

## Arbovirus Infections in Ceylon

J. VESENJAK-HIRJAN,<sup>1</sup> Y. HERMON<sup>2</sup> & T. VITARANA<sup>3</sup>

*The haemagglutination-inhibition test has been used to assess the frequency of arbovirus infections in the population of Ceylon, by age-group, and also in some animals. The antigens used were: chikungunya, JE, dengue 1, 2, 3, 4, and Tahyna.*

*The percentage of positive reactors to the group-B viruses ranged from 16.3% to 84.6% and was lower in the mountainous part of the country. Infections with the chikungunya (group-A) virus were less frequent (from 0 to 37%) and were considered to be recent infections. The prevalence of antibodies was found to increase with age.*

*From a comparison of the antibody titres of the indigenous Ceylonese population with those of a group of immigrant Yugoslav workers it was concluded that a titre of 1:640 would represent a significant titre for a recent infection.*

*The animals examined included goats, cattle, bandicoots, pigs and sheep; virus infections were most frequent in the bandicoots, pigs and sheep.*

Among diseases in Ceylon "pyrexias of unknown origin" are very often quoted by clinicians and epidemiologists (Maretić et al., 1962). In 1957, for instance, a total of 225 804 such cases with 3644 deaths was reported. It is considered that arboviruses play a significant role in the etiology of these disorders. It has been known for about 20 years that dengue is endemic in the island (Mendis, 1967), but the first recorded epidemic of the disease occurred in 1965 in the south-western coastal zone. In Colombo itself 163 000 cases were recorded (Mendis, 1967). Only a few cases of haemorrhagic fever had been notified by 1967.

The first diagnosis of arbovirus infections was made at the Virus Research Centre, Poona, India, in 1956 where in 10 out of 20 patients antibodies against dengue were observed: no antibodies against chikungunya were observed in these patients. Identical results were later obtained in the first serological analyses made in Colombo (Hermon, 1967). It has been confirmed serologically that the epidemic of 1965 was caused by the chikungunya virus (Hermon, 1967; Mendis, 1967). Haemorrhagic fever cases have not been studied virologically in a satisfactory way so far for technical reasons.

The purpose of this study was to investigate the effect of arboviruses on various age-groups of the population in all geographical areas of Ceylon, as well as on a small number of animals on the island. Antibodies for Tahyna viruses were also examined.

### MATERIALS AND METHODS

The survey covered the whole of the island of Ceylon and blood was collected at the places shown on the map (Fig. 1). The central, eastern and northern zone (referred to in the figures as the east) with the towns of Anuradhapura, Jaffna, Trincomalee and Batticaloa, represented the dry zone. The south and west regions (referred to in the figures as the west) were also surveyed, including the towns of Matara, Galle, Panadura, Negombo, and Kochchikade, as well as the up-country region (the mountainous part) and mid-country region with the towns of Nuwara Eliya, Kandy, Badulla, and Ratnapura; the latter 2 regions represent the wet zone. In most regions a certain number of samples of sera were collected per age-group, as shown in Table 1, giving a total of 50 sera per region. In a few places, owing to technical difficulties, this total varied by 1 or 2 sera. From each person a sample of 10 ml of blood was collected in a vacuum container, was stored at 4°C, transported to the laboratory and stored at -20°C until the tests were performed. Sera were collected from patients in hospitals and outpatient departments (mostly from

<sup>1</sup> Head, Department of Virology, Andrija Štampar School of Public Health, Medical Faculty, University of Zagreb, Yugoslavia.

<sup>2</sup> Head, Virus Department, Medical Research Institute, Colombo, Ceylon.

<sup>3</sup> Virus Department, Medical Research Institute, Colombo Ceylon.

surgical cases), maternity and gynaecological wards, and internal-diseases wards (febrile cases were excluded). Sera were also collected from a group of Yugoslav workers who had arrived in Ceylon 3-4 months earlier and who were stationed in the mid-country. Animal sera were collected from the slaughter-house, from a quarantine station, a zoo, the Medical Research Institute animal house, all in Colombo, and also from a pig farm near Colombo, and a cattle farm near Nuwara Eliya.

The antigens used were: group A—chikungunya; group B—JE, dengue 1, 2, 3, 4, and Tahyna.

FIG. 1  
LOCATION OF SAMPLED AREAS IN CEYLON

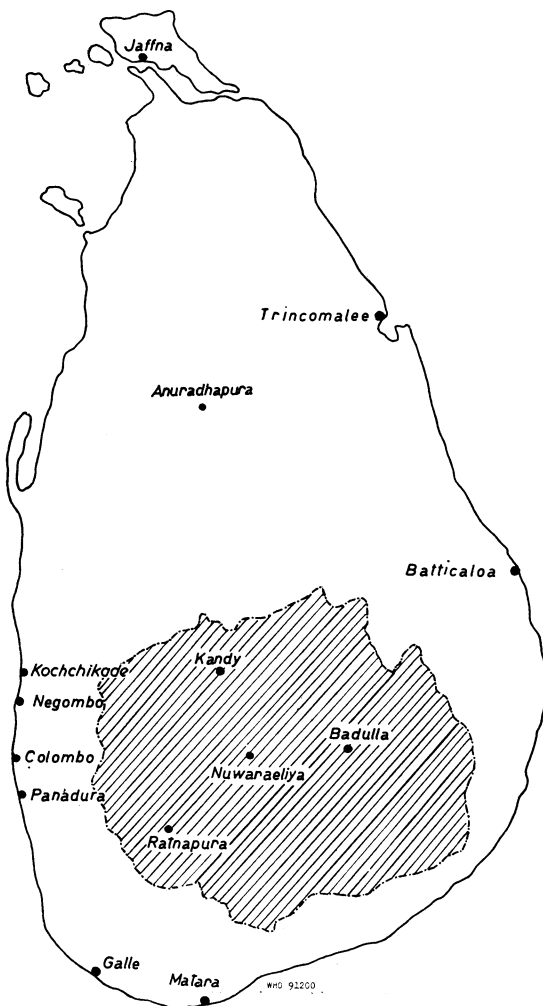


TABLE 1  
NUMBER OF SAMPLES COLLECTED PER AGE-GROUP

Age-group (years)	No. of samples
0.5-1	2
1	2
2	2
3	2
4	2
5-9	5
10-14	5
15-19	5
20-29	5
30-39	5
40-49	5
50-59	5
≥60	5
Total	50

Serological tests were carried out on plastic plates by the haemagglutination-inhibition test (HI), according to the standard method of Clarke & Casals (1958).

#### RESULTS

Table 2 shows the results of the HI tests in each of the places sampled. The highest prevalence of infections with B-group viruses was observed in Trincomalee in the east zone (84.78%) and in Panadura in the west zone (76.74%). In the mountainous zone the prevalence was much lower.

The prevalence of infections with chikungunya (group-A) virus was much lower in general, but again the highest was in Trincomalee (36.95%) and Panadura (23.25%). The prevalence of chikungunya infections was lowest in the mountainous area.

The results according to age-group and area are shown in Fig. 2. The frequency of infections with B-group viruses in the eastern and western areas was very similar, but these areas differed from the mountainous area in all age-groups. The data for the 3 areas are summarized in Fig. 3 and it is clear that, in general, the frequency of infections with group B viruses increased with age.

In the 0-14-year age-group, infections with A-group viruses were more prevalent in the east; in the

TABLE 2  
RESULTS OF HAEMAGGLUTINATION-INHIBITION TESTS BY TOWNS

Town	No. of sera examined	Positive for chikungunya		Positive for group B		Positive for chikungunya + group B	
		No.	%	No.	%	No.	%
Anuradhapura	48	7	14.58	28	58.33	7	14.58
Jaffna	52	6	11.53	27	51.92	5	9.61
Batticaloa	45	3	6.66	25	55.55	3	6.66
Trincomalee	46	17	36.95	39	84.78	16	34.78
Total	191	33	17.27	119	62.30	31	16.23
Nuwara Eliya	48	8	16.66	22	45.83	7	14.58
Kandy	46	2	4.34	16	34.78	1	2.17
Badulla	48	0	—	8	16.66	0	—
Ratnapura	48	0	—	8	16.66	0	—
Total	190	10	5.26	54	28.42	8	4.21
Galle	50	3	6.00	30	60.00	2	4.00
Matara	49	5	10.20	8	16.32	2	4.08
Negombo	42	8	19.04	25	59.52	8	19.04
Kochchikade	46	5	10.86	33	71.73	5	10.86
Panadura	43	10	23.25	33	76.74	9	20.93
Total	230	31	13.47	129	56.08	26	11.30
Grand total	611	74	12.11	302	49.42	65	10.63

15-40-year age-group they were more frequent in the east than in the mountainous area, while in the age-group of 50 years and older there was not much difference between areas. In general prevalence increased with age.

As regards combined infections with chikungunya (group-A) and group-B viruses the differences between the east and the west were not very great, while there were notably fewer combined infections in the mountainous area.

Fig. 4 shows the percentage distribution of antibody titres among the positive sera examined. Among the group-B viruses the highest number of low titres, but also the highest number of high titres were found for the JE virus. If the titres among the residents for all the group-B viruses are compared with those for chikungunya (group A) it is clear that

there is a higher percentage of low titres in the B group and a higher percentage of higher titres in the A group. The titres of the Ceylonese population were compared with a group of 76 temporary immigrants from Yugoslavia, where no group-A virus has so far been found; the TB virus from group B does exist in Yugoslavia but not in the area from which these workers came.<sup>1</sup> Thus it is justifiable to assume that the antibody titres of these workers for chikungunya and dengue indicate primary infections. Comparing the titres of the Ceylonese and non-residents there was a marked difference between the group-A and the group-B viruses. None of the non-residents had an antibody titre higher than 1 : 320 (Fig. 4).

<sup>1</sup> Vesenjck-Hirjan (unpublished data).

FIG. 2  
 POSITIVE REACTIONS TO THE HAEMAGGLUTINATION-INHIBITION TEST BY AREA AND BY AGE-GROUP

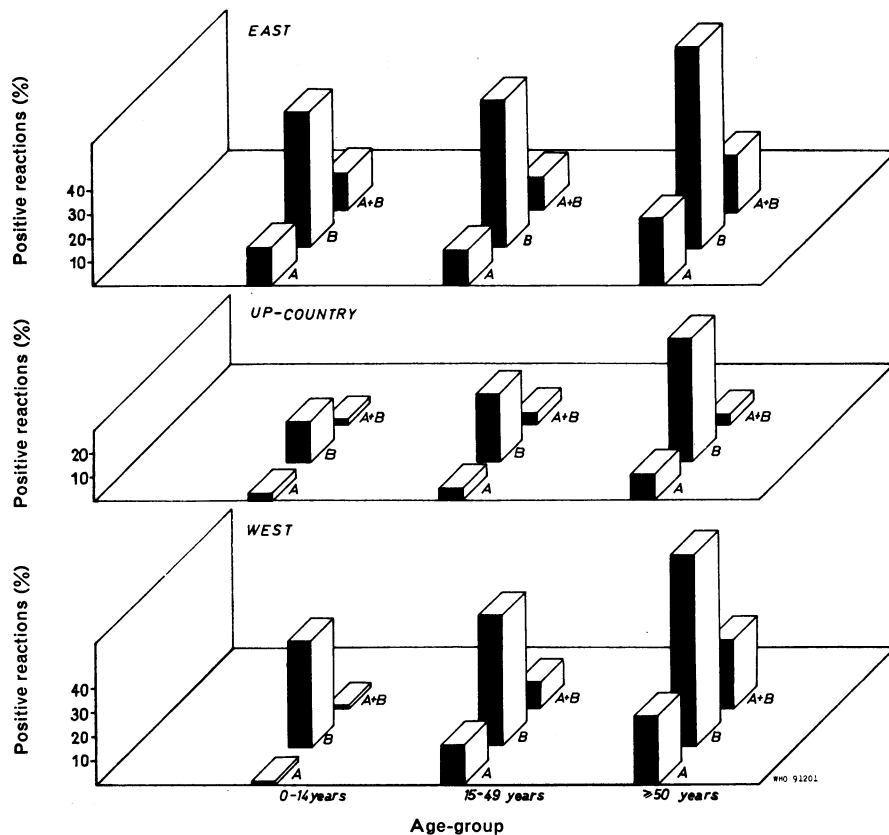


FIG. 3  
 POSITIVE REACTIONS TO THE HAEMAGGLUTINATION-INHIBITION TEST BY AGE-GROUP (ALL AREAS)

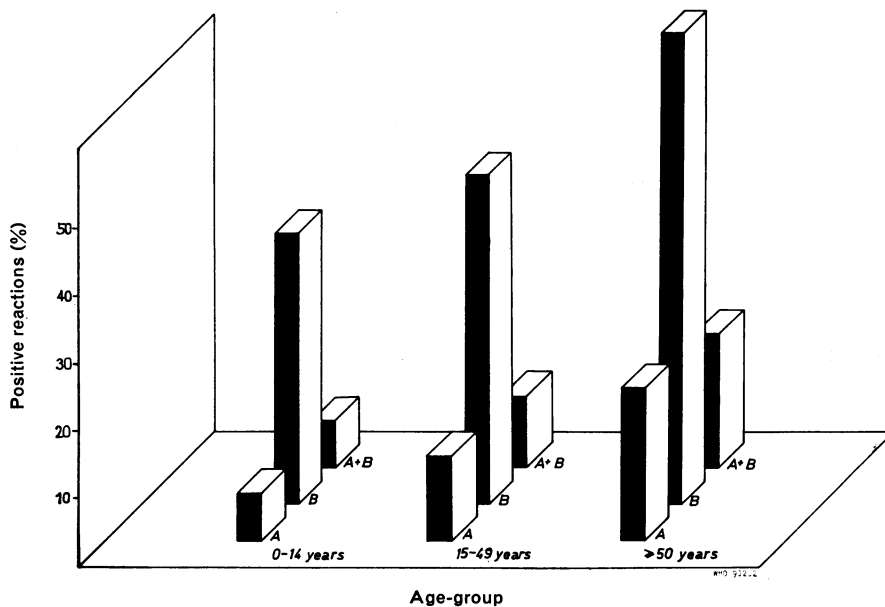


FIG. 4  
PERCENTAGE DISTRIBUTION OF ANTIBODY TITRES AMONG THE POSITIVE SERA EXAMINED

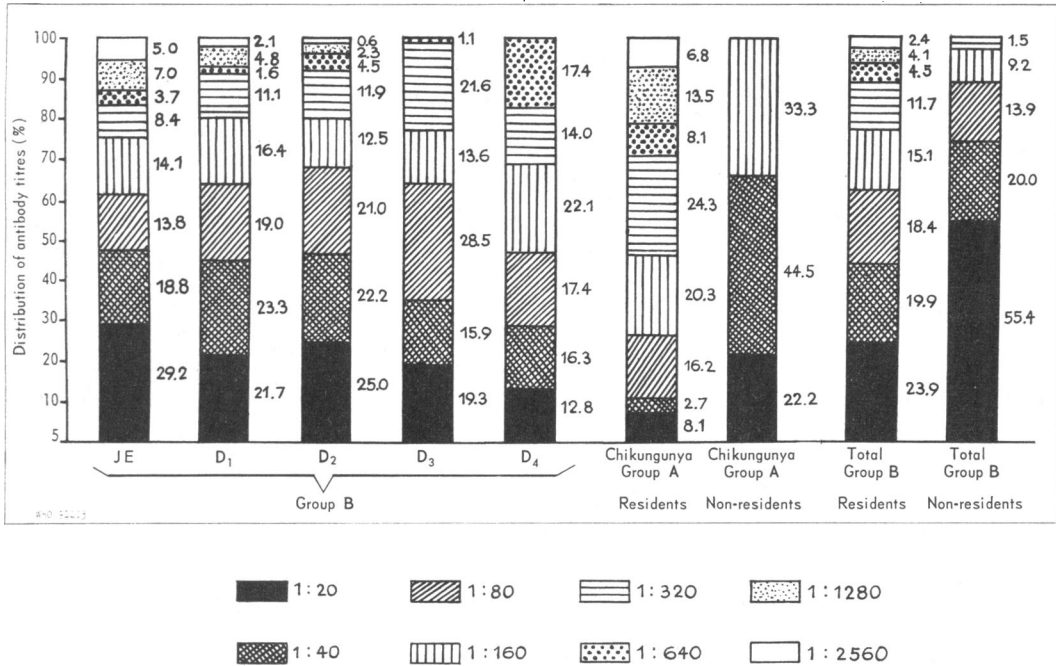


Table 3 shows the results of antibody analysis in animals. The highest percentage of positives for the group-B viruses was found among pigs and bandicoots; for chikungunya the percentages were much lower in all animals.

The actual titres (Table 4) were highest for pig; among the other animals, there were only small differences.

Altogether 358 human sera were tested for Tahyna; 11 proved positive, but in only 3 did the titre exceed 1:20.

DISCUSSION

It appears from the results that group-B arbovirus infections are endemic in the whole island of Ceylon, although they are less frequent in the mountainous areas. Chikungunya (group-A) virus infections were less numerous in general, and particularly in the mountainous regions. Arbovirus infections were also found to be less prevalent in the mountainous regions of Malaysia (Halstead & Udomsakdi, 1966); this may have been due to the smaller population of vectors in these areas, but this has not been investigated, so far, in Ceylon.

There were notable differences among the different age-groups in the percentage of positive reactors for both group-A and group-B viruses. These differences were apparently more pronounced for the chikun-

TABLE 3  
RESULTS OF HAEMAGGLUTINATION-INHIBITION TEST ON ANIMALS

Animal	No. of sera examined	Positive for chikungunya		Positive for group B		Positive for chikungunya + group B	
		No.	%	No.	%	No.	%
Goat	57	3	5.26	16	27.07	3	5.26
Sheep	39	3	7.69	10	25.64	2	5.12
Cattle	48	3	6.25	12	25.00	3	6.25
Pig	38	1	2.63	23	60.52	1	2.63
Bandicoot	21	1	4.76	13	61.90	1	4.76

TABLE 4  
ANTIBODY TITRES <sup>a</sup> IN ANIMALS

Animal	Chikungunya				Group-B viruses						
	20	40	80	160	20	40	80	160	320	640	1280
Goat	2	1			9	5	11	4			
Sheep	1		2		6	3	8	1	1		
Cattle		1	2		3	3	9	5			
Pig				1		4	20	16	11	4	4
Bandicoot	1				10	5	11	4		1	

<sup>a</sup> Titres are expressed as reciprocals of dilutions showing inhibition.

gunya (group-A) virus. It may be assumed that chikungunya or some related viruses were active many years ago and have remained inactive until the outbreak of the epidemic in 1965. The more frequent high antibody titres against the chikungunya (group-A) virus and the more frequent low titres against the group-B viruses may also be regarded as confirmation of the fact that the infection with chikungunya virus is very recent.

The high prevalence of antibodies against the group-B viruses and also against the chikungunya (group-A) virus, in Nuwara Eliya (the mountainous area) may be due to the fact that the population of this region are very largely tea-estate workers who may have had contact with southern India where epidemics occurred in 1964. Another fact that

should not be overlooked is that near Nuwara Eliya there are farms where high antibody titres have been found in some animals. The high prevalence of antibodies in Trincomalee, especially against the chikungunya (group-A) virus, may be ascribed to the fact that this is a sea-port, which has much traffic with eastern countries as well as with Colombo where the chikungunya epidemic occurred.

When the antibody titres of the Ceylonese population were compared with those of the non-resident Yugoslav workers we found that none of the latter had developed titres higher than 1 : 320; from this we can assume that a titre of 1 : 640 would represent a recent infection in Ceylon, a conclusion similar to that reached by other investigators in Thailand (Udomsakdi & Halstead, 1966).

#### ACKNOWLEDGEMENTS

We wish to thank Professor Lim Kok Ann of the Department of Bacteriology, University of Singapore, for so kindly supplying for the Virus Laboratory, Medical Research Institute, most of the arbovirus antigens used in this survey. Some antigens were also supplied by the World Health Organization and the Andrija Štampar School of Public Health, Medical Faculty, University of Zagreb.

We also wish to thank the technical staff of the Virus

Laboratory, Medical Research Institute, Colombo, for their able and willing assistance in carrying out the laboratory tests for this work. Thanks are also due to the technical staff of the Virus Laboratories, Andrija Štampar School of Public Health, and the Institute of Public Health of Croatia, Zagreb. We also greatly appreciate the assistance given us by the physicians, veterinarians, nurses and auxiliary staff in the collection of the sera.

#### RÉSUMÉ

##### LES INFECTIONS À ARBOVIRUS À CEYLAN

Les auteurs ont procédé à une enquête sérologique en vue de déterminer la fréquence des infections à arbovirus à Ceylan. Au total, 611 sérums ont été prélevés chez des personnes habitant dans diverses régions de l'île, et repré-

sentant toute la gamme des groupes d'âge. Les sérums de quelques immigrants arrivés à Ceylan depuis 3-4 mois ont été également examinés. Enfin, on a recherché les anticorps spécifiques dans les sérums prélevés chez un certain

nombre d'espèces animales domestiques et sauvages. Les tests ont été effectués par la méthode d'inhibition de l'hémagglutination en utilisant comme antigènes des arbovirus du groupe A (virus chikungunya) et du groupe B (virus de l'encéphalite japonaise (JE), de la dengue 1, 2, 3, 4 et Tahyna).

La prévalence maximale des infections à arbovirus du groupe B a été constatée dans les villes de Trincomalee (84,78%) et de Panadura (76,74%). Elle était beaucoup plus faible dans les régions montagneuses de l'île. Une situation analogue a été retrouvée en ce qui concerne le virus chikungunya, avec respectivement 36,95% et 23,25% de sérums positifs dans les deux villes précitées et une prévalence moindre dans la partie montagneuse du pays.

L'étude de la répartition des infections par groupe d'âge a fait ressortir en général une fréquence accrue de la positivité des sérums — aussi bien pour les virus du groupe B que pour le virus chikungunya — dans les tranches d'âge élevé. Le plus grand nombre de sérums positifs de faible titre ou de titre élevé a été observé avec le virus JE.

On a relevé un plus fort pourcentage de titres faibles parmi les sérums positifs pour les virus du groupe B et un pourcentage plus fort de titres élevés parmi les sérums positifs pour le virus chikungunya. Chez les immigrants de fraîche date, on n'a pas décelé de titre supérieur à 1:320 en cas de positivité des sérums, qui est considérée comme le signe d'une infection primaire.

Chez les animaux, le maximum de positivité des sérums pour les virus du groupe B a été relevé chez les porcs et les rats bandicots; les pourcentages de positivité vis-à-vis du virus chikungunya ont été beaucoup plus faibles chez tous les animaux.

L'épreuve d'inhibition de l'hémagglutination pratiquée avec le virus Tahyna sur 358 sérums humains a révélé 11 sérums positifs, dont 3 seulement avec un titre supérieur à 1:20.

Les auteurs concluent que les infections à arbovirus du groupe B sont endémiques sur toute l'étendue de Ceylan. Les infections à virus chikungunya sont moins fréquentes et d'origine plus récente.

#### REFERENCES

- Clarke, D. H. & Casals, J. (1958) *Amer. J. trop. Med. Hyg.*, **7**, 561  
 Halstead, B. S. & Udomsakdi, S. (1966) *Bull. Wld Hlth Org.*, **35**, 89  
 Hermon, Y. E. (1967) *Ceylon med. J.*, **12**, 81  
 Maretić, Z., Arumanayagam, P., Nithyananda, K., Wickramasinghe, R. L. & Ratnatunga, P. C. C. (1962) *Ceylon med. J.*, **7**, 89  
 Mendis, N. M. P. (1967) *Ceylon med. J.*, **12**, 67  
 Udomsakdi, S. & Halstead, B. S. (1966) *Bull. Wld Hlth Org.*, **35**, 70