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Determination of natural versus laboratory human infection with Mayaro virus by molecular analysis

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SUMMARY

A laboratory worker developed clinical signs of infection with Mayaro virus (Togaviridae), an arbovirus of South and Central America, 6 days after preparation of Mayaro viral antigen and 10 days after a trip to a rain forest. There was no evidence of skin lesions during the antigen preparation, and level 3 containment safety measures were followed. Therefore, molecular characterization of the virus was undertaken to identify the source of infection. RT-PCR and DNA sequence comparisons proved the infection was with the laboratory strain. Airborne Mayaro virus contamination is thus a hazard to laboratory personnel.

Mayaro virus, genus Alphavirus, family Togaviridae [1], was first isolated in Trinidad in 1954 [2]. Several outbreaks of Mayaro virus have been described in the Amazonian rain forest regions of South America, notably in Northern Brazil [3]. Available data suggest that *Haemagogus* mosquitoes are the main vectors of virus transmission [4]. The habitat of *Haemagogus* spp. is largely confined to the neotropical region and the maintenance cycle of the virus involves forest-dwelling non-human primate hosts.

Recently, Mayaro virus was isolated for the first time in French Guiana, a French overseas Department between Surinam and Brazil. In this region, Mayaro infections are currently a public health problem [5], particularly in the rain forest areas.

In 1998, a laboratory technician at the Institut Pasteur de la Guyane at Cayenne, French Guiana, contracted a febrile disease 6 days after the preparation of sucrose-acetone-extracted Mayaro viral antigen and 10 days after spending a weekend along a river in the rain forest. The symptoms were fever, headache, nausea and non-localized body pains and

thus suggestive of a virus infection. Analysis of blood samples, taken 3 and 15 days after the onset of symptoms, revealed an infection with Mayaro virus. Virus was recovered from Vero cell culture supernatants infected with the early blood sample and was identified using a mouse ascitic fluid for Mayaro virus obtained from the University of Texas, Medical Branch, Galveston, Texas. Serological testing of the acute and convalescent sera from this patient revealed that seroconversion to Mayaro virus occurred: the hemagglutination inhibition titres were < 10 in the first sample (day 3) and 320 in the convalescent sample (day 15). An IgM capture ELISA showed that the acute-phase serum diluted 1/100 was negative for Mayaro virus, but the convalescent specimen was positive (OD 450 nm: 1.55; cut-off value: 0.3).

The obligatory safety level 3 (P3) containment measures had been applied while preparing the Mayaro antigen and the work was carried out under a laminar flow hood for viruses in an officially approved, negative pressure P3 facility. The gloves worn by the technician did not tear and there was no evidence of any skin lesions due to the manipulation. However, during desiccation of the antigen preparation, the negative pressure was interrupted several times under the laminar flow hood to check that the

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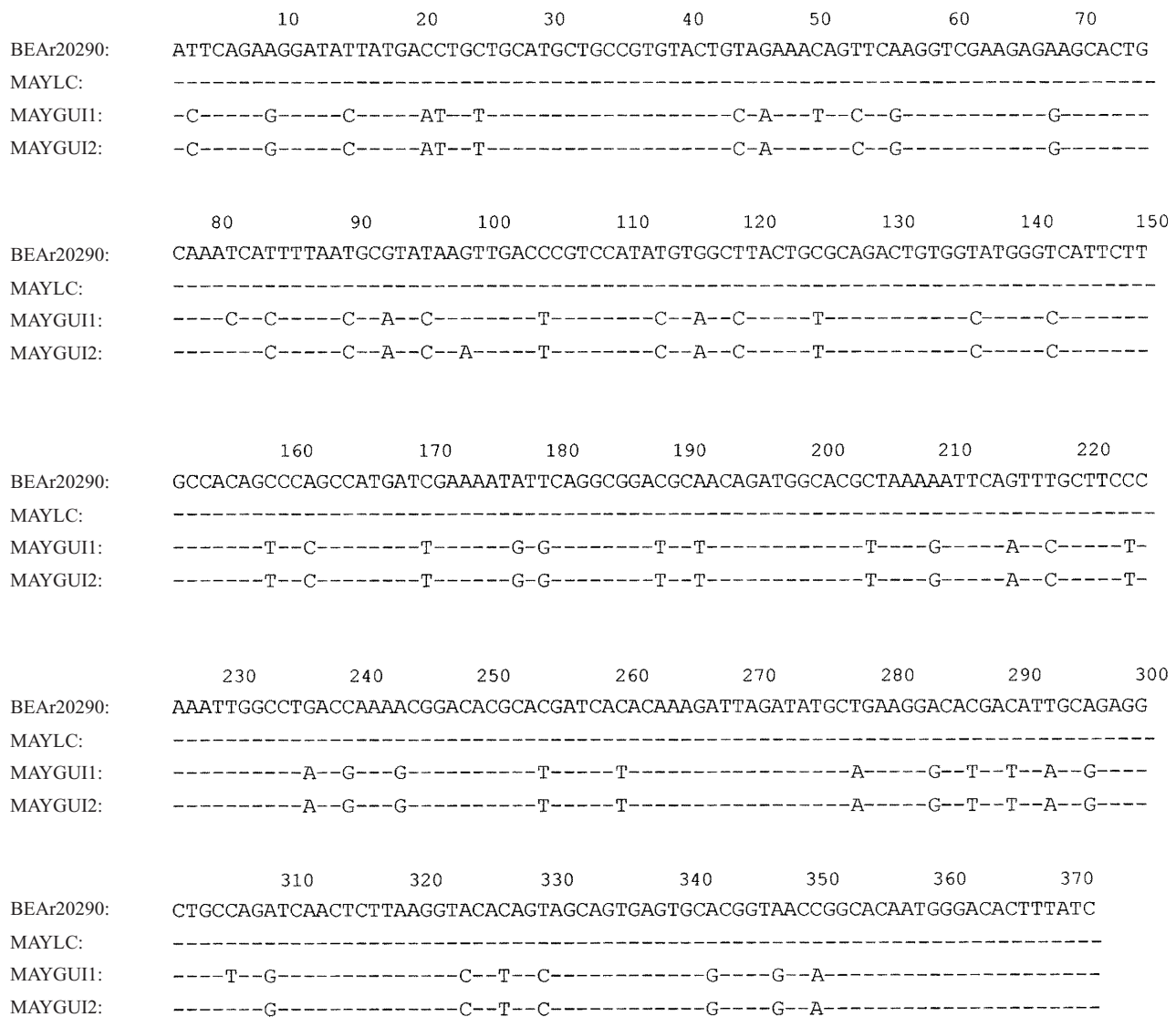


Fig. 1. Alignment of nucleotide sequences (371 bp) of the envelope E2 and E3 portion of the genome of different Mayaro virus strains. *BeAr20290* laboratory reference strain, *MayLC* Mayaro virus isolated from Vero cell cultures infected with the serum of the infected technician, 3 days after onset of symptoms, *MayGuy1* virus strain isolated in 1996 in French Guiana, *MayGuy2* virus strain isolated in 1998 in French Guiana.

vacuum pump was functioning. Therefore it was unlikely that this Mayaro virus infection was contracted by skin contact, the airborne route being the most probable. However, the Subcommittee on Arbovirus Laboratory Safety (SALS) has evaluated potential dangers for the handling of arboviruses and noted that of five laboratory infections with Mayaro virus, only one was suspected to have occurred by the airborne route [6]. Others were transmitted via an unknown route but might have been due to aerosolization of the virus.

The only means to distinguish this relatively improbable route of infection from a possible natural infection while the technician was in the rain forest

was molecular analysis of the virus. RNA was extracted from Vero cells infected with virus from the day 3 serum (strain *MayLC*). RNA was also prepared from two strains of Mayaro virus isolated in French Guiana in 1996 (strain *MayGuy1*) and 1998 (strain *MayGuy2*) and the laboratory strain *BeAr20290* isolated from *Haemagogus* mosquitoes in 1960 in the Instituto Evandro Chagas, Belem, Brazil [4], which was used for antigen preparation. All RNA samples were subjected to a Mayaro-specific RT-PCR analysis as published previously [5]. The PCR reaction amplifies a stretch of 462 bp in the envelope E3 and E2 portion of the genome. PCR products were directly sequenced with an automatic sequencing system

(ACTGene EuroSequence Gene Services, Evry, France). This technique allowed the comparison of four homologous stretches of 371 bp using the Gene Works 2.5.1. software (IntelliGenetics, Mountain View, CA). The sequence accession numbers are AF126873, AF126874, AF126875.

The sequence of BeAr20290 and the sequence of MayLC were identical. The sequence divergence between the two Mayaro viruses circulating in French Guiana was 1.1% and these viruses differed by 14% from BeAr20290 (Fig. 1). The infection of the laboratory technician was thus due to a contamination while manipulating the Mayaro virus and the airborne route of transmission was highly probable.

Transmission of arboviruses by the respiratory route has been demonstrated in laboratory animals, for example in mice for Saint-Louis encephalitis virus [7], and in rabbits for Venezuelan equine encephalitis virus [8]. Similarly airborne contamination of laboratory personnel with arboviruses has previously been strongly suspected [6, 9]. As it is very unlikely that the infection reported here was contracted through skin contact, airborne contamination is highly probable. This case is thus a further indication of the potential hazard to laboratory personnel exposed to arbovirus aerosols. The reasons for this contamination while performing routine manipulations remain obscure since all safety precaution were applied. However the problems encountered with the vacuum system during desiccation of the antigen preparation obliged the technician to stay beside the flow hood longer than usual such that exposure to the aerosol may have been greater. In light of this additional case of Mayaro laboratory infection, we wish to re-emphasize the danger to humans that can occur from exposure to aerosols from some arboviruses. After this contamination we changed our vacuum system and decided to use crude antigens instead of sucrose-acetone extracted viral antigens for MAC-ELISA and IgG-ELISA. The utilization of such antigens or of cell culture antigens is advisable to avoid the production of aerosols. When sucrose-acetone extracted antigens are required, vapours emanating from virus solutions must be bubbled

through hypochloride solution to inactivate the pathogen.

In similar cases, where the identification of laboratory infections requires molecular biological techniques because the same virus is prevalent in the region, the availability of reference strains of the virus with partially known sequences greatly facilitates analysis.

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