J. Hyg., Camb. (1966), **64**, 451
Printed in Great Britain

The isolation of Coxsackie A viruses from human sera and mosquitoes in Fiji

By T. MAGUIRE
Virus Research Unit, Medical Research Council, Dunedin
and F. N. MACNAMARA
Department of Microbiology, University of Otago, Dunedin

(*Received* 7 *June* 1966)

INTRODUCTION

In the middle and later years of the decade 1940–49 dengue was prevalent throughout the tropical islands of the Pacific. The inhabitants of Fiji were infected, a fact confirmed by a serological survey conducted in 1959–60 by Miles *et al.* (1964). Since 1950, illnesses diagnosed as dengue continued to appear, although at a much diminished rate. Illnesses diagnosed as influenza, however, were very prevalent both in epidemic years and in intervening periods. Evidence was lacking as to the proportion of these cases that were due to arboviruses, including dengue. Therefore, in 1963, attempts were made to isolate viruses from the sera of patients with febrile illnesses, and from mosquitoes caught both in dwelling houses and in the countryside.

Although no arboviruses were isolated, a number of strains of Coxsackie A6 were isolated, both from sera of patients and from mosquitoes. The isolation of Coxsackie A6 from sera has been reported before (Gear, Measroch & Prinsloo, 1956; Rodrigues *et al.* 1964), as has the isolation of Coxsackie viruses from mosquitoes (Taylor & Hurlbut, 1953), but such observations are relatively few.

METHODS

Virus isolations

The search for viruses was centred on Suva, which, with a population of 43,000, is the capital of Fiji, and is sited on the south-east corner of Viti Levu, the largest island of the group. The rainfall in this region is high, being about 130 in. per annum. The summer months, November to March, have a higher rainfall than the other months and are hot and humid. During these months mosquitoes are particularly plentiful.

During the last 3 weeks of January and the first week of February, liaison was established with the staff of a medical clinic, and samples of blood were taken at the clinic from patients suffering from ill-defined fever. Children in particular were the centre of attention.

Concurrently, mosquitoes were captured in the town and rural areas for the isolation of viruses. In the town, two Fijian mosquito inspectors visited houses

over a large area and collected, into sucking tubes, mosquitoes resting under the beds, in dark corners, wash-rooms, etc. In the rural areas the inspectors worked as a pair; one would remove his shirt and vest while the other with a sucking tube would suck off the mosquitoes alighting on him and preparing to bite.

After the mosquitoes or the blood from patients had been obtained they were taken to the laboratory in Suva. Sera were removed from the blood clots and stored at −70° C. The mosquitoes were killed by freezing, and sorted into pools of approximately 100 according to species, date and place of capture. The pools were stored at −70° C. until further processing, which consisted of trituration in buffered Hanks's solution, centrifugation at about 1000 r.p.m. for 10 min. and collection of the supernatant fluid.

Undiluted sera and the mosquito preparations were inoculated by the intracerebral and intraperitoneal routes into newborn mice. The mice were observed at least once daily for signs of illness and, if sick, they were killed and the brains removed for passage into further groups of mice.

The mosquito preparations were also inoculated on duck cell monolayers.

Serological survey

A limited number of serum samples collected from healthy children living on the three main islands of the Fiji group were available for testing for neutralizing antibodies. Intraperitoneal neutralization tests in 1-day-old mice were undertaken using a strain of Coxsackie A6 virus isolated from one of the patients in Fiji. If there was survival of more than two-thirds of the test-mice alive on the day following inoculation, the serum was considered positive for antibody content.

Table 1. *Age and sex of sixty patients examined for circulating virus*

	Age (years)						
	0–4	5–9	10+	20+	30+	40+	Unknown
Male	9	8	8	8	5	3	4
Female	6	3	3	1	0	2	
Total	15	11	11	9	5	5	4

RESULTS

Isolation of viruses from patients

Samples of sera were obtained from sixty patients whose age and sex distribution are shown in Table 1. Many of these patients complained of headache and body pains. Fever with a temperature of 100–102° F. was common. The cervical lymph nodes were frequently slightly enlarged and occasionally faucial injection was observed. No cutaneous eruptions suggestive of dengue were seen. Strains of virus were isolated from the sera of four patients (see Table 2). In every case re-isolation was successful. The four strains all had a similar behaviour in newborn mice, usually killing them in 3–4 days with widespread necrosis of the skeletal muscles, but with no appreciable damage to the brain or brown fat. They were

Table 2. *Sources and dates of virus isolations from human sera*

Specimen date	Race of patient	Age (years)	Sex	Days after onset	Remarks	Mouse inoculation: Date	Mouse inoculation: Days to death or sickness
8. i. 63	Part-European	8	F	0	Fever, 102° F, cough, tonsillar infection, total of 3 days illness	8. i. 63	1–3
14. i. 63	Indian	2½	F	0	Fever, 100° F, vomiting, ill for 3 days	14. i. 63	1–3
19. i. 63	Indian	8	M	1	Fever, 102° F, nausea cervical glands enlarged, complete recovery	21. i. 63	3
24. i. 63	Indian	6	M	1	Fever, 101·5° F, headaches and enlarged lymph glands	25. i. 63	3–10

Table 3. *Species and numbers of mosquitoes captured*

Species	Number
Aedes (*Aedimorphus*) *vexans*	1,653
A. (*Finlaya*) *fijiensis*	8
A. (*Ochlerotatus*) *vigilax*	4
A. (*Stegomyia*) *aegypti*	142
A. (*Stegomyia*) *polynesiensis*	36,007
A. (*Stegomyia*) *pseudoscutellaris*	43
Culex (*Culex*) *annulirostris*	4,187
C. (*Culex*) *quinquefasciatus*	1,505
C. (*Culex*) *sitiens*	3,273
Mansonia (*Coquilletidia*) *crassipes*	50
Total	46,872

Table 4. *Sources and dates of virus isolations from* Aedes polynesiensis *captured near Nukui village*

Pool no.	Date of capture	Date of mouse inoculation	Days to sickness or death of mice	Re-isolation
238	15. i. 63	3. v. 63	4	Not done
279	21. i. 63	23. viii. 63	2	Not done
369	22. i. 63	12. viii. 63	2	+
103	28. i. 63	7. ii. 63	4	+
233	28. i. 63	3. v. 63	5–7	+
237	28. i. 63	3. v. 63	6–8	+
305	28. i. 63	14. viii. 63	8	+
278	28. i. 63	19. viii. 63	5–7	−
267	28. i. 63	23. viii. 63	6	−

not inactivated by bile salts nor would they produce necrosis of duck embryo tissue cultures or HeLa cells. They were grouped as Coxsackie A viruses and subsequently shown by Dr L. Rosen to be type 6. This finding was confirmed in a neutralization test using specific monkey sera supplied by Dr J. Melnick.

Isolation of viruses from mosquitoes

The numbers and species of mosquitoes collected are shown in Table 3. Most of the *Aedes* (*Stegomyia*) *polynesiensis* were caught in rural areas, and the majority of the remaining species were caught in and around 100 dwelling houses in Suva. No viral isolations were made from mosquitoes caught in Suva, in spite of the fact that many contained undigested blood. Nine isolations in newborn mice, confirmed by re-isolation, were obtained from rural-caught *Ae.* (*S*) *polynesiensis*. The origin of these viruses is given in Table 4. All were derived from mosquitoes caught near the village of Nukui (lat. 18° 9′ S., long. 178° 34′ E.), situated among mangrove swamps at the southern tip of the Rewa delta. These strains have been identified as Coxsackie A 6.

Table 5. *Mouse neutralization tests on Fijian human sera using Coxsackie* A 6 (*V* 29 *strain*)

	Age group of donors								
	0–9 years			10–15 years			Total		
	No. tested	No. +	% +	No. tested	No. +	% +	No. tested	No. +	% +
Males	26	14	54	25	17	68	51	31	61
Females	19	12	63	13	12	92	32	24	75
Total	45	26	58	38	29	76	83	55	66

Serological survey

The results of the survey are shown in Table 5.

DISCUSSION

The clinical pictures of the infections were similar to those reported by Gear *et al.* (1956) and designated acute febrile lymphadenitis. Neither herpangina nor encephalitis was seen.

The isolation of the virus from the blood of four out of sixty patients suggests that an epidemic was occurring at that time, particularly when the rarity of viraemia in Coxsackie infections is recalled. Nevertheless the serological survey indicates that infections with Coxsackie A 6 virus are common in Fiji, presumably occurring in numerous small epidemics. Such a situation is to be expected with enteroviruses in tropical countries (Kalter, 1962; Rodrigues *et al.* 1964). The apparent difference in percentage of immunity between the sexes in our survey cannot be accounted for, but in any case it is hardly of statistical significance.

The frequency with which isolations were obtained from mosquitoes is striking,

and it is believed that they were not laboratory artifacts. Pools of mosquitoes and sera were stored in a low-temperature cabinet before processing, which occurred in a more or less random sequence. Some ensuing isolations were made when no previous isolations had been made for several months; and in the laboratory no grouping of isolations occurred. Yet on examining the source of the material a remarkable grouping is observed in the species of mosquitoes, and their times and place of collection. In fact all isolations were made from *Ae.* (*S*) *polynesiensis* caught near Nukui over a relatively short period.

The mosquitoes collected near Nukui could have become infected by biting the villagers or by biting the mosquito catchers who acted as bait. That the origin of the virus was the blood of the mosquito catcher is improbable for two reasons. First, the catchers took considerable care not to allow the mosquitoes, known vectors of filariasis, to probe. Secondly, the catchers remained symptomless throughout and even though symptom-free infections could have occurred it is unlikely that one or both could have carried circulating virus on four different days over a span of 14 days. Other vertebrates in the area were birds, rats and pigs and of these, pigs may have been a source of virus (Verlinde & Versteeg, 1958).

It appears that among different species of mosquitoes there is varying ability to harbour the virus. Nine isolations were made from *Ae.* (*S*) *polynesiensis*, whereas none were made from the other species captured. This could be due to the fact that only about a third as many of these others were captured. Nevertheless these were caught in houses in urban areas where the disease probably due to Coxsackie A 6 was known to be present and approximately 25 % of them contained blood.

At present it is impossible to assess the importance of the role of mosquitoes in transmitting this common infection. Further evidence is required on the degree and duration of viraemia in man and in particular on the ability of mosquitoes to transmit the virus rather than merely to harbour it. There are ample opportunities for the virus to pass from man to man other than by mosquito bites, so that in this respect mosquitoes may be of little importance.

SUMMARY

1. Coxsackie A 6 virus was isolated in January 1963 from the circulating blood of four children in Suva, Fiji, suffering from fever, lymphadenitis and pharyngitis.

2. Nine strains of the same agent were isolated from *Aedes* (*Stegomyia*) *polynesiensis* mosquitoes caught over a period of 2 weeks near or in a village situated among mangrove swamps at the mouth of the Rewa river, in January 1963.

3. No virus was isolated from mosquitoes caught in Suva.

4. A serological survey indicated that Coxsackie A 6 infections are common and widespread in Fiji.

This work was supported by funds from the U.S. Army Medical Research Command (grant No. DA–MD–49–193–63–G 84) and the New Zealand Medical Research Council.

We are grateful to the Medical Department, Fiji, for providing many facilities,

to Dr G. Hemming and the staff of the Bayly Clinic, Suva, for much co-operation in the collection of serum samples and to the staff of the Colonial War Memorial Hospital, who helped in many diverse ways. We wish to offer special thanks to the Mosquito Inspectors who did so much in collecting and sorting mosquitoes.

Grateful acknowledgement is made to Dr L. Rosen for identifying the strains and to Dr J. L. Melnick for supplying us with specific sera.

REFERENCES

GEAR, J., MEASROCH, V. & PRINSLOO, F. R. (1956). Medical and public health importance of the Coxsackie viruses. *S. Afr. med. J.* **30**, 806–10.

KALTER, S. S. (1962). A serological survey of antibodies to selected enteroviruses. *Bull. Wld Hlth Org.* **26**, 759–63.

MILES, J. A. R., MAGUIRE, T., AUSTIN, F. J., ROSS, R. W. & ORDISH, R. (1964). Evidence of arbovirus infection in Fiji. *Am. J. trop. Med. Hyg.* **13**, 327–30.

RODRIGUES, F. M., WEBB, A., ROWLAND, U., MYERS, R. M. & GEORGE, S. (1964). Isolation of Coxsackie A 6 virus from the serum of a case of herpangina in Vellore. *Indian J. med. Res.* **52**, 1221–5.

TAYLOR, R. M. & HURLBUT, H. S. (1953). The isolation of Coxsackie-like viruses from mosquitoes. *J. Egypt. med. Ass.* **36**, 489–94.

VERLINDE, J. D. & VERSTEEG, J. (1958). Coxsackie-virus-pneumonie bij biggen als smetstofbron voor de mens. *Tijdschr. Diergeneesk.* **83**, 459–68.