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A MODEL FOR DEVELOPING COUNTRIES OF MASS SEROLOGICAL SURVEY OF CHILDREN VACCINATED AGAINST DIPHTHERIA AND TETANUS (*)

F. Nazari, H. Mirchamsy, S. Alé-Agha and M. Mahinpour

An enquiry was made into the efficacy of the programmes of mass immunization in Iran and 60 000 blood samples were collected, on filter-paper discs, throughout the country, from all susceptible age groups. It was found that over 70% of the children, 3 months to 5 years old, immunized with diphtheria, tetanus and pertussis adsorbed vaccine had a satisfactory level of diphtheria and tetanus antitoxins. The boosting effect of diphtheria and tetanus adsorbed vaccine, used for maintaining immunity in older children, was to increase the antitoxin titres found in these children. Such a large survey was possible in such a short period of time because of the simplicity of the haemagglutination test used.

INTRODUCTION

In Iran immunization against diphtheria, tetanus and whooping-cough has been applied since 1950 and these diseases have practically disappeared. Mass immunization campaigns, however, started only in 1965. The official reports claim that over 70% of the susccptible age groups in the whole country accept the vaccine during annual mass campaigns. Nevertheless, small outbreaks of diphtheria with a low mortality or sporadic cases in children do still occur and occurrences such as these made it desirable to assess the level of immunity

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against diphtheria and tetanus in the country using a method suitable for mass survey.The purpose of this report is to record the level of diphtheria and tetnaus antitoxins determined by the haemagglutination test, in over 60 000 blood samples collected for this survey.

MATERIALS AND METHODS

Study groups

At the request of our laboratory,the Ministry of Health agreed to conduct a survey to ascertain the status of immunity to diphtheria and tetanus in children in the country by determining the antitoxin titres circulating in the blood. Children 3 months to 6 years old in maternity centres, kindergartens and nursing centres and older children in primary schools were randomly selected from both sexes and blood samples were collected by our trained personnel. These children were reported as being immunized, in early childhood, with combined diphtheria, tetanus and pertussis (DTP) adsorbed vaccine, prepared in our laboratories and to have received the booster doses of diphtheria and tetanus (DT) adsorbed vaccine before entering primary school as well as at every 3 years to 4 years up to the age of 12 years. Because of the inaccuracies in the answers given by respondents, our interviewers were discouraged from collecting information concerning the type and number of injections or the date of immunizations. The blood samples were collected on filter-paper squares, as described previously (Mirchamsy, Nazari, Stellman & Esterabady, 1968). Age, sex, place and date of sampling only were recorded.

Antigens

The purified diphtheria and tetanus toxiods used for sensitization of sheep red blood cells (r.b.c.) throughout this study contained 2500 Lf/mg and 2000 Lf/mg protein nitrogen of diphtheria and tetanus antigens respectively.

Preparation, tanning and sensitization of erythrocytes

Tannic acid treatment of r.b.c. and sensitization with purified antigens were carried out as described by Scheibel (1956) but with a slight modification as reported by Nazari, Alé-Agha, Mahinpoor & Mirchamsy (1972).

Formalinization of erythrocytes

The method of Butler (1962) was used with minor changes as follows. The sensitized cells were washed once with 0.5% sterile normal rabbit serum and then diluted to a 5% suspension. One vol. of the suspension was added to 1 vol. of 5% formaldehyde in a large flask and the mixture was shaken gently at room temperature overnight. The cells were then centrifuged and washed

five times with distilled water and then stored at 4° C as a 5% suspension in pH 7.2 buffer containing 0.01% merthiolate.

Diluent

Physiological saline containing 0.5% normal rabbit srum was used throughout.

Collection of finger-blood

The blood of children was absorbed onto squares of filter-paper and stored at 2-8°C. The day before the test the blood was eluted from each square of filter-paper in 1.5 ml of peptone saline in test tubes at 4°C. As determined previously, the initial dilution of the eluate was taken as a 1:10 serum dilution and each sample of eluted blood was tested for both diphtheria and tetanus antitoxin.

Performance of the hemagglutination test

The sera were titrated in twofold dilution in tubes in 0.5 ml volumes. The World Health Organization (W.H.O.) standards of diphtheria and tetanus antitoxins were also diluted in saline containing 0.01% merthiolate to contain 0.01 i.u./0.5 ml (diphtheria antitoxin)or 0.0025 i.u./0.5 ml (tetanus antitoxin) and these standard dilutions were included daily in each test. The amount of cell suspension added was 0.05 ml per test tube and the tubes were then shaken for 30 s and left overnight at room temperature (20–22°C). The reading was carried out by a mirror and magnifying glass in order to observe the pattern of haemagglutination more clearly and the end - point was chosen as the highest serum dilution giving distinct agglutination.

RESULTS

The incidence of diphtheria antitoxin

The diphtheria antitoxin titres of the 60 000 children are shown in Fig. 1, which shows the percentage with a particular antitoxin value for each age gruop. It is clear that active immunization in childhood has achieved a very high rate of antitoxin among the lower age groups. At least 74% of the age group between 3 months to 7 years have a titre of 0.01 i.u./ml which is normally enough to avoid infection. Of the same age group, 61–72% have five times more antitoxin (0.05 i.u./ml) and 40–62% have 20 times more antitoxin (0.2 i.u./ml). The data show also that the boosting effect of DT vaccine in the age group between 7 and 13 years is high. Such a boosting effect could not have been given by diphtheria infections because the natural reservoirs in children had been eliminated and disease was rare, rather it was the active immunization and the boosting

effect of DT vaccine that markedly increased the diphtheria antitoxin titre in older children; 81–91% of children between 7 and 13 years showed a titre of 0.01 i.u./ml and 62–86% had a titre of 0.2 i.u./ml.

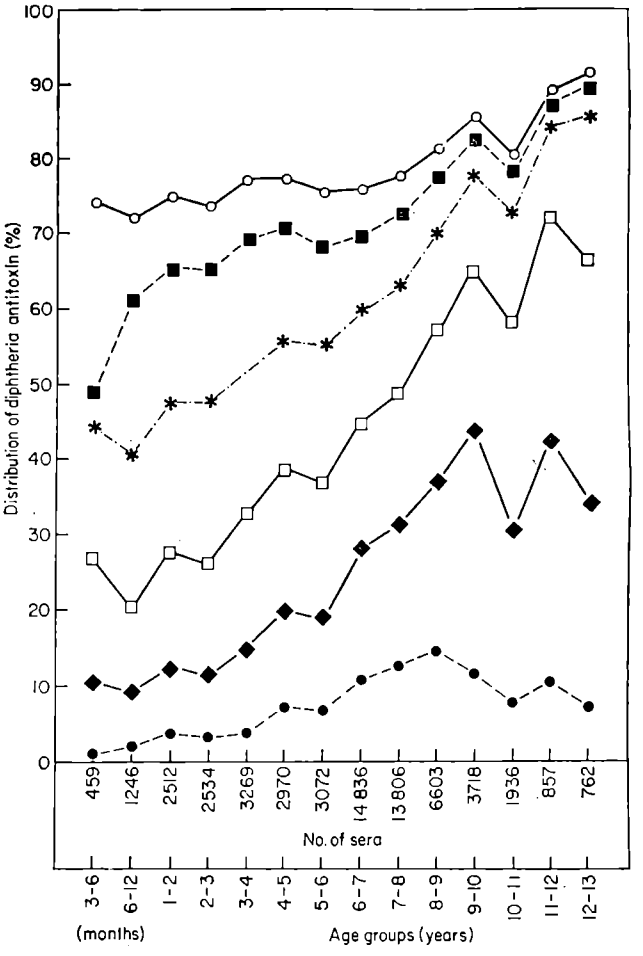

Fig. 1. Age distribution and measurable diphtheria antitoxin in sera of healthy Iranian children in 1974: ○——○, 0·01 i.u./ml; ■——■, 0·05 i.u./ml; *——*, 0·2 i.u./ml; □——□, 0·5 i.u./ml; ◆——◆, 1·5 i.u./ml; ●——●, 3·0 i.u./ml.

The incidence of tetanus antitoxin

The results of tetanus antitoxin titration of the 60 000 blood samples are shown in Fig. 2 and the serological picture here is much clearer than in cases of diphtheria. Of children aged 3 months to 7 years 74–82% had a titre of 0.005 i.u./ml and the same titre was found in 80–90% of children-aged 7–13 years.

Higher antitoxin titres were observed after the booster effect of DT vaccine in older children; 73–82% of children aged 9–13 years have a tetanus antitoxin titre of 0.5 i.u./ml and 62–71% of the same age group a titre of 1.5 i.u./ml.

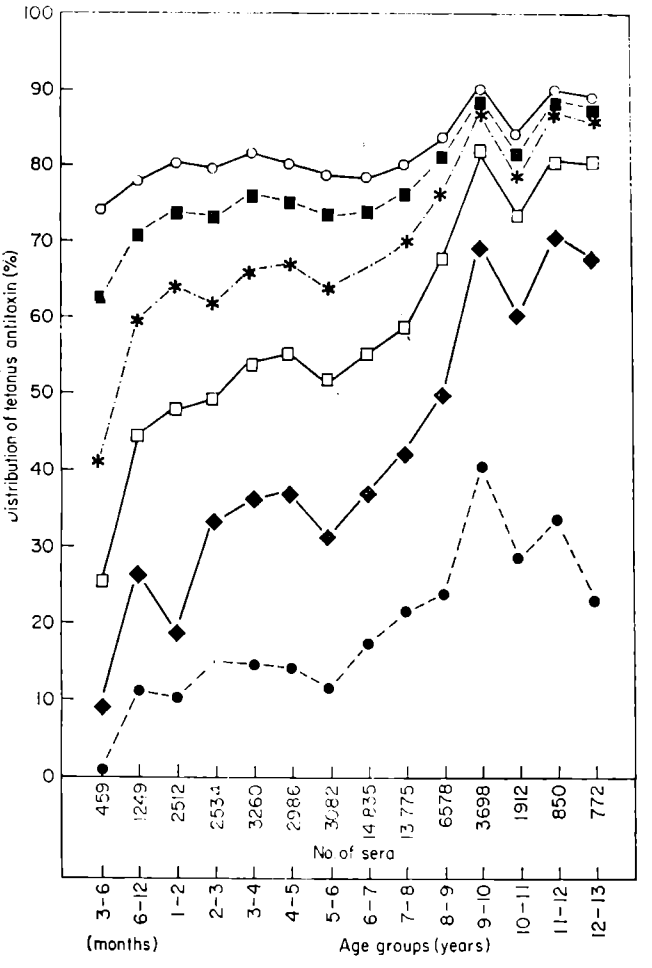

Fig. 2. Age distribution and measurable tetanus antitoxin in sera of healthy Iranian children in 1974: ○——○, 0·005 i.u./ml: ■——■, 0·02 i.u./ml: *——*, 0·1 i.u./ml; □——□, 0·5 i.u./ml; ◆——◆, 1·5 i.u./ml; ●——●, 3·0 i.u./ml.

DISCUSSION

A fully immunized child may be regarded as being protected against diphtheria but can acquire and transmit the infection as a healthy carrier. Unimmunized children therefore are always at some risk of the disease even if they live in a well-immunized community. In the United States of America, for instance, where diphtheria has been at a very low level for many years, recent outbreaks

in underimmunized populations confirm that infection with *Corynebacterium diphtheriae* is prevalent (Francis, 1973). The periodic serological survey carried out during the last decade in some regions of Iran revealed a decline in the percentage of protected children under the age of 10 and it was necessary to begin new vaccination campaigns in order to avoid new outbreaks. The reason for this decline was that the programme of mass vaccination by the mobilized teams which was started in 1965 had been replaced recently by the creation of Health Centres where people were supposed to bring their children for free vaccination. It is understandable, however, that in a rapidly growing population with many unprotected immigrants from the remote areas to the cities those people with poor living and low social conditions did not come to the centres to receive preventive care; consequently new outbreaks of limited size were anticipated. Therefore the mass campaign system was again restarted in 1970 as well as in the following years in most regions of the country.

It is also important to note that although several diphtheria outbreaks have been reported during recent years in Iran (Tahernia & Motamed, 1969) there is an exaggeration in the incidence of the disease in this country. This is due mainly to the lack of trained laboratory personnel in many cities where diphtheria-like organisms are isolated but they are reported as *C. diphtheriae*. Esterabady, Taslimi & Nategh, (1963) found that 200 out of 600 strains, isolated in the Tehran area by several private laboratories, were not *C. diphtheriae*. Amongst the positive isolates of *C. diphtheriae* it was found that *mitis* and *intermedius* strains could be typed only by adapted phages and the majority of *gravis* strains were phage type XIV, which is the epidemic type seen in other countries (Zamiri, McEntegart & Saragea, 1972).

The present survey showed the prevalence of diphtheria and tetanus antitoxins in the majority of susceptible age groups in Iran. Furthermore, the survey assessed the efficacy of the DT adsorbed vaccine used for basic immunization as well as for boosting purposes. Following mass vaccination in Iran, morbidity to diphtheria decreased in the younger age groups and incrased in adults. In the past few years cases of diphtheria have been reported in several cities in adults and a new DT adsorbed vaccine containing 2 Lf purified diphtheria and 2 Lf purified tetanus antigen per dose is now used for adults.

A significantly higher percentage of children in all age groups shows a higher tetanus antitoxin titre than diphtheria antitoxin titre. As there is no natural immunity for tetanus infection, since the reservoir of *Cl. tetani* is mainly soil, the acquired immunity in children can be attributed entirely to active immunization. During the past two decades tetanus infection of newborns was the cause of many deaths in the rural regions of Iran (Alé-Agha *et al.*, unpublished data). The disease is now much decreased due to a large campaign of vaccination of girls and young mothers with adsorbed tetanus toxoid.

For the purpose of this survey we looked for a simple technique for testing large numbers of sera. In two previous surveys (Mirchamsy, Nazari & Sadegh, 1959; Mirchamsy *et al.*, 1968) about 700 and 2000 blood samples were tested by the Jensen (1933) technique for diphtheria antitoxin and by the Ipsen (1959) method for tetanus antitoxin. The high costs of these methods, however, prohibit their use on a very large number of sera. Among the *in vitro* tests, the cell culture technique of Kritz, Sladky, Burianova, Vyro ka Mottolova & Roth (1974), microcell cultrures of Quevillon & Chagnon (1973) and Miyamura, Tajiri, Ito, Murata & Kono (1974) were experimentally compared with the *in vivo* Jensen technique (1933) with a good correlation between these methods. It was felt, however, that for testing over 60 000 samples of sera where individual serum titres are not important a simpler *in vitro* technique should be used. Tasman, Van Ramshorst & Smith (1960) found a close correlation between the values of diphtheria and tetanus antitoxins by haemagglutination in sera from human sources and the standard *in vivo* tests. In agreement with Van Ramshorst (1971) the haemagglutination test was found very suitable for the screening purpose. It is also important to note that this type of screening test for mass serological surveys can be performed easily in most of the developing countries where laboratory facilities are limited and such surveys are urgently needed by epidemiologists and health planners.

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