# Reactogenicity of yellow fever vaccines in a randomized, placebo-controlled trial

# Reatogenicidade de vacinas contra febre amarela em estudo randomizado, controlado com placebo

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**Abstract**

**Objective**

To compare the reactogenicity of three yellow fever (YF) vaccines from WHO-17D and Brazilian 17DD substrains (different seed-lots) and placebo.

**Methods**

The study involved 1,087 adults eligible for YF vaccine in Rio de Janeiro, Brazil. Vaccines produced by Bio-Manguinhos, Fiocruz (Rio de Janeiro, Brazil) were administered ("day 0") following standardized procedures adapted to allow blinding and blocked randomization of participants to coded vaccine types. Adverse events after immunization were ascertained in an interview and in diary forms filled in by each participant. Liver enzymes were measured on days 0, 4-20 and 30 of the study. Viremia levels were measured on days 4 to 20 of follow-up. The immune response was verified through serologic tests.

**Results**

Participants were mostly young males. The seroconversion rate was above 98% among those seronegative before immunization. Compared to placebo, the excess risk of any local adverse events ranged from 0.9% to 2.5%, whereas for any systemic adverse events it ranged from 3.5% to 7.4% across vaccine groups. The excess risk of events leading to search for medical care or to interruption of work activities ranged from 2% to 4.5%. Viremia was detected in 3%-6% of vaccinees up to 10 days after vaccination. Variations in liver enzyme levels after vaccination were similar in placebo and vaccine recipients.

**Conclusions**

The frequency of adverse events post-immunization against YF, accounting for the background occurrence of nonspecific signs and symptoms, was shown for the first time to be similar for vaccines from 17D and 17DD substrains. The data also provided evidence against viscerotropism of vaccine virus.

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*Descritores*

Vacina contra febre amarela. Efeitos
adversos. Ensaios controlados
aleatórios. Brasil.

*Resumo*

*Objetivo*

*Comparar a reatogenicidade de três vacinas contra a febre amarela (FA) das sub-cepas WHO-17D e 17DD (diferentes lotes-semente), e placebo.*

*Métodos*

*Foram recrutados 1.087 adultos elegíveis para vacinação contra FA no Rio de Janeiro, RJ, Brasil. Vacinas produzidas por Bio-Manguinhos, Fiocruz (Rio de Janeiro, RJ) foram administradas ("dia zero") seguindo procedimentos adaptados para alocação randômica em blocos e "cega" para o tipo de vacina. Eventos adversos pós-vacinação foram registrados em questionários e diários preenchidos pelos participantes. Enzimas hepáticas foram medidas nos dias 0, 4-20 e 30 do estudo. A viremia foi medida nos dias 4-20. A resposta imune foi verificada em testes sorológicos nos dias 0 e 30.*

*Resultados*

*Os participantes eram predominantemente homens jovens. A taxa de soroconversão foi superior a 98% no grupo soronegativo antes da vacinação. Comparado ao placebo, a diferença de risco de eventos adversos locais variou de 0,9% a 2,5%, e de 3,5% a 7,4% para eventos adversos sistêmicos nos grupos vacinados. A diferença de risco desses eventos com assistência médica e/ou falta ao trabalho variou de 2,0% a 4,5%. Viremia foi detectada em 3% a 6% dos vacinados até 10 dias após a vacinação. As variações nos níveis de enzimas hepáticas pós-vacinação foram semelhantes nos grupos vacinados e placebo.*

*Conclusões*

*Foi demonstrada pela primeira vez a semelhança do perfil de reatogenicidade das vacinas contra FA das cepas 17D e 17DD, comparados entre si e com placebo. As variações das enzimas hepáticas constituem evidência contra o potencial de viscerotropismo do vírus vacinal.*

## INTRODUCTION

Vaccination against yellow fever (YF) constitutes the single most effective means for the control of YF. The vaccine is recommended for regular immunization in endemic and epizootic regions based on the high cost-effectiveness of the vaccine and on the severity of YF.[13] The vaccines currently available are made of the same attenuated substrains of virus developed in the late 1930's, but have been incorporating a number of improvements. The realization that continued serial passage could determine changes in the immunogenicity of the virus substrain used to produce the vaccine, led to the implementation in the 1940's of the seed lot system. It consists in preparing and storing a large amount of virus, which supply vaccine production.[15] YF vaccines recommended by the World Health Organization (WHO) are from 17D and 17DD substrains, which have high genetic similarity.[15]

Variable frequency of predominantly mild signs and symptoms following administration of YF vaccine have been reported.[18,20] As systemic reactions are usually non-specific, the proportion of adverse events explained by vaccination can only be approximated by comparing with a reference non-vaccinated group. Severe events are rare and the association with the vaccine has been suggested by clinical and pathological

evidence.[6,12,24] Although those reports did not lead to changes in immunization policies for YF, the WHO called for a revision of the safety of the vaccines.[26]

The study assessed the immunogenicity and reactogenicity of a new seed lot prepared from one additional passage at *Fundação Oswaldo Cruz* (Fiocruz) in Brazil to replace the working seed. Vaccines produced with the new and the current 17DD seed-lot and those from the WHO 17D substrain were compared among them and with a placebo. Field testing of the vaccine was performed after standard laboratory and animal tests showed that the genetic characteristics of the virus in the new seed-lot had been maintained, and that it was free of avian leucosis virus,[8] and safe in non-human primates. The reactogenicity profile of the vaccine under controlled conditions is an essential reference for routine immunization. It is here reported the results of reactogenicity component of the trial. Data on immunogenicity were published elsewhere.[3]

## METHODS

A randomized, double-blinded, placebo-controlled field trial was carried out to compare seroconversion rates, geometric mean titers (GMT), rates of adverse events, abnormalities in liver enzymes, and levels of

viremia up to 30 days after administering one of three YF vaccines. Liver enzymes and viremia levels were measured to assess viscerotropic effects of the vaccine virus, which might imply a potential for adverse events.

Further data on intervention, blinding, randomization and immunogenicity evaluation were previously described elsewhere.[3]

A physician was available for intervention on acute health problems during immunization procedures. Participants with liver enzymes levels twice or more the upper normal limit were referred for further investigation.

Relevant past and current health conditions, and all signs and symptoms and medical procedures within the 30-day post-vaccination period were ascertained. Moreover, participants were asked to record on a special diary supplied by the study all signs and symptoms occurring in the first 10 days after vaccination.

Serum levels of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT) and alkaline-phosphatase (AP) were measured just before vaccination, between day 4 and 20, and 30 days after vaccination. Viremia was checked in intermediate blood samples using tissue culture methods described elsewhere.[6]

Analyses were conducted for the complete study cohort and for those who met all protocol requirements. The proportion of seroconversion, adverse events and abnormal values for liver enzymes, and the mean of antibody and enzyme levels were compared across comparison groups after immunization. Myalgia was combined with fever and headache in a flu-like syndrome. Statistical significance of differences in proportions was assessed with the Chi-square test, and the differences in means were tested with ANOVA.

The differences between post- and pre-vaccination liver enzyme levels were plotted against pre- and post-vaccination levels, and against the time interval between intermediate and pre-vaccination blood samples to compare patterns in vaccinated and placebo groups.[2]

Determination of the sample size was primarily based on the hypothesis of equivalence in vaccine immunogenicity. For *beta* =0.20, *alpha* =0.05, difference in seroconversion rates admitted for equivalence no higher than five percentage points, 95% seroconversion rate and 10% attrition rate, the required number of participants in each group was 240.[2] With 220 individuals available in each group the study had the power to detect minimum differences of 4.5 percentage points in the proportion of adverse events, if the proportion in the placebo group was 5%. Epi Info 6.04c and SPSS 10 were used for data entry and statistical analysis.[4]

## RESULTS

A total of 1,087 volunteer subjects received one of the vaccines or placebo in 2001. For reasons unrelated to adverse events, six of them missed post-vaccination blood collection. Other three were not available for post-vaccination interview. Protocol violations occurred in four cases. Data on liver enzymes 30 days post-immunization were unavailable for 12 subjects, who were lost to follow-up or had damaged blood samples. Blood was not collected in 21 subjects: two of them, who reported adverse events, belonged to the placebo group; the others reported no adverse events nor had significant abnormalities in liver enzymes in the final test. A total of 668 participants (55% to 63% across comparison groups) returned their diaries of the first 10 days after vaccination.

Males comprised 91.5%, 91.6%, 94.1% and 94.5% of participants in groups assigned to vaccines produced from 17DD-013Z, 17DD-102/84, 17D-213/77 seed-lots and placebo, respectively. The average ages in those groups were 28.1, 29.3, 28.4 and 29.3 years. In the pre-vaccination serologic tests for YF, 22.6% (61 of 270), 28.2% (77 of 273), 21.7% (59 of 272) and 26.1% (71 of 272), respectively, were found to be seropositive.

Among participants who received one of the vaccines and were seronegative before vaccination, 98% or more seroconverted. In the complete cohort 90% or more of the vaccinees seroconverted. The geometric mean titers were similar across vaccinated groups.

There were no reports of severe or immediate adverse events. Four participants were hospitalized within 30 days of vaccination for reasons unrelated to vaccination.

Signs and symptoms on the injection site (Table 1) were reported by no more than 5% among vaccinees and by 2.6% in the placebo group. The risk difference of local signs/symptoms of vaccinees pooled together and placebo controls was 1.6% (p=0.231). Pain was the most frequent local reaction, starting typically on the first day, lasting one day both in the vaccinated and in the placebo groups, and causing no functional limitation of the arm. The risk of local reactions among vaccinees compared to placebo controls was higher among

**Table 1** - Frequency of the main local signs and symptoms following administration of yellow fever vaccines or placebo. Rio de Janeiro, 2001.

| Sign/symptom | Type of vaccine | | | | | | Placebo | | p-value |
| | 17DD-013Z* N=269***** | | 17DD-102/84** N=269***** | | 17D-213/77*** N=269***** | | N=271***** | | |
| | N | % | N | % | N | % | N | % | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Local pain | 9 | 3.3 | 9 | 3.3 | 13 | 4.8 | 7 | 2.6 | 0.550 |
| Erythema | 2 | 0.7 | 2 | 0.7 | 2 | 0.7 | - | | 0.567 |
| Total with local sign/symptoms**** | 9 | 3.3 | 11 | 4.0 | 14 | 5.1 | 7 | 2.6 | 0.443 |

Vaccines produced from:
*New seed lot
**Current seed lot
***WHO seed lot
****Some individuals had more than one sign/symptom
*****Total cohort with data available for analysis

volunteers seronegative in the pre-vaccination test (2.4) than among those who had a seropositive test (1.2) (p=0.23).

The vaccine from the 17D-213/77 seed-lot had the highest, whereas the one from the 17DD-013Z seed lot had the lowest, frequency of systemic adverse events (Table 2). The excess risk of systemic adverse events in the pool of vaccinees compared to placebo controls was 6.8% (p=0.020) (data not shown). Less than half of those events led participants to seek medical care or to interrupt work activities.

Fever, myalgia and headache were the most frequent symptoms. The frequency of flu-like syndrome differed substantially in vaccinees compared to placebo controls. Conversely, nausea/vomiting and all gastrointestinal events occurred as frequently in vaccinees as in placebo controls (Table 2). A number of other signs and symptoms were reported by 49 subjects: urinary tract symptoms (5 cases), sleepiness (4 cases) dizziness (3 cases), and others. No patterns emerged from their distribution across comparison groups.

Fever, headache and myalgia had a median duration of two days in the pool of vaccinees and 1.5 days in the placebo control. Only six participants reported axillary temperature above 38.5°C.

Compared to the placebo group, the maximum risk difference was 2.5% for local adverse events and 7.5% for systemic adverse events. Therefore, 50% of the local events and 34% of systemic events among subjects inoculated with 17D-213/77 vaccsine could have been explained by the vaccine virus. Among subjects who were seropositive before vaccination the difference between vaccinees pooled together and the placebo group was almost nil for the rate of systemic adverse events, and for the rate of local reactions (data not shown).

The frequency of signs/symptoms recorded in diaries was consistently lower, but had a similar pattern of differences vaccine-placebo shown in post-immunization questionnaires.

Viremia was detected in only 2.7% (22 of 815) of vaccinated subjects from day 3 to 7 after vaccina-

**Table 2** - Frequency of main systemic signs and symptoms after vaccination against yellow fever. Rio de Janeiro, 2001.

| Signs/symptoms | Type of vaccine | | | | | | | | p-value |
| | 17DD-013Z* N=269***** | | 17DD-102/84** N=269***** | | 17D-213/77*** N=269***** | | Placebo N=271***** | | |
| | N | % | N | % | N | % | N | % | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Fever | 15 | 5.6 | 8 | 3.0 | 17 | 6.3 | 12 | 4.4 | 0.291 |
| Fever >38.5°C | 12 | 4.5 | 7 | 2.6 | 11 | 4.1 | 6 | 2.2 | 0.170 |
| Headache | 28 | 10.4 | 35 | 12.8 | 25 | 9.2 | 19 | 7.0 | 0.131 |
| Nausea | 7 | 2.6 | 8 | 3.0 | 6 | 2.2 | 8 | 3.0 | 0.945 |
| Any gastrointestinal event | 11 | 4.1 | 13 | 4.8 | 10 | 4.5 | 12 | 3.7 | 0.933 |
| Myalgia | 14 | 5.2 | 18 | 6.7 | 20 | 7.4 | 7 | 2.6 | 0.066 |
| Flu-like syndrome**** | 14 | 5.2 | 19 | 7.1 | 20 | 7.4 | 7 | 2.6 | 0.055 |
| Other symptoms | 23 | 8.6 | 26 | 9.7 | 31 | 11.5 | 17 | 6.3 | 0.190 |
| Total with any systemic adverse event after vaccination | 48 | 17.8 | 57 | 20.9 | 59 | 21.7 | 39 | 14.3 | 0.111 |
| Number with signs/symptoms leading to medical care or work absenteeism | 19 | 7.0 | 19 | 7.0 | 26 | 9.6 | 14 | 5.1 | 0.259 |

Vaccines produced from:
*New seed lot
**Current seed lot
***WHO seed lot
****Defined as myalgia + headache or fever
*****Total cohort with data available for analysis

tion: 3.5% (21 of 596) in subjects seronegative before vaccination, and 0.5% (1 of 197) in those who were seropositive before vaccination (p=0.024). In the subgroup tested within 10 days of vaccination the rates of detectable viremia by vaccine group were 3.0% (17DD-013Z), 3.6% (17DD-102/84) and 6.0% (17D-213/77). Systemic adverse events after immunization were 1.5 times more frequent among participants who had detectable viremia (p=0.213). The levels of liver enzymes were not substantially affected in participants with viremia.

The mean serum levels and the proportions of abnormal levels of liver enzymes before vaccination were well-balanced across comparison groups. The frequency distribution of pre- and post-vaccination serum levels of liver enzymes among vaccinated subjects was indistinguishable from those in the placebo group.

The frequency of abnormal liver enzymes in post-vaccination sera varied across comparison groups as follows: ALT, 1.9%-3.8% (2.7% in placebo group); AST, 0.8%-1.9% (0.8 in placebo group); GGT, 1.5%-3.0% (2.7% in placebo group); AP, 1.5%-6.1% (6.1% in placebo group). Elevations in enzyme levels were moderate (ALT <265 IU/L, AST <420 IU/L), restricted to one enzyme, transient, not accompanied by signs/symptoms, or preceded by pre-immunization abnormal values. Mean values and 95% confidence intervals did not show clinically meaningful nor statistically significant differences. Scatterplots of the differences between post- and pre-vaccination enzyme levels against post-vaccination levels showed similar patterns for vaccinated and placebo groups. There were no apparent patterns in post-immunization enzyme levels nor in post-pre-vaccination differences in enzyme levels plotted against the time interval between vaccination and the second blood sample.

## DISCUSSION

The YF vaccine has been under closer scrutiny in the last few years as clinical and pathological evidence linked the vaccine to severe and previously unrecognized adverse events.[12,24] Safety was an essential element of the performance of vaccines in this investigation and is reported separately so as to cover aspects which have remained scanty in the literature.

The study data, which was presented elsewhere, indicated that the vaccines prepared with WHO-17D strain and the Brazilian 17DD strain induced excellent immunological response, with very high seroconversion rates and antibody titers.[3]

The frequency of signs and symptoms on the injection site reported in the literature varied from nil to 39%.[5,7,9-11,14,15,17,18,20,22,23] In all studies, pain on the site of injection was the most frequently reported local adverse event. The highest frequencies of adverse events were based on diaries, which could inflate the frequency by stimulating reports of trivial signs/symptoms.[5,7,9,10,14,16,22]

The location, timing and nature of signs and symptoms on the site of vaccine injection make it easier to associate them with immunization. Placebo components are known to be able to cause local discomfort and the proportion of local reactions in placebo controls allowed a rough approximation of the role of that vaccine component (Table 1).

There was substantial excess of systemic adverse events beyond that in the placebo group. The most frequent signs and symptoms temporally related to vaccination may have been shared by many other conditions. That may have been a source of variation in the frequency of adverse events reported in published studies, in which the contribution of general morbidity could not be determined.

The flu-like syndrome, which is a more specific condition than its component signs/symptoms, appeared to have the highest frequency beyond that of the placebo group. The magnitude of background morbidity is usually unknown in non-research settings, but routine surveillance is not as sensitive to minor events as active surveillance for research purposes.

Because of the nonspecific nature of adverse events following immunization it is not possible to determine whether signs and symptoms observed in a particular vaccinated subject have been caused by the vaccine. A group of unvaccinated (placebo) individuals provided the reference to estimate the proportion of cases of flu-like syndrome (for instance) added by immunization against YF, beyond those that would have occurred regardless of vaccination. Signs/symptoms that occurred in vaccinees as often as in unvaccinated subjects (placebo) were thought not to be related to the vaccine.

The rates of systemic adverse events reported in the literature also varied widely, from nil to 72%.[1,5,8,10,11,17,18,20-22,23] The rates of systemic adverse events generated by diaries (12%-16%) were within the range reported in some studies using the same approach.[5,10] Nevertheless, figures as high as 41% and as low as 7.6% have been reported.[9,14]

It is difficult to determine to what extent methodo-

logical differences rather than genuine reactogenicity explained the performance of YF vaccines in the studies above. They varied in the age of subjects, sample size, type of vaccine (virus content, stabilization), site of inoculation (arm, thigh, interscapular region), level of inoculation (subcutaneous injection, jet injector), method of ascertainment (questionnaire, diary, physical examination, including measurement of body temperature and inspection of site reactions and lymphadenopathy), and simultaneous administration of other vaccines (measles, cholera, smallpox, hepatitis A), or drugs (chloroquine). A plausible major difference accounting for a substantial part of the variability among studies was the frequency of intercurrent conditions with signs/symptoms temporally related to vaccination.

The effect modification of immunological status prior to vaccination on the reactogenicity of the YF vaccine, suggested by the study data, had been reported before.[16] Lower excess rates (compared to placebo) of adverse events in those subjects with preexisting YF immunity were consistent with more limited virus replication in revaccinated subjects.[15] The results for the whole cohort (intention-to-treat analysis) emphasized the safeguards of randomization against imbalances in distribution by prognostic variables and selection bias that might arise from exclusion of the subjects seropositive before vaccination.

The YF vaccines did not appear to induce abnormalities in liver enzymes. There were no apparent differences between vaccinated individuals and placebo controls regarding the frequency of abnormalities in liver enzymes, and no shifts of clinical significance in the distribution of the whole range of values, indicating alterations of liver function. That converges with data from two previous studies.[5,10] Others[14,18] found small elevations in AST, ALT and GGT, but lacked unvaccinated controls.

It was assembled a large and diverse sample, including subjects with subclinical abnormalities in liver enzymes, which are not exclusion criteria for routine vaccination against YF. In none of those subjects liver enzymes seemed to be affected by vaccination. Levels of liver enzymes varied considerably within the post-immunization period. The variability was similar across vaccination and placebo groups.

The evidence gathered did not support the hypothesis that more common sub-clinical viscetropic effect of the YF vaccines could generate rarer and more severe diseases.

The proportion of participants with detected viremia

was much lower than that found by others.[19,21,25] Viremia is likely to be transitory and thus missed by a single blood collection. Revaccinated subjects did not develop detectable viremia. Low levels, period of occurrence and duration of viremia observed in this study were in accordance with previous findings in the literature.[19,21,25] Systemic events coincided with the period of viremia.

In conclusion, it was apparent that a large proportion of signs/symptoms occurring after immunization against YF could not be attributed to vaccination. The proportion of vaccinated subjects with adverse events grossly overestimated reactogenicity of the vaccine. All signs and symptoms were analyzed so as to detect any adverse event possibly related to the vaccine. Despite the detailed inventory of post-vaccination health events it ended up with the previously known set of signs and symptoms. Unrecognized adverse events should probably require much larger sample sizes.

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## DISCLOSURE

Four of the authors were employed by the vaccine manufacturer (Bio-Manguinhos, *Fundação Oswaldo Cruz*) and three others worked in other units of *Fundação Oswaldo Cruz*. Bias from competing interest was pre-

vented by: (1) participation of members of the Army with expertise in infectious diseases, vaccines, and laboratory virological methods in the Collaborating Group, which conducted the study; and (2) having two independent university professionals knowledgeable in the field of infectious diseases and study designs and analysis examine the study protocol, the setting for laboratory and data processing and analysis.

## REFERENCES

1. Ambrosch F, Fritzel B, Gregor J, Jonas S, Kollaritsch H, Teulieres L et al. Combined vaccination against yellow fever and typhoid fever: a comparative trial. *Vaccine* 1994;12:625-8.

2. Armitage P, Berry G. Statistical methods in medical research. 3rd ed. Oxford: Blackwell Science; 1994.

3. Camacho LAB, Freire MS, Leal MLF, Aguiar SG, Nascimento JP, Iguchi T et al. Immunogenicity of WHO-17D and Brazilian 17DD yellow fever vaccines: a randomized trial. *Rev Saúde Pública* 2004;38(5):671-678.

4. Dean AG, Dean JA, Coulombier D, Burton AH, Brendel KA, Smith DC et al. Epi Info, Version 6. Atlanta, GA: Centers for Disease Control and prevention; 1994.

5. Freestone DS, Ferris RD, Weinberg AL, Kelly A. Stabilized 17D strain yellow fever vaccine: dose response studies, clinical reactions and effects on hepatic function. *J Biol Stand* 1977;5:181-6.

6. Galler R, Pugachev KV, Santos CLS, Ocran SW, Jabor AV, Rodrigues SG, et al. Phenotypic and a molecular analyses of yellow fever 17DD vaccine viruses associated with serious adverse events in Brazil. *Virology* 2001;290:309-19.

7. Gil A, González A, Dal-ré R, Calero JR. Interference assessment of yellow fever vaccine with the immune response to a single-dose inactivated hepatitis A vaccine (144 EL.U.). A controlled study in adults. *Vaccine* 1996;14:1028-30.

8. Hussain AI, Johnson JA, Freire MS, Heneine W. Identification and characterization of avian retroviruses in chicken embryo-derived yellow fever vaccines: investigations of transmission to vaccine recipients. *J Virol* 2003;77:1105-11.

9. Kouwenaar W. The reaction to yellow fever vaccine (17D), particularly in allergic individuals. *Doc Med Geogr Trop* 1953;5:75-84.

10. Lang J, Zuckerman J, Clarke P, Barret P, Kirkpatrick C, Blondeau C. Comparison of the immunogenicity and safery fo two 17D yellow fever vaccines. *Am J Trop Med Hyg* 1999;60:1045-50.

11. Lhuillier M, Mazzariol MJ, Zadi S, Le Cam N, Bentejac MC, Adamowicz L et al. Study of combined vaccination against yellow fever and measles in infants from six to nine months. *J Biol Stand* 1989;17:9-15.

12. Martin M, Tsai TF, Cropp B, Chang GJ, Holmes DA, Tseng J et al. Fever and multisystem organ failure associated with 17D-204 yellow fever vacination: a report of four cases. *Lancet* 2001;358:98-104.

13. Ministério da Saúde. Fundação Nacional de Saúde. Plano de intensificação das ações de prevenção e controle da febre amarela [Plan for intensification of yellow fever prevention and Control activities]. Disponível em URL: http://www.funasa.gov.br/epi/fa/fa0.htm [20 jun 2002]

14. Monath TP, Nichols R, Archambault WT, Moore L, Marchesani R, Tian J et al. Comparative safety and immunogenicity of two yellow fever 17D vaccines (Arilvax and IF-Vax) in a Phase III Multicenter, double-blind clinical trial. *Am J Trop Med Hyg* 2002;66:533-41.

15. Monath TP. Yellow fever. In: Plotkin AS, Orenstein WA, editors. Vaccines. 3rd ed. Philadelphia: W. B. Saunders; 1999. p. 815-79.

16. Moss-Blundell AJ, Bernstein S, Shepherd WM, Langford DT, Ferris R, Kelly A. A clinical study of stabilized 17D strain live attenuated yellow fever vaccine. *J Biol Stand* 1981;9:445-52.

17. Mouchon D, Pignon D, Vicens R, Thanh TH, Tekaia F, Teulières L et al. Étude de la vaccination combinée rougeole-fièvre jaune chez l'énfant Africaine agé de 6 a 10 mois. *Bull Soc Pathol Exot* 1990;83:537-51.

18. Pivetaud JP, Raccurt CP, M'Bailara L, Le Vigouroux A, Le Bras M. Réactions post-vaccinales a la vaccination anti-amarile. *Bull Soc Pathol Exot* 1986;79:772-6.

19. Reinhardt B, Jaspert R, Niedrig M, Kostner C, L'age-Stehr J. Development of Viremia and humoral and cellular parameters of immune activation after vaccination with yellow fever virus strain 17D: a model of human flavivirus infection. *J Med Virol* 1998;56:159-67.

20. Roche JC, Jouan A, Brisou B, Rodhain R, Fritzell B, Hannoun C. Comparative clinical study of a new 17D thermostable yellow fever vaccine. *Vaccine* 1986;4:163-5.

21. Smith HH, Pena HA, Paoliello A. Yellow fever vaccination with cultured virus (17D) without immune serum. *Am J Trop Med* 1938;18:437-68.

22. Soula G, Sylla A, Pichard E, Kodio B, Bentejac MC, Teulières L et al. Étude d'un nouveau vaccin combiné contre la fièvre jaune et la rougeole chez des enfants agés de 6 a 24 mois au Mali. *Bull Soc Pathol Exot* 1991;84:885-97.

23. Tauraso NM, Coultrip RL, Legters LJ, Richman AV, Rosenberg DM, Savadge TO et al. Yellow fever vaccine. IV. Reactogenicity and antibody response in volunteers inoculated with a vaccine free from contaminating avian leukosis viruses. *Proc Soc Exp Biol Med* 1972;139:439-46.

24. Vasconcelos PFC, Luna EJ, Galler R, Silva LJ, Coimbra TL, Barros VLRS et al. Serious adverse events associated with yellow fever 17DD vaccine in Brazil: a report of two cases. *Lancet* 2001;358:91-7.

25. Wheelock EF, Sibley WA. Circulating virus, interferon and antibody after vaccination with the 17-D strain of yellow-fever virus. *N Engl J Med* 1965;273:194-8.

26. World Health Organization -WHO. Adverse events following yellow fever vaccination. *Wkly Epidemiol Rec* 2001;76:217-8.