THE OCCURRENCE OF ENTEROVIRUSES IN CHILDREN FROM MODERN FLATS AND SQUATTER-DWELLINGS IN SINGAPORE

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Concurrently with a study on the influence of rehousing on the prevalence of infections by soil-transmitted helminths (Kleevens, 1966)[1], it was thought appropriate to investigate stools of children for the occurrence of enteroviruses. Such a study will provide information on the types of enteroviruses prevalent in Singapore and show whether environmental conditions influence their distribution. This paper is a report of the findings.

The study was made during August—December 1964 in an urban area (Bukit Ho Swee) in Singapore. The survey area, population and sampling methods have been described in detail previously (Kleevens, 1966)[1]. For this study on the occurrence of enteroviruses only children under the age of seven years were included.

LABORATORY METHODS

Stool samples were sent to the Department of Bacteriology for virus studies within two hours of collection and approximately three hours after being passed.

Specimens of stool were processed on the day of collection for virus isolation. A 10% suspension was made in distilled water containing 2,000 units of penicillin and 2000 ug. of streptomycin per ml. The suspension was spun at 3,000 r.p.m. for 30 minutes, the supernatant was removed and stored frozen at -20°C until ready for use.

Monkey kidney cell cultures were used for virus isolation. Two culture tubes were each inoculated with 0·1 ml. of clarified suspension. The cultures were examined daily for cytopathogenic changes. Cultures that were negative after 10 days were discarded. Cytopathogenic agents recovered were identified by neutralization tests with antiserum pools containing immune sera for polioviruses 1, 2 and 3, coxsackieviruses B 1-5 and A9, and echoviruses 1-15, 17-19, 22-23 and 26.

RESULTS

The results of virus isolation from the stools of the children of the two study groups are summarized in Table 1. The overall rate of enterovirus circulation for Bukit Ho Swee is 21·7%. One-half of the positive isolations were echoviruses, another quarter coxsackieviruses, one-tenth polioviruses and the rest untyped viruses. The enterovirus rate was 23·1% for the flat-dwellers and 20·0% for the squatters. This difference is not statistically significant ($0{\cdot}50 > P > 0{\cdot}30$).

The rate of enterovirus circulation in the various age-groups of the flat-dwellers showed little variation. For the squatter children the rate was higher for those under 1 year of age (28·6%) than for the 4-6 year-olds (15·2%).

The isolation rates of coxsackieviruses and echoviruses in the two study groups were closely similar. The isolation rate of polioviruses for the flat-dwellers was twice that for the squatters and of the untyped viruses one and a half times. However, these differences were not statistically significant (Polio $0{\cdot}30 > P > 0{\cdot}20$; Untyped $0{\cdot}50 > P > 0{\cdot}30$).

At first glance it appeared that in both the study groups the isolation rates for polioviruses and echoviruses were higher in the younger age-groups but showed less variation with the

TABLE I

VIRUS ISOLATIONS FROM STOOLS OF CHILDREN, BUKIT HO SWEE AREA, SINGAPORE

Study Group	No. Tested	Negative	Positive Isolations				
			Polio	Cox-sackie	Echo	Untyped	Total
Flat Dwellers							
Under 1 year	58	44 (75·9)	2 (3·4)	3 (5·2)	7 (12·1)	2 (3·4)	14 (24·1)
1 - 3 years	153	114 (74·5)	6 (3·9)	7 (4·6)	19 (12·4)	7 (4·6)	39 (25·5)
4 - 6 years	162	129 (79·6)	2 (1·2)	9 (5·5)	15 (9·3)	7 (4·3)	33 (20·4)
Total:	373	287 (76·9)	10 (2·7)	19 (5·1)	41 (11·0)	16 (4·3)	86 (23·1)
Squatters							
Under 1 year	28	20 (71·4)	1 (3·4)	1 (3·4)	5 (17·9)	1 (3·4)	8 (28·6)
1 - 3 years	121	92 (76·0)	2 (1·6)	7 (5·8)	16 (13·2)	4 (3·3)	29 (23·9)
4 - 6 years	151	128 (84·7)	1 (0·7)	8 (5·3)	11 (7·3)	3 (2·0)	23 (15·2)
Total:	300	240 (80·0)	4 (1·3)	16 (5·3)	32 (10·6)	8 (2·6)	60 (20·0)
Grand Total	673	527 (78·3)	14 (2·1)	35 (5·2)	73 (10·8)	24 (3·5)	146 (21·7)

(Percentages are given in brackets)

coxsackie and untyped viruses. Statistical analysis again proved these differences to be not significant.

Types of enteroviruses isolated

The frequency of the types of viruses isolated in each of the study group is given in Table II. All three poliovirus types were found in the flat-dwellers. Poliovirus type 2 was predominant, constituting six out of the ten poliovirus isolations. The squatters produced four polioviruses, one of type 1 and three of type 3. The youngest child from whom poliovirus was isolated was an infant less than a month old from the squatter area.

Of the coxsackieviruses, only types B3, B4 and B5 were recovered. Coxsackievirus B3 was isolated from the flat-dwellers but not from the squatters. Both coxsackieviruses B4 and B5 were present in the two study groups. Coxsackievirus B4 was particularly prominent, there being 26 isolations of this virus for the whole area. It is worth mentioning that the children from whom these coxsackieviruses were isolated showed no evidence of disease or illness associated with these agents.

There was a greater variety of types of echoviruses in the flat-dwellers than in the squatters. Of the 12 types of echoviruses found in the flat-dwellers, echo 6 and echo 13 provided the highest number of isolations. Only 5 types of echoviruses were recovered from the squatters, with echo 7 as the predominant type, accounting for 22 out of a total of 32 echovirus isolations. The high incidence of echo 7, however, was not associated with an outbreak of disease as they were all recovered from apparently normal children. The isolation of echo 7 was made from all age-groups.

Infection was seen to occur early in childhood as evidenced by the isolation of an echo 13 from a 3-weeks old infant living in the flat area.

The untyped viruses represented agents which grew in monkey kidney cells causing cytopathogenic changes but which so far had resisted routine identification. They could be enteroviruses not included in the antiserum pools used in the test or strains of adenoviruses or a mixture of more than one viruses.

TABLE II

TYPES OF ENTEROVIRUSES ISOLATED, BUKIT HO SWEE AREA, SINGAPORE

Virus		Number Isolated from:		Total
		Flat-dwellers	Squatters	
Polio	1	1	1	2
	2	6	0	6
	3	3	3	6
Coxsackie	B 3	4	0	4
	B 4	13	13	26
	B 5	2	3	5
Echo	1	1	0	1
	2	1	1	2
	6	12	3	15
	7	2	22	24
	8	1	0	1
	9	1	0	1
	13	14	3	17
	14	5	0	5
	15	1	0	1
	18	1	0	1
	19	1	0	1
	26	1	3	4
Untyped		16	8	24
Total		86	60	146

Analysis of the poliovirus isolations

In Singapore a poliomyelitis immunization programme with live oral vaccine has been in operation since 1962 (Lee et. al. 1964, 1965)[2,3]. In considering the data on poliovirus isolations it is necessary to take into account that some of the isolates are derived from vaccinees and not wild strains circulating naturally. It may be presumed that a virus isolated within 4-8 weeks of vaccination is a vaccine strain. It was not considered economic or worthwhile for the purpose of this study to carry out test for distinguishing between vaccine virus and wild viruses.

Table III shows the distribution of the poliovirus isolations by age-groups in the two study groups together with their vaccination status.

Flat-dwellers Poliovirus type 2 was isolated from a child belonging to the under 1-year age-group of those vaccinated. This child had the third dose (trivalent) vaccine only 2 weeks previously. Two other children in the 1-3 year age-group also excreted poliovirus type 2 after they had the third dose (trivalent) vaccine 4 weeks and 8 weeks respectively prior to the stool sampling. These three isolations could be considered to be vaccine viruses. The remaining positive isolation from a vaccinee was of poliovirus type 1 from a child just over a year old who had completed the full course of live

TABLE III

DISTRIBUTION OF POLIOVIRUS ISOLATIONS BY VACCINATION STATUS AND AGE-GROUP

Vaccination Status	Age-group	Flat-dwellers		Squatters	
		No. tested	Positive	No. tested	Positive
Vaccinated	Under 1 yr.	13	1	4	0
	1-3 yrs.	66	3	21	1
	4-6 yrs.	49	0	6	0
Unvaccinated	Under 1 yr.	33	1	13	1
	1-3 yrs.	53	2	31	1
	4-6 yrs.	73	2	41	1
Vaccination unconfirmed or unknown	Under 1 yr.	12	0	11	0
	1-3 yrs.	34	1	69	0
	4-6 yrs.	40	0	104	0
Total		373	10	300	4

vaccine (2 bivalent and 1 trivalent) approximately 42 weeks ago. Assuming that the vaccination procedure was satisfactory, this child could be acting as a carrier of a wild poliovirus, a manifestation of insufficient resistance of the alimentary tract.

Among the non-vaccinees, poliovirus type 2 was isolated from a child in each of the three age-groups. The older two of these belonged to the same household as the infant vaccinee-excreter mentioned above. Thus the viruses isolated from them were presumably vaccine strains. Such dissemination of a vaccine virus among contacts in the same household is common and expected. The two other non-vaccinees with positive virus isolation as well as the child whose vaccination status could not be confirmed all excreted poliovirus type 3. These were probably wild polioviruses.

Squatters: The poliovirus isolated from the solitary positive vaccinee excreter was a type 3. As this child had the second dose (bivalent) of live vaccine 54 weeks ago it was unlikely that vaccine virus was still being excreted. The three isolations from the non-vaccinees consisted of one poliovirus type 1 and two type 3, presumably all wild polioviruses.

DISCUSSION

The prevalence of many types of enteroviruses circulating freely in an urban area (Bukit Ho Swee) in Singapore was revealed by this study. The rate (21·7%) of natural enterovirus circulation for this community was in accord with the finding of Lee et. al. (1964)[2] who reported a rate of 19·9% in an earlier survey of City-dwellers (Chinese, 0-4 years) in Singapore. It should be noted that the virus studies were made using only one tissue culture system, namely monkey kidney. The true prevalence rate could possibly be higher if additional tissue culture systems were included. particularly for the isolation of those enteroviruses which normally do not grow well or at all in monkey kidney cells (Lee et. al. 1965)[4].

The prevalence rates for the squatters showed a downward trend with increasing age but did not vary much for the flat-dwellers. This could be a reflection of earlier infection and faster development of immunity under less favourable sanitary conditions among the squatters.

Of interest is the greater variety of echovirus types found among the flat-dwellers as

compared to the squatters. It might be suggested that this was because the flat-dwellers came from different parts of town bringing with them their complement of enteroviruses. But 80% of these isolations were made from residents who were rehoused a year or more ago, and the types found were not much different from those recovered from families who recently moved in. A plausible explanation would be to attribute this discrepancy to the different periods in which the stools were sampled. For the flat-dwellers this was from August to October and for the squatters from October to December 1964, and it is known that the enteroviruses circulate in waves in a community.

The analysis of the poliovirus isolations indicated that of the ten positive isolations from the flat-dwellers, five (all of type 2) were in all probability vaccine viruses whereas the four positive isolations from the squatters were wild viruses. The poliovirus rate of 2·1% for the study area, or 1·3% after correction for vaccine strains, compared favourably with the rate of 2·7% obtained in a survey in 1960 (Lee et al. 1964)[2].

Enteroviruses are transmitted predominantly by the direct contact route, either man-to-man or by means of the immediate environment. Spread occurs by respiratory droplets, by faecal contamination (fingers, utensils, foodstuffs) and by flies and cockroaches acting as mechanical carriers. Infants and children are primarily affected. Thus high prevalence is associated with poor sanitary conditions, overcrowding and bad personal hygiene.

This study showed that there was no significant difference between the two groups, flat-dwellers and squatters, with regard to the prevalence of enteroviruses. As far as sanitary facilities were concerned the flat-dwellers lived more favourably compared to the squatters. Better provision of sanitation however did not necessarily imply a change in attitudes towards personal hygiene, at least not within a short period of time. The flat-dwellers were originally from the squatter area or from similar insanitary parts of the town. Although they had been rehoused for less than two years (with the average of sixteen months), there were factors in the new environment which prevailed and hindered a rapid change of attitudes (Kleevens, 1966)[1]. Thus bad hygiene habits continued to operate and the flat-dwellers still lived under overcrowded conditions. Moreover, living in the flats was more costly. Higher rents had to be paid. Water was chargeable to the individual family and hence the tendency to use it less in contrast to the squatters most of whom could obtain their water free from public stand-pipes. Under these circumstances it was not surprising to find that the occurrence of enteroviruses in children living in the urban area of Bukit Ho Swee was not influenced by the change in environmental conditions.

SUMMARY

In an urban area (Bukit Ho Swee) of Singapore, stools of Chinese children (0—6 years of age) from families of low income bracket, living under two different environmental conditions (modern flats and squatter dwellings) were examined for the occurrence of enteroviruses. The overall prevalence rate of enteroviruses for this community was 21·7%, consisting of 2·1% polioviruses, 5·2% coxsackieviruses, 10·8% echoviruses and 3·5% untyped viruses. Coxsackievirus B4 and echoviruses 6, 7 and 13 were particularly prominent.

No significant difference was found between the flat-dwellers and squatters as regards the rate of prevalence and distribution of enteroviruses in these two study groups. The reason for this was discussed.

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REFERENCES

1. Kleevens, Jan W. L. (1966): "Re-housing and Infections by Soil-transmitted Helminths", Singapore Med. J. 1, 12.
2. Lee, L.H., Lim, K.A., Tye, C.Y. (1964): "Prevention of Poliomyelitis in Singapore by Live Vaccine", Brit. med. J. 1, 1077-1080.
3. Lee, L.H., Lim, K.A., Kanagaratnam, K. (1965): "The Poliomyelitis Immunization Programme in Singapore", Singapore Med. J. 6/2, 89-95.
4. Lee, L.H., Phillips, C. Alan, South, Mary Ann, Melnick, Joseph L., Yow, Martha D. (1965): "Enteric Virus Isolation in Different Cell Cultures", Bull. Wld. Hlth. Org. 32. 657-663.