監視活動を強化すべきであり、全国的に行われている善意の献血現場で抗体スクリーニングを実施すべく、体制の整備を行う必要がある。

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遷延する関節痛を主訴に来院したチクングニヤ熱の3例

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チクングニヤ熱(Chikungunya fever; CHIKF) は発熱、関節炎、発疹を主症状とする熱性疾患であり、臨床症状や検査所見はデング熱に類似するが、遷延する関節症状が特徴的である。本年になり東南アジア地域を中心に再びCHIKF流行が拡大しており、当センターにおいても2009年5月から6月にかけての2ヶ月間で、東南アジアから帰国後に遷延する関節痛を主訴に来院した3例を血清学的にCHIKFと診断したのでその概要を報告する。(症例1) 52歳日本人男性。2009年3月26日から4月5日までインドネシア・スマトラ島へ蝶の採集目的で滞在。3月31日に39.5度の発熱、関節痛 (両手足首、両膝) が出現。翌日には解熱したものの関節痛は持続したため、帰国後5月上旬に近医整形外科受診。関節リウマチ、痛風検査を実施されるも陰性であったため、精査目的で当センターを受診した。(症例2) 30歳日本人男性。2009年4月16日より6月14日までインドネシア・ジャワ島へ舞台公演目的で滞在。5月13日に発熱、関節痛 (右足首、左膝、右肩)、頭痛、発疹が出現。4日後に解熱したものの関節痛は持続したため6月22日に当センターを受診した。(症例3) 39歳日本人女性。2009年4月4日より6月28日までマレーシア・クアラルンプール郊外に帯同家族として滞在。5月12日に39.5度の発熱、関節痛 (両手足首)、発疹、歯肉炎が出現。現地の病院で膠原病スクリーニング等の精査を受け、異常所見は認められなかったものの関節痛が持続するため、6月30日に当センターを受診した。いずれの症例も来院時の検査でチクングニヤウイルスIgM抗体及び中和抗体陽性であり、血清学的にCHIKFと確定診断した。流行地から帰国した後、遷延する関節症状を訴える患者を診療する場合には、リウマチ性疾患との鑑別の上でもCHIKFの可能性を考慮に入れた正確な血清診断を行うべきである。

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	Holzmann H, Aberle SW, Stiasny K, Werner P, Mischak A, Zainer B, et al. Emerg Infect Dis. 2009 Oct. Available from http://www.cdc.gov/EID/content/15/10/1672.htm	公表国 オーストリア
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

⊙オーストリア山岳地域におけるヤギ・チーズ摂食によるダニ媒介性脳炎
オーストリアの海抜1,500m域の山岳地域において、2008年7月に、ヒト6名とブタ4頭に非殺菌ヤギ乳を介して伝播したダニ媒介性脳炎ウイルス(TBEV)を報告する。
初発患者は43歳の羊飼いで、高山牧草地に24日間滞在した後、非細菌性尿道炎とインフルエンザ様症状で入院し髄膜炎の臨床症状を発症した。ELISAによって血清学的にTBEV感染陽性と確認された。患者はダニに咬まれた記憶はなかったが、発症8～11日前に非殺菌ヤギ乳及び牛乳から製造された自家製チーズを食べていた。さらに調査したところ、同じチーズを食べた6名中5名が血清学的にTBEV感染と診断された。
チーズ原料の乳を搾ったヤギはHI及び中和抗体検査でTBEV陽性と確認された。ウシ3頭は抗体陰性であり、問題のチーズは検査用に入手できなかった。牧草地で飼育されていたブタ4頭がホエイとヤギ乳を与えられており、TBEV抗体陽性となったが、臨床症状は見られなかった。
このアウトブレイクは、中央ヨーロッパ高地におけるTBEVの新興と、TBEVの経口感染の高い効率性を示している。感染した6名については、ワクチンを接種していれば感染を予防できただろう。

報告企業の意見	今後の対応
オーストリア山岳地域において、ヒト6名とブタ4頭に非殺菌ヤギ乳を介してダニ媒介性脳炎ウイルスが伝播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。

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

④

Tick-borne Encephalitis from Eating Goat Cheese in a Mountain Region of Austria

Heidemarie Holzmann, Stephan W. Aberle, Karin Stiasny, Philipp Werner, Andreas Mischak, Bernhard Zainer, Markus Netzer, Stefan Koppi, Elmar Bechter, and Franz X. Heinz

We report transmission of tick-borne encephalitis virus (TBEV) in July 2008 through nonpasteurized goat milk to 6 humans and 4 domestic pigs in an alpine pasture 1,500 m above sea level. This outbreak indicates the emergence of ticks and TBEV at increasing altitudes in central Europe and the efficiency of oral transmission of TBEV.

Tick-borne encephalitis virus (TBEV) is a human pathogenic flavivirus that is endemic to many European countries and to parts of central and eastern Asia (*1*). Even though vaccination can effectively prevent TBE (*2*), >10,000 cases are reported annually for hospitalized persons in areas of Europe and Asia to which TBE is endemic. TBEV occurs in natural foci characterized by ecologic habitats favorable for ticks, especially in wooded areas within the 7°C isotherm (*3*). The major route of virus transmission is tick bites, but TBEV also can be transmitted during consumption of nonpasteurized milk and milk products from infected animals, primarily goats (*3*). Outbreaks resulting from oral virus transmission are rare in central Europe but more common in eastern Europe and the Baltic states (*3*). Our investigation of TBEV transmitted by milk from a goat in an alpine pasture in a mountainous region provides evidence for a changing TBEV epidemiology in central Europe and the expansion of ticks and TBEV to higher regions.

The Study

We investigated a TBE outbreak, comprising 6 cases, in a mountain region in western Austria in July 2008. The index case occurred in a 43-year-old shepherd who had stayed for 24 days at his alpine pasture (1,564 m above sea level) before he was hospitalized for nonbacterial urethritis and nonspecific influenza-like symptoms (including pain in the lower abdomen and legs), followed by clinical signs of meningitis. TBEV infection was confirmed serologically by ELISA demonstration of specific immunoglobulin (Ig) M and IgG in serum and cerebrospinal fluid. The patient did not remember a tick bite but had eaten self-made cheese prepared from a mixture of nonpasteurized goat milk and cow milk 8–11 days before illness onset; further investigation found 6 additional persons who had eaten the same cheese (Figure). For 5 of them, recent TBEV infection was serologically proven (Table). For 3 of these persons (2 men, 44 and 65 years of age; and 1 woman, 60 years of age), similar to the index patient, a typical biphasic course and symptoms of TBE (nonspecific flu-like symptoms followed by fever, cephalea, meningism, and ataxia after 4–10 days) developed and they were hospitalized. The 2 other persons who had eaten the cheese (female, 37 and 7 years of age) were clinically asymptomatic. The noninfected person had vomited shortly after eating the cheese because of a gastric banding. None of the infected persons had been vaccinated against TBEV.

The cheese was prepared from a mixture of fresh milk from 1 goat and 3 cows and was eaten shortly after production. Detection of TBEV-specific hemagglutination inhibiting (HI) and neutralizing antibodies in the goat's serum proved infection in the goat; the 3 cows were seronegative for TBEV. At the time of this investigation (1 month after cheese production), TBEV was already undetectable by PCR in serum and milk of the goat. Cheese from the 3 batches produced after the contaminated batch was TBEV negative by PCR. The original cheese was no longer available for testing.

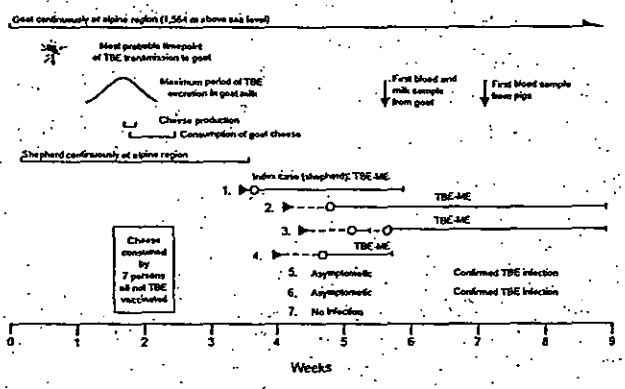

Figure. Time course and series of events of a tick-borne encephalitis (TBE) outbreak from cheese made with goat milk. Week 0, transport of goat to high altitude; ▶, onset of disease; O—I, hospitalization period; TBEV, tick-borne encephalitis virus; ME, meningoencephalitis.

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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				TBEV infection confirmed
					Material	TBEV ELISA IgM	TBEV ELISA IgG	TBEV NT	
M/43	11	Fever, cephalea, meningism, aseptic urethritis; CSF: pleocytosis	ME	18	Serum CSF	Pos Bor	Pos Pos	Pos	Yes
M/65	10	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	30	Serum CSF	Pos Bor	Pos Bor	Pos	Yes
F/60	14	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	25	Serum CSF	Pos Pos	Pos Pos	Pos	Yes
M/44	9	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	9	Serum CSF	Pos Pos	Pos Bor	Pos	Yes
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 Benes 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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Dr Holzmann is a virologist at the Clinical Institute of Virology, Medical University of Vienna, Austria. Her research interests focus on flaviviruses, hepatitis C virus, and antiviral vaccines.

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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. 10. 14	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		公表国
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)		Bondre VP, Sapkal GN, Yergolkar PN, Fulmali PV, Sankararaman V, Ayachit VM, Mishra AC, Gore MM. J Gen Virol. 2009 Nov;90(Pt 11):2644-9.	インド

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。

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。

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」
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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)

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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

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

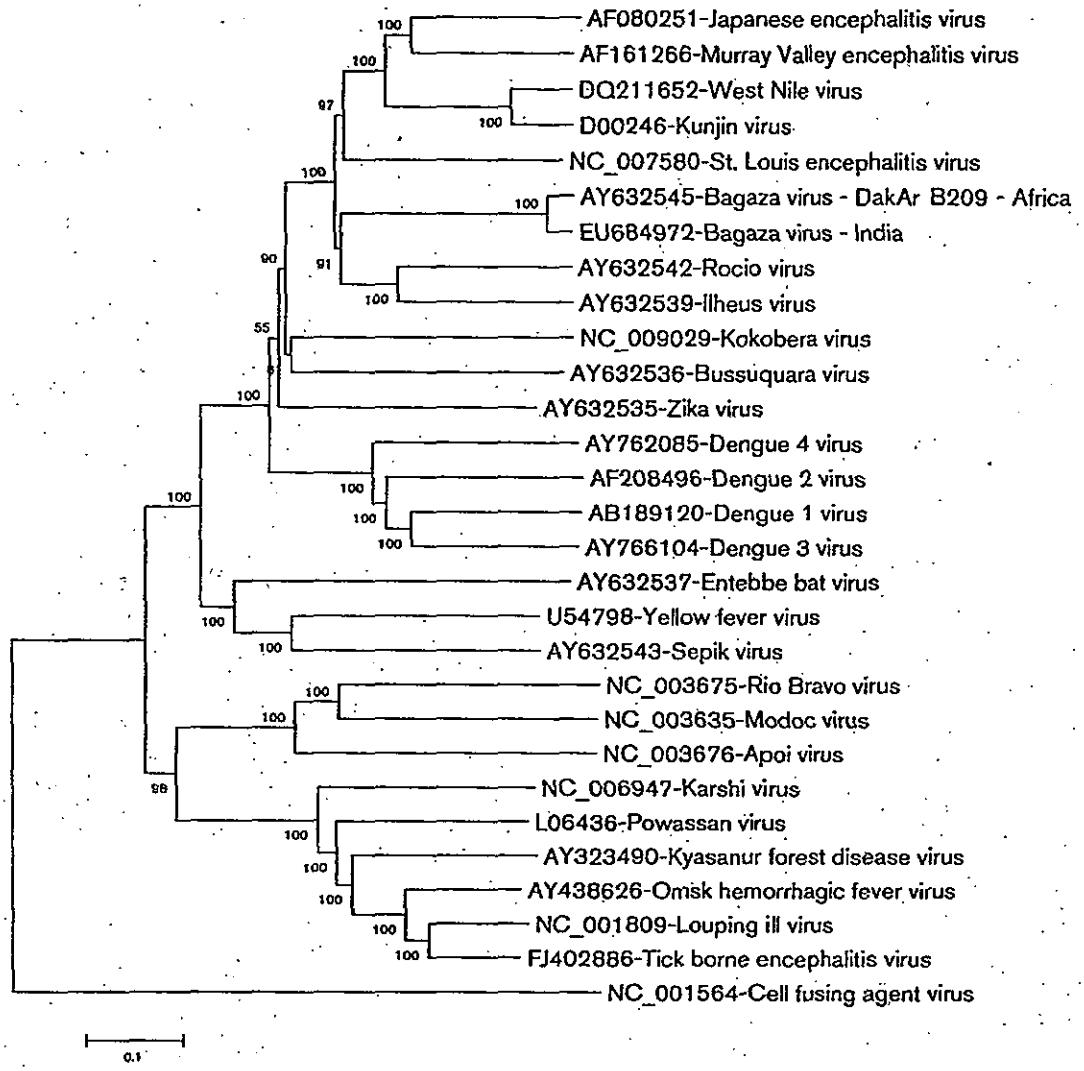

Fig. 1. Phylogenetic analysis of the BAGV complete ORF sequence using the nucleotide maximum composite likelihood model of the neighbour-joining algorithm. Cell fusing agent virus was used as an outgroup in phylogenetic analysis. GenBank accession numbers are given on the figure. Numbers at the nodes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

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

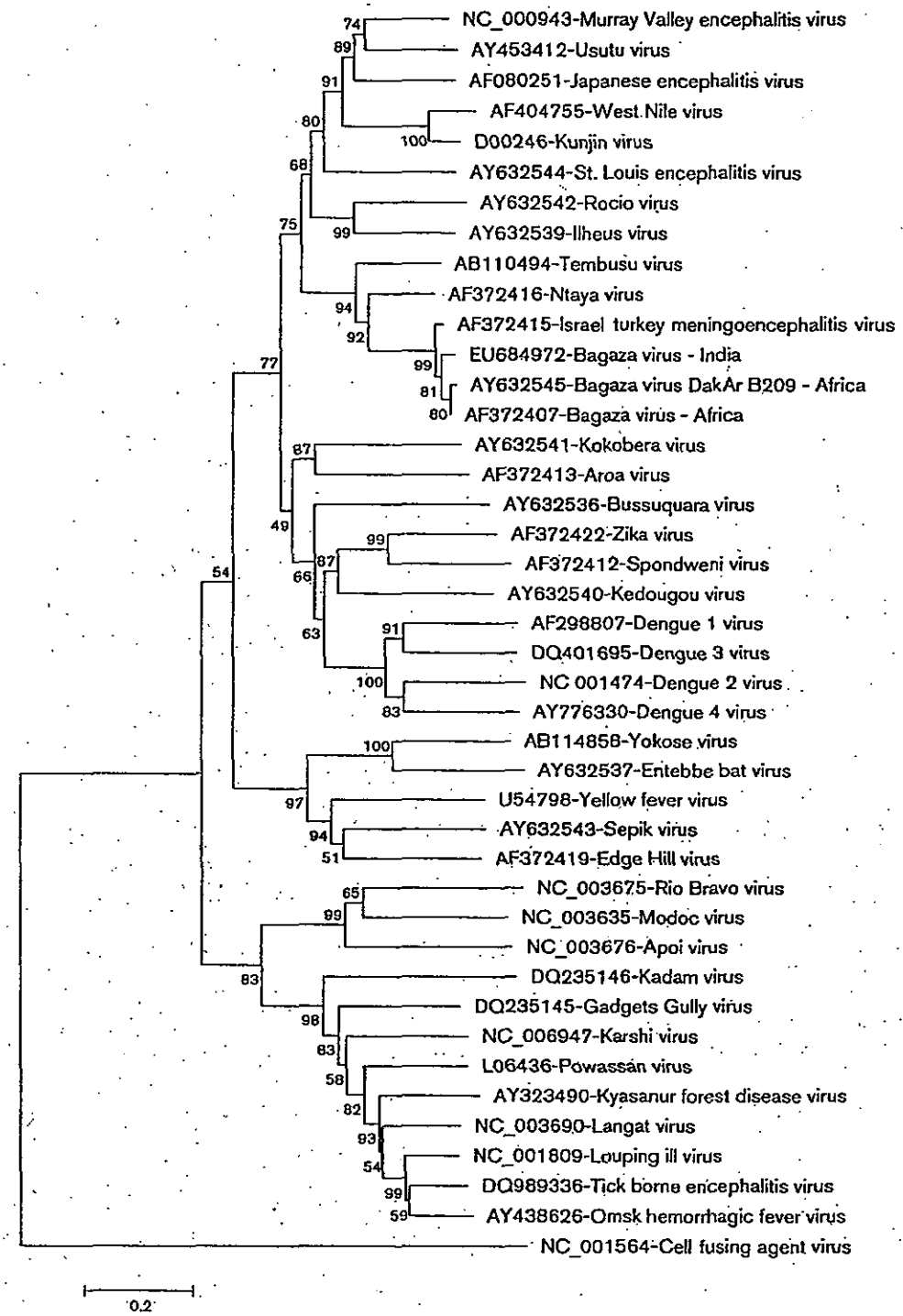

Fig. 2. Phylogenetic analysis of BAGV based on partial envelope sequences. The tree was constructed by using MEGA, by the neighbour-joining with nucleotide maximum composite likelihood model. Bootstrap confidence level (1000 replicates) and a confidence probability value based on the standard error test 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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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		公表国
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)		Planitzer CB, Modrof J, Yu MY, Kreil TR. Emerg Infect Dis. 2009 Oct. Available from http://www.cdc.gov/EID/content/15/10/1668.htm	米国

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。

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。

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

(7)

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 ($r^2 = 0.9793$).