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Routine administration of oral polio vaccine in a subtropical area. Factors possibly influencing sero-conversion rates*

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SUMMARY

Poliomyelitis is an important problem of public health in warm-climate countries. Studies of serological responses to vaccination in these countries have given conflicting results but in many investigations the rates have been considerably less than in countries with temperate climates. In this study three possible factors influencing sero-conversion were investigated – the season of the year when vaccine was given, the social status of the mother (as indicated by the number of years of schooling) and the presence of non-poliomyelitis viruses (NPV) in the gut when vaccine was given.

Over 200 children about 2 months of age were included in the study. Each was given three doses of trivalent vaccine at 6-week intervals.

The sero-conversion rates of the groups fed in winter were excellent but were slightly less good in summer. The differences were greatest in children in the lower socio-economic groups and in children excreting other enteroviruses.

The conclusions are that, provided a potent vaccine is used, the factors which diminish the effectiveness of immunization in warm-climate countries can be overcome: (1) by giving three doses of trivalent vaccine; (2) by beginning vaccination at the earliest possible age (when enteroviruses are fewest); (3) by concentrating special attention on the lower socio-economic groups and if necessary by giving a reinforcing dose several months after the third dose has been given – preferably in the colder months.

INTRODUCTION

In the past twenty years paralytic poliomyelitis has been recognized as a problem of increasing importance in the public health of numerous countries with warm climates. Clinical and epidemiological observations have been complemented by serological investigations and by isolations of polioviruses. In several

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tropical and subtropical countries the incidence in recent years has followed trends similar to those observed in countries with temperate climates before the introduction of vaccination. The age distribution has so far shown little change from the classical pattern, most cases occurring in the 0–4 age group, and though outbreaks and sporadic cases due to types 2 and 3 are not uncommon, most cases are due to type 1 (Drozdov & Cockburn, 1967, 1971).

Vaccination with live oral poliomyelitis vaccine has resulted in control of the disease in those tropical countries in which a sufficient proportion of the susceptible age groups has been given adequate dosage (Sabin *et al.* 1960), but studies based on sero-conversion rates have often indicated that the titres are much less satisfactory than in temperate countries (Cockburn & Drozdov, 1970). Among several possible reasons for the poor serological conversion rates in warm climates interference between natural enteroviral infections and the live vaccine viruses has been considered important (Montefiore, 1971) and it is well known that the incidence of gastrointestinal infections in tropical countries is high and is related both to social conditions and to the hot season. Routine poliomyelitis vaccination with oral vaccine has since 1961 reduced the incidence of the disease to a very low level in Israel (Ministry of Health, 1968). However, the considerable range in temperature between summer and winter and differences in the socio-economic conditions of certain groups in Israel provided an opportunity to study the effects of these factors on the excretion of enteroviruses and sero-conversion rates after poliovirus vaccination.

METHODS AND MATERIALS

Field procedures

The studies were made from December 1969 to April 1970 (winter trial) and May to September 1970 (summer trial). In the Tel-Aviv area the lowest average day temperatures for each month from December to March were between 13·2° and 15·6° C. and the highest from May to October were between 20·7° and 26·4 °C.

Infants living under normal home conditions and registered at six well-baby clinics in or near Tel-Aviv and Ashdod were included. The clinics were selected without prior knowledge of the incidence of acute gastrointestinal infections in the surrounding areas. The winter trial comprised 117 infants born in September and October 1969 and the summer trial 109 infants born in February and March 1970. They were allocated to one or other of two socio-economic groups according to the number of years the mothers had attended school. In Group A were children whose mothers had 12 or more years at school (12 years is the minimum before higher education can begin in Israel) and in Group B children whose mothers had less than 12 years at school. It is believed that a mother's knowledge of health and hygiene is related to her educational level, and presumably has a direct influence on the health of the child especially in the first year of life.

Administration of the vaccine

Two drops of commercially prepared trivalent live oral poliovirus vaccine* (OPV) were given to each child in a spoon containing sugared water. Beginning at

* Donated by the Wellcome Research Laboratories.

2 months of age three doses were given with an interval of 6 weeks between doses. The vaccine was held at 4° C until administered and the infectivity titres, expressed as TCID 50 per dose, were: type 1, 10^6; type 2, 10^5; type 3, $10^{5\cdot5}$.

Collection of samples

Stool samples were collected from each infant immediately before and 1 week after each dose of vaccine. They were sent to the laboratory on the same day and stored at −20° C. until investigated.

Capillary blood was collected on filter-paper disks (Reed & Brody, 1965) from about half the children in each group before each of the three vaccinations and 2 months after the third dose, when venous blood was obtained at the same time from a sample of 36 children.

Laboratory methods

The stool specimens were examined for the presence of polioviruses and other enteroviruses which in this paper are described as non-polioviruses (NPV).

For the tests attenuated strains of polioviruses* types 1, 2 and 3 were used. Virus stocks were prepared in primary cell cultures of African green monkey kidney and stored at −20° C. until required. Monkey kidney tissue cultures were employed throughout for the isolation and identification of strains.

Ten per cent. stool suspensions were centrifuged at 2500 rev./min. and 0·2 ml. of supernatant was inoculated into each of six culture tubes (3 undiluted and 3 diluted 10^{-2}). Inoculated cultures were incubated and examined for cytopathic effects (CPE) for a period of 10 days. The cultures were harvested for typing if CPE occurred. Tissue cultures harvested after 6 days of incubation were passaged once before titration. Typing of all viruses isolated in monkey kidney cell cultures was done by neutralization with antisera against the three types of poliovirus. A calculated dose of 100 TCID 50 was used in all neutralization tests.

For the serological tests three disks were placed in 1 ml. phosphate-buffered saline containing penicillin and streptomycin and held at 4° C. overnight. The soaked disks were then dropped into the chamber of a disposable syringe and the fluid squeezed back into the eluate. About 0·75 ml. of eluate, considered to be a 1/5 dilution, was obtained and was centrifuged for 10 min. at 2500 rev./min. to sediment particles of paper. The supernatant fluid was then poured off and used in the test.

The neutralization test was done in primary vervet monkey kidney tissue culture in tubes. Serial fourfold dilutions of the eluate ranging from 1/10 to 1/160 were tested against approximately 100 TCID 50 per 0·1 ml. of each of the poliovirus types.

The virus-serum mixtures were allowed to react during 4 hr. at 37° C. and overnight at 4° C., and were then inoculated in 0·2 ml. amounts into each of two monkey kidney tissue culture tubes.

Control virus was titrated with 0·1 ml. per tube (six tubes per ten-fold dilution).

Tests were read first when all virus control tubes containing 100 TCID 50

* Donated by the Wellcome Research Laboratories.

Table 1. *Subjects in study according to the social group and season of trial*

	Winter		Summer	
Social group	Virological investigations	Serological investigations	Virological investigations	Serological investigations
A	51	28	51	27
B	66	38	58	28

Table 2. *Preimmunization neutralizing antibody in the study groups*

	Neutralizing antibody					
	Type 1		Type 2		Type 3	
Subjects in study	% with antibody	G.M.*	% with antibody	G.M.	% with antibody	G.M.
Winter						
Social group A	60·0	12·3	70·0	16·2	45·0	7·3
Social group B	36·4	8·3	63·6	11·3	27·3	6·6
Summer						
Social group A	50·0	9·3	75·0	14·6	35·0	6·8
Social group B	62·5	12·2	66·6	14·1	30·9	9·0

* Geometric mean antibody titre.

exhibited a definite cytopathic effect (CPE). The titration and neutralization tests were usually completed by 7 days. The antibody titre was expressed as the reciprocal of the highest dilution of serum giving complete neutralization of the virus.

Titration of neutralizing antibody in the capillary and venous blood specimens obtained at the final bleeding was carried out simultaneously.

A neutralizing antibody titre of $\geqslant 1/10$ was considered as indicating immunity. Titres of $< 1/10$ were considered as evidence of lack of immunity. For the calculation of geometric means (GM), titres of $< 1/10$ were considered as 5, and those $\geqslant 1/160$ were considered as 160.

Distribution of the infants

The distribution of the 226 infants by social grouping and study season is given in Table 1.

RESULTS

Measurement of neutralizing antibody in simultaneously drawn capillary and venous blood specimens from 36 children gave similar antibody titres for each of the three types of poliovirus.

The state of immunity of the infants at 2 months of age, immediately before their first dose of vaccine, is shown in Table 2. The percentage of infants with antibodies (presumably maternal) varies, but in each subgroup the geometric mean titre (GM) is low for each of the three types of poliovirus.

Table 3. *Percentage of seroconversion after administration of live poliovirus vaccine*

Subjects in study	After one dose. Type 1		After two doses. Type 1		After three doses					
					Type 1		Type 2		Type 3	
	%	G.M.*	%	G.M.	%	G.M.	%	G.M.	%	G.M.
Winter										
Social group A	81·2	24·0	85·7	26·2	100·0	152·0	100·0	144·9	100·0	129·2
Social group B	67·1	15·2	92·5	46·0	100·0	115·0	100·0	142·9	100·0	132·8
Summer										
Social group A	77·7	25·9	85·7	91·3	96·4	109·4	100·0	144·9	92·8	82·7
Social group B	64·2	13·1	80·6	40·6	86·3	70·6	95·4	105·1	81·8	52·6

* Geometric mean antibody titre.

Table 4. *NPV excretion*

Study group	NPV excretion (%) in samples taken at time of administration of	
Winter		
Social group A	First dose	1·9
	Second dose	0·0
	Third dose	2·4
Social group B	First dose	9·3
	Second dose	5·1
	Third dose	8·9
Summer		
Social group A	First dose	23·0
	Second dose	15·3
	Third dose	7·6
Social group B	First dose	33·3
	Second dose	20·0
	Third dose	13·3

Excellent sero-conversion was observed after three vaccinations in the winter season in both groups (Table 3). High GM titres to the three poliovirus types were also recorded, though the GM titres to poliovirus type 1 were somewhat lower in group B.

In the summer trial the sero-conversion rates and GM titres in both groups A and B are lower, except for the GM titres to poliovirus type 2 in group A which were the same in both trials.

Also in Table 3 differences are shown between the groups in the sero-conversion rates to each of the three types of poliovirus in the summer trial. The differences are emphasized by comparing the corresponding GM titres which are much lower in group B than in group A.

In both groups many more children excreted NPV in summer than in winter and the incidence of NPV excretion was higher in group B than in group A in both seasons (Table 4).

In children who did not excrete NPV (Table 5) only slight differences in the

Table 5. *Relationship of polio neutralizing antibody to N.P.V. excretion*

	Distribution of polio neutralizing antibody											
	Without N.P.V. excretion						With N.P.V. excretion					
	Type 1		Type 2		Type 3		Type 1		Type 2		Type 3	
Study group	%	G.M.*	%	G.M.	%	G.M.	%	G.M.	%	G.M.	%	G.M.
Winter												
Social group A	100·0	127	100·0	151	100·0	151	100·0	108	100·0	160	100·0	108
Social group B	100·0	116	100·0	147	97·0	118	100·0	108	100·0	108	100·0	108
Summer												
Social group A	95·0	109	100·0	149	100·0	149	83·3	45	100·0	127	83·3	45
Social group B	94·1	102	100·0	160	94·1	129	50·0	20	83·3	61	65·0	30

* Geometric mean antibody titre.

Table 6. *Excretion of polioviruses*

Study group	Stools collected 7 days after	Percentage of stools yielding virus belonging to Type 1	Type 2	Type 3
Winter				
Social group A	First dose	40·0	77·7	42·2
	Second dose	48·8	9·3	62·7
	Third dose	14·6	4·8	26·8
Social group B	First dose	51·6	85·4	33·8
	Second dose	45·1	9·8	49·0
	Third dose	5·3	3·5	14·3
Summer				
Social group A	First dose	53·8	61·5	53·8
	Second dose	38·0	15·3	30·7
	Third dose	23·0	15·3	7·7
Social group B	First dose	40·0	80·2	40·0
	Second dose	33·3	13·3	6·7
	Third dose	6·7	6·7	6·7

sero-conversion rates and GM titres were observed between groups A and B in either season. In contrast, in NPV excreters the sero-conversion rates and GM titres in the two groups were definitely lower in summer than in winter, with the lowest values in group B in summer.

Information on the isolation of polioviruses from stools collected 7 days after each dose of vaccine was administered is given in Table 6.

In contrast to the correlation between NPV excretion and serological response, there was no consistent difference between groups or between seasons as measured by the excretion of the vaccine viruses.

DISCUSSION

The sero-conversion rates obtained in this study were excellent in winter and good in summer. Such high rates have been reported in only a few earlier studies in warm climates (e.g. Sabin *et al.* 1960). They are in distinct contrast to those in many other investigations in some of which the conversion rate for type 1 has been below 30 % in children previously without antibody (Report, 1966).

Despite the overall good results, conversion rates and GM titres were better in children vaccinated in winter than in summer and in the higher social group (A) than in the lower social group (B). Excretion of NPV was more frequent in summer than in winter and in group B than in group A.

Also the GM titres in children not excreting NPV were generally much higher than in children excreting NPV both in the winter and summer trials, and this is perhaps the most interesting observation arising from this study. It supports the conclusions of workers who have laid great stress on interference between NPV and the vaccine strains of polioviruses. At the same time it appears quite clear that three doses of well-spaced potent oral vaccine will give satisfactory sero-conversion rates even in hot weather. In practice, however, it would be advisable

to vaccinate in the cool season as far as possible, and to take particular care that infants in the lower social groups are given at least three doses. Where vaccination is done in the warm season a fourth dose might be given, preferably in the cool season.

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