

SEROLOGIC SURVEY OF DENGUE AND OTHER ARBOVIRUSES IN CURAÇAO AND ARUBA, 1973¹

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Sera obtained principally from children on the Caribbean islands of Aruba and Curaçao were tested for antibodies to various arboviruses. The tests indicated that dengue 2 was endemic on Curaçao but not on Aruba, perhaps because of more effective vector control measures on Aruba. Neutralization tests performed with a small number of sera suggest that dengue infections may have afforded some level of protection against yellow fever to a portion of the Curaçao population.

Introduction

Dengue infection is known to have been widespread in the Caribbean for many years. A comprehensive review by Theiler and Downs (1) states that the disease is usually clinically inapparent or mild in this area and that *Aedes aegypti* is the vector.

Dengue is caused by four closely related group B arboviruses, three of which (dengue 1, 2, and 3) have been found in the Caribbean. However, dengue-like fevers may be caused by other viruses—including influenza virus, coxsackie virus, adenovirus, and various arboviruses—as well as other infective agents. Thus a definite diagnosis of dengue requires either isolation of the virus or detection of a significant rise of dengue antibodies in paired (acute and convalescent) serum samples.

Facilities for these virus studies are not widely available, so exact diagnosis of dengue is difficult, the disease is frequently not diagnosed, and most knowledge of dengue distribution in the Caribbean consequently comes from serologic surveys.

Interest in dengue has increased because of the severe and sometimes fatal hemorrhagic complications associated with dengue infections in South-East Asia (2). There have also been reports of hemorrhagic complications in the Caribbean—notably those associated with dengue 2 infections in Curaçao in 1968 (3) and in Puerto Rico in 1975 (4). Moreover, the eradication of *A. aegypti* from the Americas has proceeded more slowly than anticipated; areas previously reported free have been reinfested, and in many parts of the Caribbean the mosquito still occurs in numbers sufficient to permit dengue transmission.

Because of such developments, it was decided several years ago to carry out a serologic survey of dengue and other selected arboviruses in the Netherlands Antilles. A summary of results for the Windward Islands (St. Maarten, Saba, and St. Eustatius) has been provided by van der Sar (5). The results of a survey conducted in Curaçao and Aruba in 1973, previously reported in summary form (6), are described here in more detail.

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Survey Area

Curaçao and Aruba, with respective areas of approximately 440 and 190 km², are two of the three Leeward Islands of the Netherlands Antilles situated near the Venezuelan coast. Their climate is tropical, with little

variation in temperature throughout the year. The islands are relatively dry, however, with the rainy period extending only from October to January. Rainfall on Aruba averages 43.3 cm per year, which is about three-quarters of the average rainfall on Curaçao.

Geologically, the islands are composed of an old volcanic rock series covered partly by quaternary coral limestone. The vegetation of both islands has a pronounced xerophytic character, but Aruba's landscape is more barren than Curaçao's and its vegetation is poorer.

The respective populations of Aruba and Curaçao were approximately 61,000 and 152,000 in 1973. Most residents of Curaçao live in the city of Willemstad, and most residents of Aruba live in the towns of Oranjestad and San Nicolas.

Aedes aegypti Control Measures

Several attempts have been made to control and eradicate *A. aegypti* in Aruba and Curaçao, where it has been present for over half a century. Control measures, using dieldrin as a residual spray and DDT as a perifocal treatment, started in Curaçao in 1951 and in Aruba in 1952. The campaigns were intensified in 1955 after the Trinidad yellow fever epidemic of 1954 (1).

As a result of this work, Aruba became practically free of *A. aegypti* until 1965. The campaign on Curaçao was less successful, the only considerable reductions in *A. aegypti* infestation indices being recorded in 1957-1960. Dieldrin and DDT began to lose their effectiveness in 1962, and the use of these materials was discontinued in 1963.

During 1970 an eradication campaign was begun on Aruba using malathion as an intradomiciliary residual spray and Abate as a focal treatment. This greatly reduced the *A. aegypti* house-index. Most of Aruba is now free of *A. aegypti*, except for a few localities that are believed to have been reinfested from outside the island.

In May 1975 a vector control program using the perifocal method was instituted on Curaçao. The initial survey found practically all localities to be positive for *A. aegypti*.

Yellow Fever Vaccination

There have been no mass vaccination programs. Yellow fever vaccinations have been given to those requiring them for international travel.

Materials and Methods

Sampling Procedures

A total of 3,044 sera (1,221 from Aruba and 1,823 from Curaçao) were collected during 1973 from children 4 to 12 years of age who were attending kindergarten or primary school. The children were selected using a two-stage sampling frame.

Stage I. Schools in Curaçao were initially selected from an alphabetic list in a systematic manner; but half of the schools selected in each group did not participate, so other schools willing to cooperate were added. The ultimate result was as follows:

Type	Number of Schools			
	Listed	Selected	Dropped	Added
Kindergartens	45	10	5	1
Primary schools	71	20	10	7

On Aruba, one of the larger primary schools and one or two of the kindergartens were selected from schools willing to participate in each of two towns (Oranjestad and San Nicolas) and two villages (Noord and Santa Cruz) where about 80 per cent of the inhabitants reside.

Stage II. Complete lists of children attending each school were received, and the children to be tested were selected in a manner that was free from systematic bias

but not truly random. Forms explaining the purpose of the survey were sent to the parents of all children selected. Serum samples were taken from the children after written parental consent was received.

The rate of parental refusal was low. Unfortunately, records of nonresponses and additions to the list of test subjects were not kept, though it is known that many deviations from the original sample occurred. Despite these circumstances, however, it is thought that the samples collected were reasonably representative of the areas concerned.

Adult samples. A collection of 252 sera from adults on Curaçao and Aruba between the ages of 25 and 40, which had been submitted for VDRL serology, were subsequently tested.

Serologic Techniques

Hemagglutination inhibition (HI) tests were performed using a microtechnique with methods similar to those described by Clark and Casals (7) and Severs (8). Antigens were prepared from the following arboviruses: Venezuelan, western, and eastern equine encephalitis; Mayaro; dengue 1, 2, and 3; Ilheus; yellow fever (YF); St. Louis encephalitis (SLE); Caraparu; Restan; and Oriboca.

Neutralization (N) tests that were conducted have been described by Spence *et al.* (9).

Results

The number of sera tested, by age group, are indicated in Table 1. This table also shows the population figures for children in the 4-12 age group (as reported by the Government Bureau of Statistics, Department of Economic Affairs) and the percentages testing positively for group B arbovirus antibodies. Overall, more than 60 per cent of the sera failed to give a positive HI response to any of the agents tested. The

only positive responses were to group B antigens, the other groups yielding negative results.

The proportion of group B positives, among both children and adults, was much higher in Curaçao than Aruba. The differences between the two islands' children was especially marked, the percentage of children found positive on Curaçao (58.3) being about 10 times greater than the percentage found positive on Aruba (5.9).

The sera positive to group B antigens varied in terms of both the antigens eliciting a response and the titer of that response. Of the 417 sera positive for a single group B antigen, only 20 were not positive for dengue 2 (see Table 2). In addition, all the children's sera which were positive for more than one antigen were positive for dengue 2.

Table 3 shows the children's sera that were positive for dengue 2 and other antigens in both Curaçao and Aruba. Looking at the Curaçao sera, it is noteworthy that 87 per cent (73/84) of the sera positive for only two antigens were positive for dengue 1. In Aruba, where few sera were positive, 55 per cent (6/11) of the sera positive for only two antigens were positive for dengue 1. Table 4 shows the results obtained with children's sera from the four Aruba towns and villages involved.

The various sera yielding a positive response with only one antigen had the following HI titers:

Viral source of antigen	Titers (Curaçao)		
	1/10	1/20	1/40
Dengue 2	58	296	17
YF	2	1	—
SLE	2	—	—
	Titers (Aruba)		
	1/10	1/20	1/40
Dengue 2	5	21	—
YF	11	3	1
SLE	—	—	—

Table 1. A comparison of HI test results for child and adult sera, showing the ages of children tested, the numbers of children with the same age in the population at large, and the percentages of sera testing positively for group B arbovirus antibodies.

Age (years)	Curaçao						Aruba					
	1973 population		Sera Tested				1973 population		Sera Tested			
	% of over- all 4-12 age group	No.	% of total sample tested	No.	HI-positive for group B antibodies		% of over- all 4-12 age group	No.	% of total age group tested	No.	HI-positive for group B antibodies	
				No.	No.	%				No.	No.	%
4	10.1	3,440	4.1	75	22	29.3	9.1	1,172	10.9	133	3	2.3
5	10.1	3,400	7.2	132	47	35.6	9.2	1,179	10.8	132	5	3.8
6	9.8	3,355	12.3	224	118	52.7	9.4	1,204	12.0	147	5	3.4
7	11.2	3,814	13.2	240	125	52.1	10.5	1,350	10.7	131	5	3.8
8	11.5	3,934	13.3	242	144	59.5	11.1	1,430	10.5	128	10	7.8
9	11.9	4,043	12.9	236	155	65.7	11.8	1,523	11.0	134	8	6.0
10	11.9	4,068	12.9	235	158	67.2	12.6	1,628	11.1	136	15	11.0
11	11.8	4,023	12.0	219	148	67.6	13.0	1,667	11.8	144	13	9.0
12	11.7	4,000	12.1	220	146	66.4	13.3	1,710	11.1	136	8	5.9
Total (children)	100.0	34,077	100.0	1,823	1,063	58.3	100.0	12,863	99.9	1,221	72	5.9
(adults)				140	134	95.7				95	32	33.7

Table 2. Group B virus antigens yielding a positive HI response.

Group B HI antigens		Curaçao		Aruba		Total
No. of antigens testing positively	Source of antigens	Children	Adults	Children	Adults	
1	Dengue 2 (d2)	367	4	26	0	397
	YF	0	3	1	14	18
	SLE	2	0	0	0	2
2-3	d2 included	161	5	17	3	186
	d2 not included	0	4	0	2	6
4-5	d2 included	130	6	11	4	151
	d2 not included	0	0	0	0	0
6		403	112	17	9	541
Total		1,063	134	72	32	1,301

Table 3. Children's sera that were HI-positive for different virus antigens.

No. of antigens evoking a positive response	Curaçao (children)					Aruba (children)				
	2	3	4	5	Total	2	3	4	5	Total
No. of sera responding positively to 2 or more antigens	84	77	64	66	291	11	6	4	7	28
<i>Viral sources of antigens:</i>										
Dengue 1	73	71	63	66	273	6	6	4	6	22
Dengue 2	84	77	64	66	291	11	6	4	7	28
Dengue 3	0	8	0	36	44	0	0	0	1	1
Ilheus	3	11	35	61	110	0	0	2	7	9
YF	2	19	5	41	67	2	5	4	7	18
SLE	6	45	60	60	171	4	1	2	6	13

The geometric mean titers (GMT) for all HI-positive sera, by antigen, age group, and island are shown in Table 5. The GMT for dengue 2 was found to increase with age on Curaçao. Among Curaçao adults, the observed GMT was highest for YF (1.63),

though the dengue 2 GMT (1.52) was almost as high.

To estimate potential immunity to yellow fever (YF), 69 sera selected to include YF HI-positives and a few negatives were tested. Because many sera were found to contain

Table 4. Results obtained with Aruba children's sera, showing the numbers of sera tested and the proportions yielding a positive HI response to one or more group B arbovirus antigens.

Age (years)	Oranjestad		San Nicolas		Santa Cruz		Noord		Total	
	% positive	No. pos./ No. tested	% positive	No. pos./ No. tested	% positive	No. pos./ No. tested	% positive	No. pos./ No. tested	% positive	No. pos./ No. tested
4-6	5.5	8/145	3.4	5/148	0	0/52	0	0/67	3.2	13/412
7-9	13.9	17/122	3.2	4/125	0	0/74	1.4	1/72	5.6	22/393
10-12	20.6	26/126	6.8	9/133	0	0/81	2.6	2/76	8.9	37/416
Total (children)	13.0	51/393	4.4	18/406	0	0/207	1.4	3/215	5.9	72/1,221

Table 5. The geometric mean titers of all HI-positive sera, by age group and virus, in Curaçao (C) and Aruba (A).

Test subjects	Dengue 1		Dengue 2		Dengue 3		Ilheus		YF		SLE	
	C	A	C	A	C	A	C	A	C	A	C	A
<i>Children:</i>												
4-6 years	0.53	0.74	1.36	1.30	0.28	0.25	0.38	0.36	0.33	0.55	0.45	0.95
7-9 years	0.74	0.67	1.41	1.25	0.42	0.32	0.56	0.44	0.51	0.54	0.60	0.44
10-12 years	1.03	0.68	1.45	1.25	0.62	0.23	0.75	0.42	0.78	0.62	0.86	0.38
<i>Adults:</i>	1.31	0.37	1.52	0.70	1.07	0.33	1.41	0.67	1.63	1.23	1.45	0.59

antibodies to both yellow fever and dengue, the selected sera were challenged with 1,000 LD₅₀ of a YF strain (TRVL 2943) in an infant mouse neutralization test. This test yielded the following results:

Neutralization	No. of sera	HI GMT (YF)	HI GMT (dengue)
positive	33	1.39	1.54
uncertain	16	1.26	1.90
negative	20	1.33	1.97

The donors of the 18 sera that showed only YF HI-antibodies were traced, as were 22 other donors whose sera showed high levels of YF HI-antibodies. None of these people gave a history of YF vaccination or of travel to areas where yellow fever was endemic.

Discussion

When interpreting the results, it should be noted that a number of changes were made in the sampling on Curaçao and Aruba; and the representation of children 4 and 5 years old was less than expected on Curaçao. Despite these circumstances, however, the samples are thought to have been reasonably representative.

Another point to note is that the interpretation of surveys and diagnostic serology for dengue infection may be complicated by heterologous antibody reactions to antigenically closely related group B arboviruses. The Curaçao group B results, however, show an antibody pattern compatible with endemic dengue 2. There is a definite increasing percentage of positives, a broadening of antibody patterns, and an increase in the GMTs with age that are similar to the findings reported by Theiler and Downs (1) in a survey in Trinidad in the 1950's. Similar results were reported for 1972 for St. Maarten and St. Eustatius, with a slightly lower percentage of positives being found in Saba by van der Sar (3).

In contrast, a survey of Curaçao in 1959

and 1960 by Downs et al. (10) found only 3 per cent (3/100) of the sera from subjects 5 through 14 years of age to be positive for group B, whereas 65 per cent (13/20) of the sera from subjects 20 through 39 years of age were positive for group B. The authors suggested that the low level of group B antibodies in the children might be associated with a successful *A. aegypti* control program. (As previously mentioned, considerable reductions in *A. aegypti* infestation indices were recorded on Curaçao in 1957-1960.)

The results for children in Aruba show little arbovirus activity; but they also show considerable differences from area to area (see Table 4), a point that should be kept in mind when interpreting the results for Aruba in Table 1. The lower rates of positivity among adults on Aruba suggest that there was also less dengue transmission on that island in the past. As there is regular movement of people between Aruba and Curaçao, it seems likely that a proportion of the dengue infections of Aruba residents were transmitted on Curaçao.

The results give no indication that dengue types other than dengue 2 were recently present on either island; however, the presence of dengue 1 cannot be ruled out.

Cross-reactions were probably responsible for the very few cases in which sera only showed antibodies to SLE virus, though it is possible that occasional introduction of the virus to the islands may have occurred. The Ilheus antibodies detected may all have been associated with dengue infections. Similar observations were made in a study of 278 sera from Curaçao children and adults collected in 1959 and 1960 (10).

Antibody patterns showing only antibodies to YF or relatively high titers to it could possibly indicate that yellow fever may have occasionally been introduced in recent decades, but the disease has not actually been observed in the Netherlands Antilles since 1914 (11). Our follow-up

studies on the 40 selected YF HI-positive persons were negative for a history of yellow fever immunization, and no large-scale vaccination has been done in the area. Nevertheless, as stated by Theiler and Downs (1), the presence of yellow fever can easily go unnoticed in areas where other group B arboviruses are endemic.

In view of this, it was felt that further investigation of the sera positive for YF and dengue HI antibodies was needed to determine if YF N antibodies, which are normally associated with protection against yellow fever, were present. The finding—

that 33/69 (48 per cent) of the sera were positive in a YF neutralization test—suggests that endemic dengue infections may have conveyed some level of protection against yellow fever to a proportion of the Curaçao population. This finding is in accord with the work of Theiler and Anderson (12), who studied the relative resistance of dengue-immune monkeys to yellow fever virus; they found that the viremia levels of challenge yellow fever virus were significantly lower in dengue-immune animals than in controls.

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SUMMARY

A total of 3,044 sera were collected from children 4-12 years of age on Curaçao and Aruba during 1973. The sera were tested for hemagglutinating antibodies to the following arboviruses: Venezuelan, western, and eastern equine encephalitis; Mayaro; dengue 1, 2, and 3; Ilheus; yellow fever; St. Louis encephalitis; Caraparu; Restan; and Oriboca. Antibodies were found only to group B viruses, 1,063 of 1,823 sera and 72 of 1,221 sera from residents of Curaçao and Aruba, respectively, showing detectable levels of antibodies.

Subsequently, 252 sera from adults 20-40 years old were tested. Ninety-six per cent of those from Curaçao and 34 per cent of those from Aruba were positive for group B antibodies.

The results show that dengue 2 is endemic on Curaçao but not on Aruba. The much lower positivity rates on Aruba may be related to more effective *A. aegypti* control measures.

Mouse neutralization tests on a small proportion of the sera suggest that the dengue infections involved may have afforded some level of protection against yellow fever.

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