

Observational study on immune response to yellow fever and measles vaccines in 9 to 15-month old children. Is it necessary to wait 4 weeks between two live attenuated vaccines?



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ABSTRACT

Background: The use of 2 live attenuated vaccines (LAV) is recommended to be simultaneous or after an interval of at least four weeks between injections. The primary objective of this study was to compare the humoral response to yellow fever (YF) and measles vaccines among children vaccinated against these two diseases, either simultaneously or separated by an interval of 7–28 days.

Subjects and methods: A prospective, multicenter observational study was conducted among children aged 9–15 months. The primary endpoint was the occurrence of positive yellow fever antibodies after YF vaccine by estimating the titers of neutralizing antibodies from venous blood samples. Children vaccinated against YF 7–28 days after receiving the vaccine against measles (test group) were compared with children vaccinated the same day against these two diseases (referent group).

Results: Analysis was performed on 284 children. Of them, fifty-four belonged to the test group. Measles serology was positive in 91.7% of children. Neutralizing antibodies against YF were detected in 90.7% of the test group and 92.9 of the referent group ($p=0.6$). In addition, quantitative analysis of the immune response did not show a lower response to YF vaccination when it took place 1–28 days after measles vaccination.

Discussion: In 1965, Petralli showed a lower response to the smallpox vaccine when injected 4–20 days after measles vaccination. Since then, recommendations are to observe an interval of four weeks between LAV not injected on the same day. Other published studies failed to show a significant difference in the immune response to a LAV injected 1–28 days after another LAV. These results suggest that the usual recommendations for immunization with two LAV may not be correct.

Conclusion: In low income countries, the current policy should be re-evaluated. This re-evaluation should also be applied to travelers to yellow fever endemic countries.

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1. Introduction

Yellow fever (YF) can be prevented by a 17D strain live attenuated vaccine (LAV). After vaccination, the neutralizing antibody response is obtained in 80–95% of immunocompetent subjects

[1–6] and is considered protective from 10 days after vaccination [7] for several decades [8,9].

The YF vaccine is well tolerated [10,11]. Rare cases of the viscerotropic form of the disease following vaccination have been described [12–16] and groups at risk identified [17]. Except during epidemics, the vaccine is not recommended before 9 months. It is contraindicated for below 6 month children, in case of egg allergy [18] and in severely immunocompromised subjects [19,20].

In Senegal, the YF vaccine is administered as part of the expanded program on immunization (EPI) to 9 month-old children simultaneously with the first dose of monovalent measles vaccine.

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In French Guiana, the YF vaccine is compulsory at the age of 12 months.

Measles can also be prevented by a LAV, administered standalone or in combination with vaccines against rubella, mumps and rubella or varicella. In Africa, widespread vaccination against measles has significantly dropped mortality rates in countries where the EPI has been strengthened [21,22].

Two different LAV should be administered simultaneously or at least after 4 weeks interval [23]. This recommendation is based on the assumption that the interferon response following the injection of the first LAV could reduce the response to the second LAV [24,25]. In some circumstances, this recommendation cannot be followed: vaccine stock shortages, unplanned travel to YF endemic areas for example.

In Brazil, a study evaluating the effectiveness of a YF vaccine administered 1–28 days after measles vaccination showed no difference on antibodies titers [26].

The present study aimed to compare the humoral response to YF and measles vaccines in children aged from 9 to 15 months, routinely vaccinated against these two diseases, either with an interval of 7–28 days (Group1=test group) or simultaneously (Group2=referent group).

2. Subjects and methods

2.1. Study design

This prospective, multicenter (French Guiana, Senegal) observational study compared groups of children vaccinated against YF and measles either simultaneously or at an interval of 7–28 days in the context of every day practice.

All children 9–15 months old, resident in Senegal or French Guiana not vaccinated against YF and consent form signed by the legal guardian were eligible.

The day of immunization against YF was the day of inclusion in the study. The children who, for any reason, had been vaccinated against measles 7–28 days before inclusion were assigned to test group. Children vaccinated the same day against YF and measles were assigned to referent group. Exclusion criteria were a contraindication to one of the vaccines used, a known chronic disease or an acute infection present on the day of vaccination.

The main endpoint was the YF neutralizing antibody titer and the comparison of antibody response in the two groups of children (correlate of protection = titer $\geq 1:10$ for YF). These titrations studies were performed on venous blood samples taken between 28 and 60 days after YF vaccination. The occurrence of adverse events following YF vaccination was also documented.

2.2. Study conduct

The study was carried out at Cayenne (French Guiana) and Dakar (Senegal). The vaccines used for YF vaccination were Stamaryl® (Sanofi Pasteur, France) in French Guiana and the YF vaccine produced by the Institut Pasteur in Dakar (Vaccin Amaril Stabilise®) in Senegal. For measles, trivalent vaccines against measles, mumps and rubella (Priorix® or M-M-Rvaxpro®) were used in French Guiana. A monovalent vaccine (Rouvax®) was used in Senegal.

For every child included, two visits were planned: one at inclusion (V1), the day of vaccination with the YF vaccine, and a final follow-up visit 28–60 days after vaccination (V2). During V1, the physician had to check for the absence of exclusion criteria and perform anthropometric measurements. At V2, he reviewed the occurrence of adverse events and took a sample of venous blood for the YF and measles serologies. 1–2 ml of whole blood were

collected. Serological analysis were performed by the Virology units of the Institut Pasteur in Dakar and in French Guiana.

2.3. Serological tests

The two laboratories used standardized plaque reduction neutralization test (PRNT) protocol and interpretation criteria. PRNT represents a sensitive, reproducible and functional method to measure YF neutralizing antibodies [27,28]. Briefly, employing a cell culture and carboxymethyl cellulose overlay procedure, a defined virus test dose (d) of 10^3 plaque-forming units (PFU)/ml was used to prepare the following dilution range of virus: d50, d70, d90, d95 and d99. The neutralizing capacity was calculated from virus test dose (d) yielding 30 plaque-forming units of virus (PFUVs) and the cut-off for protection was defined at d90 virus dilution. First we tested capability of diluted serum (1:10) to neutralize YFV dilution d90. In a second step we determined, using two-fold serum dilutions (from 1:20 to 1:640), the last dilution of serum that neutralized YFV dilution d90. This limiting dilution represents the antibody concentration causing a 90% reduction. Antibody results for YFV PRNT were expressed quantitatively using the titration of neutralizing antibodies.

For measles, a commercially available indirect ELISA kit (Captia™ Measles IgG, TRINITY Biotech, Bray, Ireland) was used to detect anti-measles IgG antibodies.

Antibody results for MV and YFV were expressed qualitatively (positive/negative) for statistical analysis.

2.4. Adverse events

Adverse events were searched at V2, using a standardized questionnaire (fever, nausea, pain at injection site, etc.). Serious adverse event, linked or not to the vaccination, were reported using a standardized form of the French National Agency for the Safety of Medications and Health Products – ANSM [23]. For Senegal, serious adverse events were reported to the Department of Pharmacies and Laboratories and the National Committee on Health Research Ethics. In French Guiana, they were reported to the Regional Center for Drug Safety Monitoring.

2.5. Sample size calculation

The sample size calculation was based on YFV seroconversion rate of 95% in the referent group and of 80% in the test group. Based on the ratio Group1/Group2 = 1/5, the total number of children to include was estimated at 270 (45 for Group1 and 225 for Group2), for a power $1 - \beta = 80\%$.

Expecting a proportion of subjects lost to follow up of 20%, 60 children in Group1 and 280 children in Group2 had to be included.

2.6. Processing and statistical analysis of data

Data were recorded on a standardized case report form then entered in computerized database using Epi-Info™ 3.5.1 software (CDC, Atlanta, USA). Data analysis was performed using Stata® 10 software. The Chi-2 test or Fisher's exact test were used to compare qualitative variables. Student's *t*-test or the Mann–Whitney test were used to compare quantitative variables. Univariate and multivariate models (logistic regression) were used to identify covariates associated with a negative response to the YFV measured by PRNT. For the multivariate analysis, a step-by-step backward method was used. Nested models were compared using the likelihood ratio test. The adequacy of the models was measured using the Hosmer–Lemeshow test.

Subgroups analyses were performed. First, the children of the test group were divided into two subgroups: children vaccinated

against YF 7 or 14 days after measles vaccination and children vaccinated against YF 21 or 28 days after measles vaccination. The first subgroup analysis consisted of a successive comparison of immune responses against YF in these different subgroups. In a second phase, the children of the test group were divided into two subgroups, one composed of children vaccinated against YF 7 days after measles vaccination, and the other composed of children vaccinated against YF more than 7 days after measles vaccination.

For statistical analyses, the significance level was set at 5%.

2.7. Ethics and regulatory aspects

The parents or legal guardian of the children were informed about the objectives and constraints of the study, the possible risks incurred, their right to refuse to participate in the study and the possibility of withdrawing from the study at any time. All this information was presented on a consent form. In French Guyana, the questionnaires and informed consent forms were translated into French, English, Spanish and Portuguese. In Senegal, these documents were only in French. Written informed consent of the parents or legal guardian of the child was obtained before inclusion.

The study was approved by the Institutional Review Board of the Institut Pasteur and by the ethics committees of each of the countries concerned. Ethical and administrative authorizations were obtained from the competent authorities (the National Council for Studies and Research on Health in Senegal, *Commission Nationale de l'Informatique et des Libertés* and the competent regional ethical committee for French Guiana).

3. Results

Out of the 417 children consulting for yellow fever vaccination, 374 (89.7%) were initially included from July to December

2009 for Senegal and from November 2009 to May 2010 for French Guiana. Statistical analysis of the collected data included 284 children (75.9% of the children initially included, Fig. 1).

3.1. Sample description

Of the 284 children included in the statistical analysis, 255 (89.9%) were included in Senegal and 29 in French Guiana. Fifty-four (19.0%) children belonged to the test group.

The sample was made up of 135 boys (47.5%) and 149 girls (52.5%). The distribution of the sample according to gender was not associated to the group (test vs. referent: see complementary tables) nor to the inclusion site ($p=0.48$). Age at inclusion varied from 9 to 14 months. The anthropometric measurements performed at baseline indicate that the children from French Guiana were older (mean: 10.5 vs. 9.2 month; $p < 10^{-3}$), heavier (9.6 vs. 8.9 kg; $p=0.01$), longer (74.3 vs. 69.7 cm; $p < 10^{-3}$) and had a lower body mass index (17.4 vs. 18.4; $p=0.04$) than the Senegalese children.

3.2. Groups comparison

The results of the comparative analysis of the groups are summarized in complementary tables.

None of the adverse events investigated in the questionnaire was associated with the group. Comparison of neutralizing antibody titers did not show any significant difference between the groups ($p=0.6$). For the measles serology, a sensitivity analysis was performed which first considered that the 6 questionable results were positive, and then, that these 6 questionable results were negative. None of these two simulations showed significant association between the group and the measles serology results ($p=0.54$ and $p=0.17$, respectively).

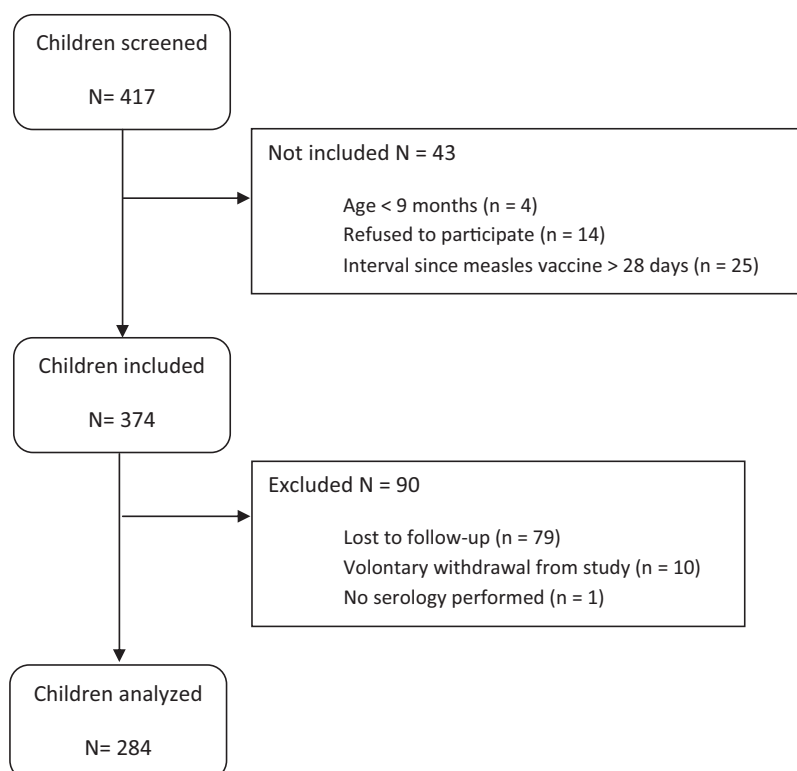


Fig. 1. Flowchart of children included.

3.3. Vaccines, tolerance and immunogenicity

The occurrence of at least one adverse event was reported in 11 children (3.9%). Local adverse reactions (LAR) were reported in 5 children, and generalized adverse reactions (GAR) in 8 children: nausea ($n = 3$), fever ($n = 2$), cutaneous rash or pruritus ($n = 2$), allergy ($n = 1$). Two children presented both one LAR and one GAR. The occurrence of a LAR was not associated with the inclusion site ($p = 0.08$). However, 7 GARs were reported in children included in French Guiana compared to one reported in Senegal ($p < 10^{-3}$). All these adverse events were considered to be non-serious by the physician who carried out the follow-up visit.

For YF, the quantity of blood collected was insufficient for 5 children. The test for neutralizing antibodies was then performed among 279 children and positive in 92.5% (258/279). The proportion of positive tests was not statistically linked to the inclusion site (91.8% in Senegal vs. 100% in French Guiana – $p = 0.14$).

The average interval between YF vaccination (V1) and the follow-up visit, during which the blood sample was taken, was 35

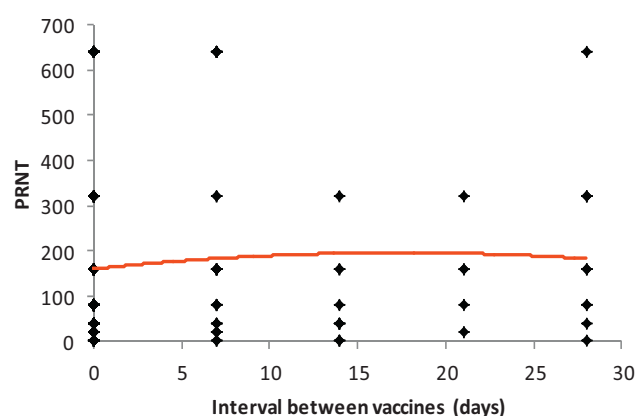


Fig. 2. Qualitative Plaque reduction neutralization tests (PRNT) to yellow fever by vaccination interval between vaccinations and trend line.

Table 1

Qualitative PRNTs^a to yellow fever by study site and vaccination group.

Serum dilution	Study site				p value	Group				p value
	French Guiana		Senegal			Test		Control		
	n	%	n	%		n	%	n	%	
0	0	0	21	8.2	0.40	5	9.3	16	7.1	0.30
20	2	8.3	37	14.5		5	9.3	34	15.1	
40	6	25.0	34	13.3		7	13.0	33	14.7	
80	5	20.8	55	21.6		8	14.8	52	23.1	
160	6	25.0	51	20.0		14	26.0	43	19.1	
320	3	12.5	28	11.0		7	13.0	24	10.7	
640	0	0	19	7.4		7	13.0	12	5.3	
>640	2	8.3	10	3.9	1	1.8	11	4.9		

^a Plaque reduction neutralization tests.

Table 2

Study of factors associated with the results of plaque reduction neutralization test (PRNT) against yellow fever.

	PRNT				Univariate analysis		Multivariate analysis		p value
	Positive		Negative		Odds ratio	p value	Odds ratio	95% CI	
	N	%	N	%					
Group						0.59			0.50
Test	49	90.7	5	9.3	0.75		0.70	0.5–4.1	
Referent	209	92.9	16	7.1	1		1	–	
Inclusion site						0.14			
French Guiana	24	100	0	0.0	–				
Senegal	234	91.7	21	8.2					
Gender						0.97			
Male	124	92.5	10	7.5	1				
Female	134	92.4	11	7.6	0.98				
Local adverse reaction						0.52			
No	253	92.3	21	7.7	–				
Yes	5	100	0	0.0					
General adverse reaction						0.45			
No	251	92.3	21	7.7	–				
Yes	7	100	0	0.0					
Breastfeeding						0.26			
No	15	100	0	0.0	–				
Yes ^c	243	92.0	21	8.0	–				
Measles serology ^a						0.45			
Negative	14	87.5	2	12.5	1				
Positive	240	92.7	19	7.3	1.80				
Measles serology ^b						0.79			
Negative	20	90.9	19	9.1	1				
Positive	234	92.5	2	7.5	1.23				

^a Simulation a: questionable serologies reclassified as positive serologies.

^b Simulation b: questionable serologies reclassified as negative serologies.

^c Mixed or exclusive breastfeeding.

Table 3

Study of factors associated with the results of plaque reduction neutralization test (PRNT) against yellow fever.

	PRNT				Univariate analysis		Multivariate analysis		
	Positive		Negative		Odds ratio ^b	p value	Odds ratio ^b	95% CI	p value
	A	SD ^a	A	SD ^a					
Age (months)	9.3	0.9	9.2	0.6	1.08	0.77			
Weight (kg)	9.0	1.3	8.7	1.2	1.23	0.23			
Length (cm)	70.2	3.2	69.1	2.8	1.12	0.11	1.34	0.98–1.31	0.09
BMI ^a	18.3	2.6	18.1	2.1	1.03	0.74			
Head circumference (cm)	45.4	1.5	45.7	1.4	0.89	0.44			
Brachial circumference (cm)	15.7	1.4	15.7	1.5	1.03	0.82			
Interval YFV – serology (days)	34.7	7.6	36.4	7.4	0.97	0.30			
Interval MV – YFV (days)	2.6	7.2	3.3	6.5	0.98	0.62			

^a A, average; SD, standard deviation.^b Measures the increased likelihood of obtaining a positive result for an increase of one unit of the variable studied.

days (28–61 days). This interval was statistically longer in French Guiana (38.3 days) than in Senegal (34.6 days – $p=0.02$). Nevertheless, the results of the test for neutralizing antibodies were not associated with the interval between the two visits ($p=0.30$).

The quantitative analysis of the immune response against YF (PRNT following successive dilutions of the serum) is summarized in Table 1 and Fig. 2. It did not show any statistically significant difference between inclusion sites ($p=0.14$), or between groups ($p=0.60$).

Results for the anti-measles IgG test were available for 278 children. Serology was positive in 91.7% of the children (255/278), negative in 6.1% of cases (17/278) and questionable in 6 children.

3.4. Factors associated with PRNT results

3.4.1. For the entire sample

Results are presented in Tables 2 and 3. Only the group (variable kept in the model whatever the statistical results) and length variables were kept in the final model. The length of the child was close to the $p=0.05$ significance threshold. The final model was poorly predictive of the immune response measured by a positive neutralization test (area under ROC curve: AUC=0.60). By keeping only the group variable, the AUC dropped to 0.53. This means that this statistical analysis did not objectivize any weaker response to YF vaccination when it took place 7–28 days after measles vaccination.

3.4.2. Subgroup analysis

The number of children of the test group vaccinated against YF 7, 14, 21 or 28 days after measles was 29, 9, 5 and 11, respectively. There was no significant difference in PRNT results between the subgroup of children vaccinated against YF 7 or 14 days after measles vaccination ($N=38$) and the subgroup of children vaccinated after 21 or 28 days ($N=16$) ($p=1.0$). There was

no significant difference in PRNT results between the subgroup of children vaccinated at day 7 and the subgroup of children vaccinated at an interval of 14–28 days ($p=0.65$). There was no significant difference in PRNT results between the subgroup of children vaccinated at day 7 or day 28 and the subgroup of children vaccinated at day 14 or day 21 ($p=0.59$).

PRNT results for each subgroup of the test group vs. referent group are presented in Table 4. None of the subgroup analyses observed a difference in the immune response against YF (PRNT) compared to the reference group.

4. Discussion

In 1965, Petralli et al. showed that live attenuated vaccines induced the production of interferon in recently vaccinated subjects [24]. They also showed that the response to a smallpox vaccine administered 4–20 days after measles vaccination was weaker [29]. Since then, recommendations have been to observe an interval of four weeks between two LAV not injected on the same day [30]. These recommendations can be problematic both in individual cases and during mass vaccination campaigns carried out in response to an epidemic situation. A study published in 1987, which aimed at evaluating the interference between the oral poliomyelitis vaccine and the measles vaccine injected at an interval of less than two weeks, did not show any decrease in the humoral response to the measles vaccine [31]. Similarly, Stefano et al. concluded that a recent immunization against measles did not interfere with the humoral response to the YF vaccine [26]. However, in that study, the seroconversion rate against YF (77.5%) was lower than in the rest of the literature, which calls into question the validity of the results. In our study, the seroconversion rate was approximately 92% for both measles and yellow fever vaccines and coincided with published data [11], which constitutes a strong point compared with the Brazilian study [26].

Whichever way the data of our study were analyzed, either for the full sample or in subgroup analyses, no significant difference in the antibody response against YF was observed between the test and the control groups.

Among the limits to our study, it should be noted that it was decided, for ethical reasons, not to perform a pre-vaccination serology, since in Senegal, venous samples were obtained from the femoral vein (unfavorable benefit/risk ratio). Another limit is linked to the fact that in French Guiana, only one child was included in the test group. This was due to the fact that, in French Guiana, physicians at the study site strictly follow the recommendations in everyday practice.

In addition, because immunizations were performed once a week, the present study only assessed humoral response to YF and measles in children vaccinated with an interval of 7–28 days instead of 1–28 days. Last, adverse effects were captured only at

Table 4Qualitative PRNTs^a to yellow fever by group and vaccination interval.

	Numbers ^b ($N=279$)	Positive PRNT (%)	p value ^c
Referent group (simultaneous vaccinations)	225	92.9	
Test group (7–28-day vaccination interval)	54	90.7	0.60
Subgroups			
Vaccination interval < 15 days	38	89.5	0.50
Vaccination interval $\geq$ 15 days	16	93.7	1.0
Vaccination interval = 7 days	29	93.1	1.0
Vaccination interval > 7 days	25	88.0	

^a Plaque reduction neutralization tests.^b For which results of PRNT are known = $284 - 5$ (insufficient quantity of serum).^c Comparison between the different subgroups vs. the referent group.

V2 and may result in an underestimation of these adverse effects. The higher prevalence of side effects notified in French Guiana can be explained by the fact that in French Guiana parents are probably more available, have better access to medical care and are also more aware of the importance of reporting side effects than in Senegal.

The main strong point of this study is the fact that vaccination practices at the participating study centers were not modified for the study. It presents a true picture of the difficulties encountered on a daily basis in health centers in YF endemic countries. The serological results, though obtained in two different laboratories, were comparable thanks to the standardization of laboratory methods used at the two study sites. In particular, the PRNT technique used for YF was developed and used in the two virological laboratories.

5. Conclusion

The present study did not show any difference in the humoral immune response to vaccination against YF according to whether it was injected on the same day or within 7–28 days following measles vaccination. These results suggest that the usual recommendations concerning vaccination with two LAV are perhaps not relevant and that the current policy should be re-evaluated.

By proposing the hypothesis that the interval between two vaccinations with LAV has no consequence on the humoral immune response, this study could make it possible to bypass current recommendations and thus facilitate the management of subjects who need to be vaccinated quickly (epidemics, unplanned travels).

By allowing flexible vaccination schedules with LAV, such an approach could contribute to improving vaccine coverage in YF endemic countries or among travelers.

It is important to note that the present study is an observational study. Consequently, the results presented in this paper need to be confirmed by complementary studies. It would be useful to perform studies aiming to evaluate the antibody response at some point during the ten years following YF vaccination, by comparing subjects vaccinated simultaneously with others vaccinated with two LAV separated by a 7–28-day interval. Randomized controlled clinical trials may also be required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2015.03.069>.

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