POLIOMYELITIS AND MEASLES SEROSURVEY IN NORTHERN MALAYSIA

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Abstract. In 1990 the Institute for Medical Research carried out a serosurvey in the state of Kelantan to study the age stratified immune prevalence rates for measles and poliomyelitis. Our findings indicate that 981 out of 1,097 (89%) of the population screened had measles antibodies and more than 90% (366 out of 400) had antibodies to all three serotypes of poliovirus. The susceptible group for measles was infants below one year of age, of whom 53.3% (8/15) did not have measles antibody. Of 400 subjects, 125 (31.3%) who were either incompletely vaccinated or had not been vaccinated against poliomyelitis, had polio neutralizing antibodies to all three poliovirus serotypes, suggesting herd immunity in the population. No high risk age group could be identified for poliomyelitis.

INTRODUCTION

Immunization is a vital activity for the prevention and control of childhood diseases. In Malaysia, routine immunization is available for the major childhood diseases, namely diphtheria, pertussis, tetanus, poliomyelitis, measles, tuberculosis and hepatitis B. The Government health care system is the main provider of immunization for children and antenatal woman and this is implemented through the Maternal and Child Health program (Ministry of Health, 1989).

Over the last decade the Malaysian Ministry of Health (MOH) has intensified the Expanded Programme of Immunization (EPI) through numerous strategies, resulting in impressive improvements in the immunization coverage and a dramatic reduction in incidence of diphtheria, whooping cough, tetanus neonatorum, poliomyelitis and measles (Ministry of Health, 1991).

Between 1990 and 1991, the Institute for Medical Research (IMR) in Kuala Lumpur carried out a serosurvey for poliomyelitis and measles with the objectives of reviewing the effectiveness of the EPI and to study age stratified immune prevalence rates in the population. This paper reports the findings of a polio and measles serosurvey carried out in Northern Malaysia.

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MATERIALS AND METHODS

Samples

The serosurvey was carried out in the state of Kelantan and covered three districts viz Pasir Mas, Pasir Putih and Macang. Using sociodemographic data of the population in these districts, subjects were randomly selected, age stratified and their complete immunization history where available were recorded. Three ml of blood was collected from the subjects by venepuncture. The sera were used for measurement of measles and polio antibodies.

Measles antibody assay

1,097 sera were analysed for measles antibody. The measles antibody assay was performed on commercially available indirect ELISA by Behring (Enzygnost® Anti MeaslesVirus/IgG). Each strip in the test plate consisted of two rows of 8 wells, one row being coated with human cell cultures infected with measles virus which had been subsequently inactivated;

the coating on the second row (control) of wells consists of uninfected cells.

0.02 ml of sera was used in the assay and the test performed according to the manufacturer's instructions. The serum dilution used in the assay was 1:44. Absorbance was measured at 405 nm.

A positive reaction is indicated when the difference in absorbance obtained between the first row of antigen well and second row of control well is more than or equal to 0.2.

Controls

A WHO control reference serum of known titer in international units, kindly supplied by the Statens Serum Institute in Denmark, was titrated first for measles antibody and a standard curve plotted for absorbance versus antibody in IU. Positive and negative control sera provided in the kit were incorporated into each assay of test samples.

Polio antibody assay

400 sera were analysed for antibodies to poliovirus types 1, 2 and 3 using the microneutralization test (WHO, 1990). Cell cultures utilized in the assay were LLC-MK_2 cell cultures from our laboratory stock held in liquid nitrogen at passage 200. The LLC-MK_2 cell cultures were grown in monolayers in 72 cm^2 tissue culture flasks in Earles Basal medium, Eagles (BME) supplemented with 25 mM Hepes buffer pH 7.2, 1.2g/l sodium bicarbonate, 5% fetal calf serum, 100 IU/ml penicillin G and 100 µg/ml streptomycin sulphate. Cells from 4 or 5 day confluent cultures were resuspended in maintenance medium and concentration adjusted to 6×10^5 cells/ml for use in the assay.

Poliovirus titration

The initial step in the assay involved titration of Sabin strains of poliovirus types 1, 2 and 3 in microtiter plate wells to determine the 50% Tissue Culture Infective Dose ($TCID_{50}$) of virus for use in the microneutralization test. 0.025ml samples of virus dilutions from 10^{-1} to 10^{-8} were added to wells of a microtiter plate followed by 0.025 ml of Eagles Growth medium and 0.025 ml of cell suspension. The plates were incubated at 34°C in a CO_2 incubator. The titration end point was calculated by Spearman Karber Method (Finney, 1952) and expressed as log $TCID_{50}$. Cell controls and medium controls were incorporated in each assay.

Microneutralization test procedure

The virus suspension, containing 100 $TCID_{50}$ per 0.025ml in Eagle's growth medium, was used in the assay. Test sera were filtered through a 0.2 micron Seitz filter, inactivated at 56°C for 1 hour, then diluted to 1:8 in Eagle's growth medium.

Starting with 0.05 ml test serum, serial two fold dilutions were made from 1:8 through to 1:1024. A back titration using 100 $TCID_{50}$ of virus suspension at dilutions 10^{-1} to 10^{-4} was included.

The plates were covered and incubated at 34°C in a CO_2 incubator for 3 hours. After incubation 0.025 ml of MK_2 cell culture suspension (6×10^5) were added to all wells except media control wells. The covered plates were incubated at 34°C in a CO_2 incubator for 4 days. On the fourth day the plates were read for neutralization of virus infectivity of cells.

Controls

The controls incorporated in the assay included serum control to detect serum toxicity for cells, cell control to check for normal cell morphology and positive control sera of known titer for poliovirus Type 1 (1:640), Type 2 (1:320) and Type 3 (1:320) (Denka Seiken Co, Tokyo, Japan).

RESULTS

The data for measles antibody titers and polio antibody titers for the different age groups of the population studied are tabulated in Table 1 and Table 2. The protective immunity status for measles was measured at 1:44 serum dilution and the protective immunity status for polio was measured at 1:8 dilution. Our findings indicate that 981 out of 1,097 people screened had measles antibody. 8 out of 15 infants below 1 year of age did not have measles antibody and the 6 out of 8 infants in this group had not received measles vaccination. For polio serosurvey more than 90% (360 out of 400) had antibody to all three poliovirus types.

Table 1

Measles serosurvey, age stratified.

Age group (yr)	Sample No.	Positive	Negative	% positive
< 1	15	7	8	46.7%
1	60	45	15	75%
2	77	69	8	89.6%
3	66	52	14	78.8%
4	83	67	16	80.7%
5	93	77	16	82.8%
6	104	92	12	88.4%
7	78	67	11	85.9%
8	99	93	6	93.9%
9	16	14	2	87.5%
10-19	117	110	7	94.0%
20-39	166	165	1	99.4%
> 40	123	123	–	100%
Total	1,097	981	116	89.4%

Table 2

Polio serosurvey.

		Neutralizing poliovirus antibodies					
Age	No.	Type 1		Type 2		Type 3	
group	screened	+ ve	– ve	+ ve	–ve	+ ve	– ve
< 1	18	17	1	17	1	16	2
1 -2	46	44	2	44	2	44	2
2 - 5	185	167	18	182	3	173	12
5 - 10	112	101	11	112	–	107	5
10 - 20	19	18	1	19	–	18	1
20 - 40	20	20	–	20	–	20	–
Total	400	367	33	394	6	378	22
Percent		91.8	8.2	98.5	1.5	94.5	5.5

The seroconversion rate differed among the 3 different poliovirus serotypes. 8.2% of the population studied were negative for poliovirus type 1 antibody; 5.5% were negative for poliovirus type 3 antibody and 1.5% were negative for poliovirus type 2 antibody. 1 out of 400 did not have antibodies to all three poliovirus types despite oral poliomyelitis vaccination (OPV).

DISCUSSION

In Malaysia, the overall incidence of poliomyelitis and measles had declined since the licensing and introduction of the OPV and live attenuated measles vaccine in the childhood immunization program. After OPV was introduced in 1972 no reported cases of poliomyelitis were observed between 1986 and 1991. Similarly, for measles, nearly 40% reduction of disease incidence was noted since inception of vaccination in 1982 (Ministry of Health, 1991). These achievements are attributed to well planned strategies of the EPI which is coordinated and supervised at the national level by the Maternal and Child Health and Epidemiology Unit in the Ministry of Health. The serological survey was carried out to evaluate the effectiveness of the EPI and relate incidence of poliomyelitis and measles with the antibody levels in the population for these diseases. Our findings indicate that more than 89% of the population screened had measles antibody. The high risk age group were infants below one year of age, of whom almost 50% were susceptible or non-immune for measles. This reflected the vaccination schedule prior to 1990, when measles vaccination was recommended between 9 to 24 months of age, which in practise was given at 15 months of age or even later. Since 1991 efforts have been made to encourage measles vaccination at an earlier age between 9 months to 12 months.

For polio no definite high risk age group could be identified. More than 90% (366 out of 400) had antibody to all three poliovirus types. It is interesting to note that 125 out of 400 subjects who were incompletely vaccinated or had not been vaccinated had polio neutralizing antibodies to all three poliovirus serotypes suggesting herd immunity in the population. This achievement was one of the main objectives of the WHO's EPI in the use of OPV to eliminate circulation of wild poliovirus in the population by herd immunity (Melnick, 1989). One individual, however, did not have antibodies to the three poliovirus types despite 3 doses of OPV. This failure to seroconvert could be due to interference phenomena of enterovirus infections in the gut. The immunity of OPV is dependent on replication of polioviruses in the gut; if the vaccine virus does not multiply because of interference by gut flora or other enterovirus infections, the individual will not become immunized. Poor seroconversion rates with OPV has been reported in many tropical countries and presumed to be caused by interference of enterovirus infections in the gut (John and Christopher, 1975; Hardas *et al,* 1978).

The serological survey although carried out on a small scale is an important part of the epidemiological surveillance system for monitoring and evaluating the immunization program. Data from the survey would assist health program managers and policy makers plan improved strategies for implementing the immunization services and review the effectivencess of our present immunization schedule.

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