Antibody response to 17D yellow fever vaccine in Ghanaian infants

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Objectives To assess the seroresponses to yellow fever vaccination at 6 and 9 months of age; assess any possible adverse effects of immunization with the 17D yellow fever vaccine in infants, particularly at 6 months of age.
Methods Four hundred and twenty infants who had completed BCG, OPV and DPT immunizations were randomized to receive yellow fever immunization at either 6 or 9 months. A single dose of 0.5 ml of the reconstituted vaccine was administered to each infant by subcutaneous injection. To determine the yellow fever antibody levels of the infants, each donated 1 ml whole blood prior to immunization and 3 months post-immunization. Each serum sample was titred on Vero cells against the vaccine virus.
Findings The most common adverse reactions reported were fever, cough, diarrhoea and mild reactions at the inoculation site. The incidences of adverse reactions were not statistically different in both groups. None of the pre-immunization sera in both age groups had detectable yellow fever antibodies. Infants immunized at 6 months recorded seroconversion of 98.6% and those immunized at 9 months recorded 98% seroconversion. The GMT of their antibodies were 158.5 and 129.8, respectively.
Conclusions The results indicate that seroresponses to yellow fever immunization at 6 and 9 months as determined by seroconversion and GMTs of antibodies are similar. The findings of good seroresponses at 6 months without significant adverse effects would suggest that the 17D yellow fever vaccine could be recommended for use in children at 6 months in outbreak situations or in high risk endemic areas.

Keywords Yellow fever vaccine/immunology/adverse effects; Antibody formation/immunology; Infant; Randomized controlled trials; Ghana (*source: MeSH*).

Mots clés Vaccin anti-fièvre jaune/immunologie/effets indésirables; Formation anticorps/immunologie; Nourrisson; Essai clinique randomisé; Ghana (*source: INSERM*).

Palabras clave Vacuna contra la fiebre amarilla/inmunología/efectos adversos; Formación de anticuerpos/inmunología; Lactante; Ensayos controlados aleatorios; Ghana (*fuente: BIREME*).

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Voir page 1058 le résumé en français. En la página 1059 figura un resumen en español.

Introduction

Epidemiological data over the past two decades attest to the resurgence of yellow fever outbreaks in sub-Saharan Africa (*1*), with more than 1 million cases between 1986 and 1990 (*2*). In Africa, children under the age of 15 years now account for 70–90% of the cases (*3*–*7*). As a result, WHO recommended that yellow fever vaccination be included in national EPI (Expanded Programme on Immunization) activities in endemic countries and that it be given to infants at the same time as measles immunization at 9 months of age (*8*). Although this schedule of immunization will not prevent some infants from contracting yellow fever infection during epidemics, immunization of infants younger than 4 months old with yellow fever (17D strain) vaccine can produce serious side-effects, such as encephalitis (*9*, *10*), and WHO recommends that infants younger than 6 months should not be vaccinated (*11*).

As part of a study to assess the immunogenicity of AIK-C measles vaccine (Kitasato strain) in infants aged 6 months (*12*), yellow fever (17D strain) vaccine was administered in parallel. The goals were to evaluate immune responses in infants at 6 and 9 months of age, and to assess adverse effects of the yellow fever vaccine in infants, particularly at 6 months of age. The yellow fever study also served as a randomization control in the AIK-C study.

Methods

Study area and population

The study was conducted in Asamankese, the capital of East Akim District in Southern Ghana. Yellow

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fever is not endemic in the district (population approximately 100 000) and no yellow fever has been reported in the last 10 years. Routine immunization is carried out as recommended by the Ghana Ministry of Health and infants receive yellow fever immunization at 9 months of age. Yellow fever immunization coverage was 28% in Asamankese during the study, slightly higher than the national average of 24% (*13*). There were no confirmed or suspected cases of yellow fever in the area during the study.

Study design

Infants aged 6 months (24–27 weeks) who had been attending the Asamankese mother and child health clinic regularly and had received all the required immunizations (BCG at birth, three doses of oral poliovirus vaccine, and three of diphtheria-pertussis-tetanus) were identified during routine clinic visits. The study was explained to the parents and their verbal consent obtained for participation. Children were randomly allocated to one of two groups that received yellow fever immunization at 6 months or at 9 months. To blind the study, infants receiving the yellow fever vaccine at 9 months received the AIK-C measles vaccine at 6 months (group A), and those receiving the yellow fever vaccine at 6 months received the Schwartz measles vaccine at 9 months (group B). A total of 420 infants were enrolled in the study, with 200 infants in group A and 220 in group B. A child was excluded from the study if he/she was malnourished (<80% of expected weight for age), or febrile (temperature $\geqslant 38$ °C), or had a previous history of measles (according to mother's report).

Immunization and blood sampling

A single 0.5 ml dose of reconstituted 17D yellow fever vaccine was administered to each infant by subcutaneous injection. Approximately 1 ml of whole blood was taken prior to immunization and 3 months post-immunization. Infants who received yellow fever immunization at 9 months had two pre-immunization blood samples taken (at 6 and 9 months) to exclude possible seroconversion due to contact with the wild virus. Blood samples were collected in labelled polystyrene tubes, transported chilled to the Noguchi Institute on the same day, centrifuged, separated and the sera stored at –20 °C until used.

Vaccine details

The vaccine for the study was manufactured by the Institute Pasteur, Dakar (Lot number E 5263) and obtained from EPI of the Ghanaian Ministry of Health. Each vaccine contained 20 doses and potency tests were randomly performed on vials from cold storage to ensure they conformed to the manufacturer's data. In addition, remaining vaccine from the field was also randomly tested for potency throughout the study.

Monitoring for adverse reactions

Mothers were instructed to come to the clinic if the child became unwell after immunization. They were also requested to return to the clinic with the infants on day 10 after immunization. At these visits, the infants were examined, axillary temperature measured and information on adverse reactions obtained with a questionnaire. Those defaulting were visited at home on days 11 or 12. Health care providers and the laboratory technicians did not know to which vaccination schedule a child belonged.

Serology

Duplicate 50 µl aliquots of diluted sera (serially diluted two-fold from 1:4 to 1:2048) were pipetted into flat bottomed 96-well microtitre plates. An equal volume of yellow fever virus (17D vaccine strain) suspension containing 100 infectious particles per 50 µl was added to each well, except wells A1–H1 which served as serum control. Wells A11–H11 and A12–H12 received only Vero cells and served as cell controls. The suspension was incubated for 2 hours, after which 100 µl of Vero cells (10^4/ml) were added to all wells of the plates. The plates were incubated at 36.5 °C under 5% CO_2 atmosphere and observed for 14 days for cytopathic effect. Serum antibody titres were calculated by the method of Reed & Munch (*14*) and the geometric mean titre (GMT) of yellow fever neutralizing antibodies calculated using the following relationship:

$$\mathrm{GMT} = ((\log X_1 + \log X_2 + \log X_3..)/n)$$

A child was considered to have seroconverted when yellow fever virus neutralizing antibodies were detected at a serum dilution of 1:4 or more.

Statistical analysis

Proportions were compared using the χ^2 test, and continuous variables with Student's *t*-test, after log transformation of non-normally distributed data. The statistical difference was set at $P \leqslant 0.05$.

Results

Of the 220 infants in the group that received yellow fever vaccine at 6 months of age, 193 presented for bleeding at 3 months post-immunization. Of the 200 who should have received the vaccine at 9 months, only 164 turned up for the immunization. Parents rescinding their consent accounted for most of the dropouts; others had travelled outside the study area. Sera from 139 of the infants immunized at 6 months of age, and from 150 for those immunized at 9 months, were available for serological studies. This was because some of the sera had been exhausted in the parallel study to assess the immunogenicity of AIK-C measles vaccine (*12*).

Adverse reactions

The most common adverse reactions reported were fever, cough, diarrhoea and mild reactions at the

inoculation site. In the 10-day period following immunization, 51 infants (23.18%) vaccinated at 6 months of age had fever, compared with 43 infants (21.5%) immunized at 9 months. Among infants that received immunization at 9 months, three experienced acute fevers 2–6 weeks post-immunization. Blood film examinations showed all three to have been infected with malaria parasites. Adverse reactions, such as jaundice and encephalitis, were not noted or reported in the two groups within 10 days after immunization, or on the AIK-C study (*12*). The adverse reactions are summarized in Table 1.

Serological results

None of the pre-immunization sera obtained from the vaccinees in either age group had detectable yellow fever antibodies. Of the 139 sera from infants immunized at 6 months, 137 (98.6%) had seroconverted, compared with 147/150 (98%) from those immunized at 9 months of age (Table 2). The seroconversion rates in the two groups were not statistically different (P = 0.922086). The GMT of antibodies in infants immunized at 6 months (158.5) was not significantly different from that in infants immunized at 9 months (129.8; P = 0.998582).

Table 1. **Adverse reactions with 17D yellow fever vaccine**

Reaction	Immunization at 6 months (n = 220)	Immunization at 9 months (n = 164)
Systemic reactions		
History of fever	51 (23.2)[a]	42 (25.6)
Cough	21 (9.5)	13 (7.9)
Rash	13 (6.5)	8 (4.9)
Running nose	5 (2.3)	11 (6.7)
Diarrhoea	9 (4.5)	12 (7.3)
Vomiting	3 (1.5)	0 (0.0)
Local reactions		
Redness at injection site	36 (18.0)	29 (17.7)
Swelling of injection site	1 (0.5)	0 (0.0)

[a] Figures in parentheses are percentages.

Table 2. **Seroconversion rates and GMTs of yellow fever virus neutralizing antibodies after immunization with 17D yellow fever vaccine.**

Age at immunization (months)	Seroconversion rates	GMTs of neutralizing antibodies
6	98.6% (137/139)	158.5
9	98.0% (147/150)	129.8

Discussion

The primary objective of vaccinating infants early in life is to induce active immunity before protective maternal antibodies are lost. Studies in West Africa have shown that the mortality rate in infants younger than 6 months of age who contract yellow fever is higher than 70%, and that young children are disproportionately infected in unimmunized populations during yellow fever outbreaks (*3–6*). Since a single dose of yellow fever vaccine probably provides lifelong protection (*10*), immunization early in life in endemic countries is highly desirable. However, vaccination of children at 4 months of age or younger is not recommended, since there is a high risk of post-vaccination encephalitis (0.5–4 per 1000 infants) (*8–10*). WHO recommends that yellow fever vaccine should not be given to infants younger than 6 months old (*11*).

The results of this study indicate that responses to yellow fever immunization in children 6 months of age are qualitatively (seroconversion) and quantitatively (GMTs of antibodies) similar to those immunized at 9 months of age. The absence of antibodies in all pre-immunization sera is an indication that infants in the study population are at risk in a yellow fever outbreak. Indeed, recent yellow fever outbreaks have involved a significant number of children, including infants below the age of 6 months (*3–6*). The results of this study, that infants aged 6 months gave good immune responses to yellow fever vaccine without significant adverse effects, suggest that the 17D yellow fever vaccine could be used in children at 6 months of age during fever outbreaks, or in high risk areas where yellow fever is endemic. ■

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Conflicts of interest: none declared.

Résumé

Réponse en anticorps après administration de vaccin antiamaril 17D chez des nourrissons ghanéens

Objectif Evaluer la réponse sérologique à la vaccination antiamarile pratiquée à l'âge de 6 mois ou de 9 mois ; évaluer tout effet indésirable du vaccin antiamaril 17D chez le nourrisson, en particulier à l'âge de 6 mois.

Méthodes Après tirage au sort, 420 nourrissons déjà vaccinés par le BCG, le VPO et le DTC ont reçu le vaccin antiamaril à l'âge de 6 mois ou de 9 mois. Le vaccin était administré par injection sous-cutanée d'une dose unique

de 0,5 ml de vaccin reconstitué. Pour déterminer le taux d'anticorps antiamarils chez les nourrissons vaccinés, on a effectué chez chacun d'entre eux un prélèvement de 1 ml de sang total avant la vaccination et trois mois après. Les échantillons de sérum ont été titrés sur cellules Vero en présence de virus vaccin.

Résultats Les réactions indésirables les plus couramment rapportées consistaient en fièvre, toux, diarrhée et réaction locale bénigne au point d'injection. Il n'y avait pas de différence statistiquement significative d'incidence des réactions entre les deux groupes. Aucun des sérums prévaccinaux ne contenait d'anticorps antiamarils décelables. Le taux de séroconversion était de 98,6 % chez les nourrissons vaccinés à 6 mois et de 98 % chez ceux vaccinés à 9 mois. Le titre moyen géométrique d'anticorps était de 158,5 dans le premier groupe et 129,8 dans le deuxième.

Conclusion D'après les résultats, la réponse sérologique à la vaccination antiamarile pratiquée à l'âge de 6 ou de 9 mois et déterminée par le titre moyen géométrique d'anticorps est identique. Etant donné la bonne réponse sérologique obtenue à l'âge de 6 mois sans effets indésirables notables, le vaccin antiamaril 17D pourrait être recommandé pour la vaccination des nourrissons de 6 mois lors d'épidémies ou dans les zones d'endémie à haut risque.

Resumen

Respuesta de producción de anticuerpos a la vacuna antiamarílica 17D en lactantes de Ghana

Objetivo Evaluar la respuesta serológica a la vacunación contra la fiebre amarilla a los 6 y 9 meses de edad, y evaluar los posibles efectos adversos de la inmunización con la vacuna antiamarílica 17D en los lactantes, en particular a los 6 meses de edad.

Métodos 420 lactantes que habían recibido todas las dosis necesarias de las vacunas BCG, OPV y DPT fueron distribuidos aleatoriamente para recibir inmunización antiamarílica bien a los 6 meses o bien a los 9 meses de edad. Mediante inyección subcutánea se administró a cada lactante una dosis única de 0,5 ml de la vacuna reconstituida. A fin de determinar los niveles de anticuerpos antiamarílicos de los lactantes, se obtuvo 1 ml de sangre entera antes de la inmunización y a los 3 meses de la misma. Los títulos de cada muestra de suero se determinaron utilizando células Vero frente al virus vacunal.

Resultados Las reacciones adversas notificadas más frecuentemente fueron fiebre, tos, diarrea y manifestaciones leves en el punto de inoculación. La incidencia de esas reacciones no difirió de forma significativa entre un grupo y otro. Ninguno de los sueros preinmunización obtenidos en los dos grupos de edad presentó anticuerpos antiamarílicos detectables. Se observó seroconversión en el 98,6% de los lactantes inmunizados a los 6 meses, y en el 98% de los inmunizados a los 9 meses. La media geométrica de sus títulos de anticuerpos fue de 158,5 y 129,8 respectivamente.

Conclusión Los resultados indican que la respuesta serológica a la inmunización antiamarílica a los 6 y 9 meses de edad, según cabe deducir de la seroconversión y de la media geométrica de los títulos de anticuerpos, es similar. La observación de una buena respuesta a los 6 meses, no acompañada de efectos adversos importantes, lleva a pensar que se podría recomendar el uso de la vacuna 17D en los niños de 6 meses en los casos de declaración de brotes y en las zonas endémicas de alto riesgo.

References

1. **Expanded Programme on Immunization.** The resurgence of deadly yellow fever. Geneva, World Health Organization, March 1992 (EPI update No. 21).
2. **Robertson SE.** *Yellow fever: the immunological basis for immunization.* Geneva, World Health Organization, 1993 (unpublished document WHO/EPI/GEN/93.18).
3. **Monath TP.** Yellow fever in the Gambia, 1978-1979: epidemiologic aspects with observations on the occurrence of Orungo virus infections. *American Journal of Tropical Medicine and Hygiene*, 1980, **29** (5): 912–928.
4. **World Health Organization.** Yellow fever in 1983. *Weekly Epidemiological Record*, 1984, **59** (43): 329–335.
5. **Addy PAK, Minami K, Agadzi VK.** Recent yellow fever epidemics in Ghana (1969-1983). *East African Medical Journal*, 1986, **63** (6): 422–434.
6. **Nasidi A et al.** Urban yellow fever epidemic in western Nigeria, 1987. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1989, **83**: 401–406.
7. **World Health Organization.** Yellow fever, 1996–1997. *Weekly Epidemiological Record*, 1998, **73** (46–47): 354–357 and 370–372.
8. **Joint UNICEF/WHO Technical Group on Immunization for the African Region.** Report of the third meeting, Nairobi, Kenya, August 1988.
9. **Monath TP.** Yellow fever. In :Plotkin SA, Orenstein WA, eds. *Vaccine,* 3rd edition. Philadelphia, W.B. Saunders, 1999: 815–879.
10. **Tsai TF.** Quibbling over yellow fever vaccine: operational research issues. In: *Proceedings of the International Seminar on Yellow Fever in Africa, Dakar, Senegal, 25–27 June 1998.* Poitiers, France, Aubin Imprimeur, 1999: 68–71.
11. *WHO Expert Committee on Biological Standardization. Thirty-eighth report.* World Health Organization, Geneva,1988: 208–209 (WHO Technical Report Series No. 872).
12. **Nkrumah FK et al.** Comparison of AIK-C measles vaccine in infants at 6 months with Schwarz vaccine at 9 months: a randomized controlled trial in Ghana. *Bulletin of the World Health Organization*, 1998, **76** (4): 353–359.
13. **Department of Vaccines and Biologicals.** *WHO Vaccine Preventable Diseases Monitoring System: 1999 global summary.* Geneva, World Health Organization, 1999 (unpublished document WHO/V&B/99.17).
14. **Reed LJ, Muench H.** A simple method of estimation of 50% endpoints. *American Journal of Hygiene*, 1938, **27**: 493–497.