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Duration of immunity in recipients of two doses of 17DD yellow fever vaccine

Collaborative group for studies on yellow fever vaccines *

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ABSTRACT

Antibody levels following yellow fever vaccination (YFV) decrease considerably with time, leaving a substantial proportion of single-dose recipients seronegative after 10 years. Little is known about the persistence of immunity after two or more doses of the vaccine. This study was designed to verify the current immune status of adults who had received two or more doses of YFV.

A cross-sectional study assessed the immune status for yellow fever according to time since the latest YFV: second dose at 30–45 days (reference group), 1–5 years, 6–9 years and 10 years or more; and three or more doses. Volunteers had their vaccination cards checked and were interviewed before having a blood sample drawn for titration of neutralizing YF antibodies, and being tested for dengue IgG. The proportion seropositive (titer $\geq$ 1:50) and the geometric mean titers (GMT) were compared among the subgroups of time since the latest YFV.

Participants (n = 323) were predominantly female (80.5%) and had a median age of 34 years (range 18–76). In the reference group (n = 99), 69% were seropositive before the second dose. After revaccination, the proportions seropositive (95% C.I.) were 100% (96–100%) in the reference subgroup, 90% (83–95%) in the 1–5 year subgroup (n = 109), 86% (77–92%) in the 6–9 year subgroup (n = 92) and 86% (57–98%) in the 10+ years subgroup (n = 14). Only 9 participants with more than 2 previous doses of YFV were eligible for the study, and 8 of them were seropositive. Sociodemographic variables, age at vaccination and previous dengue infection did not confound the association of seropositivity to time since the last YFV.

The results support the need of booster doses of the YFV to maintain antibody levels consistent with protection, and indicate that a small proportion of individuals may need more than two doses, provided that priority is given to primovaccination of all susceptibles.

1. Introduction

Yellow Fever (YF) is an acute viral disease with a clinical spectrum ranging from asymptomatic infections to severe disease with 20–50% case-fatality rates [1]. It is endemic in the tropical areas of South America and sub-Saharan Africa, being one of the diseases under the International Health Regulations subject to international notification and vaccination requirement for travelers [2].

YF can be prevented by a live attenuated virus vaccine, which induces seroconversion in more than 95% of vaccinated adults [3]. The National Immunization Program of the Brazilian Ministry of Health, consistent with WHO recommendations, recommends immunization against YF of residents of and travelers to areas with endemic or epizootic yellow fever virus transmission [4–7].

The duration of immunity induced by the YF vaccine (YFV) in primo-vaccinated adults appears to be lifelong for most vaccinees [8,9], and the World Health Organization in 2013 discontinued the recommendation for booster doses [7]. However, a study among adults in Brazil showed that the proportion seropositive dropped considerably after 4 years of vaccination, leaving 24% of single-dose recipients seronegative after 10–11 years [10]. Consistently, the data for cellular immunity showed that the effector memory $CD4^+$ and $CD8^+$ T-cells, the classical memory B-cells as well as the median PRNT titers decreased after vaccination, leading to limited memory responses after 10 years of primary vaccination against YF [11]. After considering thee available scientific evidence [7,8] and the epidemiological context of yellow fever in Brazil, the Brazilian Ministry of Health maintained its recommendation for a booster dose after 10 years of primovaccination in adults, and at 4 years of age in children who had been vaccinated in their first year of life [12]. After the 2016 YF epidemics in Brazil, the Ministry

* A list of the members and their role is presented at the end of the paper.

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0264-410X/

of Health adhered to the single dose policy, to cope with the increased demand for vaccines [13].

Considering the persistence of immunity in a substantial proportion of individuals with one dose of the vaccine, one might hypothesize that one booster dose would suffice for lifetime protection. This study was designed to verify the immune status of adults who had received two or more doses of YFV, according to the time of the last dose. The results may have important implications for residents and travelers to endemic areas and for immunization programs in many countries.

2. Subjects and methods

This was a cross-sectional study of the immune status for YF in a sample with subgroups defined by number of previous doses and the time interval between the latest YF vaccination and blood collection: newly revaccinated (second dose 30–45 days before); second dose 1–5 years before; second dose 6–9 years before; second dose 10 years or more before; three or more doses, with the latest dose received at least one year before (Fig. 1). The subgroup of newly revaccinated individuals had received the first dose at least 10 years before. They were taken as a reference as a recent booster dose was expected to have the maximum antibody levels. Potential confounders, such as age, sex, other vaccines, comorbidity and use of medication and previous dengue infection were considered in data analyses.

Participants were recruited in an Army unit and a research institution in Rio de Janeiro where the YFV is an occupational health procedure, and in primary health care units in the State of Minas Gerais. In that region preemptive vaccination against YF had been ongoing for more than ten years despite lack of evidence of epizootics.

2.1. Eligibility criteria

Men and women aged 18–59 years for the reference group and aged ≥18 years for other groups, who had a vaccination card or proof of vaccination on functional or health services records were screened for the number of doses and date of last dose. The interval between the two doses was not less than 5 years. For individuals who had received more than two doses of vaccines, any interval between the second and the other doses was accepted.

Individuals were not included if (1) they resided in, or travelled to endemic areas and regions considered at risk; or (2) had a contraindication for YFV (immunodepression, severe adverse reaction on previous doses, severe allergy to chicken egg), (3) use of hyperimmune serum or any other vaccination within 30 days; or (4) had occupational exposures, such as missions in jungles or accidents while handling the YF virus in laboratories.

2.2. Data collection

Individuals who provided informed consent were interviewed by a nurse, who checked immunization cards and records of previous YFV doses and respective dates, asked about places where the participant had lived or visited, and recorded sociodemographic data and medical history.

On the day of the enrollment 10 mL of blood was collected for antibody titration. Those with only one dose of YFV 10 years of more before were revaccinated, according to current recommendation. This subgroup had a second blood sample collected 30–45 days after revaccination. Aliquots of sera were coded and stored at −70 °C.

Vaccination was not part of the research, as it just took advantage of the opportunity provided by revaccination that was recommended after10 years or more of the first dose. Post-vaccination adverse events were recorded according to recommendations of the Brazilian Ministry of Health by professionals that administered the vaccine [14].

2.3. Laboratory tests

Neutralizing antibodies to YF were titrated in sera using the plaque reduction neutralization test (PRNT) performed at the Virologic Technology Laboratory at Bio-Manguinhos, Fiocruz, according to the standard procedures detailed elsewhere [15]. Briefly, the PRNT test was conducted in serial 2-fold dilutions starting at 1:5, in volumes of 20 μl of inactivated serum samples in 96 well tissue culture plates. About thirty plaque-forming units (pfu) of YF virus (strain 17D 213/77, lot UEXVFB01) in 50 μl were dispensed into all wells. A positive serum sample with YF antibody content calibrated by a WHO International Reference Preparation was included in each set of tests [16]. The dilution of the test and standard serum, which reduced the plaque numbers by 50% relative to the virus control, was determined by linear regression and the results were given in reciprocal of dilution. Seropositivity to YF was defined as antibody titer ≥1:50. Serum samples were also tested for dengue IgG using a dengue IgG Elisa developed by the Laboratory of Flavivirus, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil [17].

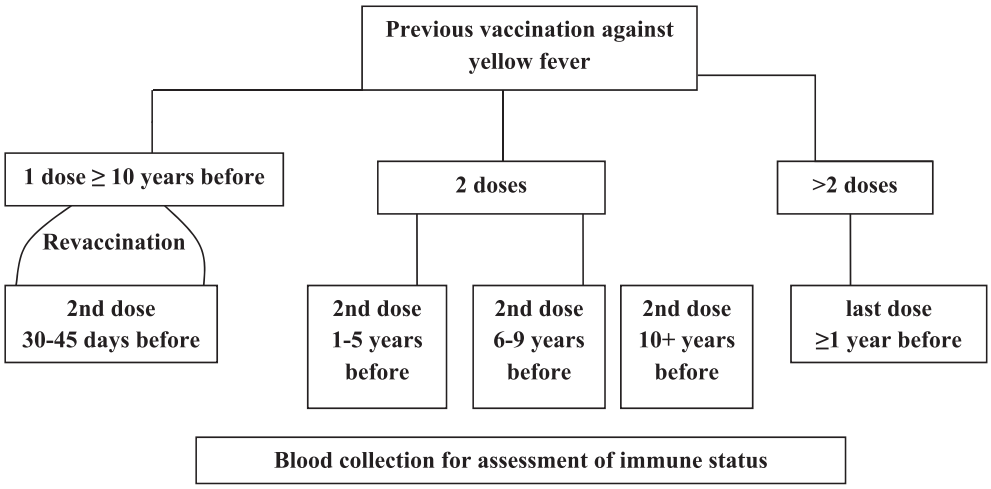

Fig. 1. Approach to assess immune status for yellow fever according to number of previous doses of yellow fever vaccine.

2.4. Statistical analysis

The response variable was the log 10-transformed reciprocal neutralizing antibody titer. Antibody titers equal to or greater than 50 were considered seropositive. The proportion of seropositivity and the geometric mean antibody titers were estimated (with 95% confidence intervals) for each subgroup defined by time since the last dose: 30–45 days, 1–5 years, 6–9 years, 10 years or more, and at least one year for those with three doses or more. For subgroup analyses, a category "6 years or more" was used, to manage the scarcity of participants with 10 years or more. The seropositivity among those newly revaccinated was the reference for comparisons with the other subgroups. For geometric mean computations, antibody titers presented as <5 (reciprocal dilution) were computed as 2.5 and titers >640 were computed as 640. The statistical significance of the differences between groups was assessed with the chi-squared test for proportions, and the *t*-test for means.

Antibody titers (Log 10) were plotted against time since the last dose, with different symbols for the number of previous doses. Data points were fitted to a line (loess Kernel functions: epanechnikov) representing the trend of the data.

The proportion seropositive for YF was calculated within categories defined by previous dengue infection. The other covariates for analysis were age (continuous variable, in years) at enrolment, at the first dose and at last dose of YFV, sex, vaccination history, history of severe illness (hospitalization, sequela, disability), comorbidity and medication at the time of blood collection. In the analysis of vaccination history, we considered the live attenuated virus vaccines (measles, rubella, mumps and varicella) applied simultaneously or less than 30 days in relation to the YFV. Multivariate analysis (logistic regression) was used to identify associations between the covariates and serological status according to the time of vaccination. Statistical analysis was carried out with SPSS® 18 (SPSS Inc, Chicago, Illinois) and Winpepi [18]. A 5% significance level was adopted in all analyses.

2.5. Sample size calculation

This was a convenience sample of adults from different regions, but immunized with the same vaccine. Considering 98% seropositivity after 30 days of revaccination as reference, 110 volunteers were needed in each group to detect a minimum difference of 10 percentage points with 80% power and significance level of 5%. The total of 440 volunteers in four subgroups allowed for 10% of withdrawals and missing data.

2.6. Ethical considerations

The fundamental ethical principles that guide research involving human beings were considered in the development of the study [19]. Brazilian National standards were followed [20] and the study team adhered to international scientific integrity and ethical aspects in the design, conduct, recording and reporting of scientific studies. The study protocol was approved by the Research Ethics Committee of the National School of Public Health/Fiocruz) (CAAE: 15752013.1.0000.5240).

3. Results

A total of 355 individuals consented to participate in the research from May 2014 to January 2015 in four sites: Immunobiological clinical trials unit in Rio de Janeiro-RJ (n = 26), and primary care units in three municipalities of the State of Minas Gerais: Alfenas (n = 229), Contagem (n = 74) and Ribeirão das Neves (n = 26). However, 23 individuals were found after enrolment to be ineligible for having resided in areas of potential transmission of YF. Of the 332 eligible, it was not possible to obtain blood samples in 9 individuals.

All 99 participants in the reference subgroup were enrolled in the study on the day of revaccination (second dose) (Table 1). Overall, participants were predominantly females and young adults. 95% of the study participants were 4 or more years of age at the time of their first dose of YFV (data not shown). Only 14 individuals with two doses or more had received the last dose of YFV 10 years or more before enrolment. Overall, more than ¼ had a previous dengue infection. The medical conditions reported most often were hypertension (29), diabetes (12), hypothyroidism (12) and chronic respiratory diseases (bronchitis, asthma etc.: 8). Few participants reported current medical treatment for conditions relevant for immunogenicity: antiretroviral medication, methotrexate and prednisolone for arthritis. All individuals with past or current medical conditions showed strong immune response after revaccination. Previous hospitalizations appeared relevant only in a case of "removal of carcinoma" (non-specified) 3 years before, and the participant was seropositive for YF.

None of the participants had received another vaccine 30 days before YF revaccination or before blood collection. Only 19 participants had received a vaccine 60 days or less prior to the serological test, 13 of them being against influenza. In 43 of the participants the last dose of YF was concomitant to other vaccines, mostly diphtheria-tetanus, hepatitis B and influenza. Only 2 indi-

Table 1
Sociodemographic characteristics, dengue serology (Elisa, IgG) and medical history of participants according to time since last vaccine dose.

	Time (years) after last vaccine dose								Total	
	0*		1–5		6–9		10+			
	n	%	n	%	n	%	n	%	n	%
Total	99	30.7	117	36.2	93	28.8	14	4.3	323	100.0
Sex (Male)	23	23.2	20	17.1	15	16.1	5	35.7	63	19.5
Age (years) Median, min. – max.	36	18–62	35	18–76	32	18–67	39	22–69	34	18–76
Dengue (IgG) Seropositive	24	24.2	20	17.1	44	47.3	4	28.6	92	28.5
Other vaccines concomitant to YF	1	1.0	30	25.6	12	12.9	0	0	43	13.3
History of hospitalization	61	61.6	57	48.7	42	45.2	9	64.3	169	52.3
Current medical treatment	20	20.2	34	29.1	12	12.9	6	42.9	72	22.3

* Reference subgroup comprising individuals enrolled in the study on the same day of revaccination.

viduals had the measles-mumps-rubella vaccine administered simultaneous to the last dose of YFV, and both were seropositive to YF.

Among study subjects who had been vaccinated once and were eligible for revaccination 31% were seronegative to YF, whereas 12% of those with two doses were seronegative (Table 2). Subgroups of number of previous doses differed widely in the distribution of time since the last dose of YFV, though (Fig. 2). Only 9 individuals with three or more previous doses of YF were eligible as most candidates had had multiple vaccine doses to travel or live in endemic areas, which was an exclusion criterion.

Antibody levels appeared to decrease with time after the first dose of YFV (individuals tested immediately before the second dose), although the time span of the group did not seem wide enough for a clear pattern to emerge (Fig. 2). Conversely, no trend with time since the second dose was apparent. The scatter plot omitted two individuals with outlier values of time since the last dose of YFV (22 and 21 years), as their leverage was thought to exaggerate the downward trend in the subgroup with one previous vaccine dose. However, the fitted lines showed almost the same inclination of the plot that included the two outliers (data not shown).

All participants who had only one previous dose of YFV were seropositive shortly after revaccination (Table 3), and 49% had a four-fold or higher rise in their antibody titers. Only 5 individuals failed to respond to the booster dose and remained weakly seropositive. Individuals previously seronegative had the highest post-vaccination titers whereas 5 participants with very high pre-vaccination levels showed a decrease of post-vaccination titers up to 60% (data not shown). The time between the second dose and blood collection varied from 30 to 327 days (median 35 days, interquartile range 10 days).

Compared to the newly revaccinated, the proportion seropositive and the GMT were substantially lower in the subgroup revaccinated 1–5 years before (Table 3). A further 4 percentage point decrease was observed in the subgroup that had a second dose of the vaccine 6–9 years before. Consistently, the geometric mean titer after 1–5 years was 60% lower compared to those newly revaccinated (subgroup "<1", Table 3). Differences between the reference and the other subgroups, as well as the downward trend, were statistically significant (Table 3). The estimates in Table 3 excluded 9 participants who had three or more doses of YFV, as they comprised a subgroup too small to add meaningful information. The estimates of the proportion seropositive were not substantially affected by the exclusion of the subgroup with 3 doses.

The proportion seropositive with different times of vaccination varied substantially across sex and age groups, and slightly across categories of previous dengue infection (Table 4) but the differ-

Table 2
Immune status of participants upon enrolment, according to the number of previous doses of yellow fever vaccine (YFV).

Number of previous doses of yellow fever vaccine								
	1		2		3 or more		Total	
Total	99		215		9		323	
Years since last YFV Median, min.-max.	14	10 – 22	5	1 – 16	4	2–6	6	1–22
YF antibodies								
% Seropositive		68.7		87.9		88.9		82.0
Geometric mean titer (reciprocal dilution)	87		162		175		134	

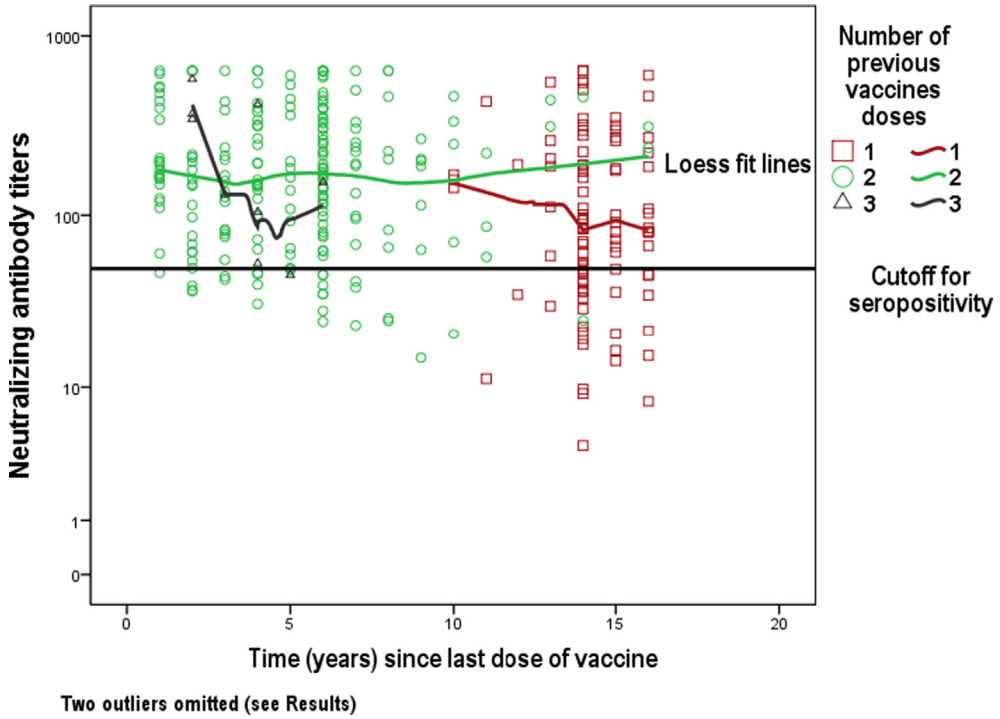

Fig. 2. Neutralizing antibody titers (Log 10) by time since last dose of yellow fever vaccine and number of previous doses.

Table 3
Proportion seropositive to yellow fever and geometric mean titer (GMT) of neutralizing antibodies according to time since last dose of yellow fever vaccine.

	Time (years) after last vaccine dose[#]							
	< 1[@]		1–5		6–9		10+	
Total (n, %)	99	30.7	109	36.2	92	28.8	14	4.3
Yellow fever PRNT % Seropositive)		100.0		89.9		85.9		85.7[*]
95% C.I.	96.3–100.0		82.7–94.8		77.0–92.3		57.2 – 98.2	
GMT, 95%C.I.	406.6		162.0		162.3		160.4[&]	
	358.8; 460.8		140.2; 189.6		134.4; 195.9		91.7; 280.5	

[#] Estimates did not include 9 participants with 3 doses of yellow vaccine;
[@] Newly revaccinated;
[*] p = 0.0003 (linear trend);
[&] p < 0.001.

Table 4
Proportion seropositive to yellow fever according to time since last dose of yellow fever vaccine, by age, sex and previous dengue infection [a].

Subtotal; % seropositive	Years since last dose of YFV					
	< 1[b]		1–5		6 +	
	n	%	n	%	n	%
Age < 50 years	77	100	90	91.1	91	84.6
Age ≥ 50 years	22	100	19	84.2	15	93.3
Male	23	100	16	93.8	20	95.0
Female	76	100	93	89.2	86	83.7
Dengue [c] seronegative	74	100	93	90.3	59	84.7
Dengue [c] seropositive	24	100	14	92.9	47	87.2

[a] Estimates did not include 9 participants with 3 doses of yellow fever vaccine;
[b] Newly revaccinated;
[c] Three cases with inconclusive serology for dengue were disregarded.

ences were not statistically significant (test for heterogeneity: P = 0.894, p = 0.983, p = 0.948, respectively). However, a linear downward trend across 4 categories of time since vaccination (Table 3) was statistically significant for women (p < 0.001), for individuals < 50 years old (p < 0.001) and seronegativity for dengue (p < 0.001).

Apart from the time after the second dose of YFV, none of the covariates was significant in the multiple regression model for the log10 reciprocal dilution of YF antibody titers. In the logistic regression model, association of seropositivity to residence or travel to other municipalities or states was also statistically significant, although it did not change substantially the magnitude of the association to the time after the second dose of YFV (data not shown). Association with sex, age at study enrolment, age at first dose, age at second dose, seropositivity (IgG) to dengue, history of severe illness (hospitalization, sequela, disability), other vaccines and co-morbidity (medicines used at the time of blood collection) were not statistically significant (data not shown).

4. Discussion

Available evidence supported the recommendation of booster doses of the YFV to avert primary and secondary failures [10,12]. In fact, 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 [21]. To the extent that undetectable antibodies relate to susceptibility to yellow fever, the substantial proportion of seronegative participants among those with one previous dose of YFV in the present study may have public health significance. It might indicate waning of immunity, consistent with the long time since vaccination (median 14 years), and was also in agreement with the results of previous studies [10,22,23]. Other live viral vaccines, such as measles and rubella, require booster doses to avert primary and secondary failure. Despite waning of measles vaccine-induced immunity vaccine failures do not appear to increase with time since vaccination [24]. However, measles vaccine effectiveness after two doses has been shown to be higher than with one dose [25].

A downward trend of antibody levels with time after the first dose was suggested by the fit line in the scatter plot, although a clear pattern was not disclosed with these data. On the other hand, for those who had received two doses of the vaccine, the fit line suggested sustained higher antibody levels. Although it seemed plausible that a few booster doses should sustain seropositivity, this study showed that a sizable proportion of individuals with two doses were seronegative after a few years of the latest dose. The subgroup with three or more doses was too small to give meaningful information about the benefits of a third dose of the vaccine.

The subgroups in the present study represented cohorts with increasing time since the second dose, and disclosed the impact of time in seropositivity. Compared to a previous study with single dose recipients of the same vaccine assessed with similar methods [10], the proportion of seropositive individuals decreased similarly up to 6–9 years. Nevertheless, after 10 years or more, the drop in seropositivity was much less pronounced among the two-dose recipients than in the single-dose ones (86% vs. 76%), with the caveat that the sample was small and estimates were imprecise.

The decline in seropositivity after booster doses had already been reported [26] in 1029 laboratory workers, tested annually and given booster doses whenever the antibody levels fell below 1:40 ($PRNT_{80}$). The authors concluded that most individuals will not maintain titers above the limit defining failure (1:40) for more than 3–5 years, indicating that booster doses might be necessary before completing 10 years of the last dose, unless the participant had already high levels. Conversely, in a study in retiring U.S. Navy and Marine personnel, Rosenzweig et al.[27] found that both neutralizing (intracerebral inoculation in mice) and hemaglutinin inhibition antibodies persisted in all 9 individuals 0–8 years after revaccination. None of those subjects had antibodies against other

arboviruses. Reinhardt et al. [28] observed a two-fold increase in neutralizing antibodies ($PRNT_{90}$) after revaccination of 5 seropositive subjects previously vaccinated at least 10 years before, and a return to pre-booster antibody levels 7 months later. In contrast to previous studies, our observations were based on a sizable sample with longer intervals since the last dose.

The direct comparison of subgroups in the present study considered that the vaccine and immunization practices have not changed in Brazil over the last several decades, which is a strength of the present study. On the other hand, taking seropositivity as a surrogate for protection, for lack of a proper serological correlate, may be a limitation of the study. However, for public health purposes the evidence of protection of seropositive individuals is supported by the undisputed effectiveness of vaccination in the control of YF.

Seropositivity also implies the caveat that the PRNT has a limited reliability and lack standardization, which hampers comparison across studies. In the present study, seropositivity was based on a serum-dilution plaque-neutralization test with a 50% end point that has been widely used in studies of yellow fever vaccines and is accepted by the WHO. A surrogate for protection in humans is available from a constant serum, varying virus, neutralization test, from which a Log Neutralization Index (LNI) $\geq$0.7 was shown to correlate with protection in a study in monkeys challenged in laboratory [29]. However, the LNI test may be less sensitive than the PRNT and appears to be influenced by antibody affinity, which can decrease over time [24].

Partial protection in individuals with borderline antibody levels seems plausible on the basis of observations in animal models [30]. Moreover, activation of the cellular immune system led by the increase in circulating $CD8^+$ T-cells has been shown by some [11,26] to play a significant role in the immune response and possibly afford protection in vaccinees without detectable neutralizing antibodies, a view that had been disputed in a paper published before new evidence came up [31,11]. Analyses of cell-mediated immunity in participants of the present study will be presented in another paper.

One of the main challenges of field work was the selection of participants for whom the risk of exposure to natural infection was unlikely. That was the case of the municipalities where the participants lived, which had not reported YF cases for several decades. Moreover, surveillance of epizootics had not detected circulation of YF among non human primates in the State of Minas Gerais. Therefore, natural infection did not seem to have interfered in the results.

Despite being a non-probabilistic sample, gathered in different regions, we argue that the result is a fair representation of the immune status in recipients of a YFV from the same manufacturer, administered in primary health care units.

The results of this study cannot be directly extrapolated to individuals that had their first YFV dose before completing 2 years of age, when the immune response is known to be lower than in adults. It seems plausible that antibody levels decline even further in individuals who got their first dose in the first two years of life.

In conclusion, the results provided additional evidence of the need of booster doses of the YFV to maintain antibody levels thought to be protective. The optimum number of doses to sustain long term protection remains unclear. The appropriate timing of revaccination depends on the risk of exposure. For residents and travelers to areas with ongoing epidemics or epizootics a booster dose should be considered soon after the first dose. Although previous studies have indicated some putative predictors of the persistence of immunity, such as the antibody levels immediately following vaccination, age and some medical conditions at the time of vaccination, it is not practical to screen for candidates to revaccination. From a pragmatic individual and public health perspectives it seems more convenient to revaccinate all individuals living in endemic areas, considering the potential for exposure to infection, the low cost of the vaccine and that the booster dose is much safer than primary vaccination. Nevertheless, the overriding consideration for a national immunization program is the availability of vaccines, especially in epidemic or epizootic areas where priority should be given to individuals who have not been vaccinated. Ultimately, vaccine stockpiles will dictate when and where booster doses can be implemented.

Declaration of Competing Interest

Researchers and collaborators include employees of several units of Oswaldo Cruz Foundation (FIOCRUZ, linked to Brazilian Ministry of Health), including Bio-Manguinhos, which is responsible for the production of the YFV used in Brazil.

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Appendix A. Collaborative group for studies on yellow fever vaccines.

The members of the Collaborative Group include professionals involved in the conception and design of the study, data ascertainment and analysis, interpretation of results, drafting the article, revising it critically for important intellectual content, and giving the final approval of the version to be submitted.

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Administrative Support Group (Asclin, Bio-Manguinhos, Fiocruz).

Planning and Management – Contracts and Agreements – Ailson Dias Castilho, Sandro Ricardo Vieira de Moura.

Executive Administrative Support: Renata Silveira Thomazini de Oliveira.

Selection and Recruitment:–Dayana Cristina Vieira de Souza – Executive Administrative Support:– Maria Leticia Borges dos Santos

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