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A controlled field trial of live oral typhoid vaccine Ty21a

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A controlled field trial of a live oral typhoid vaccine was carried out in Alexandria, Egypt, in 1978–79. A total of 32 388 children were included in the study. They were divided in two comparable groups, one given 3 doses of the vaccine and the other 3 doses of the placebo. Each active dose contained 1 x 10^9–8 x 10^9 live Ty21a bacteria. From March 1978 to March 1979, the population studied was followed up and suspected typhoid cases were investigated bacteriologically and serologically. The effectiveness of the vaccine was assessed by analysis of the incidence of typhoid fever in the two groups. The results of the follow-up indicate that, in the dosage schedule used, the Ty21a mutant strain, found previously to be stable and safe, is protective against typhoid fever for at least one year.

Typhoid fever remains a serious public health problem in many regions of the world, and the typhoid vaccines at present available are not wholly satisfactory. Parenteral vaccines have been shown in several large-scale controlled field trials to confer significant protection to persons living in endemic areas (*1–4*). However, they frequently cause adverse reactions, such as fever, intense local inflammation, and headache. Oral inactivated vaccines are non-reactogenic but their efficacy in man has not been demonstrated in field trials (*5*). Challenge studies in adult volunteers with a streptomycin-dependent live vaccine gave variable results (*6, 7*).

More recently, a galactose epimerase (gal E) mutant of *Salmonella typhi* (Ty21a) was isolated (*8*) and results obtained with an animal model indicated that this strain has the potential for use as a live vaccine. Mutant Ty21a was evaluated as an attenuated live vaccine at the University of Maryland; 5–8 doses of vaccine containing 3–10 x 10^{10} live organisms were ingested without significant side effects (*9*). The efficacy of the vaccine was tested in experimental challenge studies in volunteers. It gave a protection of 87% against a challenge dose that caused typhoid fever in 53% of unimmunized control volunteers (*9, 10*). In earlier studies, parenteral vaccines that had previously been found effective in the field gave less protection in experimental challenge (*11*).

The encouraging results obtained with the vaccine Ty21a warranted a controlled field trial in an area endemic for typhoid fever. The purpose of this trial was to check, on a large scale, the safety and stability of the mutant strain and to test the protection against typhoid fever afforded by 3 oral doses. This report presents the results obtained after one year of observation of the first field trial carried out with the oral typhoid vaccine Ty21a.

MATERIALS AND METHODS

Oral typhoid vaccine

The vaccine was prepared from the *S. typhi* mutant strain Ty21a (*8*). The parent and working seed lots consisted of dried identical cultures kept at 4 °C. The identity of the strain in both parent and working seed lots was checked as described by Germanier et al. (*8*):

- When plated on Endo agar containing galactose instead of lactose, only pale, translucent, concave, non-galactose-fermenting colonies, characteristic of strain Ty21a, were formed.

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- When plated on Kligler agar, only pale colonies that did not produce H_2S were formed. (This characteristic can be used as a marker to differentiate galactose-fermenting revertants from the wild type).
- When the vaccine strain was grown on heart infusion and galactose was added at a concentration of 0.1%, the growing culture underwent severe lysis within 1–2 hours.
- Ty21a bacteria grown on brain-heart infusion containing 0.001% of galactose agglutinated with O- and H-antisera but did not agglutinate with Vi-antiserum.
- Ty21a bacteria had no detectable UDP-galactose-4-epimerase activity. With Ty21a, the activities of the two other Leloir enzymes, galactokinase and galactose-1-phosphate-uridyltransferase, were reduced to 10% and 24%, respectively, of the levels with the wild type Ty2.
- Galactose added (final concentration 0.001%) to a growing culture of Ty21a was readily taken up, 50% becoming incorporated into the cell wall and 10% accumulating in the cytoplasm.
- The LD_{50} of strain Ty21a was $>10^8$ when the cells were suspended either in 0.85% saline or in gastric mucin.
- Ty21a bacteria injected intraperitoneally in a concentration of 10^8 live cells/mouse were eliminated within one week from the liver and spleen of the animals.
- Mice immunized intraperitoneally with 10^7 Ty21a cells were protected 4 weeks later against an intraperitoneal challenge with 10^6 live Ty2 cells.

The vaccine was prepared by growing strain Ty21a on brain-heart infusion containing 0.001% galactose in a 50-litre fermenter. Growth was measured by recording the optical density of the culture. At harvest the number of viable cells was determined by plating the culture on brain-heart infusion agar plates. The bacteria were harvested by centrifugation, resuspended in appropriate concentration in a sucrose solution, and lyophilized in 1-ml portions in 3-ml glass vials. Each vial represented one dose. All vials prepared from one fermentation and one lyophilization represented one final lot.

From each final lot, five randomly selected vials were suspended in 3 ml of saline and plated in serial dilutions on 3 brain-heart infusion agar plates, 4 blood agar plates, and 10 galactose-containing Endo agar plates. Each vial contained $1–8 \times 10^9$ viable Ty21a bacteria. No colony other than Ty21a was demonstrable on the different plates. All final lots not satisfying these requirements were eliminated. The number of inactivated Ty21a bacteria was estimated from colony counts in the culture at harvest and in the final vials. They ranged between 2×10^{10} and 1×10^{11}.

After vaccination had been completed, the remaining vaccine was controlled again and shown to contain on average 2.7×10^9 viable cells per dose.

The safety of the vaccine was checked by intraperitoneal injection of 1% of one dose into five mice (each weighing 20 g) and 10% of one dose into 3 guinea-pigs (each weighing 250–300 g) and by oral administration of 10% of one dose to five mice, one dose to 3 guinea-pigs, and 3 times one dose at two-day intervals to 2 rabbits. All animals survived and no adverse reaction was noted.

Placebo

The placebo was prepared in a similar manner from a milk powder and sugar solution.

The vaccine and placebo were indistinguishable in lyophilized as well as in reconstituted form. They were supplied by the Swiss Serum and Vaccine Institute and were given the code signs O and X, respectively.

The freeze-dried preparations were reconstituted using a diluent (prepared daily) containing disodium phosphate, monosodium phosphate, and sucrose.

Since live vaccine has to be protected against gastric acidity and as it was administered in a provisional form, sweetened, aromatized sodium bicarbonate tablets were prepared to be chewed before each administration of the vaccine or placebo.

Trial area and population sample studied

The study was carried out in Alexandria where typhoid fever is endemic. Because of the high incidence of typhoid fever among schoolchildren and because they could be followed up easily, the trial was carried out on first-year primary schoolchildren, aged 6–7 years.

In the light of previous studies on the incidence of typhoid fever in this area (*12*) and on the basis of an estimated rate of about 0.1–0.2% in this population, two groups of 15 000 children each were considered suitable for demonstrating a statistically significant difference between the vaccinated and control (placebo) group.

Considering that typhoid incidence, even during the summer peak, is almost uniform throughout the city and that the total number of non-vaccinated new school entrants amounted to 57 016 in 328 schools in Alexandria, 219 public primary schools situated in the 11 different districts of the city, were chosen for the study. Of the 37 506 first-year children enrolled in these schools, 5118 were absent on the day of the beginning of the trial (due to an exceptionally bad spell of rain). Thus, 32 388 children were involved in the study. The 5118 absentees and 20 510 children in other

schools not included (i.e., 25 628 children) were subjected to the same follow-up.

In each class, the children were listed on a vaccination card. The classes were marked at random with the code letter X or O using a permutation table and balancing every 6 classes to contain 3 of each designation.

Parents were informed that their consent was required for inclusion of their children in the trial. Their free acceptance was obtained before the study and they were free to withdraw their children from the trial at any time.

Consequently, the study population of newly enrolled primary schoolchildren comprised 3 groups:

— group O receiving oral vaccine (16 486 children)
— group X receiving oral placebo (15 902 children)
— a non-vaccinated group (25 628 children)

Vaccination procedure

Three oral doses of vaccine or placebo were given, one dose every other day. Each dose was suspended in 20–30 ml of diluent and given orally after ensuring that the child had chewed the bicarbonate tablet.

The vaccine and the placebo were both stored at 4 °C. The amounts needed were taken from the refrigerator only on the day of vaccination.

Conduct and planning of the trial

The trial began in March 1978 when the incidence of typhoid was still low, and was carried out in four stages: (1) preparation and formation of vaccination teams; (2) conduct of the pilot study; (3) main vaccination; (4) one-year follow-up (from March 1978 to March 1979).

Vaccination teams

During several meetings held with physicians and nurses of the school health department, the vaccination programme and the vaccination and follow-up procedures were thoroughly explained. Teams consisting of one physician and at least three nurses were set up, each team being in charge of a number of schools in one of the 11 districts of Alexandria. Altogether, 86 physicians and 390 nurses assisted in the vaccination programme.

Pilot study

As requested by local authorities, a pilot study was performed prior to the main vaccination. The aims of the pilot study were to test acceptance of the oral vaccine and bicarbonate tablets by the children, to detect any side-effects of the oral vaccine or placebo, and to investigate the excretion of the mutant strain in the stools. In addition, it was intended as a practical test of the recommended procedures.

The pilot study was undertaken on 24 classes of the selected schools on 7–11 March 1978. Among the 884 children who were chosen for this pilot trial, 413 received the oral vaccine and 471 received the oral placebo.

Main vaccination

The main vaccination was carried out on 11–16 March 1978. Half of the children received vaccine or placebo on 11, 13, and 15 March and the other half, on 12, 14, and 16 March, a total of 92 675 doses being administered.

The distribution of children included in the study is shown by vaccination status in Table 1. The O and X groups were compared with regard to their distribution within each of the 11 school health districts and were found to be well balanced.

Table 1. Distribution by vaccination status of children included in the study

Group	Number of children in group	Number of children who received:		
		3 doses	2 doses	1 dose
O group (vaccine)	16 486	14 735	1 081	670
X group (placebo)	15 902	14 557	622	723

One-year follow-up

In Alexandria, schoolchildren with unexplained fever persisting more than three days are systematically referred to the communicable diseases hospital for admision. Arrangements were made for daily checking of all admissions of children 5–9 years of age as regards schooling. All those who were in first year of primary school at the time of vaccination were checked both bacteriologically and serologically. The school and class of these children were identified and recorded on a card, together with the clinical symptoms. These data were then sent to the epidemiology department. There, the serial number assigned to the child for vaccination was checked and the blood and stool samples that had been collected were given the same number and sent to the laboratory for diagnosis. The vaccination status of the patient was then checked and recorded on the admission card, on which the laboratory results were also reported when received.

Criteria for diagnosis of typhoid fever

In the protocol, it was decided that only those cases with positive blood culture were to be considered as

proved typhoid cases. Patients with typical clinical illness, who had a negative blood culture but either a positive stool culture or high titres (1:400 or above) of anti-O and anti-H antibodies were to be considered as probable cases and studied separately.

RESULTS

Adverse reactions

Digestive and general reactions observed in the pilot study and those observed in the main trial were recorded and are shown in Table 2. In the pilot study, of the 12 children from O-group who vomited, 9 were in the same class.

With the exception of this observation, reactions were minimal in the pilot study and in the main trial. No complaint of diarrhoea was recorded and no difference was observed between reactions after the first, second, and third doses.

Excretion of vaccine strain

From the 884 students in the pilot study, it was possible to collect 510 stool samples 7 days after the first dose (i.e., 2 days after the third dose) and 475 stool samples after another two weeks, for detecting *S. typhi*, including Ty21a. The vaccine strain was not isolated from any of these stools, but two healthy *S. typhi* carriers were identified; both were in the X-group.

Incidence of typhoid fever

Of the 57 016 children who were followed up from March 1978 to the end of March 1979, 79 patients were investigated bacteriologically and serologically for typhoid fever. Of 22 suspected cases from the O-group, 18 children had received three doses and 4 children one or two doses. Of 57 suspected cases from the X-group, 53 children had received three doses and 4 children one or two doses.

The distribution of confirmed and probable cases of typhoid fever detected during the follow up is given in Tables 3 and 4. There was no proved case of typhoid fever in the O-group but there were 7 cases in the X-group.

Table 2. Number of adverse reactions observed following vaccination

Group	No. of doses	Vomiting	Fever	Nausea/ abdominal pain
Pilot trial				
O group (vaccine)	1 159	12	1	3
X group (placebo)	1 313	2	1	0
Total	2 472	14	2	3
Main vaccination				
O group (vaccine)	47 037	49	1	14
X group (placebo)	45 638	21	3	2
Total	92 675	70	4	16

Table 3. Number of confirmed typhoid fever cases in the experimental groups during one year of follow-up

Group	No. of doses given	Population	Positive blood culture	Incidence/ 10 000
O group (vaccine)	3	14 735	0	0[a]
	1 or 2	1 751	0	0
	Total	16 486	0	0
X group (placebo)	3	14 557	7	4.80[a]
	1 or 2	1 345	0	0
	Total	15 902	7	4.40

[a] The difference in attack rates between the two groups is statistically significant ($0.02 < P < 0.05$).

Table 4. Number of probable typhoid cases in the experimental groups during one year of follow-up

Group	No. of doses given	Positive stool culture	Positive sero-diagnosis	Total no. of probable cases	Incidence/ 10 000
O group (vaccine)	3	0	0	0	0[a]
	1 or 2	0	0	0	0
	Total	0	0	0	0
X group (placebo)	3	1	12	13	8.93[a]
	1 or 2	0	0	0	0
	Total	1	12	13	8.17

[a] The difference in attack rates between the two groups is statistically significant ($P < 0.001$)

To permit a comparison of the respective incidences among the children belonging to the control (X) group and the non-vaccinated children who were not involved in the trial but subjected to the same follow-up, the number of cases of typhoid fever (confirmed and probable cases) that occurred in this non-vaccinated group is given in Table 5.

Table 5. Typhoid fever incidence in the non-vaccinated group

Population followed	No. of confirmed cases (positive blood culture)	Attack rate/ 10 000[a]	No. of probable cases: Positive stool culture	No. of probable cases: Positive sero-diagnosis	Incidence/ 10 000
25 628	14	5.46	3	17	7.80

As previously mentioned, the non-vaccinated group included 5118 absentees from the classes from which the vaccinees were drawn and it is interesting to note that of the 14 confirmed cases of typhoid fever that occurred in this group, two were among the absentees.

Effectiveness of the vaccine

Evaluation of the vaccine based on the number of cases of typhoid fever diagnosed by positive blood culture revealed that 7 cases occurred in the control group while no case was observed in the vaccinated group (Table 3). This is an incidence of 4.80/10 000 in the control group and none in the vaccinated group. The difference is statistically significant ($0.02 < P < 0.05$).

Similar analysis of probable cases of typhoid fever revealed incidences of 8.93/10 000 in the control group and none in the vaccinated group (Table 4). This difference is also statistically significant ($P < 0.001$).

Combining the confirmed and probable cases, the incidence is 12.57/10 000 in the control group and none among the vaccinees.

The incidence of typhoid fever noted in the non-vaccinated group is 5.46/10 000 for confirmed cases and 7.80/10 000 for probable cases (Table 5); for confirmed and probable cases combined the incidence is 13.27/10 000.

There is no statistically significant difference between the morbidity rate observed in the control group (X-group) and in the non-vaccinated group (i.e., those receiving neither vaccine nor placebo). This comparability confirms that the selection of schools involved in the study was satisfactory.

Although the incidence of typhoid fever in the population under study was low, analysis of the data obtained in the one year of follow-up, proved that effective protection against *S. typhi* infection was given by this oral vaccine containing strain Ty21a, at the dosage schedule used.

The number of children who received one or two doses of vaccine was too limited to allow any conclusion to be drawn on the effectiveness of less than three doses and, hence, on the optimal vaccination dose.

In order to confirm the identification of each *S. typhi* isolate, the phage type was determined. Eleven different phage types (Vi^+) were identified.[a]

Spread of the vaccine strain could not be detected in any suspected case.

The results indicate that in the form and the dosage schedule used, the live oral vaccine, Ty21a, is safe, stable, and effective against typhoid fever for at least one year.

Continuation of follow-up is now being conducted on the same population to evaluate the duration of protection induced by vaccination.

[a] Phage typing was done by J. F. Vieu at the National Salmonella Centre, Pasteur Institute, Paris.

RÉSUMÉ

ESSAI PRATIQUE CONTRÔLÉ D'UN VACCIN ANTITYPHOÏDIQUE ORAL PRÉPARÉ AVEC LA SOUCHE ATTÉNUÉE Ty21a

Un essai pratique contrôlé a été organisé à Alexandrie (Egypte) en 1978–1979, afin d'évaluer le pouvoir protecteur d'un vaccin antityphoïdique atténué administré par voie orale. Au total, 32 388 enfants de 6 à 7 ans, non antérieurement vaccinés, y ont participé. Trois doses d'un vaccin renfermant 1×10^9 à 8×10^9 germes vivants de la souche Ty21a par dose ont été données par voie orale à 14 735 enfants; 14 557 autres enfants (témoins) ont reçu 3 doses de placebo. Pour diverses raisons, 3096 enfants n'ont reçu qu'une ou deux doses de l'une ou l'autre préparation. Chacune des doses de ce vaccin lyophilisé a été reconstituée à l'aide d'un diluant spécial et a été administrée, à 48 heures d'intervalle, après la prise d'un comprimé de bicarbonate de sodium pour neutraliser l'acidité gastrique. La vaccination a été précédée d'un essai préliminaire, destiné à tester l'innocuité et la stabilité de la souche vaccinale par une étude des réactions post-vaccinales éventuelles et par un contrôle de l'excrétion de la souche Ty21a dans les selles.

La surveillance épidémiologique de l'effectif a été appliquée de mars 1978 à mars 1979, par l'intermédiaire de l'Hôpital des Maladies Infectieuses où tous les cas de fièvre persistante (3 à 4 jours) sont systématiquement adressés. Hémoculture, coproculture et sérodiagnostic ont été pratiqués sur tous les cas suspects de fièvre typhoïde. Seuls les cas de fièvre typhoïde confirmés par hémoculture ont été retenus pour l'analyse des résultats. Les cas présentant seule-

ment une coproculture positive et/ou un sérodiagnostic positif ont été considérés comme des cas probables et analysés séparément. Un groupe d'enfants du même âge et vivant dans les mêmes lieux et conditions que les enfants inclus dans l'essai a été également surveillé de mars 1978 à mars 1979.

Sur les 79 cas suspects de fièvre typhoïde, on a isolé 7 fois *Salmonella typhi* par hémoculture dans le groupe témoin. Aucune hémoculture positive n'a été enregistrée dans le groupe des vaccinés. La différence est significative ($0{,}02 < P < 0{,}05$). La répartition des cas probables est de 13 pour le groupe témoin contre 0 parmi les vaccinés, où aucun des 22 cas suspects n'a présenté de coproculture ou de sérodiagnostic positif. La différence est également significative ($P < 0{,}001$).

L'incidence de la fièvre typhoïde constatée dans le groupe d'enfants non vaccinés, où 14 hémocultures positives, 3 coprocultures et 17 sérodiagnostics positifs ont été notés, a été globalement de 13,27 pour 10 000. Ce taux, voisin de celui de 12,57 pour 10 000 enregistré dans le groupe témoin, vient à l'appui des résultats significatifs obtenus en comparant les deux groupes de l'étude. L'ensemble de ces éléments indique que le vaccin antityphoïdique atténué Ty21a, administré par voie orale aux doses utilisées dans le présent essai, est bien toléré, stable et qu'il confère une protection contre la fièvre typhoïde.

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