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Article

Randomised Study of the Possible Adjuvant Effect of BCG Vaccine on the Immunogenicity of Diphtheria-Tetanus-Acellular Pertussis Vaccine in Senegalese Infants

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Abstract Following a study in Senegal (1990–1995) in which the relative efficacy of a diphtheria-tetanus-acellular pertussis vaccine (DTaP) was compared with that of a diphtheria-tetanus-whole-cell pertussis vaccine in children given a simultaneous injection of Bacille Calmette-Guérin (BCG) vaccine, this subsequent study was conducted to evaluate the possible adjuvant effect of the BCG vaccine on acellular pertussis vaccine components. A second objective was to compare the immunogenicity of these components when administered in accordance with a 2–4–6-month (spaced) schedule or an accelerated 2–3–4-month schedule. In all, 390 healthy Senegalese infants were randomly divided into three groups of 130 infants. Antibodies to acellular pertussis components were measured in serum samples obtained within 2 days of the first DTaP dose and 1 month after the third dose. BCG vaccine, given simultaneously with the DTaP vaccine, did not influence the immunogenicity of the acellular pertussis vaccine components when compared with separate administration of the two vaccines. Infants immunised according to a 2–4–6-month schedule had a significantly higher immune response than those immunised according to a 2–3–4-month schedule with respect to the response to pertussis toxoid assessed by seroneutralisation on Chinese hamster ovary cells ($P<0.0001$). These results suggest that BCG and DTaP vaccines can be given simultaneously without interference or enhancement and that more optimal immunogenicity is achieved with an extended than with an accelerated schedule.

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Introduction

Recent studies have demonstrated the efficacy and the safety [1–6] of various types of acellular pertussis vaccines in combination with diphtheria and tetanus toxoids (DTaP) compared with those of whole-cell pertussis vaccines (DTwP), which are known to be more reactogenic [7, 8]. These results have stimulated the development of DTaP vaccines, which have been licensed in certain European countries and in the USA for use in primary series and booster vaccination [9]. It was important to investigate any factors capable of influencing the immunogenicity and efficacy of these acellular pertussis vaccines. One such potential factor is BCG vaccination, which is known to exert immunopotentiating adjuvant effects in the treatment of some cancers [10, 11], in the prevention and control of some

parasitic infections [12] such as leishmaniasis [13], and against leprosy when administered in combination with killed *Mycobacterium leprae* vaccine [14]. In routine vaccination, although recommended at birth as part of the Expanded Programme on Immunization (EPI) of the World Health Organization (WHO), for logistic reasons, BCG is often given with the first dose of DTaP vaccine.

The immunisation schedule is known to influence the immunogenicity of combination vaccines. Studies investigating DTwP vaccines have demonstrated that a 2–3–4-month schedule was less immunogenic for several antigens than a 3–5–9-month schedule, although these differences in immunogenicity tend to diminish over the 12-month period following the primary vaccination series [15–19]. Data suggest that the immunogenicity of acellular pertussis vaccines may be schedule-influenced to a greater extent than that of whole-cell pertussis [17].

The objectives of the present study were twofold: first, to assess the possible adjuvant effect of BCG vaccine given concomitantly with the first dose of DTaP at 2 months of age; and second, to compare the immunogenicity of the acellular pertussis components of DTaP administered according to two vaccine schedules: the 2–3–4-month schedule, which is close to the 6–10–14-week schedule recommended by the EPI of the WHO and used in France and the UK, and the 2–4–6-month schedule, widely used in the USA and certain European countries. The study was performed in a malaria-endemic area. Given that there is a possible interaction between malaria infection and the immune response to vaccination in infancy [20, 21]), we examined the impact of malaria infection on the immunogenicity of the pertussis components of DTaP vaccine.

Materials and Methods

Ethics. The study was conducted in compliance with the Helsinki Declaration and its 1989 Hong Kong Amendment, Good Clinical Practices, and local regulations. The study protocol was approved by the Ministry of Health in Dakar, Senegal, the Ethics Committee of the Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) in Dakar, and the Institutional Review Board of the National Institute of Allergy and Infectious Diseases in Bethesda, USA, prior to study initiation.

Population. This study was conducted in Niakhar, a rural farming area 150 km from Dakar, Senegal. The population comprises approximately 28 000 inhabitants living in 30 villages. ORSTOM has conducted several epidemiological and demographic studies in the Niakhar area starting in 1983 [22]. Under the EPI, pertussis vaccination was routinely introduced in late 1986 and led to a decline in pertussis incidence and morbidity.

Eligible infants were those born between January and May 1996 and whose mothers resided permanently in the study area. A total of 390 infants were recruited from five consecutive birth cohorts. The first (BCG) vaccinations took place in March 1996 and the last (yellow fever and measles) vaccinations were performed in February 1997. The first doses of DTaP and inactivated polio vaccine (IPV) were administered in April 1996 and the last doses were given in December 1996.

To be included in the study, infants had to be aged 1–2 months, and verbal informed consent had to be obtained from one or both parents. Severe congenital or chronic disease, previous vaccination with one or more of the DTP, IPV, or BCG vaccines, a history of any of these diseases, a history of seizures or an evolving neurological disorder, a rectal temperature $\geq 38.5\,^{\circ}C$, and cachexia were considered noninclusion criteria. Infants meeting all eligibility and inclusion criteria were enrolled in the study.

Study Design. Infants were randomly allocated to one of three administration groups: group A, BCG and DTaP simultaneously at separate sites at 2 months of age, then DTaP at 4 and 6 months of age ($n=130$); group B, BCG during the second month of life, followed at least 3 weeks later by DTaP at age 2 months, then DTaP at 4 and 6 months ($n=130$); or group C, BCG during the second month of life, followed at least 3 weeks later by DTaP at age 2 months, then DTaP at 3 and 4 months ($n=130$).

Vaccines were administered according to the schedules shown in Table 1. After one or both parents had given verbal consent, all infants received a dose of IPV with each dose of DTaP but at a different injection site. At visit 5 (age 9–10 months), measles and yellow fever vaccines were administered to all subjects.

Blood samples were obtained by finger prick at visit 1 (age 2–3 months) and at visit 4 (age 5–6 months in the accelerated schedule group and age 7–8 months in the spaced schedule groups). In order to obtain optimal compliance and for logistic reasons, blood sampling was performed in the infants' homes [within 2 days of the first dose of DTaP and 1 month (21–42 days) after the third dose of DTaP], and verbal consent was obtained from one or both parents before each blood sample was taken. At each sampling a blood smear test was also performed in order to determine malaria status. All samples were centrifuged at the field station, and sera were stored at $-20\,^{\circ}C$ until assay.

Characteristics of the Vaccines. DTaP vaccine [Pasteur Mérieux Connaught (PMC), France; lot S2964] consisted of at least 30 IU of purified diphtheria toxoid, at least 40 IU of purified tetanus toxoid, 25 μg of glutaraldehyde-detoxified purified pertussis toxoid (PT), and 25 μg of native filamentous haemagglutinin (FHA) per 0.5 ml dose adsorbed on aluminium hydroxide (aluminium content ≤ 1.25 mg).

BCG vaccine (PMC, France, lot K6025) contained freeze-dried viable bacteria, Mérieux strain derived from strain 1077, 800 000 to 3 200 000 units; and excipient (dextran, anhydrous glucose, Triton WR 1339, human albumin). Half a dose (i.e., 0.05 ml) was administered.

Each 0.5 ml dose of IPV vaccine (PMC, France) was presented in a prefilled syringe and contained 40-D, 8-D, and 32-D antigen units of inactivated poliovirus types 1, 2, and 3, respectively.

Administration. All vaccines were administered during vaccination sessions held during the first week of the month. Verbal consent was obtained from the parent or the person acting with parental power before each dose of vaccine. DTaP and IPV were administered intramuscularly in the anterolateral aspect of the right and left thighs, respectively. BCG vaccine was administered intradermally in the lower deltoid area.

Safety Assessment. Over the 2-week period following each vaccination, interviewers visited all vaccinated infants weekly, in their homes. Mothers were asked about drowsiness or convulsions, and in cases in which such reactions had occurred, the interviewers

Table 1 Vaccine administration schedules

Schedule[a]	Age (in months)						
	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7	7 to 8
Group A		BCG					
BCG (simultaneous) given with the first dose of		DTaP		DTaP		DTaP	
DTaP; 2-4-6-month (spaced) DTaP schedule.		BS 1					BS2
Group B	BCG						
BCG (previous) given 3 weeks before the first dose		DTaP		DTaP		DTaP	
of DTaP; 2-4-6-month (spaced) DTaP schedule.		BS1					BS 2
Group C	BCG						
BCG (previous) given 3 weeks before the first dose		DTaP	DTaP	DTaP			
of DTaP; 2-3-4-month (accelerated) DTaP schedule.		BS 1			BS 2		

[a] Inactivated polio vaccine was given at the same time as DTaP, at a separate injection site; yellow fever and measles vaccines were given at 9–10 months of age

BS, blood sample; DTaP, diphtheria-tetanus-acellular pertussis vaccine

asked the physician to visit the infant. Investigators also monitored the infants at each vaccination visit. Serious adverse events (death, life-threatening or neurologic events) were recorded throughout the entire study period. The verbal autopsy method [23], in which interviewers questioned the parents about the circumstances of the child's death, was used to determine the cause of death and to complete certificates.

Immunogenicity Assessment. Serum samples were obtained at the time of the first dose of DTaP and 1 month after the third dose of DTaP. Due to insufficient blood, prevaccination FHA titres were not investigated. Neutralising antibodies to PT were measured by seroneutralisation on Chinese hamster ovary (CHO) cells [24] by the Karolinska Institute, Stockholm, Sweden. Results were expressed as reciprocal dilutions (lower limit of detection=2). Antibodies to PT and FHA were measured in triplicate enzyme-linked immunosorbent assays (EIA) [25] at the Clinical Sero-Immunology Laboratory, PMC, Val de Reuil, France. Results were expressed as EIA units (EU)/ml by comparison with an internal reference serum calibrated against the FDA human serum lot 3. The lower limit of detection for both of these assays was 2 EU/ml. In the absence of a serological correlate of protection for antibodies against PT and FHA, infants were considered to have seroconverted if their antibody titres increased fourfold from pre-immunisation levels.

Malaria Parasitaemia. Blood smears were defibrinated, dried, and stained with Giemsa. Readings were performed by the same trained technician. All thick blood film readings were standardised. A total of 200 microscopic oil-immersion fields were examined on each slide (about 0.5 µl of blood).

Nutritional Assessment. At each vaccination session, the weight and height of each infant were measured. Infants were weighed naked on scales (R. Seca, Germany) to the nearest 20 g; length was measured to the nearest millimetre on a wooden portable scale.

Data Analysis. Data analysis was performed using SAS software (SAS version 6.11; SAS Institute, USA). Nutritional status, assessed by weight-for-height Z-score, was computed using an Anthro1 (WHO-CDC, Switzerland) system.

Geometric mean titres (GMTs) and their 95% confidence intervals (95% CI) were calculated for PT antibodies (CHO cell assay and EIA) and for FHA antibodies (EIA) at each blood sampling time. The percentage of infants in whom a fourfold increase in PT and FHA antibody titres occurred was calculated, as was the percentage of infants in whom postimmunisation neutralising antibody titres for PT were above the arbitrary threshold of 32 [26].

Statistical Analysis. In order to test the above hypothesis with an α-risk of 2.5% at a power ($1-\beta$) of 80%, and using a standard deviation of 0.6 based on previous immunogenicity studies, it was calculated that 63 subjects per group were required. On the basis of estimated rates of migration and deaths and dropout rates, it was planned to enroll 130 subjects per group.

The possible adjuvant effect of BCG vaccination was assessed by comparing post-immunisation GMTs to PT (assayed by CHO and EIA) and FHA (EIA) between groups A and B. BCG was considered to have a clinically adjuvant effect if post-immunisation GMTs to the pertussis antigens from group A were more than twice as high as those from group B (Table 2). The influence of the vaccination schedule was assessed in the same manner: postimmunisation GMTs to PT (CHO and EIA) and FHA were compared using a variation factor of two between GMTs in groups B and C (Table 2).

A Student's *t* test was performed for each comparison. Alternatively, the Kruskall Wallis test was performed when variances were not homogeneous. Serological data calculations were performed on logarithmically transformed data, reporting the antilogarithm.

Both intention-to-treat and per-protocol analyses were performed. Infants who did not receive vaccinations according to the appropriate group schedule; infants from whom a blood sample was obtained more than 6 weeks after the third dose of vaccine; infants for whom an interval of more than 1 month (>28 days) separated the second and third doses of vaccine in group C, and infants for whom an interval of more than 2 months (>56 days) separated the same doses in groups A and B were excluded from the per-protocol analysis of immunogenicity.

The comparability of the vaccine groups for analysis was assessed for factors such as gender, weight-for-height Z-score at 2 months of age, and preimmunisation titres for PT.

Safety analysis was performed by descriptive analysis of serious adverse events occurring during the study period.

Results

Population. Of the 390 infants included in the trial, 277 (71%) completed the three-dose DTaP primary series. Of the 113 infants who did not complete the primary series, 48 were not present for a scheduled dose (16 at dose one, 19 at dose two, and 13 at dose three); 21 were withdrawn from the study by their parents; 12 died

Table 2 Sample size, comparability between groups, and geometric mean titres (GMT) for pertussis toxoid (PT) and filamentous haemagglutinin (FHA) antibodies

	Group A BCG given simultaneously; 2-4-6-month schedule			Group B BCG given previously; 2-4-6-month schedule			Group C BCG given previously; 2-3-4-month schedule		
	No.	Percent, means ±SD or GMT (95% CI)	No.; Percent >4-fold rise	No.	Percent, means ±SD or GMT (95% CI)	No.; Percent >4-fold rise	No.	Percent, means ±SD or GMT (95% CI)	No.; Percent >4-fold rise
Percent female	62	61.3		65	60.0		65	52.3	
Age (months)	62	2.7±0.9		65	2.8±1.7		65	2.7±0.9	
Weight for height (Z-score)	60	0.1±2.3		65	0.3±1.8		63	0.1±1.7	
Malaria[a]	62	41.9		64	43.8		65	20.0	
Prevaccination titres									
PT (CHO)	48	1.6 (1.3–2.1)		51	1.6 (1.3–2.0)		48	1.7 (1.4–2.2)	
PT (EIA)	40	1.4 (1.2–1.9)		45	1.7 (1.6–2.7)		38	1.6 (1.2–1.9)	
Postvaccination titres									
PT (CHO)	61	76.8 (60.2–97.8)	47; 95.7	62	73.2 (61.5–87.1)[b]	47; 97.9	64	42.9 (36.7–50.1)[b]	47; 96
PT (EIA)	57	97 (83.4–113)	38; 100	63	91.9 (81.6–103)	44; 97.7	62	82.6 (72–94.7)	37; 100
FHA (EIA)	55	250 (207–302)	NC	61	258 (224–297)	NC	61	244 (205–289)	NC

[a] Global comparison of groups A, B, C, $P<0.01$
[b] Comparison of group B versus group C, $P<0.0001$
CHO, Chinese hamster ovary; EIA, enzyme immunoassay; NC, not calculated

before completing the primary series; five emigrated from the study area; 23 experienced a contraindication precluding further vaccination (before dose one, 3 infants had fever ≥38.5 °C and 1 had experienced seizures; before dose two, 3 infants had fever ≥38.5 °C and 1 had clinical pertussis in the context of exposure; and before dose three, 14 infants had fever ≥38.5 °C, 1 had concomitant seizures, and 2 had experienced isolated seizures). Three infants, although presenting for dose two, were not given this dose because parental consent was not obtained. One child was given the incorrect vaccine according to the randomisation code. The vaccine groups were comparable in terms of gender, weight-for-height Z-score at 2 months of age, and preimmunisation titres for PT (Table 2).

All recipients of all three doses of DTaP had also received BCG vaccine. Among them, 192 (49.2%) who accepted the second serum sample were included in intention-to-treat analysis and 191 (49%) in the per-protocol analysis of immunogenicity data. As only one infant received the wrong vaccine and no immunogenicity results were available for him, both types of analysis gave identical results.

Twenty percent of the infants aged 5–6 months were already infected by malaria (group C); at 7–8 months of age, 40% were infected (Table 2).

Immune Response. Preimmunisation antibody titres for PT were comparable between groups (Table 2). The antibody response to PT measured by EIA and CHO assays and to FHA was equivalent whether BCG was administered at the same time as the first dose of DTaP (group A) or at least 3 weeks before the latter (group B) (Table 2).

No difference in the time interval between the third vaccination and blood sampling (mean 34.5 days, range 21 to 42 days) could be observed between groups. The postimmunisation GMT for PT neutralising antibodies (assessed by CHO assay) was significantly higher ($P<0.0001$) in infants vaccinated according to the spaced 2–4–6-month schedule (group B) than in infants vaccinated according to the accelerated 2–3–4-month schedule (group C). There were, however, no significant differences between groups B and C for PT and FHA antibodies measured by EIA. Similar results were found when comparing groups A and B versus group C (data not shown). The percentage of infants displaying a fourfold rise in neutralising PT antibodies assessed by CHO assay was high (>95%), and similar in all three vaccination groups (Table 2). However, the percentage of infants achieving neutralising antibody titres for PT above the threshold of 32 was lower in those children who were immunised according to the accelerated schedule (85.9%) compared with those immunised according to the spaced schedule (96.8%) (chi-square test, $P=0.031$).

The antibody response to PT and FHA in terms of GMTs was somewhat lower in infants who were found to be infected with malaria than in infants not infected with malaria, regardless of their vaccination group (Table 3). This difference was significant for PT (assessed by EIA) ($P<0.05$); however, the proportion of infants with CHO titres above the threshold of 32 was not significantly lower in children with malaria (56%) than in those not infected (65%).

Serious Adverse Events. A total of 35 serious adverse events occurred in 35 infants (9% of the study population). These events were equally distributed among the

Table 3 Geometric mean antibody titres (GMT) after DTaP vaccination in infants infected and not infected with malaria

	Group A BCG given simultaneously; 2-4-6-month schedule				Group B BCG given previously; 2-4-6-month schedule				Group C BCG given previously; 2-3-4-month schedule				All groups			
	No.	No malaria GMT (95% CI)	No.	Malaria GMT (95% CI)	No.	No malaria GMT (95% CI)	No.	Malaria GMT (95% CI)	No.	No malaria GMT (95% CI)	No.	Malaria GMT (95% CI)	No.	No malaria GMT (95% CI)	No.	Malaria GMT (95% CI)
PT (CHO)	35	86.5 (61.7–130.2)	26	63.1 (46.7–85.7)	33	86.3 (69.2–107.2)	28	58.6[a] (45.7–75.9)	51	43.2 (36.3–51.3)	13	39.1 (30.9–48.9)	119	64.3 (54.9–74.1)	67	55.7 (46.8–66.1)
PT (EIA)	32	105.2 (85.1–128.8)	25	88.7 (72.4–109.6)	34	107.7 (93.3–123.0)	28	77.5[b] (64.6–93.3)	49	85.3 (66.1–100.0)	13	73.9 (58.8–91.2)	115	97.3 (87.1–107.1)	66	81.1[c] (72.4–91.2)
FHA (EIA)	30	277.3 (213.8–363.1)	25	224.9 (173.7–295.1)	33	279.9 (234.4–331.1)	27	230.6 (181.9–288.4)	50	247.7 (204.2–301.9)	13	230.1 (158.5–338.8)	114	265.5 (234.4–302.0)	65	230.7 (199.5–269.1)

PT, pertussis toxoid; CHO, Chinese hamster ovary; FHA, filamentous haemagglutinin; EIA, enzyme immunoassay

[a] $P<0.05$
[b] $P<0.01$
[c] $P<0.05$

three groups, and none were considered related to vaccination. Deaths accounted for 21 of these events (5 in group A, 8 in group B, and 8 in group C), and causes were as follows: malaria ($n=7$), pneumonia ($n=6$), fever of unknown aetiology ($n=2$), diarrhoea accompanied by dehydration ($n=4$), cutaneous infection ($n=1$), and meningeal irritation ($n=1$). Two deaths occurred within 3 days of vaccination; in one case death was due to pneumonia and occurred 2 days after the third dose, and in the other case death was due to malaria and occurred 3 days after the second dose.

The other events comprised febrile convulsions (5 in group A, 3 in group B, and 4 in group C), monoplegia following intramuscular administration of a drug (probably quinine) (1 in group A), and pneumonia (1 in group C).

Discussion

The present study demonstrated that the simultaneous administration of BCG vaccine and the first dose of DTaP vaccine, in comparison with separate administration of these two vaccines at different times, did not significantly enhance antibody responses to PT (assessed by CHO and EIA) and FHA (assessed by EIA) in terms of GMT values obtained 1 month after primary immunisation according to a 2–4–6-month schedule. BCG vaccine can thus be administered at the same time as DTaP vaccine without influencing the immunogenicity of the pertussis components.

These findings concur with those of a study investigating the immunogenicity of the whole-cell DTP-IPV vaccine administered simultaneously with BCG and hepatitis B vaccines [27]. In the latter study, BCG vaccine was given with the third dose of DTP-IPV vaccine at 4 months of age and did not influence the immunogenicity of DTP-IPV when compared with alternating administration of the same vaccines. This DTaP vaccine has previously been found safe and immunogenic in this setting [3], and its efficacy was further assessed with reference to the DTwP PMC vaccine in a double-blind randomised trial from 1990 to 1995 involving over 4000 infants [18]. The absolute efficacy of both vaccines was evaluated using a household case-contact analysis. The protective efficacy afforded by the DTaP vaccine was lower than that of DTwP vaccine, as expressed by a relative efficacy of 1.54 (95% CI 1.23–1.93). The absolute efficacies were 96% (95% CI 86–99%) and 85% (95% CI 66–93%) for the whole-cell and acellular vaccines, respectively, using the WHO case definition supplemented by confirmation of pertussis exposure using polymerase chain reaction. In the efficacy trial, BCG vaccine was administered with the first dose of DTaP or DTwP vaccine, and a 2–4–6-month schedule was used. The results of the present study strongly suggest that the efficacy data

from the DTaP clinical trial can be relevant for other settings.

A more marked immune response to PT (assessed by CHO) was obtained in infants immunised with the spaced schedule compared with those immunised with the accelerated schedule. However, antibody responses to PT assessed by EIA and to FHA were comparable with both schedules. Studies exploring schedule influence have shown that a 2–3–4-month schedule was less immunogenic than a 3–5–9-month spaced schedule in terms of diphtheria, tetanus, and, to a lesser extent, pertussis antibody response following whole-cell DTP immunisation [15, 16].

Data suggest that the immunisation schedule also influences the immune response to pertussis components of acellular pertussis-containing vaccines, and it has been demonstrated that a 3–5–12-month schedule is more immunogenic than a 2–4–6-month schedule [17, 27–29]. Although in the present study an enhanced immune response, in terms of PT neutralising antibody titres, was obtained with the spaced schedule compared with the accelerated schedule, this effect may not have clinical significance, since the proportion of infants with seroconversion to PT (both assays) did not differ by group. However, the percentage of children displaying postimmunisation neutralising antibody titres for PT above the threshold of 32, considered as a possible threshold for long-term protection, was significantly higher after a primary series at 2, 4, and 6 months than at 2, 3 and 4 months [26].

Although there is no agreed-upon correlate of protection in pertussis, this study indicates that better immunogenicity is achieved with an extended than with an accelerated schedule (as recommended by the WHO in the EPI), and hence, that the immunogenicity of pertussis components should be borne in mind when considering evaluation or revision of immunisation schedules.

None of the serious adverse events reported were considered related to vaccination. Causes of deaths, mainly malaria and pneumonia, were similar to those reported during the previous studies conducted in Niakhar, and the death rate during this study was slightly lower than that usually observed in this study area, i.e., 85 per 1000 [3, 18, 30].

The inhibitory effect of malaria infection observed in this study confirmed previous observations in animals following pertussis vaccination [20] and in infants following tetanus vaccination [21]. The prevalence of malaria parasitaemia increased with the age of the infants, as is classically observed in such settings (H. Whittle, personal communication), although the prevalence of malaria infection found in the present study was remarkably high. As a consequence, the inhibitory effect of malaria on the immune response to pertussis following vaccination lowered the advantage of the spaced schedule over the accelerated schedule, since malaria was more prevalent at 7 months than at 5 months of age. The clinical significance of this is unknown but deserves further evaluation regarding vaccination studies and practice in endemic areas.

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