**Title:** Isolation of Japanese encephalitis Virus Strains from Sentinel Pigs in Northern Thailand, 1982.

1. **Performing Organization Name and Address:**
   Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

2. **Author(s):**
   Donald S. Burke, Michael A. Ussery, M.R. Elwell, Ananda Nisalak, Colin J. Leake, Thanom Laorpongse

3. **ABSTRACT:**
   Pigs were placed at the homes of patients with viral encephalitis in Thailand in 1982 in an effort to isolate the causative virus. The studies showed that transmission of Japanese encephalitis virus to pigs was intense, and suggest that the peak rate of transmission to pigs was two weeks before the peak rate of transmission of humans.
These studies (i) show the utility of using sentinel pigs to isolate strains of JEV, (ii) indicate that transmission of JEV to pigs was intense and widespread during the 1982 epidemic, and (iii) suggest that the peak rate of JEV transmission to pigs occurred early, before the peak of human encephalitis cases.

DONALD S. BURKE1
MICHAEL A. USSERTY1
MICHAEL R. ELWELL2
ANANDA NISALAK3
COLIN LEAKE3
THANOM LAORAKHONGSE4

Depts. of 1Virology and 2Veterinary Medicine of the US Component of the Armed Forces Research Institute of Medical Sciences, Rajprapap Road, Bangkok 10400, Thailand; 3Arbovirus Research Unit, London School of Hygiene and Tropical Medicine, Winches Farm Field Station, 355 Hatfield Road, St. Albans, AL4 OXU; 4Kampangphet Provincial Hospital, Kampangphet, Thailand.

References

Accepted for publication 12th November, 1984.

Leishmaniasis or Aids?
The recent description of the acquired immunodeficiency syndrome (AIDS) has had a great psychosocial impact. However, its correct diagnosis remains difficult (CURRAN, 1983). We present here a case which posed serious doubts in diagnosis, and which may be of interest to Mediterranean countries, as well as to Northern European countries, because of the ever growing problem of imported diseases.

A 20-year-old heterosexual man with type A haemophilia, who had been treated with factor VIII concentrates, was admitted to hospital in April 1983 with a history of several months of fever and diarrhoea. His previous clinical history showed pulmonary tuberculosis in 1973, and diabetes mellitus in 1981. While in hospital, chest X-rays revealed two epithelial bronchopneumonia.

In July 1983, persistent cough, weight loss, general malaise and minimal hepatosplenomegaly were added to his initial symptoms. Chest X-rays, blood cultures, and microbiological studies of respiratory samples, gave no indication of recent infection. Candida albicans was isolated from the oral mucosa, faeces and urine, and was treated with ketoconazole. Leukopenia was noted, 0.5-10^6/l as well as anaemia haemoglobin 8 g/dl and 25% haematocrit. A steat bone marrow puncture revealed few cells, a decrease in helper T lymphocytes (3-7%) and an inverted helper suppressor ratio (0-05). Hepatosplenomegaly increased from July to September, and generalized lymphadenopathy was noted for several days. A second bone marrow puncture performed on 6th September showed Leishmania.

Treatment was initiated with Glucantime (nimethyglycine antimoniate, 100 mg/kg by intramuscular injection, daily for 10 days). Two subsequent bone marrow aspirates, performed a month apart, showed no Leishmaniasis. However, as the patient's hepatic biochemistry was not yet normal, a third Glucantime treatment was administered from 8th November. The patient showed signs of hepatic pre-coma in early January, and died days later. Microbiological study of the necropsy samples showed "lodging" of Leishmania in the spleen, and cytomegalovirus infection of the lungs and liver. Retrospective serological studies by indirect immunofluorescence and radioimmunoassay (in the Kusin Centre, Hadassah University, WHO International Reference Centre for Leishmaniasis), confirmed L. donovani infection before May 1983. These data indicate that L. donovani infection was present several months before the diagnosis was made. Until then we strongly suspected a case of pre-AIDS (FAUCI et al., 1984).

Doubts were raised again when the patient did not recover after several courses of Glucantime and repeated negative bone marrow aspirates. The subsequent confirmation of the persistence of Leishmania in the patient's spleen does not permit this case to be classified as AIDS according to CDC criteria (FAUCI et al., 1984), although we cannot exclude the simultaneous existence of both diseases. The late treatment and/or a certain resistance of the parasite to Glucantime may explain these results (MURPHY & BONG, 1981). The isolation of cytomegalovirus may be a reactivation of the virus, secondary to the cellular immunodeficiency which accompanies visceral leishmaniasis (MUSEMECH et al., 1981).

For these reasons, we would like to point out the need to exclude the possibility of L. donovani infection in all patients with cellular immunodeficiency in countries with visceral leishmaniasis or in those who have visited such countries.

A. DE LA LOMA1
J. ALVAR1
E. MARTINEZ GALLANO1
J. BLAZQUEZ1
A. ALCAI MUNOZ2
R. NAJERA1

1Centro Nacional de Microbiologia, Virologia e Inmunologia Santarras, Majadahonda, Madrid, Spain
2Residencia Santarras "Capitan Cortes", Jaca, Spain
present. Paired acute and convalescent sera from 959 haemorrhagic fever patients were tested for CHIK HAI antibodies (Clarke & Casals, 1958). None showed a reproducible four-fold or greater rise in titre. (ii) Among paired sera from 457 children with undifferentiated febrile illnesses who presented to the outpatient department in 1979-80, none showed a four-fold or greater rise in serum CHIK HAI titre, although 109 (24%) pairs did show a diagnostic rise in dengue HAI titre. (iii) Among 195 out-patients with various mild illnesses in 1979-80 who were more than six months old but who were born in greater Bangkok after 1st January 1976, only one had serum CHIK HAI antibodies. Thus, the incidence of CHIK infections in this group was no more than one infection per 403 child-years at risk. (iv) Paired sera from 33 suspect haemorrhagic fever cases in 1981-82, which were proved not to be due to dengue virus, were assayed for CHIK antibodies by plaque reduction neutralization method (Russell et al., 1967). None were diagnostic of acute CHIK infection.

These findings suggest that CHIK has virtually disappeared from Bangkok. Known vector species (Aedes aegypti) are obviously present in abundance as In early June, four-to six-month-old Bangkok pigs were screened for plaque reduction neutralizing antibodies (Clarke & Casals, 1958). Sera obtained from four of the nine seroconverting animals along each route. HAI antibodies (Clarke & Casals, 1958). Sera obtained from four of the nine seroconverting pigs were examined for plaque reduction neutralizing antibodies to JEV (Russell et al., 1967), and seronegative animals were trucked to Kampangphet Province for use as sentinel pigs. 22 pigs were set out along three routes at homes of persons previously admitted to hospital with encephalitis (10 of whom were admitted during the preceding epidemic in 1981 and 12 during the current 1982 epidemic). Pigs were bled every three days (all the pigs on one route each day) until the end of the epidemic in mid-August. Sera were separated and stored frozen on dry ice. Later, all pig sera were extracted with acetone and tested for JEV haemagglutination inhibiting (HAI) antibodies (Clarke & Casals, 1958). Sera obtained two or three bleeds before HAI seroconversion were inoculated on to C6-36 Aedes albopictus cells (Ito & Cherry, 1971), and 10- to 14-day culture fluids were tested by plaque assay on LLC-MK2 cells (Yuill et al., 1968).

Among 17 sentinel pigs which were monitored for at least 21 days, nine seroconverted with the first HAI antibodies detectable 12±4±5±2 days after placement. JEV strains were obtained from four of the nine seroconverting sentinel pigs from specimens obtained 10±7±4±2 days after placement. The seroconversion rate was related to the date of placement: 5/6 placed on 21 June; 3/5 placed on 8 July; and one/6 placed on 22 July. The peak of confirmed human cases of encephalitis occurred during the third week of July in 1982. The time to first detection of antibody among seroconverting pigs was also related to the date of placement: 10±2±3±6 days for pigs (n=5) placed on 21 June; 12±3±0±6 for pigs (n=3) placed on 8 July; and 24 days for pigs (n=1) placed on 22 July. Sentinel pigs along all three routes seroconverted 3/6, 4/4 and 2/5, and at least one isolate of JEV was obtained from animals along each route.

The opinions expressed in this article are those of the authors and do not purport to reflect the opinions or viewpoints of the US Army or the Department of Defense.