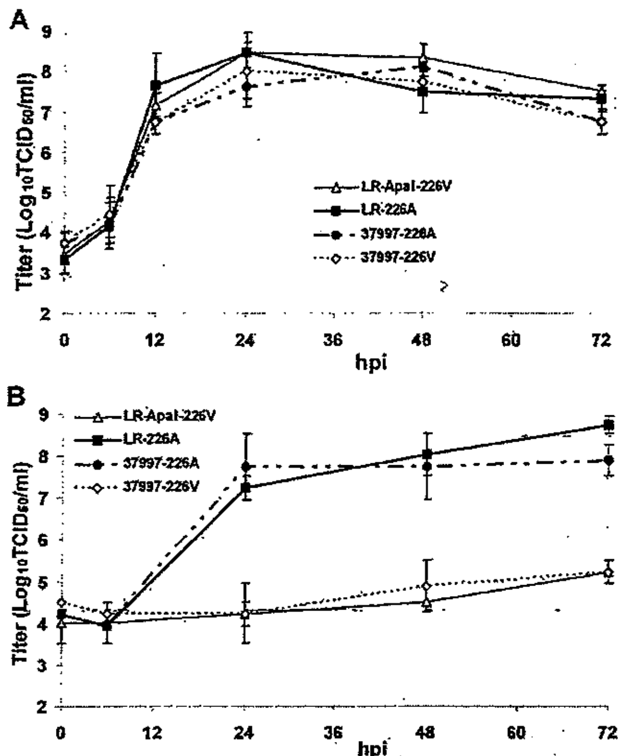

Figure 6. Effect of E1-A226V Mutation on *In Vitro* Growth of CHIKV in Standard (A) and Cholesterol-Depleted (B) C6/36 Cells

Cholesterol-depleted C6/36 cells were produced by five passages in L-15 medium containing 10% FBS treated with 2% CAB-O-Sil for 12 h at room temperature as previously described [52]. Confluent monolayers of standard (A) and cholesterol-depleted (B) C6/36 cells were infected with LR-Apal-226V, LR-226A, 37997–226A and 37997–226V viruses at an MOI of 1.0 (A) and an MOI of 0.1 (B). Cells were washed three times with L-15 medium, and 5.5 ml of fresh L-15 supplied with 10% of standard or CAB-O-Sil-treated FBS were added to the flask. Cells were maintained at 28 °C. At the indicated times post-infection, 0.5 ml of medium was removed and stored at −80 °C for later titration on Vero cells. Viral titers are estimated as average $Log_{10}TCID_{50}$/ml ± standard deviation of two independent experiments.
hpi - hours post-infection.
doi:10.1371/journal.ppat.0030201.g006

with E1-226A viruses. This increased opportunity for *Ae. albopictus* infection, would perpetuate the selection and transmission of the mutant virus.

During transmission competition assays, only E1-226V virus was transmitted to suckling mice by *Ae. albopictus*, although in these experiments, titers of E1-226V and E1-226A viruses were of a high enough magnitude to allow both of these viruses to efficiently infect this mosquitoes species. This indicates that there are additional mechanisms that could ensure evolutional success of the E1-A226V viruses transmitted by *Ae. albopictus*. It is possible that one of these mechanisms is associated with more efficient dissemination of the E1-226V as compared with E1-226A viruses. This could shorten the extrinsic incubation period (EIP)—the time from mosquito infection to transmission—and could have contributed to the evolutionary success of CHIKV during the Reunion outbreak because vectors infected with the LR-226V virus would transmit it more quickly than those infected with LR-226A viruses. Additionally, with relatively short-lived vectors such as mosquitoes [43], longer EIPs reduce transmission efficiency simply because fewer mosquitoes survive long enough to transmit the virus.

Our current studies do not provide data to determine if dissemination efficiency of the E1-226V viruses into the salivary glands is a consequence of more efficient midgut infectivity or if these two phenomena are independent. In this regard, it will be of particular interest to investigate the effect of the E1-A226V mutation on CHIKV transmission by orally or intrathoracically infected *Ae. albopictus* mosquitoes.

Although the CHIKV E1-A226V mutation gives a selective advantage in *Ae. albopictus*, there was not a corresponding advantage in *Ae. aegypti*. The OID_{50} and midgut competition assay data indicate that E1-226V viruses were slightly less infectious for midgut cells of *Ae. aegypti* mosquitoes (Figures 1C, 1D, and 2C; Table 2). Additionally, in contrast to *Ae. albopictus*, E1-226V viruses do not have a detectable advantage for dissemination into salivary glands and heads of *Ae. aegypti*. In transmission competition experiments from *Ae aegypti* to suckling mice, E1-226V conferred a slight competitive advantage over E1-226A (Figure 5C). However, five out of six mice exposed to CHIKV infected *Ae aegypti* had equivalent amounts of both E1-226A and E1-226V viral RNAs. These results are markedly different compared to the results obtained in similar experiments using *Ae. albopictus* mosquitoes and further support the hypothesis that this E1-A226V was specifically selected as a result of adaptation of CHIKV to *Ae. albopictus* mosquitoes. To explain the small fitness advantage associated with the E1-A226V mutation which was observed in transmission experiments, we hypothesize that, similarly to *Ae. albopictus*, E1-226A and E1-226V viruses colonize different *Ae. aegypti* organs at different efficiencies. E1-226A appears to colonize midgut cells of *Ae aegypti* better than E1-226V viruses; however, following dissemination into salivary glands, the E1-226V virus gains an advantage for transmission to vertebrates.

The E1-A226V mutation was found to have a slightly negative effect on infectivity, a negligible effect on dissemination, but a slight positive effect on transmissibility of CHIKV by *Ae. aegypti* in the competition experiment. We suggest that these small (as compared with *Ae. albopictus*) differences associated with the E1-A226V mutation would not be sufficient to have a significant effect on the evolution of CHIKV transmitted by *Ae. aegypti* and would not result in accumulation of this mutation in the regions where *Ae. aegypti* serves as a primary vector for CHIKV. This may explain the lack of emergence of the E1-226V genotype in previous outbreaks and the predominance of E1-226A viruses during the 2006 CHIKV epidemic in India, in which *Ae. aegypti* is considered to be the main vector species [44]. Adaptation of African strains of CHIKV from forest dwelling mosquitoes species to *Ae. aegypti* has never been shown to be associated with any particular mutations, therefore we believe that the same negative impact of E1-A226V would be seen in African mosquito vectors which were responsible for transmission of CHIKV strains ancestral to Reunion isolates.

Our data does not exclude the possibility that the E1-A226V mutation might have a negative effect on the evolution of CHIKV transmitted by *Ae. aegypti*. Since our dissemination and transmission studies were performed using blood meal titers that were 1–2 $Log_{10}TCID_{50}$/ml higher than $Log_{10}OID_{50}$/ml values we suggest that the negative effect of decreased midgut infectivity of E1-A226V on virus trans-

missibility would be almost completely missed, simply because, under this condition, almost 100% of mosquitoes could become infected. In general, CHIKV requires significantly higher blood meal titers for infection of *Ae. aegypti* compared to *Ae. albopictus* [36,37] (Tables 1 and 2), which suggests that the slight decrease in midgut infectivity of E1-226V viruses would have a more profound effect on the evolution of CHIKV transmitted by *Ae. aegypti*, compared to the effect of a small advantage in the ability to compete with E1-226A viruses for transmission to suckling mice. Therefore, if the E1-A226V mutation occurred in CHIKV transmitted by *Ae. aegypti*, it would have a weak negative effect on viral fitness and would most likely not be preferentially selected. Additional experiments are required to evaluate this hypothesis.

Available data cannot exclude the possibility that E1-226A viruses may have an unknown beneficial effect on the fitness of CHIKV in vertebrate hosts over E1-226V viruses, and that the minor negative effect of E1-226A observed in transmission experiments by *Ae. aegypti* can be compensated for by more efficient viral replication in the vertebrate host, leading to an overall more efficient adaptation to the transmission cycle. However, comparison of the different effects of A or V residues at position E1-226 on CHIKV infectivity for, and transmission by *Ae. aegypti* and *Ae. albopictus* mosquitoes clearly suggests that polymorphisms at this position may determine the host range of the alphaviruses and may play an important role in adaptation of the viruses to a particular mosquito vector.

An interesting observation, which should be studied in more detail, was that adaptation of CHIKV to *Ae. albopictus* mosquitoes coincided with the acquisition of CHIKV dependence on cholesterol in the target membrane. It has been previously shown that various mutations in the same region of the E1 protein of SFV and Sindbis virus can modulate the cholesterol dependence of these viruses [33,45] and that SFV independence from cholesterol coincides with more rapid growth of the virus in *Ae. albopictus* [34]. Although there is an apparent association, it is currently unknown if cholesterol dependence of alphaviruses is directly responsible for modulation of fitness of alphaviruses in mosquito vectors. A possible explanation for the opposite effects of the cholesterol-dependent phenotype of SFV and CHIKV on fitness in *Ae. albopictus* may reflect the use of different techniques for mosquito infection. In our study, mosquitoes were orally infected via cholesterol rich blood meals, whereas in the previous study SFV was intrathoracically inoculated into the mosquito [34]. It is also possible that cholesterol-dependent and -independent viruses would replicate differently in different mosquito organs. As such, our data indicate that more efficient colonization of *Ae. albopictus* midgut cells by cholesterol-dependent LR-ApaI-226V is followed by relatively more rapid growth of cholesterol-independent LR –226A virus in mosquito bodies between 3 and 5 dpi (Figure 4B). Three to 5 dpi coincides with virus escape from the mosquito midgut.

Alignment of amino acid sequences that constitute the ij loop of E1 protein from different members of the alphaviruses genus revealed that position E1–226 is not conserved ([33] and data not shown) and can vary even between different strains of the same virus. In this regard, it would be reasonable to determine the cholesterol requirement of other clinically important alphaviruses, especially Venezuelan equine encephalitis virus (VEEV) and eastern equine encephalitis virus (EEEV), which show significant intra-strain variation at position E1–226 among natural isolates of these viruses, and determine mutations which can modulate their cholesterol dependence. In recent studies by Kolokoltsov et al. [46], it was suggested that VEEV, a New world alphavirus, might be cholesterol independent, although the use of Vero cells instead of C6/36 cells, and the use of different protocols for cell membrane cholesterol depletion, make it difficult to compare the results of this study with our findings. Also it would be of interest to determine possible relationships between mutations which modulate cholesterol dependence of alphaviruses other than CHIKV and on their infectivity for *Ae. aegypti* and *Ae. albopictus* mosquitoes and perhaps other epidemiologically important mosquito vectors.

The molecular mechanisms responsible for the association between host range and cholesterol dependence of CHIKV are unknown [47]. It has been proposed that upon exposure to low pH, the E1 protein of cholesterol-dependent viruses senses the target membrane lipid composition and goes through a cholesterol-dependent priming recognition reaction [48] which is not required for cholesterol-independent viruses. It is possible that CHIKV infects *Ae. aegypti* and *Ae. albopictus* midgut cells using different endocytic pathways, which targets virus to cellular compartments with different lipid contents in which fusion occurs. Specific lipids such as cholesterol may differentially affect fusion of cholesterol-dependent and cholesterol-independent CHIKV strains in these compartments and therefore define the outcome of infection. Although our observations are suggestive, more comprehensive studies should be completed to determine the exact molecular mechanisms responsible for penetration of E1-226A and E1-226V viruses into *Ae. aegypti* and *Ae. albopictus* cells.

Although previous laboratory studies have demonstrated susceptibility of *Ae. albopictus* to CHIKV infection [36,37], our data demonstrate that the E1-A226V mutation promoted infection and accelerated dissemination of CHIKV in *Ae. albopictus* mosquitoes and conferred a selective advantage over infection of *Ae. aegypti*. Whilst the mutation did not increase the maximum viral titer attainable in the mosquitoes, the synergistic effects of increased infectivity and faster dissemination of the E1-A226V virus in *Ae. albopictus* would accelerate virus transmission to a naïve human population which would have contributed to initiating and sustaining the 2005–2006 CHIKV epidemic on Reunion island. That a single amino acid change can act through multiple phenotypic effects to create an epidemic situation has implications for other arthropod-transmitted viruses and the evolution of human infectious diseases [49].

Methods

Viruses and plasmids. The viruses and plasmids encoding full-length infectious clones of the LR2006 OPY1 strain CHIK-LR ic (GenBank accession number EU224268; http://www.ncbi.nlm.nih.gov/Genbank/index.html) and GFP-expressing full-length clone LR-GFP-226V (CHIK-LR 5'GFP, GenBank accession number EU224269) have been previously described [15,35]. The plasmids 37997-226A (pCHIK-37997ic, GenBank accession number EU224270) encoding full-length infectious clones of the West African strain of CHIKV 37997 and a GFP-expressing full-length clone 37997-GFP-226A (pCHIK-37997-5GFP, GenBank accession number EU224271) were derived from previously described plasmids pCHIKic and 5'CHIK EGFP [35] by

introducing CHIKV encoding cDNA into a modified pSinRep5 (Invitrogen) at positions 8055–9930. Viruses derived from 37997-226A and 37997-GFP-226A are identical to viruses derived form pCHIKic and 5'CHIK EGFP. To facilitate rapid screening of viruses in mosquitoes, the gene encoding enhance green fluorescent protein (eGFP), that is known not to compromise CHIKV phenotype in mosquitoes [15], was incorporated into clones as previously described [15]. Plasmids were constructed and propagated using conventional PCR-based cloning methods [50]. The entire PCR-generated regions of all constructs were verified by sequence analysis. The maps, sequences and detailed description of the clones are available from the authors upon request. For studies comparing the relative fitness of the mutant (E1-226V) virus and the pre-epidemic genotype (E1-226A), a silent mutation (6454C) was introduced into the CHIK-LR ic, to add an *ApaI* restriction site into the coding sequence of CHIK-LR ic. The resultant plasmid was designated LR-ApaI-226V. The E1-V226A mutation was introduced into CHIK-LR ic and LR-GFP-226V to generate plasmids designated as LR-226A and LR-GFP-226A, respectively. The mutation E1-A226V was also introduced into plasmids 37997–226A and 37997-GFP-226A. The resulted plasmids were designated 37997–226V and 37997-GFP-226V.

All plasmids were purified by centrifugation in CsCl gradients, linearized with *NotI* and *in vitro* transcribed from the minimal SP6 promoter using the mMESSAGE mMACHINE kit (Ambion) following the manufacturer's instructions. The yield and integrity of synthesized RNA were analyzed by agarose gel electrophoresis in the presence of 0.25 µg/ml of ethidium bromide. RNA (10 µg) was transfected into $1x10^7$ BHK-21 cells by electroporation as previously described [15]. Cells were transferred to 25 cm^2 tissue culture flasks with 10 ml of Leibovitz L-15 (L-15) medium, and supernatants were collected at 24 and 48 h post-electroporation and stored at −80 °C. In parallel, $1x10^5$ electroporated BHK-21 cells were serially 10-fold diluted and seeded in six-well plates for infectious centers assay as previously described [15].

Cells and mosquitoes. BHK-21 (baby hamster kidney) cells were maintained at 37 °C in L-15 medium supplemented with 10% fetal bovine serum (FBS), 100 U penicillin, and 100 µg/ml streptomycin. C6/36 cells (*Ae. albopictus*) were grown in the same medium at 28 °C. *Ae. aegypti* (white-eyed Higgs variant of the Rexville D strain) and *Ae. albopictus* (Galveston strain) were reared at 27 °C and 80% relative humidity under a 16h light: 8h dark photoperiod, as previously described [35]. Adults were kept in paper cartons supplied with 10% sucrose on cotton balls. To promote egg production females were fed on anaesthetized hamsters once per week.

Rexville D strain of *Ae. aegypti* mosquitoes were originally selected for susceptibility to flavivirus infection [51]. Since there are no known consequences of this original selection with respect to susceptibility to CHIKV, a white eyed variant of the strain that facilitates detection of GFP was used in our experiments.

***In vitro* virus growth of CHIKV in standard and cholesterol-depleted C6/36 cells.** To investigate if the mutation influenced cholesterol dependence of the virus, cholesterol-depleted C6/36 cells were prepared by five passages in L-15 medium containing 10% FBS treated with 2% CAB-O-Sil (Acros Organics) for 12 h at room temperature as previously described [52]. CHIKV growth curves were determined by infecting cholesterol-depleted and normal C6/36 cells at a multiplicity of infection (MOI) of 0.1 and 1.0, respectively, by rocking for 1 h at 25 °C. The cells were washed three times with L-15 medium and 5.5 ml of fresh L-15 supplied with 10% of standard or CAB-O-Sil treated FBS was added to the flask. At the indicated times post-infection, 0.5 ml of medium was removed and stored at −80 °C until titrated. The volume of medium was then restored by adding 0.5 ml of appropriate medium.

Titrations. Viral titers from mosquito samples and from tissue culture supernatant were determined using Vero cells and expressed as tissue culture infectious dose 50 percent endpoint titers ($Log_{10}TCID_{50}$) as previously described [53]. Additionally, for viral competition experiments, titers of LR-Apa-226V LR-226A viruses were determined using standard plaque assay on Vero cells as previously described [54].

Oral infection of mosquitoes. *Ae. aegypti* and *Ae. albopictus* were infected in an Arthropod Containment Level 3 insectary as described previously [35,55]. To make infectious blood meals for the viruses lacking eGFP, viral stocks derived from electroporated BHK-21 cells were mixed with an equal volume of defibrinated sheep blood and supplemented with 3 mM ATP as a phago-stimulant. To produce infectious blood meals for the eGFP-expressing viruses, the viruses were additionally passed on BHK-21 cells. The cells were infected at a MOI ≈ 1.0 with virus derived from electroporation. At 2 dpi, cell culture supernatants were mixed with an equal volume of defibrinated sheep blood and presented to 4- to 5-day-old female mosquitoes that had been starved for 24 h, using a Hemotek membrane feeding system (Discovery Workshops) and hamster skin membrane. Mosquitoes were allowed to feed for 45 min, and engorged mosquitoes (stage ≥3+ [56]) were sorted and returned to a cage for maintenance. Blood meals and three to four mosquitoes were immediately removed for titration and/or RNA extraction. Depending on the purpose of the experiments, mosquitoes were collected at different days post-infection and either titrated to determine viral titer, dissected for analysis of eGFP expression in the midguts or salivary glands [15], or used for RNA extraction in competition experiments.

To estimate the Oral Infectious Dose 50% values (OID_{50}), serial 10-fold dilutions of viruses were made in L-15 medium followed by mixing the samples with defibrinated sheep blood. Mosquitoes were dissected at 7 dpi and eGFP expression in infected midguts was analyzed by fluorescence microscopy. A mosquito was considered infected if at least one foci of eGFP-expressing cells was present in the midgut. The experiments were performed twice for each virus. OID_{50} values and confidence intervals were calculated using PriProbit (version 1.63).

Viral competition experiments. To test the hypothesis that the E1-A226V mutation might be associated with a competitive advantage in mosquito vectors, competition assays were designed similar to those described previously in mice [57], with minor modifications (Figure 2A). Both *Ae. aegypti* and *Ae. albopictus* mosquitoes were presented with a blood meal containing 10^7 plaque-forming units (pfu)/ml of LR-Apa-226V and 10^7 pfu/ml of LR-226A viruses. It had been previously found that for these two viruses the ratio of viral RNAs corresponds to the ratio of viral titers (data not shown). Midguts were collected at 7 dpi and analyzed in pools of eight to ten, and heads were collected at 12 dpi and analyzed in pools of five. RNA was extracted from the tissue pools using TRIzol reagent (Invitrogen) followed by additional purification using a Viral RNA mini kit (QIAGEN). RNAs from blood meal samples were extracted using Viral RNA Mini Kit followed by treatment with DNAse (Ambion) to destroy any residual plasmid DNA contaminant in the viral samples. RNA was reversed transcribed from random hexamer primers using Superscript III (Invitrogen) according to the manufacturer's instructions. cDNA was amplified from 41855ns-F5 (5'- ATATCTAGACATGGTGGAC) and 41855ns-R1 (5'-TATCAAAGGAGGCTATGTC) primers using Taq DNA polymerase (New England Biolabs). PCR products were purified using Zymo clean columns (Zymo Research) and were quantified by spectrophotometry. Equal amount of PCR products were digested with *ApaI*, separated in 2% agarose gels that were stained using ethidium bromide. Thus the LR-Apa-226V and LR-226A viruses could be distinguished by size on an agarose gel (Figure 2A). Gel images were analyzed using TolaLab (version 2.01). Relative fitness of LR-Apa-226V and LR-226A viruses was calculated as a ratio between 226V and 226A bands in the sample, divided by the control ratio of 226V and 226A in the blood meal.

Virus competition in an animal transmission model. *Ae. aegypti* and *Ae. albopictus* mosquitoes were presented with a blood meal containing 10^7 pfu/ml of LR-Apa-226V and 10^7 pfu/ml of LR-226A viruses. At 13 dpi, ten to 15 mosquitoes were placed in separate paper cartons and starved for 24 h. The next day the mosquitoes in each carton were presented with individual 2- to 3-day-old suckling mouse (Swiss Webster). Feeding continued until 2–3 mosquitoes per carton were fully engorged (stage ≥3+[56]). In a parallel experiment six 2- to 3-day-old suckling mice were subcutaneously infected with 20 µl of mixture containing ≈ 25 pfu of LR-Apa-226V and ≈ 25 pfu of LR-226A viruses. Mice were returned to their cage and sacrificed on day 3 post-exposure. Blood from each individual mouse (≈ 50 µl) was collected and immediately mixed with 450 µl of TRIzol reagent for RNA extraction. The RNA was processed as described above. All animal manipulations were conducted in accordance with federal laws, regulations, and in compliance with National Institutes of Health and University of Texas Medical Branch Institutional Animal Care and Use Committee guidelines and with the Association for Assessment and Accreditation of Laboratory Animal Care standards.

Supporting Information

Figure S1. Schematic Representation of the Viruses Used in This Study

Found at doi:10.1371/journal.ppat.0030201.sg001 (917 KB PDF).

Figure S2. Growth of the eGFP-Expressing Viruses in BHK-21(A, C) and C6/36 (B, D) Cells

Confluent monolayers of BHK-21 and C6/36 cells in T25 tissue culture flasks were infected with LR-GFP-226V and LR-GFP-226A (A,

B) or 37997-GFP-226A and 37997-GFP-226V viruses derived from electroporation at a MOI of 0.1. At the indicated times post-infection, 0.5 ml of medium was removed and stored at −80 °C for later titration on Vero cells. Viral titers are expressed as $Log_{10}TCID_{50}$/ml.

Found at doi:10.1371/journal.ppat.0030201.sg002 (372 KB PDF).

Figure S3. Growth of the CHIK-LR ic, LR-ApaI-226V and LR-226A Viruses in BHK-21(A) and C6/36 (B) Cells

Confluent monolayers of BHK-21 and C6/36 cells in T25 tissue culture flacks were infected with LR-GFP-226V and LR-GFP-226A (A, B) or 37997-GFP-226A and 37997-GFP-226V viruses derived from electroporation at a MOI of 1.0. At the indicated times post-infection, 0.5 ml of medium was removed and stored at −80 °C until titrated on Vero cells. Viral titers are expressed as $Log_{10}TCID_{50}$/ml ± standard deviation of three independent experiments.
hpi - hours post-infection.

Found at doi:10.1371/journal.ppat.0030201.sg003 (177 KB PDF).

Figure S4. Competition between CHIK-LR ic and LR-ApaI-226V for Growth in BHK-21 and C6/36 Cells

Cells were infected with a 1:1 mixture of both viruses at a MOI of 0.001. 2 dpi, cell culture supernatant was collected and samples proceeded as described. The experiment was repeated three times for each of the cell types.
inoc - initial ratio of CHIK-LR ic and LR-ApaI-226V in the inoculum used for infection of cells.
Relative fitness (RF) of CHIK-LR ic and LR-ApaI-226V was calculated as an average ratio between CHIK-LR ic and LR-ApaI-226V bands in the supernatant obtained from BHK-21 cells (RF_1) and C6/36 cells (RF_2), divided by the control ratio between CHIK-LR ic and LR-ApaI-226V in the inoculum.

Found at doi:10.1371/journal.ppat.0030201.sg004 (3.6 MB PDF).

Table S1. Specific Infectivity and Virus Titers after Electroporation

a - amino acids at position of E1–226.
b - Specific infectivity of *in vitro* transcribed RNA. 10^7 BHK-21 cells were transfected with 10 µg of RNA. Electroporated BHK-21 cells were 10-fold serially diluted, seeded in 6-well tissue culture plates containing $5x10^5$ naive BHK-21 cells per well and covered with 0.5% agarose in L-15. Plaques were scored on day 2 post-transfection.
c - Supernatants of electroporated BHK-21 cells were collected on days 1 and 2. Virus titers were determined by titration on Vero cells and expressed as $Log_{10}TCID_{50}$/ml.
hpi - hours post-infection.

Found at doi:10.1371/journal.ppat.0030201.st001 (34 KB DOC).

Table S2. Infection Rates and Average Titers of CHIKV-LR ic or LR-ApaI-226V in Orally Infected *Ae. aegypti* and *Ae. albopictus*

Ae. aegypti mosquitoes were orally presented with 7.24±0.4 $Log_{10}TCID_{50}$/ml of CHIKV-LR ic (summary of two experiments) and 6.52 $Log_{10}TCID_{50}$/ml of LR-ApaI-226V.
Ae. albopictus mosquitoes were orally presented with 7.24±0.4 $Log_{10}TCID_{50}$/ml of CHIKV-LR ic (summary of two experiments) and 7.52 $Log_{10}TCID_{50}$/ml LR-ApaI-226V.
At 7 and 14 dpi, mosquitoes were collected and triturated in 1mL of L-15 medium for titration on Vero cells.
Titers are reported as $Log_{10}TCID_{50}$/ml ± standard deviation.

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Competing interests. The authors have declared that no competing interests exist.

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No. 33

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	機構処理欄
			2007. 10. 5	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	ProMED 20071001-3237, 2007 Oct 1. 情報源:[1]China Daily, Xinhua News Agency report, 2007 Sep 30. [2]VietNamNet Bridge, 2007 Sep 26 [3]Daily Times, 2007 Sep 27. [4]Associated Press, 2007 Sep 29.	公表国 [1]中国[2]ベトナム[3]パキスタン[4]汎米保健機構	
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

○デングウイルス最新情報

[1]中国(福建省):9月30日、保健当局は福建省莆田市でデング熱症例39例を確認したと発表した。感染拡大を防ぐ為の総合的予防対策が実施され、医療機関でのモニタリングが強化されている。市民には、蚊の増殖を防ぐ為に衛生状態改善が呼びかけられている。

[2]ベトナム:2007年のデング熱発生件数は、昨年と比べて50%増加したと保健省が報告した。9月24日時点で患者68,000人が報告され、60人が死亡した。感染例のほとんどは南部で発生している。患者は通常10歳以下の子どもが多いが、2007年は成人患者も増加している。

[3]パキスタン(カラチ):保健省のデング熱サーベイランス班によると、カラチ市の4つの病院で22例の新規デング熱疑い症例が報告された。うち20人が陽性、2人が検査中となっている。2007年はこれまでに170例の疑い症例が報告された。

[4]ラテンアメリカ:デング熱がラテンアメリカとカリブ海諸国に感染拡大しており、この10年で最も深刻な事態になっている。2007年はこれまでに630,356人の患者が主にブラジル・ベネズエラ・コロンビアから報告され、うち12,147人が出血熱を発症、183人が死亡した。このまま拡大が続けば2002年の1,015,000例を超える可能性がある。流行が沈静化しないと社会的、経済的に大きな影響が出るだろうと汎米保健機構の専門家は述べている。観光や移住によって4系統のウイルス株が地域内で循環しているために、患者が重症化しやすくなっていると考えられている。

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
中国福建省、ベトナム、パキスタン、ラテンアメリカでデング熱が流行しているとの報告である。	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。問診でデング熱の既往があった場合には、治癒後1ヶ月間献血不可としている。今後も引き続き情報の収集に努める。

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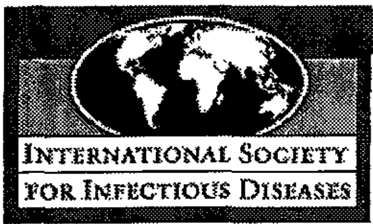

Archive Number 20071001.3237
Published Date 01-OCT-2007
Subject PRO/EDR> Dengue/DHF update 2007 (37)

DENGUE/DHF UPDATE 2007 (37)
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

In this update:
[1] China (Fujian)
[2] Viet Nam
[3] Pakistan (Karachi)
[4] Latin America

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[1] China (Fujian)
Date: Sun 30 Sep 2007
Source: China Daily, Xinhua News Agency report [edited]
<http://www.chinadaily.com.cn/china/2007-09/30/content_6149071.htm>

On Sunday [30 Sep 2007], health authorities said 39 dengue fever cases have been confirmed in Putian City of east China's Fujian Province. Thus far, 26 of the 39 patients in Hanjiang District of Putian City have been cured and the others are in stable condition, said the provincial health department.

The city has adopted "comprehensive prevention and control measures" to curb the spread of the disease, said the department. All medical and health institutions in the province have also strengthened monitoring on the disease, it added.

The department reminded citizens of household sanitation and the prevention of proliferation of mosquitoes, which transmit the disease [v us]

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Communicated by:
ProMED-mail Rapporteur Brent Barrett

[Putian City is situated in the central part of the coastal area of Fujian Province. Putian neighbors Fuzhou in the northeast and Quanzhou in the southeast, and is separated from Taiwan by the Taiwan Strait.

[A zoomable map of Fujian Province showing the location of Putian city can be accessed at
<http://encarta.msn.com/map_701510630/Fujian.html>. - Mod.TY]

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[2] Viet Nam
Date: Wed 26 Sep 2007
Source: VietNamNet Bridge [edited]
<http://english.vietnamnet.vn/social/2007/09/745035/>

The incidence of dengue fever in Viet Nam has risen by almost 50 percent this year [2007] against last year [2006], reports the Health Minist

A medical worker instructs Dao ethnic minorities in the northern mountainous province of 94 en Bai's Quang Minh Commune to dip mosquito

nets in chemicals to prevent dengue fever.

About 68 000 people had been stricken with the mosquito-borne disease, Preventative Health Department director Nguyen Huy Nga said on Monday [24 Sep 2007]; 60 had died.

Most infections had occurred in southern Dong Thap, An Giang, Tien Giang, and Ben Tre provinces and the total increase was about 48 percent, he said.

Ho Chi Minh [HCM] City-based Pasteur Institute National Dengue Fever Programme representative Luong Chan Quang said more than 58 000 people had been infected in the Cuu Long (Mekong) Delta provinces by the end of August [2007]. Deaths were put at 54-40 percent more than last year [2006].

Infections in Tien Giang Province totalled 9800 with 9 deaths, Dong Thap 8700 with 9 deaths, and An Giang 6000 with 6 deaths.

In HCM City, almost 5400 people had been stricken with dengue fever -- 40 percent more than last year [2006 -- and 6 had died.

Quang warned that another serious outbreak was likely in the southern delta before the end of the year [2007] if effective preventive measures were not taken because people regularly stored water to prepare for the dry season.

The _Aedes_ mosquito, which carries dengue fever, breeds in still or stagnant water.

HCM City Preventive Health Department deputy director Nguyen Dac Tho said about 350 people were being admitted to hospital each week with dengue fever -- 50 more than last year [2006].

Inner city districts 8, 10, 11, Binh Thanh, and Binh Tan were the worst affected. People in densely populated precincts stored more water for their own use than others as did construction projects, said Dr Tho.

Dengue fever is most common among children under 10 but the number of afflicted adults has increased this year [2007].

HCM City Tropical Diseases Hospital figures show that of about 150 people admitted to the hospital with dengue fever each week, more than 100 were adults.

There are 4 types of the dengue fever virus that often result in similar symptoms. This year [2007], the transmitted virus was usually type 1 or type 2.

Haemorrhagic fever is a severe, often fatal, complication of dengue fever.

The HCM City People's Committee has mobilised measures to prevent dengue fever across the city. Citizens are encouraged to clean around their residences every Sunday and spray mosquito killer [insecticides].

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Communicated by:
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[A map of Viet Nam can be accessed at
<http://www.lib.utexas.edu/maps/middle_east_and_asia/vietnam_admin01.jpg>.
- Mod.TY]

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[3] Pakistan (Karachi)
Date: Thu 27 Sep 2007
Source: Daily Times [edited]
<http://www.dailytimes.com.pk/default.asp?page=2007%5C09%5C27%5Cstory_27-9-200

The Sindh Health Department's Dengue Fever Surveillance Cell reported 22 fresh cases of the disease in select hospitals across Karachi on Wednesday [26 Sep 2007].