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Studies on Vaccination against Bacillary Dysentery

6. Protection of Children by Oral Immunization with Streptomycin-Dependent *Shigella* Strains *

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A field trial of oral streptomycin-dependent mutant Shigella *vaccines in five hyperendemic areas of Yugoslavia in 1969 confirmed the findings of earlier studies by demonstrating the effectiveness of these vaccines against dysentery. For the first time, a high degree of protection was demonstrated in children. The vaccines induced serotype-specific immunity against* Shigella flexneri *1 and 2a and* S. sonnei. *Postvaccinal reactions were minor and consisted of vomiting or diarrhoea, or both, in a small proportion of children within several hours of the administration of the vaccine. These reactions, seen mainly after the first dose, were dose-dependent and could be decreased by reducing the number of live organisms. Reactions to subsequent doses were much fewer. Pretreatment with sodium bicarbonate was necessary. Under the conditions of this study, the vaccines proved to be stable with no evidence of reversion of the mutant strains to the virulent parent.*

An oral attenuated dysentery vaccine prepared from streptomycin-dependent (S^d) strains of *S. flexneri* 2a administered in 5 doses to military recruits conferred a high degree of protection against dysentery in a controlled field trial conducted in Yugoslavia in 1963 (Mel et al., 1965). In a field trial in 1964, the same high degree of protection was demonstrated with similar vaccines freshly prepared from other *Shigella* serotypes (Mel et al., 1968). In 1965 and 1966 lyophilized vaccines were used for the first time, with equal effectiveness, in three different garrisons comprising 8 500 men. A high degree of serotype-specific immunity was demonstrated in all these trials.

The field trials evaluated the effectiveness of the vaccine in soldiers, but the highest morbidity from enterocolitis-dysentery occurs in the 2-7-year age group. The problem of dysentery in children is particularly acute in many developing countries and in certain high-risk groups in the more advanced countries. For this reason, a field trial was conducted in 1969 in five hyperendemic areas of Yugoslavia to determine whether similar specific protection could be achieved in children by administering live oral S^d lyophilized vaccines; the subjects were 7 281 children aged 2–8 years.

MATERIALS AND METHODS

Five known hyperendemic areas in Yugoslavia were selected for the study; two were in the town of Skoplje and the other three were in Prizren, Bitolj, and Niš. The parts of these towns included in the study were divided into units, each containing about 80 children. Responsibility for dysentery surveillance of each unit was assigned to a field nurse. A senior, more experienced, nurse supervised the work of 5 or 6 field nurses. The senior nurses were supervised by a nursing officer reporting to an epidemiologist who had overall responsibility for the local team. In addition to this surveillance team, a paediatrician and a specialist in infectious diseases were assigned to each area to provide clinical services

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for the children included in the study. A senior epidemiologist supervised the work of the local team in each area, while overall co-ordination of the five areas was provided by the senior investigator.

Mothers in each unit were informed in writing by nurses exactly when and where the 4-dose vaccination regimen would be given. On the eve of vaccination, and during the hours of vaccination, mothers were again visited and urged to have their children vaccinated. Children were randomly assigned to one of two vaccine groups. Stratification by age was used in the random system. Participation was strictly voluntary.

In 4 of the 5 areas, each child in the care of a parent reporting to the vaccination centre was given a prevaccination dose of 1.5 g of sodium bicarbonate in 20 ml of water 5 minutes before being given each dose of vaccine. At the same time, the nurse located the child's record card and an epidemiologist assigned the child to the A or B vaccine group. The child remained in his designated group throughout the study. An average of 500 children were vaccinated per day in each area. Since there were about 1 500 children in each area, it took 3 days to administer each dose of the vaccine. On the fourth day, children were called who had failed to report during the three vaccination days.

A physician was present to document possible vaccine reactions, to eliminate from the study those children with underlying diseases, and to answer questions. Nurses visited each child after every dose of vaccine to record any postvaccinal reactions.

The vaccines used in this study consisted of streptomycin-dependent *Shigella* strains lyophilized at the Torlak Institute of Immunology and Virology, Belgrade, Yugoslavia, in vials containing 5 ml of *Shigella* suspension. There were $90–203 \times 10^9$ live organisms per ml of suspension before lyophilization, 45–85% of which survived after rehydration. There was an additional loss of 10–15% immediately before vaccination and 35–80 days after lyophilization. Four serotypes were included in this study—namely, *S. flexneri* 1, 2a, and 3, and *S. sonnei*.

Vaccines had been lyophilized in different batches at different times. Vaccines rehydrated to their original volumes varied in the final concentration of organisms present. For example, the *S. flexneri* 1 vaccine was prepared in three different batches containing, respectively, 64, 70, and 75×10^9 live organisms per ml of rehydrated vaccine; the *S. flexneri* 2a vaccine was prepared in four different batches containing 24, 36, 40, and 54×10^9 live organisms per ml of rehydrated vaccine; the *S. flexneri* 3 vaccine was prepared in two batches with 64 and 79×10^9 live shigellae per ml of rehydrated vaccine; and one batch of *S. sonnei* had 147×10^9 live organisms in 1 ml of rehydrated vaccine. The vaccine was distributed in the field trial in such a way that the number of organisms in each dose and identical batches were equally represented in each of the five areas.

These strains were used as bivalent vaccines by mixing, at the time of rehydration, two combinations: *S. flexneri* 1 and 2a were designated " vaccine A ", and *S. flexneri* 3 and *S. sonnei* were coded " vaccine B ". Each bivaccine contained the same number of live organisms. Because of previously demonstrated serotype-specific immunity (Mel et al., 1965), a placebo control was not employed in this study.

The vaccine was rehydrated with 7% sucrose. The quantity specified for one dose (20 ml) was measured by an automatic pipette dispenser in small glasses from which the children drank the vaccine. The vaccine was kept on ice after rehydration. Only that quantity necessary for daily use was rehydrated; unused portions were discarded.

The vaccination programme was started in the town of Prizren; the vaccine was administered in 4 doses at 3-day intervals, as follows: 5–8 April, first dose with 28×10^9; 9–12 April, second dose with 34×10^9; 13–16 April, third dose with 36×10^9; 17–20 April, fourth dose with 42×10^9. These doses refer to the total number of live organisms in the bivaccine, which means that each serotype was represented by only half or 14, 17, 18, and 21×10^9 live organisms per dose, respectively.

There were two study areas in the town of Skoplje, referred to as Skoplje I and II. The same procedure and doses were used as in Prizren except that pretreatment with sodium bicarbonate was not given in Skoplje II, to determine whether protection could be achieved without sodium bicarbonate. Vaccination in both study areas I and II began on 15 April and lasted to 29 April 1969.

Because the postvaccinal reaction rate in Prizren and Skoplje I and II was quite high, the number of live shigellae in initial doses of the vaccine given in Bitolj and Niš was diminished. Vaccination in those towns was carried out concurrently from 9 May to 24 May 1969. The schedule and doses administered were as follows: 9–12 May, first dose with 20×10^9; 13–16 May, second dose with 28×10^9; 17–20 May, third dose with 34×10^9; and 21–24 May, fourth dose with 40×10^9.

Stool cultures were obtained from all children who developed diarrhoea after vaccination to search for reversions of the vaccine strains. In addition, stool samples from 22 children who received vaccine A and 22 who received vaccine B were cultured daily for 4 days after the second and subsequent doses to determine the duration of excretion of the vaccine strains. During the 3½ months of surveillance, from 1 June to 15 September, each child was visited in his home at least three times each week by a nurse. Stool specimens were obtained from all children with diarrhoea and were cultured for *Shigella*.

Cultures were seeded in agar containing streptomycin and in SS medium and were incubated overnight. Colonies on plates containing streptomycin were examined under a stereoscopic microscope; those with typical morphology were confirmed by slide agglutination. Suspicious colonies from SS medium were inoculated on Russell's double sugar slants and reincubated overnight for further study.

RESULTS

The numbers of children who received 3 or 4 doses of vaccine are shown in Table 1 by age, sex, and type of vaccine.

Postvaccinal reactions that occurred in the form of vomiting and diarrhoea are listed in Table 2. There were almost no elevations of temperature. The exceptions were noted in children with unrelated infections.

Altogether, 459 children (6.3%) vomited once or twice on the day the vaccine was given; none required treatment, and all except two were cheerful and played normally. Two children who vomited became drowsy and pale, and were covered with cold sweat, but they recovered after bedrest the same day without treatment. Eight children vomited immediately after the vaccine was administered.

A total of 248 children (3.4%) developed diarrhoea within 6 hours after the administration of vaccine. Most had only 1 or 2 liquid stools, a few had a maximum of 5–6. In all but a few cases, diarrhoea had ceased by the following morning, and in all it ceased spontaneously and without therapy. Mucus or blood was not detected. Cultures from children with diarrhoea failed to show reversions of the vaccine strains.

In Table 2 it can be seen that reactions decrease with age. This was noted for both vomiting and diarrhoea in each of the five study areas in groups treated with both vaccine A and vaccine B. The incidence of vomiting decreased from 13.8% in 2-year-old children to 1.6% in 8-year-old children. The incidence of diarrhoea dropped from 7.1% in 2-year-old children to 1.1% in the 8-year-old group. The highest incidence of reactions occurred after the first dose, and it decreased after each subsequent dose.

Each of the vaccine serotypes except *S. flexneri* 3 was easily recovered on media containing streptomycin from stool samples obtained from vaccinated children. *S. flexneri* 3 could not be isolated from 352 stool specimens obtained from 22 vaccinated

Table 1. Number of vaccinated children by age, sex, and type of vaccine administered in 3 or 4 doses in five study areas of Yugoslavia in 1969

Age (years)	Type A			Type B		
	Male	Female	Total	Male	Female	Total
2	283	254	537	277	276	553
3	351	296	647	340	337	677
4	338	343	681	349	344	693
5	348	334	682	362	324	686
6	301	311	612	332	259	591
7	158	176	334	189	141	330
8	65	66	131	67	66	133
Total	1 844	1 780	3 624	1 916	1 747	3 663

Table 2. Postvaccinal reactions in children in five study areas of Yugoslavia

Age (years)	Vaccine type A								Total		Vaccine type B								Total	
	No. of subjects vomiting after following no. of doses:				No. of subjects with diarrhoea after following no. of doses:				Vomiting	Diarrhoea	No. of subjects vomiting after following no. of doses:				No. of subjects with diarrhoea after following no. of doses:				Vomiting	Diarrhoea
	1	2	3	4	1	2	3	4			1	2	3	4	1	2	3	4		
2	57	22	5		18	8	9	4	84	39	47	10	8	1	23	3	7	5	66	38
3	51	8	11	1	10	10	2	3	71	25	28	7	9	4	7	7	7	3	48	24
4	31	7	5	2	11	10	5	7	45	33	24	8	3	1	14	6	5	4	36	29
5	17	2	3		11	3		1	22	15	11	6	1		4	7	2	2	18	15
6	16	6	2		7	2	1		24	10	13	1	3	1	2	3	4	2	18	11
7	14	2	2	2	2				20	2			2	1		1	2	1	3	4
8	2		1				1		3	1	1				1		1		1	2
Total	188	47	29	5	59	33	18	15	269	125	124	32	26	8	51	27	28	17	190	123

children. The average duration of excretion of the other 3 serotypes was 1.2–1.9 days. There was no evidence of reversion in the course of culturing 264 stools in which this was specifically looked for.

Postvaccinal reactions decreased appreciably when the numbers of organisms were reduced in the last two study areas, Niš and Bitolj (Table 3). After the vaccination experience in Prizren and Skoplje I and II, it was clear that the doses given included a superabundance of organisms. For that reason, the number of organisms in the initial dose, i.e., 28×10^9, was reduced to 20×10^9 and the numbers of organisms in doses 2, 3, and 4 were also reduced in the vaccination of children in Niš and Bitolj. This reduction resulted in an appreciable decrease in reaction rates, as seen in the table. The higher doses given in the first three study areas resulted in vomiting reaction rates of 5.6, 1.5, 1.1, and 0.2 for doses 1–4, respectively, compared with 2.2, 0.4, 0.3, and 0.2 after reduced doses in the last two study areas. There were also decreases in the diarrhoea rates (1.6, 1.0, 0.9, and 0.5 against 1.3, 0.5, 0.4, and 0.2) and in the rates of both diarrhoea and vomiting occurring together (not shown). Clearly, postvaccinal reactions were dose-dependent. They were directly proportional to the number of live organisms in the initial dose, and they diminished from dose to dose in spite of the increase in the number of organisms in subsequent doses.

During the period of surveillance, 1 June to 15 September 1969, there were 1 432 (19.7%) cases of diarrhoea, defined as one or more bowel movements with liquid stool, which were cultured for enteric pathogens. In Prizren there were 337 cases (20.5%); in Skoplje I, 271 (24.4%); in Skoplje II, 412 (24.8%); in Bitolj, 240 (16.8%); and in Niš, 172 (11.2%). The distribution of *Shigella*-negative diarrhoeas by age, sex, and type of vaccine given is shown in Table 4. The comparability of the two groups is evident.

Shigellae were cultured in 291 of the 1 432 cases of diarrhoea. These were distributed as follows: Prizren 118 (7.1%), Skoplje I 46 (4.1%), Skoplje II 71 (4.3%), Bitolj 42 (2.9%), Niš 14 (0.09%). The most commonly isolated serotypes were *S. sonnei* and *S. flexneri* 6 (each accounting for 77 cases) followed by *S. flexneri* 2a (67 cases), *S. flexneri* 1 (12 cases), *S. flexneri* 4 and *S. schmitzi* (each with 19 cases), *S. boydii* (10 cases), and *S. flexneri* 3 and *S. dysenteriae* group A strains (5 cases each).

Tables 5–8 compare the morbidity observed in the two vaccine groups in Prizren, Skoplje I, Bitolj, and

Table 3. Incidence of reactions following the administration of vaccine according to vaccination schedule

Doses	No. of organisms ($\times 10^9$)	Skoplje I, II and Prizren				No. of organisms ($\times 10^9$)	Niš and Bitolj			
		Vomiting		Diarrhoea			Vomiting		Diarrhoea	
		Cases	Percentage	Cases	Percentage		Cases	Percentage	Cases	Percentage
1	28	248	5.6	72	1.6	20	64	2.2	38	1.3
2	34	66	1.5	46	1.0	28	13	0.4	14	0.5
3	36	47	1.1	37	0.9	34	8	0.3	12	0.4
4	42	7	0.2	22	0.5	40	6	0.2	7	0.2
Total	140	368	8.4	177	4.0	122	91	3.1	71	2.5

Table 4. Distribution of shigellae-negative diarrhoeas in the five study areas according to type of vaccine administered, age, and sex

Age (years)	No. of diarrhoeal episodes in children vaccinated						Total (A + B)
	Vaccine A			Vaccine B			
	Male	Female	All	Male	Female	All	
2	95	75	170	88	84	172	342
3	99	59	158	79	71	150	308
4	53	49	102	47	53	100	202
5	32	39	71	28	38	66	137
6	17	19	36	30	29	59	95
7	16	10	26	15	7	22	48
8	3	2	5	3	1	4	9
Total	315	253	568	290	283	573	1 141

Niš, and Table 9 summarizes the findings in these four study areas. Findings were qualitatively consistent in each of the areas, although there were quantitative differences from place to place. Table 9 shows that there were 46 cases, or an incidence rate of 1.63%, of *S. flexneri* 1 and 2a in the group vaccinated against *S. flexneri* 3 and *S. sonnei* compared with only 4 cases, or an incidence rate of 0.13%, in children vaccinated with the bivaccine containing *S. flexneri* 1 and 2a. This difference is highly significant ($\chi^2=35$; $P<0.0001$). The total morbidity due to *S. flexneri* 3 and *S. sonnei* observed in children vaccinated against *S. flexneri* 1 and 2a, i.e., 55 cases, or an incidence rate of 1.96%, was significantly higher ($\chi^2=27.5$; $P<0.0001$) than that observed in the group vaccinated with bivaccine type *S. flexneri* 3 and *S. sonnei*, which had 10 cases, or an incidence of 0.35%.

The findings are in contrast to those observed in Skoplje II, where no significant protection was observed in children who received vaccine without the routine pretreatment of sodium bicarbonate used in the four other study areas (Table 10). There were too few cases of confirmed shigellosis due to *S. flexneri* 1 and 3 to draw any conclusions, but there was minimal, if any, protection among children who received the *S. flexneri* 2a and *S. sonnei* types of vaccine without sodium bicarbonate.

Table 5. Morbidity observed in Prizren according to serotype (1 June–15 September 1969)

Diagnosis	No. of children vaccinated against:	
	S. flexneri 1 and 2a	*S. flexneri* 3 and *S. sonnei*
S. flexneri 1	0	7
S. flexneri 2a	1 (0.1 %)	8 (1.0 %)
S. flexneri 3	1 (0.1 %)	2 (0.2 %)
S. flexneri 4	8	6
S. flexneri 6	12	18
S. sonnei	32 (3.9 %)	5 (0.6 %)
S. schmitzi	5	4
S. boydii 1–15	2	2
S. dysenteriae A	3	2
diarrhoea only	107	112
all cases	171 (20.7 %)	166 (20.3 %)
without diarrhoeal symptoms	656 (79.3 %)	650 (79.7 %)
Total	827 (100.0 %)	816 (100.0 %)

Table 6. Morbidity observed in Skoplje I according to serotype (1 June–15 September 1969)

Diagnosis	No. of children vaccinated against:	
	S. flexneri 1 and 2a	*S. flexneri* 3 and *S. sonnei*
S. flexneri 1	0	1 (0.2 %)
S. flexneri 2a	1 (0.2 %)	12 (2.1 %)
S. flexneri 3	0	1 (0.2 %)
S. flexneri 4	1	0
S. flexneri 6	9	11
S. sonnei	10 (1.8 %)	0
diarrhoea only	115	110
all cases	136 (25.1 %)	135 (23.7 %)
without diarrhoeal symptoms	405 (75.0 %)	434 (76.3 %)
Total	541 (100.0 %)	569 (100.0 %)

Table 7. Morbidity observed in Bitolj according to serotype (1 June–15 September 1969)

Diagnosis	No. of children vaccinated against:	
	S. flexneri 1 and 2a	*S. flexneri* 3 and *S. sonnei*
S. flexneri 1	0	3 (0.4 %)
S. flexneri 2a	1 (0.1 %)	13 (1.9 %)
S. flexneri 3	0	0
S. flexneri 4	0	1
S. flexneri 6	2	2
S. sonnei	12 (1.7 %)	2 (0.3 %)
S. schmitzi	1	2
S. boydii 1–15	1	2
diarrhoea only	95	103
all cases	112 (15.8 %)	128 (17.9 %)
without diarrhoeal symptoms	595 (84.2 %)	588 (62.1 %)
Total	707 (100.0 %)	716 (100.0 %)

Table 8. Morbidity observed in Niš according to serotype (1 June–15 September 1969)

Diagnosis	No. of children vaccinated against:	
	S. flexneri 1 and 2a	*S. flexneri* 3 and *S. sonnei*
S. flexneri 1	1 (0.1 %)	0
S. flexneri 2a	0	2 (0.3 %)
S. flexneri 3	0	0
S. flexneri 4	1	1
S. flexneri 6	2	2
S. sonnei	2 (0.3 %)	0
S. schmitzi	0	1
S. boydii 1–15	1	1
diarrhoea only	81	77
all cases	88 (12.1 %)	84 (11.7 %)
without diarrhoeal symptoms	639 (87.9 %)	636 (88.3 %)
Total	727 (100.0 %)	720 (100.0 %)

Table 9. Summary of morbidity observed in four study areas of Yugoslavia according to serotype (1 June–15 September 1969)

Diagnosis	No. of children vaccinated against:	
	S. flexneri 1 and 2a	*S. flexneri* 3 and *S. sonnei*
S. flexneri 1	1 (0.03 %)	11 (0.04 %)
S. flexneri 2a	3 (0.1 %)	35 (1.2 %)
S. flexneri 3	1 (0.03 %)	3 (0.1 %)
S. flexneri 4	10	8
S. flexneri 6	25	33
S. sonnei	54 (1.9 %)	7 (0.2 %)
S. schmitzi	8	7
S. boydii 1–15	4	5
S. dysenteriae A	3	2
diarrhoea only	398	402
all cases	507 (18.1 %)	513 (18.2 %)
without diarrhoeal symptoms	2 295 (81.9 %)	2 308 (81.8 %)
Total	2 802 (100.0 %)	2 821 (100.0 %)

Table 10. Morbidity observed in Skoplje II according to serotype in children given vaccine without sodium bicarbonate pretreatment (1 June–15 September 1969)

Diagnosis	No. of children vaccinated against:	
	S. flexneri 1 and 2a	*S. flexneri* 3 and *S. sonnei*
S. flexneri 1	0	0
S. flexneri 2a	12 (1.5 %)	17 (2.0 %)
S. flexneri 3	0	1 (0.1 %)
S. flexneri 4	1	0
S. flexneri 6	9	10
S. sonnei	10 (1.2 %)	6 (0.7 %)
S. schmitzi	2	2
S. boydii 1–7	1	0
diarrhoea only	170	171
all cases	205 (24.9 %)	207 (24.8 %)
without diarrhoeal symptoms	617 (75.1 %)	629 (75.2 %)
Total	822 (100.0 %)	836 (100.0 %)

DISCUSSION

This study demonstrated that orally administered vaccines composed of streptomycin-dependent *Shigella* mutants conferred significant protection during 3½ months of surveillance against type-specific infection in children in four out of five hyperendemic areas of Yugoslavia in which these vaccines were tested. The bivalent vaccine composed of *S. flexneri* 1 and 2a conferred 91.2% protection, and the vaccine with *S. flexneri* 3 and *S. sonnei* gave 82% protection.

The importance of pretreatment with sodium bicarbonate in the vaccination of children was emphasized by the failure to achieve protection in one study area where only this procedure was omitted. The probable mechanism of action of sodium bicarbonate in the vaccination of children is its neutralization of gastric acid, permitting the organisms in the vaccine to reach the intestine. To immunize, the *Shigella* mutants apparently must reach the intestines alive.

Postvaccinal reactions were limited to vomiting and diarrhoea and were dose-dependent. Reaction rates were directly proportional to the number of live organisms in the initial dose and were minimized by reducing the number of live organisms from 28×10^9 to 20×10^9. In future studies it will be important to diminish the number of live organisms in the first dose to less than 20×10^9 while continuing gradually to increase them in each subsequent dose. By this means, reaction rates should be even lower than those reported here.

It was surprising to find such a high frequency of isolation of *S. flexneri* 6 in this study. In previous studies conducted in Yugoslavia we have found—as have other teams in different countries—that *S. flexneri* 6 is relatively uncommon among the group B shigellae (Aoki, 1969; Kostrzewski & Stypulkowska-Misiurewicz, 1968). If further studies corroborate the importance of the type 6 serotype, a vaccine against it may be necessary.

By the laboratory methods used in this study, no evidence of instability or tendency for reversion of the vaccine strains to the virulent parent organisms was found. Reversions *in vitro* have been observed (Linde & Koch, 1969), and it is possible that this may occur *in vivo*. If it does occur, the normal non-specific defence mechanisms of the bowel, especially the known antagonistic action of the normal flora on shigellae, might be expected to suppress or destroy the small numbers of revertants (Linde & Koch, 1969; Mel et al., 1965). It should be emphasized that there has been no evidence of reversion in over

25 000 children and adults vaccinated in this, and other, studies conducted in Yugoslavia. Although we have concluded that this theoretical risk is remote, the existence of a risk, however small, emphasizes the need to develop and test vaccine strains that are at least two steps genetically separated from the virulent parent.

Several questions of the utmost importance remain to be answered by field trials now in progress or planned for the near future. It is important to know the duration of protection of the vaccine, whether protection can be achieved by 3 or even fewer doses, and whether a booster dose can reinforce and extend the period of immunity.

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RÉSUMÉ

ÉTUDES SUR LA VACCINATION CONTRE LA DYSENTERIE BACILLAIRE: 6. IMMUNISATION D'ENFANTS PAR ADMINISTRATION ORALE DE SOUCHES DE *SHIGELLA* STREPTOMYCINODÉPENDANTES

En 1969, on a organisé dans cinq régions d'hyperendémicité de Yougoslavie un essai pratique de vaccins antidysentériques préparés à partir de mutants de *Shigella* streptomycinodépendants. Cet essai a confirmé les résultats antérieurs prouvant l'efficacité de ce type de préparation.

Pour la 1re fois, il a été démontré que les enfants vaccinés étaient immunisés dans une mesure appréciable contre l'infection: le taux de protection a été de 91,2% après administration d'un vaccin bivalent renfermant *Sh. flexneri* 1 et 2a, et de 82% après vaccination par *Sh. flexneri* 3 et *Sh. sonnei*. La réponse immunitaire a été spécifique du sérotype. Durant la période d'observation de 3½ mois, les enfants ont été visités à domicile à de multiples reprises et des coprocultures ont été effectuées dans tous les cas de diarrhée. Au total, 4 doses de vaccin ont été administrées.

Les réactions postvaccinales ont été peu intenses. Un petit nombre d'enfants ont présenté des vomissements (6,3‰) et/ou de la diarrhée (3,4‰) dans les premières heures suivant la vaccination. Ces incidents, fonction de la teneur en germes du vaccin, sont survenus surtout après la 1re dose. Le vaccin a été bien toléré lorsque la 1re dose contenait 20×10^9 organismes vivants, les doses suivantes étant progressivement augmentées jusqu'à 40×10^9 organismes vivants. Chaque dose a été donnée après ingestion de bicarbonate de sodium. Ce prétraitement est considéré comme essentiel pour assurer au vaccin sa pleine efficacité. Le bicarbonate de sodium agit probablement en neutralisant l'acidité gastrique, permettant ainsi aux germes vaccinaux d'atteindre l'intestin sans être lésés.

Aucun signe de réversion des mutants vaccinaux vers une souche mère virulente n'a été constaté durant cette étude.

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