Vaccine 37 (2019) 7147–7154

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Duration of post-vaccination humoral immunity against yellow fever in children

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ARTICLE INFO

Article history:
Received 24 June 2019
Received in revised form 11 September 2019
Accepted 12 September 2019
Available online 4 October 2019

Keywords:
Yellow fever vaccine
Immunogenicity
Vaccination policy
Duration of immunity in children

ABSTRACT

Introduction: Vaccination is the most important measure for prevention and control of yellow fever. It is recommended by the World Health Organization (WHO) for residents of endemic areas and travelers to risk areas. In 2013, the WHO discontinued the recommendation of booster doses every 10 years, indicating a single dose as sufficient for lifelong protection.
Objective: Considering the lower immune response to YF vaccine in children compared to adults, this study was set out to assess the duration of immunity to YF in children vaccinated in the first two years of life.
Methods: This cross-sectional study involved children aged 9 months to 12 years with accessible vaccination records recruited in primary care units from a metropolitan area in Southeast Brazil. The serologic status (negative, indeterminate and positive), and geometric mean titers (GMT, inverse dilution) of neutralizing antibodies against YF obtained by Plaque Reduction Neutralization Test was assessed across categories of time after YF vaccination. The strength of association of seropositivity with time was assessed by the odds ratio (OR) taking recent vaccination (1–6 months) as reference.
Results: A total of 824 children recruited from August 2010 to July 2011were tested. The proportion of seropositivity (95% C.I.) and GMT (95% C.I.) dropped markedly across time periods: from 86.7% (80.5–91.4%), GMT 47.9 (38.3–59.9) in newly vaccinated to 59.0% (49.7–67.8%), GMT 14.8 (11.6–19.1) and 42.2% (33.8–51.0), GMT 8.6 (7.1–12.1), respectively in the subgroups vaccinated 31–72 months and 73–100 months before.
Conclusions: Analogous to previous findings in adults, these data support the need for revaccination of children living in areas with yellow fever virus circulation in humans or in other primates. The data also supported the change of a booster dose to 4 years of age for those primarily vaccinated for yellow fever in the first two years of life.

1. Introduction

Yellow fever is an acute infectious disease, caused by an arbovirus (genus *Flavivirus*), with wide variability of clinical presentations. Roughly 90% of cases are mild and asymptomatic, and probably underreported. Severe forms of the disease represent about 12% of the cases, with an average lethality of 50% [1,2].

Considering the number of cases and deaths caused per year in endemic areas, besides the fact that its eradication is not feasible due to the wildlife reservoir [3], yellow fever has a considerable epidemiological relevance.

Yellow fever is endemic in 43 countries in tropical regions of Africa and Central and South America [4], reaching areas that had been free of the disease for several decades. Expansion of yellow

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https://doi.org/10.1016/j.vaccine.2019.09.051
0264-410X/

fever has been limited to those 43 endemic countries, where outbreaks have erupted sporadically in the last decades. In 2016, two linked urban yellow fever outbreaks – in Angola (3137 suspected cases, 345 deaths) and the Democratic Republic of Congo (700 suspected cases, 63 deaths) exported cases to other countries [5]. In South and Central America, it is endemic in 13 countries, its incidence is seasonal and is related to high levels of rainfall, humidity and temperature [6]. In Brazil, only sporadic cases and small outbreaks of wild yellow fever had been described in the last decades [7], but in the 2016–2017 and 2017–2018 seasonal periods, 778 human cases (262 deaths) and 1376 human cases (483 deaths), respectively, were reported. Those outbreaks of the disease occurred close to large urban centers infested by *A. aegypti*, with a considerable portion of the population vulnerable to yellow fever, thus posing a risk of urbanization if high vaccine coverage were not reached.

Considering the epidemiologic relevance of yellow fever, the International Health Regulations demands the immediate notification of yellow fever cases to the World Health Organization (WHO) and an international vaccination certificate [8]. This certificate is required for many countries worldwide as a proof of previous vaccination against yellow fever, particularly for residents and travelers to yellow fever endemic areas.

There is no specific treatment for yellow fever and vaccination is the most effective measure for its control [7]. Licensed yellow fever vaccines in the world are attenuated live virus vaccines, with safety and effectiveness evidenced by the reduction in the number of cases of the disease following mass vaccination campaigns [9]. Seroprotection correlates have not been established for humans; however, the role of neutralizing antibody titers obtained after vaccination in predicting susceptibility to infection is well known, allowing the extrapolation of immunogenicity data in the measurement of vaccine efficacy [10].

Currently, vaccination against yellow fever is indicated in a single dose for residents of disease risk areas and travelers from or to those areas, aged 9 months or older. According to a WHO recommendation published in 2013, one dose of the yellow fever vaccine would be sufficient to provide lifelong protection in the general population [6]. As a result, in 2014, the International Health Regulations were revised, removing the requirement for revaccination against yellow fever every 10 years. However, a study conducted in adults in Brazil demonstrated a gradual drop in neutralizing antibody titers 4 years after a single dose of the vaccine, so that approximately 10 years after the first vaccination, 76% of the subjects had titers considered as "protective" [11]. There are no data on the duration of immunity when the yellow fever vaccine is administered during the first two years of life, when the immunogenicity of the vaccine is known to be lower [12,13].

The present study aims to study the persistence of immunity against yellow fever after the first yellow fever vaccine dose in children aged two years or less. In this article we will present the analyzes of neutralizing antibodies. The results regarding cellular immunity will be analyzed in another article.

2. Methods

This is a sectional study designed to compare the proportion of seropositivity and the geometric mean serum titers of neutralizing antibodies against yellow fever in children aged 9 months to 12 years, vaccinated with only one dose of the yellow fever vaccine, administered in the first two years of life, and categorized into six study groups according to the time since vaccination: (I) 30–45 days, (II) 1 year, (III) 2 years, (IV) 4 years, (V) 7 years, and (VI) 10 years. These ranges were arbitrarily defined to guide the inclusion of participants, in order to obtain a representation of the variation of the serological status. There being no biologically defined limits for these categories, the ranges included times dispersed around the central value taken.

The study population consisted of children residing in areas where the yellow fever vaccine is included in the basic immunization schedule and who received the vaccine through routine care at basic immunization units in the first two years of their life, and were not revaccinated. The yellow fever vaccine available in the public health services in Brazil is the 17DD strain produced by Bio-Manguinhos and administered subcutaneously. The release of vaccine lots is calibrated so that each 0.5 mL dose contains the minimum of 1000 MLD_{50} (equivalent to approximately 5000 PFU). The dose effectively applied depends on the lot, the time of manufacture and the storage conditions, but the stability of the vaccine and the cold chain allow us to assume the doses are, overall, much higher than the minimum.

The sites for research participant selection were the municipalities of Ribeirão das Neves and Contagem, in the metropolitan region of Belo Horizonte, Minas Gerais, Brazil. These municipalities had already taken part in previous studies with the yellow fever vaccine, because they have the conditions conducive to carrying out studies of this type, such as large volume of vaccination of infants and human resources with availability and interest in participating in research projects.

2.1. Eligibility criteria

Eligible participants were children of both sexes, aged 9 months to 12 years, with primary vaccination against yellow fever between 9 and 23 months of age, with post-vaccination time of about 30–45 days, 1 year (12–15 months), 2 years (24–27 months), 4 years (48–51 months), 7 years (84–87 months), and 10 years (120–123 months), whose legal guardians signed the informed consent form. The date of vaccination was obtained from records at health facilities and in vaccination cards brought by legal guardians of research participants at the time of the enrollment interview.

Children with autoimmune diseases, transient or permanent immunosuppression, hemoglobinopathies, and children with a history of blood transfusion or treatment with hyperimmune serum up to 90 days prior to blood collection were not included.

2.2. Data collection

For each research participant, a peripheral blood sample of about 4 mL was collected in a tube containing sodium heparin as anticoagulant, to be used for cellular immunity analyzes, provided for in the research protocol.

In addition to blood collection, a questionnaire was used to collect demographic data, vaccination history, with confirmation of dates of the last doses in the vaccination card; current health problems and use of medications; pathological background; and trips made after yellow fever vaccine administration. All procedures were conducted only after Informed Consent was obtained and the form signed by legal guardians. The study was conducted in accordance with fundamental ethical principles of the Declaration of Helsinki [14] and the Brazilian National Health Council on research involving human beings [15]. The study protocol was approved by the Research Ethics Committee of the National School of Public Health - Fiocruz (CAAE: 45946915.1.0000.5240).

2.3. Laboratory testing

In order to optimize the blood collection in children, for analysis of humoral and cellular immunity, a primary tube containing anticoagulant was used and, therefore, titrations of neutralizing antibodies were obtained in heparinized plasma with the assumption

that they would render the same results as serum. However, a post hoc check comparing the titers of neutralizing antibodies obtained in serum, heparinized plasma and EDTA plasma showed that the titers were found to be higher with heparinized plasma. However, the correlation was not high enough to allow the derivation of a correction factor to compare with data from other studies that used serum. Thus, the samples were reanalyzed after establishing a plasma heparin removal protocol prior to performing the yellow fever serum neutralization tests.

In that protocol, the plasma samples were treated with the anion exchange resin Epichlorohydrin triethanolamine-cellulose (Ecteola-Cellulose®), to remove heparin and minimize its effects on the measurement of neutralizing antibodies. The protocol for the removal of heparin from plasma samples, previously applied to serum neutralization tests for yellow fever, was developed and validated by the René Rachou Research Center/Fiocruz and Bio-Manguinhos Virological Technology Laboratory/Fiocruz [16].

The neutralizing antibody titers were determined by a neutralization test set at 50% reduction of the lysis plaques (PRNT50), conducted at the Bio-Manguinhos Virological Technology Laboratory/Fiocruz according to the procedure described by Simões et al [17]. From the cut-off point established during the validation of the heparin removal method using Ecteola-Cellulose® (1:10), the study participants were classified into 3 groups according to antibody titers (reciprocal of the dilution): (i) seropositive - greater than or equal to 10; (ii) indeterminate - greater than or equal to 5 and less than 10; (iii) seronegative - below 5.

2.4. Statistical considerations

The sample size was calculated to estimate the expected 85% seropositivity rate in children [18], with 95% confidence limits set at 5 percentage points (80–90%). Approximately 200 volunteers would be required in each of the groups of time after vaccination. Assuming a 85% seropositivity in the reference group, that is, 30–45 days of vaccination, beta equal to 0.1 and alpha equal to 0.01 (to compensate for multiple analyzes), 200 individuals in each group would allow the detection of a difference of at least 20 percentage points in relation to the other subgroups.

The following were excluded from the analysis: those who did not meet the requirements of the study protocol in relation to (i) inclusion age outside the range of 9 months to 12 years; (ii) age of first vaccination against yellow fever of less than 9 months and more than 23 months; (iii) clinical eligibility criteria such as autoimmune diseases; disease-induced transient or permanent immunosuppression or treatment; hemoglobinopathies; history of blood transfusion or treatment with hyperimmune serum within 90 days prior to blood collection.

The response variable of interest is the serum titer of neutralizing antibodies, in reciprocal of the dilution, transformed into base 10 logs. Therefore, only those individuals with availability for PRNT after the heparin removal protocol with Ecteola-cellulose® were analyzed. From the response variable analysis, research participants were classified according to their serological status as "seropositive", "indeterminate" and "seronegative". The proportion of seropositivity and mean antibody titers were estimated, with their respective 95% confidence intervals, for each subgroup of time since vaccination. The geometric mean of neutralizing antibody titers against yellow fever was also calculated and, to that end, titers below the detection threshold of the method (less than "5" in the reciprocal of the dilution) were substituted for "2.5" (midpoint between "0" and "5"). Antibody titers "greater than 640" were substituted for the dilution value immediately above, 1280.

For the analysis of differences in immune response between the comparison groups, the main explanatory variable of interest was time in months after vaccination. Time was analyzed as a continuous variable and later classified into categories established from observation of the distribution of post-vaccination times, in order to accommodate the variations observed in the volunteers regarding the groups of time listed in the protocol. Thus, the categories applied to the variable time after vaccination were: "0–6 months" (approximation of the reference category from 30 to 45 days) - Group I; "7–18 months" (approximately one year after vaccination) - Group II; "19–30 months" (approximately 2 years after vaccination) - Group III; "31–72 months" (approximately 4 years after vaccination) - Group IV; "73–100 months" (approximately seven years after vaccination) - Group V; and "over 100 months" (approximately 10 years after vaccination) - Group VI. Flexibilization in the categorization of vaccination times considered that the cutoff points of this continuous variable were defined by the inflection point of the distribution curve, allowing more internally homogenous subgroups to be better delimited.

The group "0–6 months after vaccination" (Group I) was taken as a reference for comparison of the proportion of seropositive participants for the other groups of time after vaccination. Statistical significance for differences in the proportions of seropositivity between the times was assessed by Pearson's chi-square test. The distribution of the logarithms (base 10) of the antibody titers was compared in box-plot graphs. The geometric means of the antibody titers in subgroups one year and more after vaccination were compared to the geometric mean of the titers in the 0–6 months subgroup.

A multivariate analysis of serological status was used to adjust the effect of covariates of interest on the association between serological status and time of vaccination. The immunological response to the yellow fever vaccine (response variable), indicated by the log10 antibody titer (reciprocal of the dilution) in the multiple regression model, and by the seropositivity in the logistic regression model, was evaluated as a function of time since the primary vaccination (explanatory variable), presented continuously and categorically (0–6 months, 7–18 months, 19–30 months, 31–72 months, 73–100 months, over 100 months). The covariates included in the model were: age; sex; receipt of attenuated virus injectable vaccines, simultaneously or within 30 days prior to vaccination against yellow fever; age at the most recent dose of the vaccines against tuberculosis, hepatitis B, polio, diphtheria-tetanus-pertussis, measles-mumps-rubella, rotavirus and others; history of severe diseases; comorbidities; history of travel to areas at risk of yellow fever transmission, or areas with vaccination recommended, since the date the child was vaccinated against yellow fever until the date of the immunogenicity assessment of the present study. "History of severe illness" was considered to be health problems that led to hospitalization for more than one day and/or represented a risk of imminent death or permanent sequelae, according to the report of legal guardians of the research participants. In the multiple linear regression analysis, the response variable (antibody titers against yellow fever in the reciprocal of the dilution) was entered in log 10 to correct asymmetries in the distribution of the serological data. At first, there was a complete model, including the main explanatory variable "time after vaccination" and all covariates. However, some of these variables had many missing data and did not make it into the modeling. From the initial model, the variables that did not reach statistical significance ($p < 0.05$) were excluded, until the final model was selected.

Multivariate analysis was also performed by logistic regression, considering as a response variable the presence or absence of seropositivity to the yellow fever vaccine, according to the time after vaccination. The explanatory variables considered in the models were the same ones evaluated by multiple linear regression. Modeling was started with the complete model, including the categorical explanatory variable "time after vaccination"

(the reference category was the group vaccinated 30–45 days ago) and the possible covariates for insertion into the model.

Statistical analysis was performed using the statistical software IBM SPSS Statistics 20.0 [19] and R version 3.3.1 [20].

3. Results

In the period from August 2010 to July 2011, 1195 research participants were included in four health units in the municipalities of Ribeirão das Neves and Contagem (State of Minas Gerais, Brazil). Of these, 57 participants who did not comply with the study protocol were excluded from the analysis because they were older than 12 years old at the time of blood collection or older than two years at the time of the first vaccination against yellow fever (Fig. 1).

Among the 1138 eligible research participants, PRNT retest was possible after treatment of the plasma samples with Ecteola-Celulose®, in 824 children, for whom there was sufficient biological material present. There was a higher proportion of untested samples due to unavailability of biological material in the 2–4 years post-vaccination categories (Fig. 1), but the losses did not represent a major impact on the distribution of the most important covariates in the subgroup of 824 participants analyzed in relation to the total of 1138 participant who adhered to the study protocol (Supplementary table 1).

The classification into time categories (Table 1) was the one that best fit the time distribution curve between vaccination and blood collection for immunogenicity assessment.

In group I, children up to 6 months after vaccination were included, which one could assume would present antibody levels close to the maximum induced by vaccination (Table 1).

The proportion of seropositivity for the reference group was 86.7%, progressively decreasing until the 73–100 months group after vaccination against yellow fever, with a slight increase on the last group, which had been vaccinated more than 100 months prior (Table 2).

Among the 165 individuals on Group 1 (range 0–6 months after vaccination), who were eligible for the study protocol, 121 (73.3%) had a post-vaccination time of 30–45 days, which was considered the reference for analysis. Among these, the seropositivity rate was 87.6%.

In relation to the geometric means of the titers (reciprocal of the dilution) of neutralizing antibodies against yellow fever, there was a progressive decrease starting from the reference category (“0–6 months”) up to the category of 73–100 months after vaccination. For those vaccinated more than 100 months before, the geometric mean titer is slightly higher than the category immediately before it (Table 3).

The antibody titer distribution curves show a gradual shift to the left (lower titers) in the groups with longer vaccination times. The frequency in borderline categories (close to the cut-off point), representing low titers, is substantial and may have interfered with the accuracy of estimates of seropositive rates (Fig. 2).

In multiple analysis, the time since vaccination adjusted for a history of severe disease (the only covariate with statistical significance) confirmed its role as the major determinant of antibody titers (Supplementary table 2).

In the bivariate analysis, it was possible to observe a statistically significant association for the explanatory variable “Time since vaccination” and borderline significance for the covariate “Travel to other risk areas for YF”. In the multivariate analysis, the model selected for its potential to explain variations in the binary

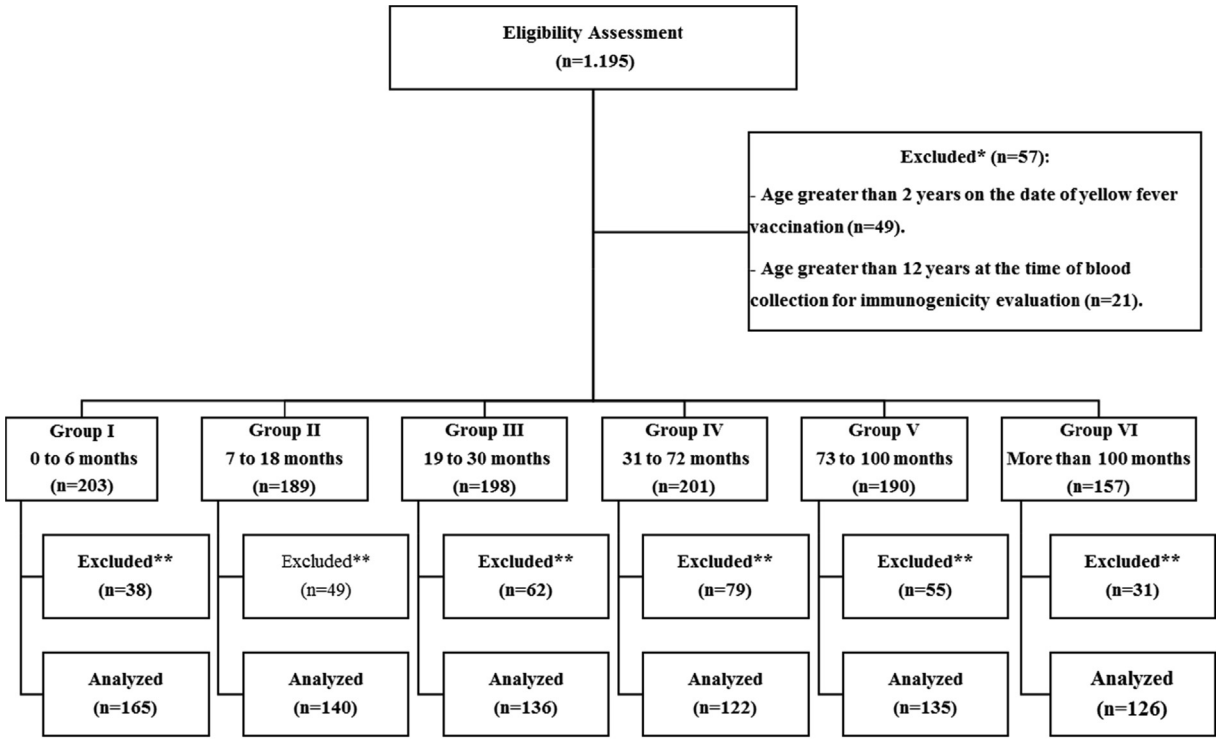

Fig. 1. Flowchart of recruitment and analysis of research participants. *Excluded for non-compliance with study protocol. ** Excluded due to serum unavailability for PRNT after treatment with Ecteola-cellulose®.

Table 1
Distribution of participants by time after vaccination against yellow fever.

Participants			Time after vaccination		
Group	Number	%	Range	Mean	Standard deviation
I	165	20.0	0–6 months*	48.5	25.6
II	140	17.0	7–18 months	13.7	2.0
III	136	16.5	19–30 months	25.4	2.5
IV	122	14.8	31–72 months	50.9	5.8
V	135	16.4	73–100 months	85.4	3.2
VI	126	15.3	More than 100 months**	120.8	5.2

* 9–182 days.
** 101–141 months.

Table 2
Serological status measured by neutralizing antibody titer (PRNT), according to the time since vaccination.

Time after vaccination	Seronegative		Indeterminate		Seropositive	
	Number	%	Number	%	Number	% (95% C.I.)
0–6 months	9	5.4	13	7.9	143	86.7 (80.5–91.4)
7–18 months	13	9.3	20	14.3	107	76.4 (68.5–83.2)
19–30 months	15	11.0	24	17.7	97	71.3 (62.9–78.7)
31–72 months	28	23.0	22	18.0	72	59.0 (49.7–67.8)
73–100 months	48	35.6	30	22.2	57	42.2 (33.8–51.0)
More than 100 months	36	28.6	32	25.4	58	46.0 (37.1–55.1)

Seronegative: Titers less than 1:5 in the reciprocal of the dilution.
Indeterminate: Titers greater than or equal to 1:5 and less than 1:10 in the reciprocal of the dilution.
Seropositive: Titers greater than 1:10 in the reciprocal of the dilution.

Table 3
Mean geometric titers of antibodies and their 95% confidence intervals in the reciprocal of the dilution, according to time after vaccination.

Time after vaccination	Number	GMT[a]	LL[b]	UL[c]
0–6 months	165	47.9	38.3	59.9
7–18 months	140	33.2	25.9	42.5
19–30 months	136	25.0	20.0	31.2
31–72 months	122	14.8	11.6	19.1
73–100 months	135	8.6	7.1	10.5
More than 100 months	126	10.0	8.2	12.1
Total	824	20.2	18.3	22.3

[a] GMT: geometric mean titres.
[b] LL: lower limit of the 95% confidence interval.
[c] UL: upper limit of the 95% confidence interval.

response variable (absence or presence of post-vaccinal seropositivity) was the one that included only the explanatory variable "Time since vaccination", indicating that the chances of seropositivity decreased with time after vaccination (Table 4).

4. Discussion

In 2013, the WHO withdrew the recommendation to boost yellow fever vaccine every 10 years, considering, as a general rule, a single dose of the vaccine sufficient for lifelong protection [6]. However, this decision to eliminate booster doses of the yellow fever vaccine is controversial and still requires robust scientific evidence [11]. The present study filled a gap in information on the duration of immunity against yellow fever in vaccinated children in the first two years of life, which is the target age range for routine vaccination in endemic areas, as recommended by the WHO [6]. The lower seropositivity in newly vaccinated infants, compared to that observed in adults, was followed by a substantial reduction in the proportion of seropositivity to yellow fever, as well as the geometric mean titers, over the years after vaccination. The reduction was more pronounced than in the study in adults [11]. Assuming all subgroups had similar seropositivity rates when they were newly vaccinated, the proportion seropositive and GMT dropped 28% after 31–72 months (median of 4.2 years) and 51% after 73–100 months (median of 7 years), leaving virtually unprotected a substantial proportion of children. For the group with a median time since vaccination of 120 months (or 10 years) the proportion of seropositivity was 46%, slightly higher than the immediately preceding time group, possibly justified by a second unrecorded dose of the yellow fever vaccine in some of the participants, which was an exclusion criterion for the present study. A similar result to that found for the proportions of seropositivity was observed in the analysis of the geometric mean titers, which showed progressive reduction in the comparative analysis of the successive time intervals between the primary vaccination against yellow fever and the collection of blood for the evaluation of immunogenicity, reaching a decrease of approximately 5-fold in titers of those vaccinated about 85 months (7 years) before, compared to the newly vaccinated group (30–45 days). Bivariate and multivariate analyzes, with some limitations, demonstrated the effect of time between yellow fever vaccination and blood collection for immunogenicity evaluation, in the explanation of changes in yellow fever antibody titers.

In Brazil, the study on duration of immunity in adults, with humoral immunity data, concluded that revaccination against yellow fever for this group would not only be necessary, but should also be anticipated because of the high proportion of seronegative individuals and low titers of neutralizing antibodies against yellow fever observed around five years after vaccination, with progressive decline to about 10–11 years, compared with individuals vaccinated up to 45 days prior to the evaluation [11,21].

Blood collection with heparin, required by the parallel analysis of cellular immunity, represented an initial limitation to the study, due to the incoherence found in the initial results of humoral immunity analysis. We consider that these limitations were solved by the heparin removal protocol of the plasma samples treated with Ecteola-Celulose® [16], which allowed the humoral immunity analysis to be performed and generated the results presented here. The plasma treatment procedure with an exchange enzyme for heparin removal led to a need to establish a new cutoff for PRNT

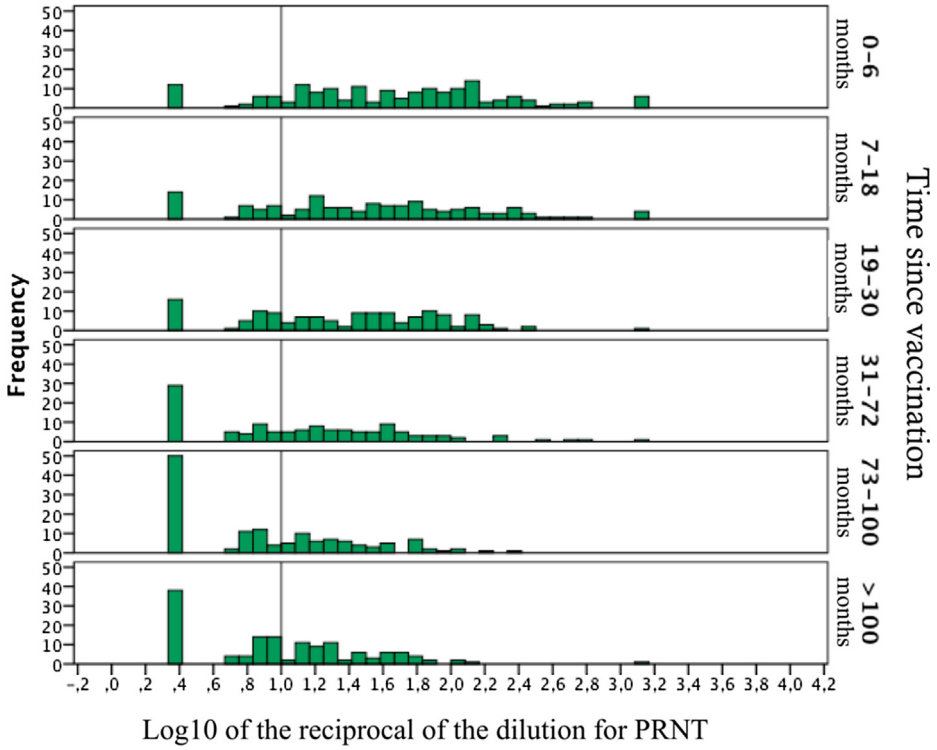

Fig. 2. Distribution of antibody titers (log10 of the reciprocal of the dilution for PRNT), per time after vaccination.

Table 4
Measure of association (Odds Ratio, OR) between seropositivity to yellow fever and time since vacination (final model).

Categorical explanatory variable		OR	CI 95%	
			Lower	Upper
Time since vaccination	0–6 months	1.00	.	.
	7–18 months	0.50	0.28	0.90
	19–30 months	0.38	0.21	0.69
	31–72 months	0.22	0.13	0.39
	73–100 months	0.11	0.06	0.20
	More than 100 months	0.13	0.07	0.23

for yellow fever, different from that used in the analyzes of the Collaborative Group for Studies on Yellow Fever Vaccines for adults [11]. This new cut-off point was validated by Campi-Azevedo and colleagues [16] in a study that used pre- and post-vaccinal blood samples, with control over the time between them and the vaccination procedure, following the same collection procedures and processing. After the heparin removal procedure with Ecteola-Celulose® and PRNT testing, considering the seropositivity cut-off point of 10 in the reciprocal of the dilution, Campi-Azevedo and colleagues [16] found, for children under two years of age, who had only received a dose of the vaccine, a seropositivity rate of 87.5%, about 30 and 45 days after vaccination. This value is similar to that described in the literature for the same age group. In a multicenter study of immunogenicity carried out in Brazil, a seroconversion rate of 88% was observed in children 12–23 months after vaccination against yellow fever [13]. A similar result was observed in an immunogenicity study in Peru, which demonstrated a 88.5% seroconversion rate after vaccination against yellow fever (17D vaccine) in children aged 9–18 months [22]. In another multicenter immunogenicity study conducted in Brazil, 83% of children vaccinated in the first year of life showed a doubling of antibody titers 30 days or more after vaccination [18].

In addition to collection of plasma samples with heparin and the need for its treatment with Ecteola-Celulose® and sample retesting, other limitations of the present study are: (i) several cohorts were represented in the sectional study with the assumption that immunization practices and the immunogenicity of the vaccine did not change significantly over time, which seems acceptable since there were no changes in the vaccine and vaccination practices in this period; (ii) limitations on the reproducibility of the serological method (PRNT) are well known and the comparison with previous studies was limited because we did not have titers in International Units; (iii) for convenience to obtain a sample of vaccinated children, the study was conducted in an area with recommendation of routine vaccination against yellow fever. With high vaccine coverage, wild-type virus circulation in non-human primates would hardly determine the occurrence of cases, but could cause a "natural booster" that would confound the results on post-vaccination antibody levels. However, the study was conducted in the urban metropolitan area of Belo Horizonte, where human cases were no longer occurring for several decades prior to routine vaccination, and surveillance of epizootics in the State of Minas Gerais had not detected yellow fever in non-human primates (unpublished data, State Department of Health of Minas Gerais). Despite the above limitations, the results presented here are of great relevance to fill the information gap regarding the duration of immunity against yellow fever in children. As previously mentioned, the yellow fever vaccine has a lower performance

in children than in adults. Considering the vaccination of the 9 to 12-month-old age group included in routine immunization schedules as a priority strategy by WHO for yellow fever control in endemic areas [6], special attention should be given to this age group for decision-making on the need for revaccination. According to Hepburn et al. [22], the duration of immunity would be associated with the magnitude of post-vaccine antibody titers. Fox & Cabral [23] had already drawn attention to less intense and shorter responses in children younger than 10 years of age, while Anderson & Gast-Galvis [24] observed proportions greater than 90% of children over 6 years of age and adults with persistent neutralizing antibodies up to 5 years after vaccination. However, these studies have not specifically addressed the duration of immunity in individuals vaccinated in the first two years of life, which accounts for the majority of primary vaccination in areas where the vaccine is part of the regular childhood vaccination schedule. The present study looked specifically at this age group, seeking scientific evidence of the medium and long-term behavior of immune status after vaccination against yellow fever in order to guide decisions about revaccination needs and the opportunities to do so.

In relation to those who had been vaccinated for more than 100 months, and showed a slight increase in the proportion of seropositivity and average geometric titers (compared to the 73-to-100-month subgroup), it is possible that some children received a second dose of the yellow fever vaccine, not included in their records or omitted by the research participant's legal guardian (Table 3).

Statistical modeling supported the role of post-vaccination time to explain the variation in antibody titers, with the caveat that the control of confounding by relevant covariates may have been limited by the accuracy of the response variable, the distribution of post-vaccination time clustered around the pre-defined times (departing from the normal distribution required by the model), and other unknown confounders.

Knowledge about the duration of immunity against yellow fever is crucial for making decisions on the need for revaccination and the best time to do so. Serological correlates of protection for yellow fever are not established in humans; however, evidence of effectiveness of immunization programs in the control of yellow fever suggests that seropositive individuals after vaccination are protected against the disease [10]. Although seronegativity does not mean lack of protection, the increase in the occurrence of primary failures and secondary failures is plausible with the reduction of antibody levels. In Brazil, a study of 831 cases of yellow fever found that 52% of the reported cases had been vaccinated >10 years earlier [25]. Also, in 2017–2018, the largest YF epidemic in recent times in Brazil disclosed 16 cases that had a documented vaccination history, in a Brazilian state where YFV had been used both in campaigns and routine vaccination [26].

External validity of the study results is limited by participants selection based on non-probabilistic sample. However, they constituted a typical clientele of primary health units from the Brazilian unified health system, with standard vaccination procedures carried out nationwide with the same vaccine. That does not ensure generalizability of the findings, but it can be argued that there is nothing special about the study group or the study setting that could hamper the application of the results.

5. Conclusion

The results of this study demonstrated a progressive decrease in antibody titers and a reduction in the proportion of seropositivity over time after vaccination, in a similar way to that already described for adults with primary vaccination, especially the early decrease starting from 31 months post-vaccination. These data together with previous publications appear to support the need for booster doses, especially in children living within the risk areas for yellow fever, who receive the first dose of the vaccine between 9 and 23 months of age. The data also questions the 10-year interval previously recommended by WHO for the booster dose. Considering the expansion of the areas with recommended vaccination against yellow fever due to epizootics, the high rates of urban infestation of the *Aedes aegypti* vector, the ease of movement between the areas and the high lethality of the disease, the data presented here draw attention to the potential negative impact on public health of the absence of the yellow fever vaccine booster dose in children, especially in endemic areas or areas with viral circulation in non-human primates. In addition, they indicate that revaccination should be ideally recommended at an interval shorter than 10 years after the first vaccination, up to 4 years after the initial dose administered in the first two years of life. Nevertheless, scientific evidence on the need for booster doses of yellow fever vaccine must be reconciled with epidemiological (outbreaks and epizootics), logistic (vaccine availability) and programmatic (prioritizing primary vaccination to maximize vaccine coverage) aspects.

Funding

Health Surveillance Department, Ministry of Health; Institute of Technology for Immunobiologicals of Bio-Manguinhos – FIOCRUZ; Brazilian National Research Council-CNPq; FAPERJ (Acronym for the Foundation of support to scientific research in the State of Rio de Janeiro); FAPEMIG (Acronym for the Foundation of support to scientific research in the State of Minas Gerais; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [The Collaborative Group for the Study of Yellow Fever Vaccines includes several professionals who either work for or collaborate with Bio-Manguinhos-Fiocruz, which is a technical unit of the Brazilian Ministry of Health that manufactures the yellow fever vaccine and is the major supplier of that vaccine for the Brazilian National Immunization Program.].

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.09.051.

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