Controlled field trial on the effectiveness of one and two doses of acetone-inactivated and dried typhoid vaccine

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A controlled field trial was carried out with acetone-dried vaccine on about 35 000 people on the Tongan islands of Tongatapu and 'Eua where typhoid fever is endemic. Volunteers were distributed at random into 3 groups, 1 of which received 1 dose of typhoid vaccine and a second 2 doses; the third, a control group, received tetanus toxoid. The population was vaccinated in 1966 and was studied until the end of 1973. Evaluation of the effectiveness of the vaccine was based on cases of typhoid fever that were confirmed by positive blood cultures. Morbidity rates in the 3 groups indicated that the acetone-dried vaccine used was effective and that a single dose of vaccine gave reasonable protection for a short period, but that 2 doses gave greater and longer protection. The results of this trial are compared with those of earlier field and laboratory studies.

In earlier controlled field trials of monovalent typhoid vaccines acetone-dried vaccine was shown to be the most effective (1). Two doses of the vaccine were given; in one of the trials (2) a very high degree of protection was also observed in a small proportion of the schoolchildren who failed to receive the second dose. However, since this group was small and not strictly comparable with the others, no definite conclusion could be drawn as to whether two doses are needed to obtain complete and lasting immunity. In view of the importance of keeping the number of doses in large scale immunization programmes as small as possible, it was decided to compare the effectiveness of one and two doses of acetone-dried typhoid vaccine.

The study was carried out according to experience gained in previous studies of typhoid vaccines (1, 2). The acetone-dried vaccine was prepared in the same manner as for earlier studies (3) and according to the requirements of the WHO Expert Committee on Biological Standardization (4). Vaccination teams and all facilities for mass immunization and follow-up were provided by the Government of Tonga, and the World Health Organization provided the necessary laboratory and other equipment.

The Kingdom of Tonga consists of some 150 islands and has over 70 000 inhabitants. The most populated island, Tongatapu, and the nearby island of 'Eua, which together have a population of 51 311,[a] were regarded as suitable for the trial since typhoid fever was common there.

Before the recent construction of central water supply systems rain water and shallow wells were the only sources of drinking water. The shallow well water could easily be polluted from nearby latrines. The handling of food by several persons engaged in its preparation and the close family and community contacts favour the spread of typhoid fever, which has apparently been endemic on the islands for a long time.

Typhoid was previously diagnosed only on clinical grounds, and the number of actual cases in the past is uncertain. The incidence varied on Tongatapu and 'Eua from approximately 20 to 50 per 10 000. Since 1966, all cases of suspected typhoid fever have been bacteriologically examined by blood culture at the enteric laboratory in Nuku'alofa, which was established for this purpose. In 1966, 183 cases of typhoid were bacteriologically confirmed.

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[a] November 1966 census.

MATERIALS AND METHODS

Vaccine

The acetone-dried typhoid vaccine was prepared according to procedures applied in earlier controlled trials and as used in the production of international reference acetone-inactivated and dried vaccine (K). The vaccine was prepared from the Ty_2 strain of *Salmonella typhi* which was treated with acetone by a modification of the Landy method (3). The vaccine was dried in the final containers, and reconstituted each morning before use. The diluted vaccine contained 1 000 million organisms per ml. The control vaccine was alum-precipitated tetanus toxoid in containers identical to those used for the typhoid vaccine. Both vaccines were coded to prevent their identification by the field and hospital staff.

Vaccination

Children and adults up to 60 years of age were accepted for the trial provided they were healthy and had no recent history of enteric infection. Individuals with skin lesions, chronic or acute infections, or heart or kidney disease were not accepted. The nature of the trial was fully explained to the adult volunteers and to the parents of the children, and their free and informed consent to vaccination was obtained.

The vaccines were injected subcutaneously in the upper arm in doses of 0.5 ml for children between 2 and 11 years of age, and in 1-ml doses for persons of 12 years and over. Each person received 2 doses with an interval of 4 weeks. One group received 2 doses of typhoid vaccine, another received tetanus toxoid followed by typhoid vaccine, and a third, the control group, received 2 doses of tetanus toxoid. The vaccines were allocated to each volunteeer strictly at random so that the 3 groups were as similar as possible. Vaccination commenced on 21 June and ended on 17 September 1966. The number of immunized persons is shown in Table 1.

Table 1. Number of persons who received typhoid vaccine and/or tetanus toxoid

Locality	Population [a]	Volunteers who received:		Percentage of population who received 2 doses
		1 dose [b]	2 doses	
Tongatapu				
Nuku'alofa	15 545	522	9 818	63.16
other localities	32 375	1 039	21 853	67.50
'Eua	3 391	128	1 977	58.30
total	51 311	1 689	33 648	65.58

[a] 1966 census.
[b] These volunteers did not return for the second dose.

Table 2. Comparison of vaccination groups

	Vaccine		
	two doses typhoid	one dose typhoid plus one dose tetanus	two doses tetanus (control)
sex			
M	5 794	5 938	5 860
F	5 334	5 453	5 269
age group			
0–5	2 064	2 066	2 093
6–15	4 183	4 227	4 187
16–20	1 368	1 414	1 400
21–30	1 490	1 511	1 487
>30	2 032	2 173	1 962
previous disease history			
typhoid	752	819	777
pertussis	1 088	1 225	1 060
tuberculosis	62	89	87
previous history of vaccination			
BCG	1 978	2 053	2 129
DTP	999	1 052	978
TAB	1 729	1 699	1 671
water supply			
well	4 373	4 438	4 358
tank	4 497	5 003	4 815
piped	5 847	6 438	6 330
latrine			
dry pit	9 615	9 449	9 180
water sealed	2 079	2 129	2 085
flush	534	578	594
total No. vaccinated	11 128	11 391	11 129

The data in Table 2 indicate that the groups were very similar, being practically identical in respect of

Table 3. Reactions to typhoid vaccine and tetanus toxoid

Vaccine	No. of vaccinees [a]	Day after vaccination	Reaction: temperature above 98° F dose 1	temperature above 98° F dose 2	inability to work dose 1	inability to work dose 2	headache dose 1	headache dose 2	severe local pain dose 1	severe local pain dose 2	erythema (diam. ≥5 cm) dose 1	erythema (diam. ≥5 cm) dose 2	local swelling dose 1	local swelling dose 2
typhoid	159	1	14	7	21	13	30	20	6	4	28	26	40	25
		2	7	5	7	5	14	3	1	1	28	15	23	21
		3	1	0	6	5	5	1	0	0	3	3	2	5
tetanus (control)	156	1	5	1	10	3	8	6	1	0	4	4	15	10
		2	3	1	3	2	10	7	0	1	3	3	11	5
		3	2	0	3	4	3	2	0	0	0	1	1	1

[a] A few persons were not examined on all days for all reactions listed.

number, sex, age, previous history of illness and vaccination, environmental sanitation, drinking water, and waste disposal. It may therefore be assumed that all three groups were exposed to infection to the same degree.

Reactions to inoculation

A preliminary study of reactions to the batch of typhoid vaccine used in the trial was made on schoolchildren in Fiji in 1965.[a] They were generally mild and the vaccine was well accepted by the children. Reactions were also studied during a mass immunization campaign in Tongatapu on a group of randomly selected people. Reactions to acetone-dried vaccine were comparable to those observed in earlier studies (5). Table 3 shows that reactions were more severe in every respect than those to tetanus toxoid. The second dose induced a less severe reaction than the first. However, the reactions were not so severe as to cause people to refuse the second dose.

Laboratory tests

Serological investigations of agglutinating titres of H, O, and Vi antibodies were made on 202 sera collected at random 8 months after inoculation. More persons with high H titres were observed among those who received typhoid vaccine than among those who received only tetanus toxoid. Higher H titres were observed among those who received 2 doses than among those who received one dose.

Laboratory potency testing of the vaccine and its characterization were carried out according to methods used in earlier studies (1, 3). The vaccine was tested by the active mouse-protection test by four collaborating laboratories,[b] none of which found a significant difference between the vaccine used in Tonga (coded B) and the international reference vaccine (K) used in earlier trials (2).[a] Other characteristics of the 2 vaccines, such as their nitrogen and water contents, were very similar (3). It was therefore assumed that the acetone-dried typhoid vaccine used in Tonga was representative of its type.

Follow-up

The immunized population was followed up for 7½ years. Specimens of blood and faeces from individuals with pyrexia of unknown origin, and from suspected and clinical cases of enteric fever, were examined bacteriologically for the presence of *S. typhi*. All cases presented in the tables were bacteriologically proved. Phage typing of the strains isolated revealed only two phage types, viz., A and E_1.

Owing to the improvement in the water supply and in the general standard of hygiene, the incidence of typhoid declined steadily during the study. However, a number of cases occurred between 1966 and 1973, as can be seen from Table 4.

[a] HAWLEY, T. G. Reaction to typhoid vaccines in Fiji children SPM 5/11. (Mimeographed document, Government of Fiji.)

[b] Institute 'Human', Budapest, Hungary; Lister Institute of Preventive Medicine, Elstree, England; Walter Reed Army Institute of Research, Washington, DC, USA; Institute of Immunology, Zagreb, Yugoslavia.

Table 4. Bacteriologically proved cases of typhoid among vaccines

Year [a]	Vaccine		
	two doses typhoid	one dose typhoid plus one dose tetanus	two doses tetanus (control)
1966–67	8	9	18
1967–68	3	6	6
1968–69	2	8	2
1969–70	2	4	6
1970–71	5	4	14
1971–72	8	18	4
1972–73	4	8	3
total 1966–73	32	57	53

[a] Period from 1 July to 30 June except for 1973 (to 31 December).

RESULTS

The number of bacteriologically proved cases of typhoid in Tongatapu following vaccination is presented in Table 4. The number of observed cases was rather small, and there were fluctuations from year to year. However, the differences in morbidity among the 3 vaccine groups indicated that the typhoid vaccine protected for 5 years when 2 doses were given, but protected for only 1 year when 1 dose was given. There was no evidence of protection after the fifth year.

The incidence of typhoid in vaccinees of different age groups is set out in Table 5; this indicates that vaccination was more effective in the younger age groups than in the older groups. The oldest group had a lower natural occurrence of typhoid than the younger groups. The difference in morbidity between the different vaccine groups was particularly pronounced among the very young.

Table 5. Typhoid cases among vaccinees of different age groups over a period of 7½ years

Age group	Vaccine			Total
	two doses typhoid	one dose typhoid plus one dose tetanus	two doses tetanus (control)	
2–9 years	6	13	23	42
10–14 years	13	20	15	48
>14 years	13	24	15	52
total	32	57	53	142

Statistical evaluation of typhoid incidence during the first 5 years of the study in all the age groups taken together confirmed the effectiveness of the vaccine. There was a statistically significant difference at the 5% level between 1 or 2 doses of typhoid vaccine and the control. Two doses gave significantly greater protection than 1 dose only in the vaccinees aged 2–11 years.

Of those people who did not come for a second dose, and who therefore had only one dose of control or typhoid vaccine, there were 4 bacteriologically proved cases of typhoid fever among 1 112 persons who received control vaccine, and 1 among 577 persons who received typhoid vaccine.

The occurrence of typhoid among the 6 021 people who did not volunteer for vaccination was nearly 4 times higher than in the control group. This suggests that a non-cooperative, non-immunized population is more exposed to infection, which is probably due to their low level of health consciousness and low standards of hygiene as observed in earlier studies (5).

DISCUSSION

Earlier studies (1) on acetone-dried vaccine have demonstrated its high effectiveness, which this study confirms. However, possibly owing to the high infective dose in Tonga, where typhoid is mainly food-borne, the level of protection observed was lower than in other studies where the disease was more often water-borne (2, 5) and the infective dose probably lower. Experiments carried out on volunteers have shown that a high challenge dose overcomes immunity (6).

In a study carried out in Guyana (2) with acetone-dried vaccine in a small group of schoolchildren who did not receive the second dose, the protective effect of 1 dose was apparently as great as with 2 doses. Studies in the USSR, however, have shown that 2 doses of heat-killed phenol-preserved vaccine protect better than 1 dose (7). Another study with heat-killed acetone-inactivated vaccine has shown that 1 dose gives protection for only about 10 months (8). The difference in the effectiveness of 1 and 2 doses in primary immunization in younger age groups in the present trial was significant, and can perhaps be explained by a higher infective dose in

Tonga against which one dose of vaccine gave insufficient protection. The increased incidence after 5 years in the group given 1 dose indicated that immunity had ceased and that these people were then possibly more susceptible than the rest of the population.

Potency tests carried out by collaborating laboratories revealed no difference between the potency of the acetone-dried vaccine B used in these studies and the international reference vaccine K used in earlier trials (2, 3, 5). It must therefore be assumed, on the basis of the protection shown in the field and in view of laboratory tests, that the vaccine B used in Tonga was similar in potency and effectiveness to vaccine K.

It must be emphasized that these results were obtained in a highly endemic area where the people already had some immunity against typhoid. A single dose of typhoid vaccine may, therefore, have had the effect of boosting the natural immunity to some extent.

From a public health point of view this study is of particular importance because it emphasizes the need for 2 doses of vaccine for primary immunization in endemic areas, although it does also indicate that 1 dose gives some protection. Furthermore, it indicates that immunity after 2 doses lasts for 5 years and that booster doses need not be given too often.

CONCLUSIONS

This study confirmed that acetone-dried typhoid vaccine protects when given in 2 doses and also demonstrated that the vaccine is effective when only 1 dose is administered, although to a lesser degree. The effectiveness of this type of vaccine was relatively high, particularly in younger age groups in whom 2 doses also gave significantly greater protection than 1 dose. It would seem, therefore, that primary immunization in endemic areas should consist of 2 doses of acetone-dried typhoid vaccine.

ACKNOWLEDGEMENTS

The authors express their appreciation to His Majesty King Taufa'ahau Tupou IV and to His Royal Highness Prince Tu'ipelehake, Prime Minister, for their interest and support in this study, and to the populations of Tongatapu and 'Eua for their cooperation. The acetone-dried typhoid vaccine used in this study was donated by the Walter Reed Army Institute of Research, Washington, DC, USA.

RÉSUMÉ

ÉVALUATION PAR ESSAI CONTRÔLÉ SUR LE TERRAIN DE L'EFFICACITÉ EN DOSE SIMPLE OU DOUBLE D'UN VACCIN ANTITYPHOÏDIQUE DESSÉCHÉ ET INACTIVÉ PAR L'ACÉTONE

Par l'essai pratique contrôlé de divers vaccins antityphoïdiques, on savait que c'est le vaccin desséché et inactivé par l'acétone qui assure la meilleure protection. Il restait à déterminer avec précision s'il fallait administrer deux doses de ce vaccin ou si une dose unique suffirait à conférer une immunité certaine et durable.

Pour cela, les auteurs ont procédé sur le terrain, dans le Royaume des Tonga, à de nouveaux essais contrôlés sur un échantillon de 35 000 habitants des îles de Tongatapu et de 'Eua où la fièvre typhoïde est endémique. Ces volontaires ont été répartis par tirage au sort en trois groupes dont l'un a reçu une dose du vaccin, l'autre deux doses, et le troisième, servant de témoin, une injection d'anatoxine tétanique. L'échantillon de population a été vacciné en 1966 et suivi pendant une période de 7 ans et demi jusqu'à la fin de 1973.

Pour évaluer l'efficacité du vaccin antityphoïdique, les auteurs se sont basés sur le nombre de cas de fièvre typhoïde confirmés par une hémoculture positive. Pendant les deux premières années, ils en ont enregistré 24 dans le groupe témoin, 15 dans le groupe ayant reçu une dose du vaccin et seulement 11 dans le groupe ayant reçu deux doses. Au cours de la période de 5 ans pendant laquelle les effets du vaccin se sont maintenus, le nombre de cas observés dans chaque groupe a été respectivement de 46, 31 et 20, ce qui indique bien que l'administration de deux doses assure une protection de plus de 50%, tandis que la valeur protectrice d'une dose unique est inférieure à ce niveau. Si le vaccin desséché et inactivé par l'acétone a paru relativement moins efficace dans cet essai que dans les études précédentes, c'est probablement à cause de l'importance de la dose infectante dans les cas de typhoïde d'origine alimentaire au Royaume des Tonga.

REFERENCES

1. CVJETANOVIĆ, B. & UEMURA, K. *Bulletin of the World Health Organization*, **32**: 29-36 (1965).
2. TYPHOID PANEL, UK DEPARTMENT OF TECHNICAL CO-OPERATION. *Bulletin of the World Health Organization*, **30**: 631-634 (1964).
3. DIVISION OF IMMUNOLOGY, WALTER REED ARMY INSTITUTE OF RESEARCH. *Bulletin of the World Health Organization*, **30**: 635-646 (1964).
4. WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION. Nineteenth report, Geneva, World Health Organization, 1967, pp. 61-75 (*Technical report series* No. 361).
5. YUGOSLAV TYPHOID COMMISSION. *Bulletin of the World Health Organization*, **30**: 623-630 (1964).
6. HORNICK, R. B. & WOODWARD, T. E. *Transactions of the American Clinical and Climatological Association*, **78**: 70-78 (1966).
7. HEJFEC, L. B. ET AL. *Bulletin of the World Health Organization*, **38**: 907-915 (1968).
8. HEJFEC, L. B. ET AL. *Bulletin of the World Health Organization*, **40**: 903-907 (1969).