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The primary serological response to a single dose of adsorbed tetanus toxoid, high concentration type*

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Single-dose immunization against tetanus was studied in 511 previously non-immunized residents of rural villages in Upper Volta. Males and females were equally represented and a wide age range was covered. A single dose of adsorbed tetanus toxoid containing 17.5 Lf units of toxoid and 3.86 mg of aluminium phosphate per 0.5 ml dose was used. Blood samples were taken 7 days, 2 months, and 12 months after immunization, and serum antitoxin titres were determined by neutralization titrations in mice. Adverse reactions were negligible. Only 2 participants gave evidence of prior immunization by developing detectable antitoxin titres after 7 days; they were eliminated from the study. After 12 months, 59% of the participants had antitoxin titres of ⩾0.01 IU/ml, a titre usually considered protective. The mean titre and the proportion of those protected decreased substantially with increasing age; overall, females gave somewhat greater serological responses than males. Mean titre increased by 25% between 2 months and 1 year after immunization; the increase was greater in females than in males. In children under 6 years of age, 100% of females and 82% of males had protective titres after 1 year.

The preparations of tetanus toxoids at present in use, either alone or in combination with other antigens, are almost universally effective in preventing all forms of tetanus provided multiple doses are given at suitable intervals, followed by a reinforcing dose six months or more after the initial series (*1*). While multiple-dose schedules of immunization can be carried out with little difficulty in areas where health services are adequate, such immunization schedules are difficult to implement in countries in which the population is primarily rural and lacks easy access to health care facilities. Hence, efforts have been made to develop tetanus toxoids that can provide protection after a single dose (*2*, *3*).

One such toxoid, developed at the Biologic Laboratories, Boston, contains 3–5 times the concentration of toxoid and almost twice the amount of aluminium phosphate used in conventional preparations. This preparation has been shown to be well tolerated and more effective than three other formulations containing different concentrations of toxoid and adjuvant (*2*). In one study, 31 susceptible women were given a single injection of the Boston toxoid mentioned above and after 28 days antitoxin titres of at least 0.01 IU/ml were elicited from 24 of them; after 56 days 17 women were bled and only one failed to respond; the geometric mean titre was 0.032 IU/ml (*2*, *4*).

The purpose of the present study was to determine the short- and long-term serological responses in previously non-immunized persons of all ages to a single dose of the high-concentration tetanus toxoid, and to assess the effect of age and sex on the serological response.

MATERIALS AND METHODS

Location

The study was done in the rural villages of Samogohiri and Soukouraba in western Upper Volta. There was a small dispensary in Samogohiri, which is 7 kilometres from Soukouraba. Tetanus toxoid had never been administered routinely to the inhabitants. Tetanus antitoxin (equine) has been available occasionally in local health units.

Participants

Four age groups were studied: 1–5 years; 6–11 years; 12–19 years; and over 19 years. About one hundred persons were included in each group, except

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for the 12–19 year age group in which over 200 persons were registered in order to assess more precisely the response among adolescents; both sexes were equally represented in all age groups. Age was determined from birth certificates and identity cards. All subjects denied having received tetanus toxoid or antitoxin in the previous 6 months. Acutely ill individuals and those with chronic debilitating illnesses, allergies, or febrile convulsions were excluded.

Vaccine

All subjects were given a 0.5-ml dose containing 17.5 Lf units of purified adsorbed tetanus toxoid and 3.86 mg of aluminium phosphate (lot Al 39) prepared by the Biologic Laboratories, Boston, as previously described (*2*, *4*). It should be pointed out here that the apparent difference between the amount of toxoid in this vaccine and previous preparations of the same vaccine (17.5 Lf units as against 25 Lf units) is due to the use of the United States reference antitoxin in determining the amount of toxoid in this preparation, instead of the historical local reference previously used; the actual amount of tetanus toxoid is unchanged. The preparation was assayed by immunization and challenge of mice, using as reference the international standard for tetanus toxoid, adsorbed (*5*). Values of 329, 280, and 356 IU/ml were obtained, the former two in the State Laboratory Institute, Boston, the third by Dr F. W. Sheffield, National Institute for Biological Standards and Control, London. The toxoid was bottled in 10-ml (20-dose) vials and was kept under refrigeration at 4–8 °C. This product was registered as an investigational, new drug with the Food and Drug Administration, Public Health Service and the protocol for the trial was approved by the Ministry of Health of Upper Volta and the Protocol Review Committee of the Center for Disease Control, Public Health Service.

Consent

The purpose of the study was explained in detail in the local language to the subjects or their parents, and to the local administrative authorities. This procedure is customary in seeking approval for activities in villages where the procurement of individual written permission is impracticable owing to low literacy rates in the population. The participants were told in advance that the vaccine could cause fever, swelling and pain in the arm, and possibly other severe effects and that they could withdraw from the study at any time.

Immunization

On day 0 all subjects were registered and screened for contraindications. The vaccine was injected in the upper arm with the Ped-O-Jet (foot actuated, hydraulic, needleless) injector with a nozzle for subcutaneous injection. Special care was taken to suspend the adsorbed toxoid in each vial before use. Identification tickets were issued to all participants and a Polaroid photograph was taken of each of them on day 7 in order to facilitate identification; these were given to the participants after the one-year bleeding.

Reactions

One of the investigators (JGB) remained in the villages for two days after the administration of the vaccine to examine any person who became sick after the injection. A nurse assigned to the local dispensary by the Ministry of Health of Upper Volta monitored reactions when the investigators were absent. On day 7 following vaccination, the subjects were asked if they had fever, local reactions, or other health problems, and their arms were examined; at 2 months their arms were palpated.

Bleeding

Seven ml of blood were taken by aseptic venepuncture on day 7 (to detect any anamnestic response to prior vaccination), on day 56 (± 4 days), and on day 365 (± 7 days). The blood samples were allowed to clot under refrigeration for 12–36 h before the serum was removed, frozen, and forwarded to the Biologic Laboratories, Boston, for titration.

Follow-up

Visits to the villages were made at 4, 5, 9, 10, and 17 months after vaccination. Reactions to the injection were sought by discussions with participants, village elders, family heads, and the local nurse.

Serum titrations

Antitoxin titrations were performed using the toxin neutralization method at the L+/100 or L+/1000 levels (*6*). Mice were purchased from Charles River Farms, Inc.[a] The USA standard tetanus antitoxin was obtained from the Bureau of Biologics, Food and Drug Administration, Washington, DC. The sera collected on day 7 were screened for detectable antitoxin at the 0.005 IU/ml level. The serum samples taken 2 months and 12 months after immunization were screened at the 0.01 IU/ml level (a titre considered by most investigators as the protective threshold) and titrated to definite values based on 2-fold dilutions; those below 0.01 IU/ml were reported as <0.01 IU/ml. Subjects whose serum titres equalled or exceeded 0.01 IU/ml were regarded as having achieved "seroconversion" to a protected status.

[a] Mention of any commercial company or product does not constitute endorsement by the Public Health Service.

Statistical analysis of the serological data

In order to facilitate computation, the titres were logarithmically coded such that successive integers, 3, 4, 5, etc., represented titres of 0.01, 0.02, 0.04 IU/ml, etc. Frequency distributions of coded titres were plotted for each age group according to sex and month of bleeding and the resultant 16 distributions were analysed statistically as described below.

RESULTS

Participation

A total of 515 persons was registered and immunized. At day 7, all the participants were present, and on days 56 and 365, 99% and 92%, respectively, of the participants were available. All those who were absent at one year were travelling, except for an adolescent girl who died during the study from an unrelated cause. Three males less than one year of age were included in the 1–5 year group; two were aged 10 months and one 11 months.

Reactions

Six persons reported fever within the week following immunization. None reported having a painful or swollen arm, except a 16-year-old girl whose pain was not associated with swelling, warmth, or lymphadenopathy. This pain persisted intermittently for 2 months but did not limit her daily activities or require analgesics. Small non-tender nodules, estimated to measure 1, 2, 3, and 4 mm in diameter, were found in 10, 20, 8, and 1 persons, respectively. Nodules were noted on the arms of 3 male children under 5 years of age (2 aged 1 year and 1 aged 2 years), and on one boy aged 8 and one aged 17 years. Twelve adolescent females and 22 adolescent males had nodules that were not associated with local inflammation or lymphadenopathy and were thought to have been due to a small amount of vaccine that remained intradermal.

Serology

A total of 515 sera collected on day 7 were screened and all but two had antitoxin concentrations of <0.005 IU/ml. The two sera containing higher concentrations of antitoxin were titrated and were found to contain 0.01 IU/ml (male aged 15 years) and 5 IU/ml (male aged 41 years). Both subjects denied previous immunization with tetanus toxoid, but they were eliminated from the study.

A total of 511 and 473 sera were collected and titrated 2 months and 1 year, respectively, after immunization. The number and percentage of subjects developing antitoxin concentrations of $\geqslant 0.01$ IU/ml of serum by age, sex, and time after injection are given in Table 1. The overall conversion rate was 51% at 2 months and 59% at 1 year. The best result at 1 year was 100% for 43 females aged 5 years or younger, and

Table 1. Seroconversion 2 and 12 months following one dose of high concentration tetanus toxoid, by age and sex

Age (years)	Males			Females			Total		
	Number	⩾0.01 IU/ml	%	Number	⩾0.01 IU/ml	%	Number	⩾0.01 IU/ml	%
A. At 2 months									
1–5	51	46	90	45	37	82	96	83	86
6–11	49	35	71	51	37	72	100	72	72
12–19	105	40	38	110	46	42	215	86	40
>19	49	9	18	51	12	24	100	21	21
Total	254	130	51	257	132	51	511	262	51
B. At 12 months									
1–5	49	40	82	43	43	100	92	83	90
6–11	46	37	80	49	32	65	95	69	72
12–19	95	41	43	96	64	67	191	105	55
>19	47	8	17	48	16	33	95	24	25
Total	237	126	53	236	155	66	473	281	59

the poorest was 17% for 47 males aged 19 years or more. The trend indicating a decrease in seroconversion rates with age was significant at the <0.01 probability level.

When frequency distributions of the coded titres were plotted for each group according to sex and month of bleeding, the resultant curves tended to be sigmoid in shape, suggesting that they represented the right-hand segments of normal distribution curves. Thus, the missing left-hand segments of these curves would represent the distributions of the unknown titres recorded as <0.01 IU/ml. Since the number of subjects in this category was already known, it was possible to calculate the distribution of the unknown titres for each curve. This was done by plotting cumulative percentages of subjects against observed coded titres on arithmetic probability paper (7). This gave a straight line which was extended backwards so that the cumulative percentages of subjects corresponding to the lower titre intervals could be read off until all subjects with titres <0.01 IU/ml were accounted for. These calculated frequencies, along with the observed frequencies, are plotted in Fig. 1 as frequency distributions by age, sex, and month of bleeding. Since the change in immune status between the 2- and 12-month bleeding was of major interest, only the 472 subjects for whom both titres were available were considered in this part of the analysis. The 16 distributions were subjected to the standard statistical test for goodness of fit to the normal distribution curve (7). The departure from a normal distribution was statistically significant ($P<0.05$) in only 4 of the 16 curves (see P values in Fig. 1). In some curves the calculated points did not fall on the normal curves. This was due to the fact that in those cases the normal curves required a different number of subjects than were actually observed. This difference in the number observed and that required for a normal distribution was largest in the four curves having statistically significant deviations from normality.

The combined calculated and observed data were subjected to an analysis of variance in order to assess the influence of age, sex, and time after bleeding on serological response. The degrees of freedom for the analysis were based only on the 526 observed titres, rather than on the 944 combined titres (observed plus calculated). The variance between the four age groups exceeded the error variance (F test) by 70-fold. The corresponding values for sex and time-of-bleeding variance were 11.6 and 7.4, respectively. The probability that the inverse correlation between age and responsiveness was due to chance is less than 1 : 1000.

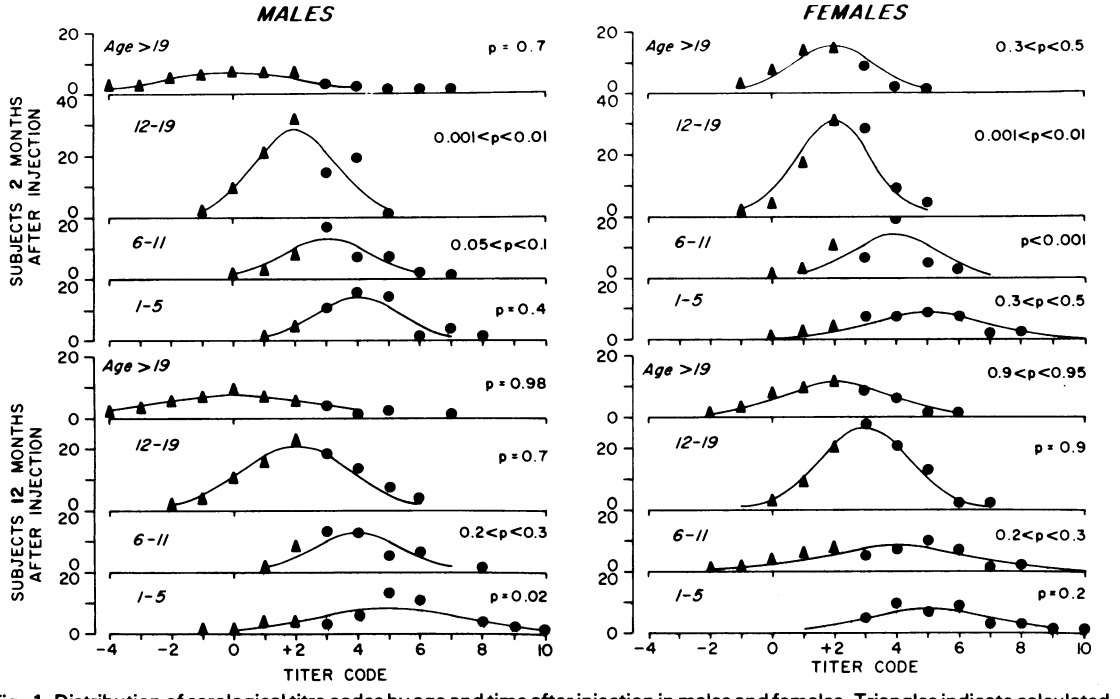

Fig. 1. Distribution of serological titre codes by age and time after injection in males and females. Triangles indicate calculated numbers of subjects and circles indicate observed numbers. (Codes 0, 1, 2, 3, 4, 5, etc., represent 0.00125, 0.0025, 0.005, 0.01, 0.02, and 0.04 IU/ml, respectively.)

Table 2. Geometric mean titres in relation to age group, sex, and time after injection[a]

Age (years)	Males			Females			Total		
	No.	2 months	12 months	No.	2 months	12 months	No.	2 months	12 months
1–5	49	0.022 (0.76)	0.034 (0.63)	42	0.025 (0.67)	0.071 (0.62)	91	0.024 (0.79)	0.048 (0.71)
6–11	46	0.012 (0.74)	0.017 (0.73)	49	0.013 (0.76)	0.015 (0.63)	95	0.013 (0.82)	0.016 (0.76)
12–19	95	0.006 (0.83)	0.006 (0.78)	96	0.006 (0.84)	0.011 (0.81)	191	0.006 (0.88)	0.008 (0.85)
>19	47	0.002 (0.57)	0.002 (0.60)	48	0.004 (0.77)	0.004 (0.71)	95	0.002 (0.73)	0.003 (0.72)
Total	237	0.007 (0.82)	0.008 (0.80)	235	0.009 (0.86)	0.013 (0.82)	472	0.008 (0.89)	0.010 (0.86)

[a] The upper and lower 95% confidence limits of the mean may be found by dividing and multiplying each mean by the adjacent figure in parenthesis.

A similar P-value was observed with regard to the greater responsiveness of females compared with males; the 12-month titres were higher than the 2-month titres ($P = 0.008$).

The above estimates of distributions of titres <0.01 IU/ml permitted the calculation of coded mean titres by age, sex, and interval after injection. These coded means were decoded to geometric mean titres (GMTs) by using the equation: log IU/ml = $0.30y - 2.903$, where y is the coded mean. These GMTs and their confidence limits are given in Table 2. The GMTs of the 472 subjects increased between 2 and 12 months from 0.008 to 0.010 IU/ml, i.e., by 25% ($0.001 < P < 0.01$). The contribution of males to this increase was only 14.3% and this was not statistically significant ($0.2 < P < 0.3$). Among females the increase was much greater, 44.4%, and this was statistically highly significant ($P < 0.001$).

Changes in the proportion of subjects whose serum contained ⩾0.01 IU of antitoxin per ml of serum between 2 and 12 months after immunization are shown in Table 3. Whereas the proportion of the younger subjects with protective titres tended to increase during the 10-month interval, that of the >19 years age group decreased. The proportion of all subjects with titres ⩾0.01 IU/ml increased, since the number of subjects in whom the titre became protective was twice the number in whom the reverse was the

Table 3. Changes in serum antitoxin titres of subjects during the interval between 2- and 12-month bleedings after immunization, by age and sex

Age (years)	Sex	Titre <0.01 at 2 months			Titre ⩾0.01 at 2 months		
		Total	No. with ⩾0.01 IU/ml at 12 months	% increase	Total	No. with <0.01 IU/ml at 12 months	% decrease
1–5	M	5	2	40	44	6	14
	F	7	7	100	35	0	0
6–11	M	12	6	50	34	3	9
	F	14	2	14	35	5	14
12–19	M	61	13	21	34	6	18
	F	53	24	45	43	3	7
>19	M	38	3	8	9	4	44
	F	36	10	28	12	6	50
Total	M	116	24	21	121	19	16
	F	110	43	39	125	14	11
All ages and sexes		226	67	30	246	33	13

case. Clearly the magnitude of this effect is influenced by the distribution of ages among the subjects.

The consistent influence of age on immune responsiveness is shown in Fig. 2, in which GMT is plotted as a function of group mean age. For both sexes and times of bleeding the mean titre decreased steadily with increasing age.

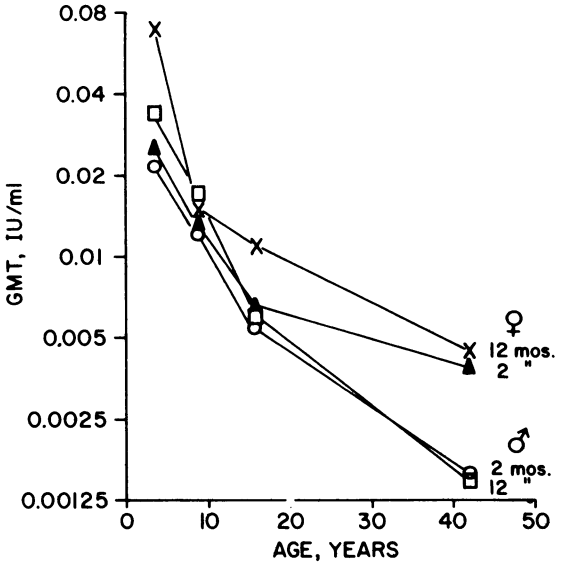

Fig. 2. Effect of mean age on geometric mean titre (GMT), by sex and time after injection.

DISCUSSION

This study has demonstrated that a single dose of an adsorbed tetanus toxoid, containing five times the concentration of the toxoid and almost twice the amount of the aluminium adjuvant present in conventional preparations, protected 59% of 473 persons in Upper Volta from tetanus for at least one year. Young children showed much higher rates of protection ranging up to 100% among females between 1 and 5 years of age. There were no significant adverse reactions to the immunization. The low incidence of even minor local reactions among all age groups was consistent with their lack of prior exposure to the antigen. Only 2 of 515 persons had to be excluded from the study because of recall responses, indicating that the "natural immunity" claimed in other regions (*8*) probably does not exist in the populations of West Africa.

Of the 3 independent variables studied, i.e., age, sex, and time after the single injection, age showed by far the greatest influence on the immune response. Perhaps this was due to a dose–response effect, with younger children receiving more toxoid per unit weight. Other explanations, such as enhanced immunological responsiveness in children or relative immunological dysfunction in older persons, are possible but have not been proved with regard to tetanus toxoid.

The distribution of antitoxin titres in each age group was found to be normal for both sexes and months of bleeding. Of the 16 distributions obtained, only four had statistically significant departures from a normal distribution ($P<0.05$), these deviations being due to one or two aberrant points on each curve. Such normal distributions were not found in another study (*2*) in Cali, Colombia, where bimodal distributions were observed 28 days after one injection of a toxoid with identical specifications. In the Cali study there were only 3 distributions with 30, 31, and 32 observations, respectively, compared with the 16 distributions in the present study, which included 42–96 observations for each. The rates of seroconversion in the two studies also appear significantly different where conditions allow a comparison. While in our study 12 out of 51 adult females showed seroconversion (24%) 2 months after immunization, the comparable Colombian group of adult females after 28 days showed seroconversion in 16 out of 17 (94%). This finding is unexplained; however, the nurses and nurse aids in Colombia may have had a younger mean age than the adult females studied in Upper Volta (mean age, 42 years) (Fig. 2). There is no basis at present for determining the relative contributions of genetic factors and various environmental influences to these differences in response.

Greater immunological responsiveness in females, as observed in this study, has also been noted with other antigens (*9*). This effect has been ascribed to hormonal differences but the mechanism remains poorly understood. The mean titre did not increase in the 49 girls in the age group 6–11 years during the 10 months between the two bleedings as was observed in two adjacent age groups; their response resembled that of their male counterparts. It seems unlikely that this is an experimental artefact, but there is no explanation for this anomaly.

The rise in mean titre during the 10 months between the two bleedings was probably due to slow release of antigen following injection, known as "depot effect", this explanation having been used to explain the superiority of adjuvant over fluid toxoids (*10*). Human and animal studies involving equally prolonged observations have generally used at least 2 doses of adsorbed antigen and these invariably showed a decline in antitoxin concentration from an early maximum. The few studies with single-dose tetanus toxoid have either included too few subjects to reveal the phenomenon (*4*), or have used the less

reliable haemagglutination titration methods (*11*).

Although the neutralization test in mice is sensitive down to 0.0025 IU/ml, for economy we terminated our titrations at the protective threshold, 0.01 IU/ml (except that day 7 sera were screened at the 0.005 IU/ml level to detect any recall response). The use of the indirect haemagglutination method for titration of the 1500 sera would have been more economical, but experience has shown that the method is not sufficiently reliable (*12*, *13*).

Although this study indicates that single-dose immunization can induce a significant level of protection, especially in the young, a second dose and periodic boosters would, however, be desirable to ensure durable immunity in essentially all recipients. For children less than 6 years of age, the second dose could be given at 1 year. For others, the recall dose should be given earlier, at 2 months if possible. This would be of special importance for pregnant women, particularly in preventing neonatal tetanus. In all cases, the effectiveness of these altered schedules must be confirmed. In those instances in which optimum schedules of immunization cannot be maintained, it seems probable that serum antitoxin titres below 0.01 IU/ml, if associated with the potential to mount a vigorous secondary response, would be associated with an increased resistance to tetanus.

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RÉSUMÉ

RÉPONSE SÉROLOGIQUE PRIMAIRE À UNE DOSE UNIQUE D'ANATOXINE TÉTANIQUE ADSORBÉE, DE FORTE CONCENTRATION

La réponse sérologique à une dose unique d'anatoxine tétanique adsorbée, de forte concentration, a été étudiée chez 511 personnes non encore immunisées, habitant dans des villages de Haute-Volta, en Afrique occidentale. Ces 511 sujets comprenaient des personnes de tous âges, depuis des petits enfants jusqu'à des personnes âgées. Ils ont été divisées en quatre groupes d'âge: 1 à 5 ans, 6 à 11 ans, 12 à 19 ans et plus de 19 ans; les sexes étaient également représentés. Une dose unique d'anatoxine tétanique adsorbée leur a été injectée à l'aide d'un injecteur sans aiguille, le Ped-O-Jet. Chaque dose de 0,5 ml contenait 17,5 Lf d'anatoxine et 3,86 mg de phosphate d'aluminium. Des échantillons de sang ont été prélevés au bout de sept jours, de deux mois et de 12 mois; les titres d'antitoxine sérique ont été déterminés par l'épreuve de neutralisation chez la souris.

Les proportions des participants qui se sont présentés à l'examen médical pour le prélèvement de sang ont été de 100% au bout de sept jours, 99% à deux mois et 92% à un an. Les réactions adverses ont été négligeables. Chez deux participants seulement, on a eu la preuve d'une immunisation antérieure parce qu'ils présentaient des titres d'antitoxine décelables au bout de sept jours; ces deux personnes ont été éliminées de l'étude. Au bout de 12 mois, 59% de tous les participants ont présenté des titres d'antitoxine $\geqslant$ 0,01 UI/ml, titre généralement considéré comme conférant une protection. La moyenne géométrique des titres et la proportion de personnes protégées diminuaient notablement avec l'âge; les femmes présentaient des réponses quelque peu supérieures à celles des hommes dans l'ensemble. Le titre moyen a augmenté de 25% entre deux mois et un an; l'augmentation était plus grande chez les femmes que chez les hommes. Parmi les enfants de moins de six ans, 100% des filles et 82% des garçons ont présenté des titres protecteurs au bout d'un an.

Bien qu'une dose unique de cette anatoxine tétanique puisse conférer un haut degré de protection, en particulier dans les groupes d'âge les plus jeunes, une deuxième dose et des rappels périodiques seraient probablement nécessaires pour assurer une immunité complète et durable chez tous les vaccinés. Cependant, l'utilisation d'anatoxines tétaniques (et d'autres antigènes) de forte concentration présente de nombreux avantages. Il semble qu'elle offre une solution pour les zones où les ressources ne permettent pas d'offrir à la population cible la série complète de trois injections ou davantage nécessaire lorsqu'on utilise des produits moins actifs. Comme la majorité de la population des pays en développement vit dans ces zones sous-desservies, cela justifie que l'on continue à chercher à mettre au point des agents immunisants plus actifs.

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