Immunity of children to diphtheria, tetanus, and poliomyelitis

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British Medical Journal, 1979, 1, 854-857

Summary and conclusions

A survey of titres of diphtheria and tetanus antitoxins and of antibodies to polioviruses in the sera of 291 schoolchildren aged 15, 11, and 7 years showed that high immunisation rates can evoke protective concentrations of tetanus antitoxin in 98% of children and protective levels of the antibodies to diphtheria and all three types of poliomyelitis in 85% of children. Reinforcing immunisation at school entry appeared to be necessary to maintain adequate titres of diphtheria antitoxin in children up to 15 years of age, not essential to maintain adequate titres of tetanus antitoxin, and to have little effect on the titres of antibodies to poliomyelitis.

Introduction

In Britain and many other countries preventive medicine has virtually eradicated diphtheria and poliomyelitis and greatly reduced the incidence of tetanus. These successes of immunoprophylaxis are continuously and precisely recorded epidemiologically but, except in the short term of a vaccine trial, are only rarely related to the serological phenomena on which they depend. Serological surveys, particularly on young people, are notoriously difficult to organise owing to problems associated with selecting population samples truly representative of the cohorts of interest, collecting blood samples, and limited facilities for titrating antibodies. Nevertheless, as immunisation programmes approach their objectives and natural infections become rare serological surveys become ever more necessary, since they are the only indicators of the continuing effectiveness of an immunisation schedule and the need to modify immunisation schedules or vaccine potency.

Recently in the county of Avon special conditions provided valid reasons for collecting blood from three precisely defined population samples and estimating the concentrations of all the serum antibodies, other than those to *Bordetella pertussis*, that may be expected consequent on primary immunisation in infancy and reinforcing immunisation at school entry. The samples consisted of children aged 15, 11, and 7 years and the results obtained thus indicated the immune states of the cohorts from which the samples were drawn some 10, six, and two years after the scheduled time for reinforcing immunisation.

Patients and methods

Children—Three samples, each comprising 120 children or about 1% of the available population, were selected at random, one sample being taken from each cohort born in 1961, 1965, and 1969 in the area of the Avon Area Health Authority. The study was publicised by local newspapers, radio, and television, and the parents of each selected child were informed by post of their child's inclusion in a sample and asked to co-operate. During November 1976 medical staff of the school health service visited the home of each selected child, provided the parents with any further information that they requested, and obtained from the child, by venepuncture, a 10 ml blood sample. Each blood sample was matched with the record card of that child and sent to the laboratory for separation of serum. The immunisation history of each child was obtained from the school health service records.

Titration of diphtheria antitoxin—Diphtheria antitoxin was measured by neutralisation tests in microtitre plates.[1] HeLa cells and a toxin test dose of 2 TCD were used in all assays. The British standard for diphtheria antitoxin was used as the reference material. The results were expressed in IU/ml serum.

Screening for tetanus antitoxin—Each serum sample was initially screened for 0·1 IU tetanus antitoxin/ml. Sera containing less than this concentration were screened for 0·01 IU/ml. Screening was done by neutralisation tests in mice according to the method of the *European Pharmacopoeia*[2] except that, to achieve the requisite sensitivity, the tests were conducted at the Lp/400 rather than the Lp/10 level. The British standard for tetanus antitoxin was used as the reference material.

Titration of poliovirus antibodies—Poliovirus antibodies were measured by neutralisation tests in microtitre plates containing cultured HEp2C cells. Each serum sample was diluted to provide a dilution series in fourfold steps from 1/4 to 1/256, and from this series three subordinate series were drawn. To each dilution of these three series were added between 30 and 200 TCD_{50} of the three Sabin attenuated polioviruses, one virus type being added to each dilution series. The mixtures of serum and virus were incubated at 35°C for two hours and then added to the wells. The plates were incubated at 35°C for seven days, and at the end of this time the cultures were inspected for cytopathic effect. The appropriate British standard for antipoliovirus serum was used as a reference material. The titres were expressed as the reciprocal of the serum dilution that neutralised the virus in 50% of the wells. Dividing the titres by 35, 21, and 73 in the cases of types 1, 2, and 3 respectively gives a good approximation of the antibody titres in international units.

Replicate samples—Serum samples from a random 10% of the children were split into two aliquots, one being identified with the number and name of the child and the other labelled with a number and a fictitious name. In each case both aliquots were submitted to the laboratory without indication of their common origin and all five antibodies assayed in both. The results included here were those obtained from the truly labelled aliquots, but in every case comparability of the results obtained with each pair was satisfactory.

Results

The three samples of 120 children contained 31 children who were known to have left Avon and a further 18 who could not be traced despite repeated attempts. Of the remaining 311 children available for the study, 19 children or their parents refused to co-operate, leaving 292 participants, an overall response rate of 94%. In only one case was the volume of blood obtained insufficient.

Immunisation records were available and complete for 246 of the 291 children from whom satisfactory blood samples were obtained, available but incomplete for 36 children, and unavailable for nine. The table summarises the immunisation histories of each sample. If an unqualified record of immunisation is assumed to signify the administration of all the vaccines and doses that were required by the national immunisation schedule, 171 children had received both primary and reinforcing immunisation and 93 had probably received only primary immunisation. Because of a steady increase in the uptake of reinforcing immunisation most of the children who had received

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Immunisation histories of 291 children from whom satisfactory blood samples were obtained

	Year of birth			Total
	1961	1965	1969	
Primary immunisation and reinforcement	35	53	83	171
Primary immunisation without reinforcement or record of reinforcement	44	35	14	93
No primary immunisation or record of primary immunisation	17	8	2	27
Total	96	96	99	291

only primary immunisation were in the 1961 and 1965 samples and almost all of the children in the 1969 sample were fully immunised. The 27 children who were incompletely immunised, not immunised, or without immunisation records were originally thought likely to provide informal but useful baseline values with which the antibody titres of their immunised peers might be meaningfully compared. Eleven of these children, however, were found to have antibodies to all vaccine antigens and so must almost certainly have been immunised.

Throughout this report the results of the antitoxin and antibody assays are recorded as frequency distribution histograms. Each serum titre indicates the immunisation history of the child from whom the sample was obtained: (1) fully immunised—a child with records of both primary and reinforcing immunisation; (2) primarily immunised only—a child with a record of primary immunisation but lacking a record of reinforcing immunisation; (3) others—a child recorded as not having been immunised or a child without records.

Fig 1 shows the diphtheria antitoxin titres of children in the three samples. There was a preponderance of low titres in the 1961 sample,

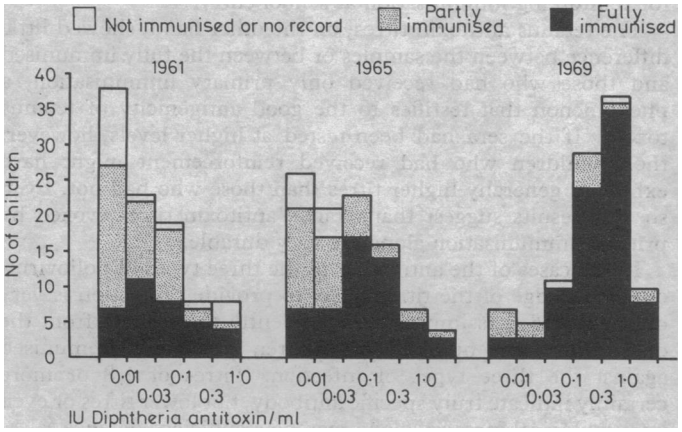

FIG 1—Frequency distributions of titres of diphtheria antitoxin found in 1976 in sera of children born in 1961, 1965, and 1969.

intermediate titres in the 1965 sample, and high titres in the 1969 sample; this pattern applied not only to each distribution as a whole but also to each distribution in respect of the fully immunised children alone. The low titres in the 1961 distribution were largely contributed by children who had failed to receive reinforcing immunisation or whose immunisation records were doubtful, and the high titres in the 1969 sample were almost wholly contributed by children who had received reinforcing immunisation within the two years before blood sampling.

If an antitoxin titre of 0·01 IU/ml—that is, about three times the concentration associated with Schick conversion—is considered to indicate adequate immunisation, the numbers of thoroughly protected children in the 1961, 1965, and 1969 samples were 58 (60%), 70 (73%), and 92 (93%) respectively. The 71 children with antitoxin titres below 0·01 IU/ml, and who may be considered to have been inadequately protected, comprised 17 who had been fully immunised, 38 who had received only primary immunisation, and 16 who were without adequate records.

Fig 2 shows the tetanus antitoxin titres in the three samples. The method used for screening for tetanus antitoxin gave titres within three ranges, but regardless of immunisation history or time since the last immunisation almost all titres were in the highest range—that is, over 0·1 IU/ml. If 0·01 IU tetanus antitoxin/ml is taken to indicate adequate immunisation there were only eight children, none of whom had been fully immunised, who may be thought to have been inadequately protected.

Figs 3, 4, and 5 show the poliovirus antibody titres in the three samples of children. In all three samples the median titres of type 1

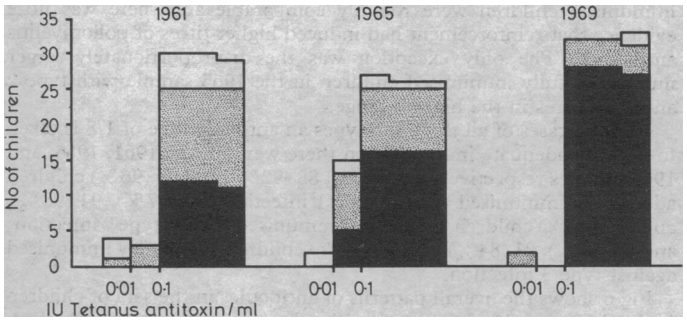

FIG 2—Frequency distributions of titres of tetanus antitoxin found in 1976 in sera of children born in 1961, 1965, and 1969. Key as in fig 1.

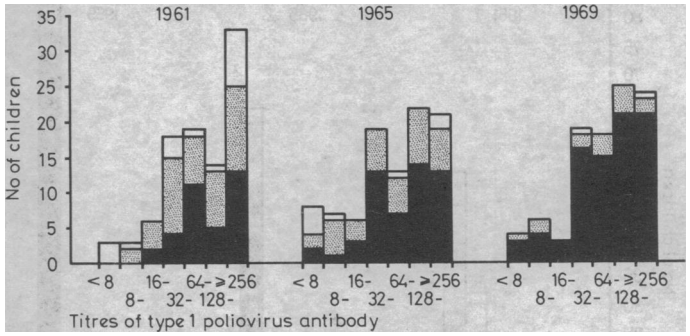

FIG 3—Frequency distributions of titres of antibodies to type 1 poliovirus found in 1976 in sera of children born in 1961, 1965, and 1969. Key as in fig 1.

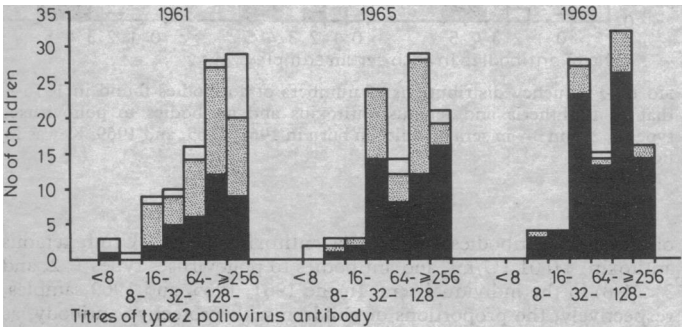

FIG 4—Frequency distributions of titres of antibodies to type 2 poliovirus found in 1976 in sera of children born in 1961, 1965, and 1969. Key as in fig 1.

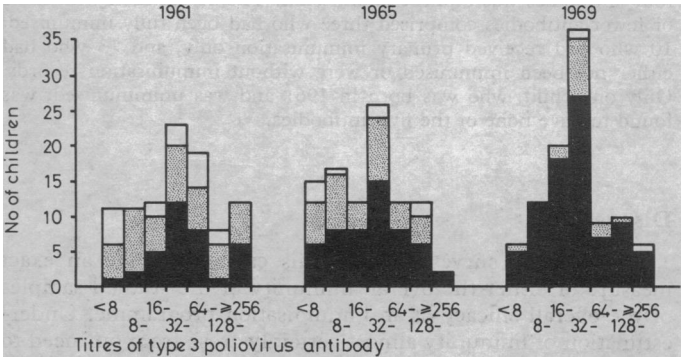

FIG 5—Frequency distributions of titres of antibodies to type 3 polioviru. found in 1976 in sera of children born in 1961, 1965, and 1969. Key as in fig 1s

and type 2 antibodies were higher than the median titres of type 3 antibody, the overall median titres for the three types being 1/48, 1/45, and 1/22 respectively. In the 1961 and 1965 samples there were enough children who had received only primary immunisation to permit a meaningful comparison of the antibody titres of the fully immunised children with those of children who had received only primary immunisation against poliomyelitis. In each distribution the contributions to each titre range made by the fully and partly immunised children were roughly comparable and there was little evidence that reinforcement had induced higher titres of poliomyelitis antibodies. The only exception was the disproportionately larger number of fully immunised children in the 1965 sample with type 2 antibody titres in the highest range.

If in the cases of all three serotypes an antibody titre of 1/8 is taken to indicate adequate immunisation there were, in the 1961, 1965, and 1969 samples respectively, 93 (97%), 88 (92%), and 95 (96%) children adequately immunised against type 1 infection; 93 (97%), 94 (98%), and 99 (100%) children adequately immunised against type 2 infection; and 85 (89%), 81 (84%), and 93 (94%) children adequately immunised against type 3 infection.

Fig 6 shows the overall patterns of antibodies in the sera of children in the three samples. In this figure the abscissae represent the numbers

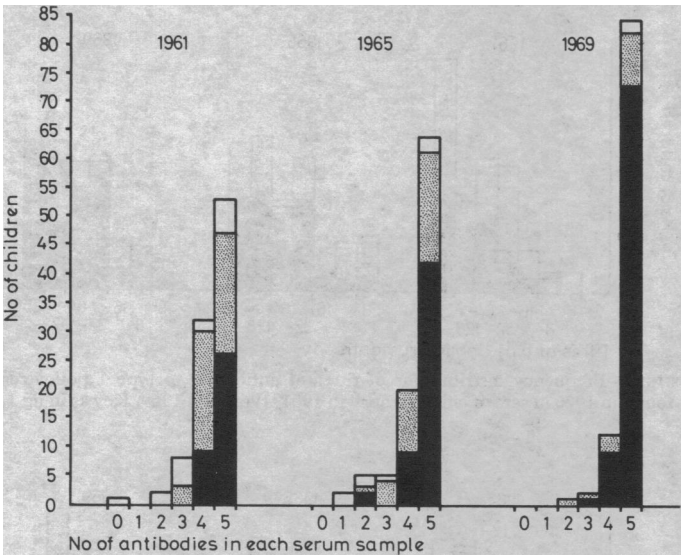

FIG 6—Frequency distributions of numbers of antibodies found in 1976—that is, diphtheria and tetanus antitoxins and antibodies to polioviruses types 1, 2, and 3—in sera of children born in 1961, 1965, and 1969. Key as in fig 1.

of different antibodies (diphtheria antitoxin $<0{\cdot}01$ IU/ml, tetanus antitoxin $<0{\cdot}01$ IU/ml, and antibodies to polioviruses types 1, 2, and 3 $<$ 1/8) in the individual sera. In the 1961, 1965, and 1969 samples, respectively, the proportions of children with protective antibody, as defined above, to diphtheria, tetanus, and the three types of poliomyelitis were 55%, 67%, and 85%, and the proportions with antibodies to at least four of the infecting antigens were 86%, 87%, and 97%. The antibody most often missing from the sera of children with four antibodies was diphtheria antitoxin. The 26 children with three or fewer antibodies comprised three who had been fully immunised, 10 who had received primary immunisation only, and 13 who had either not been immunised or were without immunisation records. Only one child, who was born in 1961 and was unimmunised, was found to have none of the five antibodies.

Discussion

An antibody survey such as this cannot provide an exact measure of either the overall immunity of the studied samples or the overall efficacy of an immunisation programme. Underestimation of immunity almost certainly arose from our need to equate protection with arbitrary antibody titres and our inability to assess the protective role of immunological memory. Conversely, overestimation of the efficacy of an immunisation programme may result from the detection of poliovirus antibodies in the sera of children who have not received vaccine but who have suffered asymptomatic natural infection or infection with attenuated vaccine virus derived from a recent vaccinee. Furthermore, tetanus antitoxin may be present as a consequence of vaccination after an injury. None the less, despite these sources of error the pattern of antibodies in a sample is a useful indicator of the immune state of a population and, more important, an indicator of any serious deficiencies.

The diphtheria antitoxin titres are of particular interest as they showed the effects of two influences: a noticeable improvement in the uptake of reinforcing immunisation between 1966 and 1974, which accounted for an increase in the fully vaccinated children from 36% in the 1961 sample to 84% in the 1969 sample; and the inevitable slow decline in antitoxin titre with time after the last antigenic stimulus.[3] Thus the predominantly low titres in the 1961 sample were attributable in part to the small proportion of children in the sample who were fully immunised but also to the long lapse of time between the last antigenic stimulus and the date of bleeding. Conversely, the predominantly high titres in the 1969 sample were attributable in part to a high acceptance of reinforcement but also to the short lapse of time since reinforcement. An intermediate position obtained in the 1965 sample. When those children who received reinforcement were considered alone the fall in titre with time became very evident indeed, but it is clear that even in the 1961 sample, 10 years after reinforcement, most of the titres exceeded 0·01 IU diphtheria antitoxin/ml. There was thus a pronounced contrast in titres between the children with reinforcement and those without reinforcement and a clear indication of the need for reinforcing immunisation at school entry.

The tetanus antitoxin titres, on the other hand, showed little difference between the samples or between the fully immunised and those who had received only primary immunisation, a phenomenon that testifies to the good antigenicity of tetanus toxoid. If the sera had been tested at higher levels, however, those children who had received reinforcement might have exhibited generally higher titres than those who had not. Even so, our results suggest that tetanus antitoxin titres evoked by primary immunisation alone are very durable.

In the cases of the antibodies to the three types of poliovirus, our knowledge of the titres likely to provide protection is very deficient and it is impossible confidently to estimate from the distributions the numbers of children adequately immunised against the three types of infection. Titres of 1/8 or more certainly indicate truly specific antibody, but lower titres or even immunological memory alone may be a sufficient defence.[4]

The similarity in the 1961 and 1965 samples of the antibody distributions of children who had been fully immunised and those who had received only primary immunisation was unexpected. Since it is unreasonable to believe that the children who were fully immunised had, before reinforcement, antibody titres that were generally lower than those who had not received reinforcing immunisation it seems that, in the case of poliovirus antibodies, reinforcement had little effect on titres. The most likely explanation is that in most cases the antibodies evoked by primary immunisation were enough to prevent intestinal reinfection, and thus no stimulus to the production of further antibody occurred.[5,6]

In general, our results show that the national immunisation programme consisting of primary immunisation in infancy followed by reinforcing immunisation at school entry continues to provide most children with adequate immunity, as assessed from serum antibody titres, to diphtheria, tetanus, and the three types of poliomyelitis. Immunity to diphtheria—that is, an antitoxin titre of at least 0·01 IU/ml—appears to be less readily evoked and maintained than immunity to the other infections, and, as indicated by the 1961 and 1965 samples, failure to achieve good coverage with reinforcing immunisation leads to a high proportion of children with inadequate or barely adequate protection. On the other hand lasting immunity to tetanus is

readily established in most children by primary immunisation alone, and the prime benefit of reinforcement at school entry may be to prolong immunity through adolescence into adulthood. In the case of poliomyelitis, reinforcing immunisation was largely without effect, probably on account of exclusion by pre-existing antibody. Thus it might be argued that, except in the case of immunity to diphtheria, the benefits of reinforcing immunisation are chiefly the immunisation against tetanus and poliomyelitis of children who have inadvertently missed primary immunisation.

Extrapolation from the samples to the cohorts from which they were drawn provides estimates of the percentages of children who, by the criteria of this study, may be considered to have been adequately protected. Thus in the 1969 cohort, in which the best immunisation coverage was achieved (84% of the children were fully immunised and a further 14% had received primary immunisation, although not reinforcement), the estimate of those protected from tetanus was 98% and of those protected from diphtheria and all three types of poliomyelitis 85%. In view of the excellence of the protection from tetanus and the additional protection provided by the herd in the cases of the communicable infections, it seems unreasonable to suppose that more rigorous implementation of an immunisation programme would provide anything other than marginal additional benefit.

We thank the children for the blood samples, and their parents for allowing them to participate; Mr L Youens and Mrs Vivienne Miller and staff of the child health service of the Avon AHA; the doctors of the Avon AHA for performing the venepunctures; Dr Suzanne Clarke, of the Bristol PHLS Laboratory, for separating the sera; and Miss Janet Bootman, Mrs Anne Johns, Miss Moira Melville, and Miss Johanna Watkins for skilled technical help.

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(*Accepted 8 February 1979*)

Campylobacter colitis

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British Medical Journal, 1979, **1**, 857-859

Summary and conclusions

Eleven consecutive patients with diarrhoea from whose stools campylobacter were isolated were investigated by sigmoidoscopy and rectal biopsy. Eight had definite proctitis, and in seven biopsy specimens were abnormal with histological changes ranging from non-specific colitis to gross colitis with goblet-cell depletion and crypt-abscess formation. Nine of the patients passed blood in their stools, and in all but one abdominal pain was a feature of the illness.

Severe campylobacter colitis may be clinically, sigmoidoscopically, and histologically difficult to differentiate from ulcerative colitis and is a differential diagnosis in acute colitis.

Introduction

Campylobacter has only recently been recognised as a cause of diarrhoea. Skirrow[1] reported that this organism could be isolated by selective culture of the faeces of over 7% of unselected patients with diarrhoea but not from control samples from subjects without diarrhoea. Other studies[2-8] have confirmed these findings. During 1977 1513 reports of the isolation of campylobacter from the faeces of patients were received by the Communicable Disease Surveillance Centre (Public Health Laboratory Service), and out of 1336 cases analysed, 95% of the patients were suffering from diarrhoea.[9] The clear correlation between symptomatic diarrhoea and the isolation of campylobacter leaves little doubt that it is potentially pathogenic, though "carrier states" probably exist.

The concept of colonic disease resulting from salmonella infection, which was previously regarded as causing "enteritis," has recently gained wide acceptance.[10,11] In view of the close similarity between the clinical features of salmonella and campylobacter infections we carried out the present study to determine whether similar colonic changes occurred in patients with diarrhoea due to campylobacter.

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Patients and methods

Eleven patients (three female, eight male) presented during a six-month period with a diarrhoeal illness and campylobacter in their faeces. They were aged 14-80 years (mean 46 years), and 10 warranted admission for inpatient management. Sigmoidoscopy was carried out in all these cases and rectal biopsy specimens taken; in eight cases this was done shortly after admission. Three patients who did not undergo sigmoidoscopy on admission were found to have campylobacter in their stools only after the diarrhoea had settled; sigmoidoscopy and biopsy were performed as soon as the diagnosis was made, so in these cases the results represent a resolving or resolved condition. A barium enema was thought to be justified in two patients. Filtrates of faecal suspensions were cultured at 43°C on Oxoid BA base No 2 with 7% lysed horse blood plus (final concentrations) vancomycin 10 μg/ml, polymixin B sulphate 2·5 IU/ml, and trimethoprim lactate 5 μg/ml, in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% hydrogen.

CLINICAL FEATURES

The patients presented with diarrhoea or abdominal pain, or both. In most cases the duration of diarrhoea before presentation was