Duration of post-vaccination immunity against yellow fever in adults

Collaborative group for studies on yellow fever vaccines

1. Introduction

Yellow fever is an acute arboviral disease with clinical presentations that include mild forms with a sudden onset of febrile symptoms and severe forms with over 30% lethality, and also asymptomatic infections [1]. Yellow fever is one of the diseases requiring immediate report to the World Health Organization (WHO) under International Health Regulations [2].

In Brazil, most cases of yellow fever occur among adult males conducting occupational, tourism, or leisure activities in forested areas, where they become exposed to infected mosquitoes, mainly the wild species Haemagogus janthinomys. Although disease transmission in urban areas have not been reported in Brazil since 1942, sporadic outbreaks of yellow fever transmitted by jungle vectors in the southern and southeastern regions of the country, close to urban zones where Aedes aegypti is abundant, poses a threat of re-urbanisation of the disease [3].

There is no specific treatment for yellow fever. Disease prevention relies on current commercially available vaccines, which are highly immunogenic and safe. Immunisation is recommended to unvaccinated residents and travellers to and from at-risk areas, aged ≥9 months [3,4].

Despite the lack of efficacy studies on yellow fever vaccines, vaccine effectiveness is evidenced by the dramatic reduction of disease incidence following mass vaccination. The duration of vaccine-induced immunity in primo-vaccinated adults appears to last for decades [5]. Previous recommendations [6] of revaccination have been revised by WHO experts in 2013 [5] and a systematic review of scientific evidence available until June 2012 [7]. The International Health Regulations have been amended in May 2014 to stipulate that a single dose of the yellow fever vaccine is valid for the duration of the vaccinee's life [2].

Data on the long-term immunity induced by yellow fever vaccine, which should guide vaccination policy are still scarce. Therefore, this study aimed to assess the level of neutralising antibodies persisting after years of primovaccination against yellow fever in adults. Moreover, the study evaluated the immune
status of adults over 1 year post-vaccination compared with those at 30 days post-vaccination using neutralising antibodies as humoral immune response biomarkers.

2. Methods

This cross-sectional study was designed to assess and compare the rate of seropositivity and the geometric mean titres (GMT) of yellow fever neutralising antibodies persisting in primo-vaccinated adults. The time since vaccination was grouped in arbitrary categories to determine the length of time that it takes for the immune response to decline and warrant the need for revaccination. Study subjects were grouped according to the length of time since vaccination as follows: 30–45 days, 1–4 years, 5–9 years, 10–11 years, and 12 years or more. In the 30–45 days vaccination subgroup, the presence of neutralising antibodies was also assessed prior to immunisation. The immune response in this newly vaccinated subgroup provided the reference to assess the variation of antibody levels over time. For the comparison subgroups, 1 year was thought to be the minimum time since vaccination, to disclose substantial decline antibody titres. In addition, the effects of anti-dengue IgG antibodies on the humoral immune status of yellow fever-vaccinated adults were also evaluated.

The study population comprised adult volunteers of both genders serving in the Army in the city of Rio de Janeiro, in addition to civilian volunteers from the “Oswaldo Cruz” Foundation (FIOCRUZ; Manguinhos campus, Rio de Janeiro) and from health centres in the municipality of Alfenas, state of Minas Gerais. All subjects either had received a single dose of the yellow fever vaccine 17DD at least 1 year before (confirmed in immunisation records) or had never been vaccinated (Fig. 1). Rio de Janeiro residents are advised to take the yellow fever vaccine only if they travel to endemic areas. The municipality of Alfenas is located in Minas Gerais, which is a large state in southeast Brazil where vaccination against yellow fever is recommended at the age of 9 months. In the Alfenas region, there are no recorded cases of yellow fever. In Brazil, infections by flaviviruses other than dengue and yellow fever have been reported, with minor public health significance.

Aliquots (5 mL) of peripheral blood were collected to measure anti-yellow fever neutralising antibodies and anti-dengue IgG antibodies. Vaccinated subjects were divided into subgroups according to the time elapsed since their last vaccination and were submitted to serological tests to quantify yellow fever antibody titres. A military subgroup with no history of yellow fever vaccination was tested for yellow fever antibodies immediately before routine vaccination required for military personnel involved in missions in the forest. It followed standard immunisation procedures for the general population, which have not undergone major changes in the last decades. The procedures followed in this study were limited to the application of questionnaires inquiring about sociodemographic data and personal medical history, in addition to blood collection for serological tests to determine the maximum antibody levels that the vaccination could achieve. This newly vaccinated subgroup provided the reference for comparison with other subgroups who were vaccinated for longer periods.

Specimens were collected after a signed informed consent was obtained from each participant, and the data collected were handled so as to protect confidentiality. The study protocol was approved by the Research Ethics Committee of the Evandro Chagas Clinical Research Institute at FIOCRUZ (Opinion No. 040/2011).

2.1. Eligibility criteria

Subjects with proof of vaccination (in vaccination card or medical records) and who agreed to the terms of the study were eligible to participate in the study. Exclusion criteria included the following: contraindications for yellow fever vaccine (e.g., pregnancy, permanent or transient immunosuppression, severe adverse reactions following previous vaccination, and severe allergy to chicken eggs), individuals who reported 2 or more previous vaccine doses (even if proof of vaccination could not be provided), lack of proof of prior vaccination, and residence in or travel to risk areas (which have been defined by the Health Surveillance Department of the Ministry of Health) until the time of the study.

The rationale for inclusion of subjects with a documented single dose of yellow fever vaccine and no potential exposure to natural infections was to avoid interference of booster on antibody levels induced by one dose. Cases with uncertain potential exposure to infection were not included. In addition, military personnel who participated in missions to endemic areas or who had been immunised more than once were excluded from the study.

2.2. Laboratory tests

The yellow fever neutralising antibody titres were quantified by PRNT\textsubscript{50} using 20 μL of heat inactivated (56°C for 30 min) serum as described by Simões and colleagues [8] in the Laboratory of Viral Technology of Bio-Manguinhos (LATEV/BIO, in Rio de Janeiro). In each set of tests, a standard serum prepared in house was included as positive control (called M7/100). This serum from Rhesus monkeys (Macaca mulatta) vaccinated against YF had been calibrated against an international reference serum from WHO and was known to contain 1115 IU/mL. Antibody concentration in IU/mL was calculated relative to the antibody content in the international reference (quotient of 1115 IU/mL and the dilution corresponding to the 50% endpoint of the reference is multiplied by the dilution equivalent to the 50% of each serum sample).

Yellow fever antibody titres (in IU/mL) were classified as follows: titres \( \geq 2.9 \log_{10} \text{IU/mL} \) or reciprocal of the dilution \( \geq 50 \) indicated positive serology; titres \(< 2.5 \log_{10} \text{IU/mL} \) or reciprocal of the dilution \(< 5 \) indicated negative serology; titres \( \geq 2.5 \) and \(< 2.9 \log_{10} \text{IU/mL} \) or reciprocal of the dilution \(\geq 5 \) and \(< 50 \) indicated undetermined serology.

The serum samples were also tested for the presence of IgG by ELISA to confirm the presence of anti-yellow fever antibodies in sera from vaccinated subjects according to previously described methods [9]. Serological tests (IgG) for dengue were performed at the Flavivirus Laboratory of the Oswaldo Cruz Institute (Rio de Janeiro) using PANBIO dengue IgG indirect Elisa (Brisbane, Australia) [10]. Dengue is a flavivirus with widespread circulation in Brazil. Neutralising antibody response to YF vaccine is highly specific with no or low-titre antibodies to other flavivirus, but evidence for interference by naturally acquired heterologous flavivirus immunity with 17D vaccine in humans is conflicting [11].

2.3. Statistical analysis

The response variable of interest was the serum neutralising antibody titres (in IU/mL), which were converted to \( \log_{10} \) values and categorised. The co-variables of interest were age (in years), gender, presence of anti-dengue virus antibodies, prior vaccination, history of severe illness (hospitalisation, disease sequelae, and disability), comorbidity and medications used at the time of blood collection. The rate of seropositivity and the geometric mean antibody titres, along with the corresponding 95% confidence intervals (CI), were estimated for each subgroup of time since vaccination.

In the multivariate analysis, the immune response (indicated by \( \log_{10} \) of titres in the multiple regression model and seropositivity in the logistic regression model) was modelled as a function of the time (in months) elapsed since vaccination as a continuous variable and categories: 30–45 days, 1–9 years, 10–11 years, and \( \geq 12 \) years.
Further eligibility assessment (n=721)

Excluded (n=30): seropositive before vaccination (n=15); previous vaccination < 1 year (n=6); missing proof of vaccination (=2); failed to attend blood collection (n=4); travelled to endemic areas (n=1); previous 2 doses of vaccine (n=2)

History of single dose (n=526)
Time since previous vaccination:
1 – 4 years (n=114)
5 – 9 years (n=83)
10 – 11 years (n=138)
12+ years (n=191)

Serological test 30-45 days after vaccination (n=165)

Analysed (n=125)
Excluded from analysis with seropositive or inconclusive pre-vaccination serology (n=40); seropositive for yellow fever, whereas 30 subjects had neutralising antibody titre ≥ 2.5 and < 2.9 \( \log_{10} \) IU/mL. The latter were excluded from the analysis as previous vaccination could not be ruled out in individuals with borderline titres (Fig. 1). Their results were disregarded to ensure the reference group contained only primovaccinated subjects. Post-vaccination seropositivity among the 40 subjects excluded because of yellow fever high or borderline titres before vaccination was 89.7%, whereas for those seronegative it was 93.7%.

As shown in Table 2, approximately 93% of volunteers in the reference group became seropositive after vaccination. The percentage of subjects with neutralising antibody titres ≥ 2.9 \( \log_{10} \) IU/mL decreased gradually from 1–4 years up to 10–11 years post-vaccination. However, there was an unexpected increase in the proportion of seropositive subjects in the subgroup vaccinated for ≥ 12 years (Table 2). The distribution of antibody titres according to the elapsed time since vaccination and the corresponding GMT showed higher titres in newly vaccinated subjects (up to 45 days) decreasing sharply in 1–4 years and slightly in 10–11 years, and followed by an unexpected slight increase in subjects at ≥ 12 years post-vaccination (Fig. 2 and Table 3).

The decreasing trend in antibody titres with the time since vaccination appeared strongly modified by age as the data showing a significant decline in antibody titres after one year were available only for 18–30-year-old subjects (Fig. 3). An increasing trend in the mean titres across age groups was disclosed in volunteers with 10–11 years and ≥ 12 years post-vaccination.

Fig. 1. Flowchart of recruitment and monitoring of the volunteers.

after primo-vaccination (categories 1–4 and 5–9 years were collapsed for multivariate analysis). The co-variables included in the model were age, gender, city of residence, and serological status for dengue. Statistical analysis was performed using the software SPSS® (SPSS Inc., Chicago, IL) and WinPEPI [12].

3. Results

The study group consisted of a non-random sample of 721 adult volunteers, which included military personnel from 7 Army units located in the city of Rio de Janeiro (50.7%), and civilians from the Manguinhos campus at Fiocruz in Rio de Janeiro (16%) and from health centres in Alfenas, Minas Gerais (33.3%). Volunteers were recruited between August 2011 and July 2012. The recruitment sites were selected based on expected numbers of eligible subjects.

Of the 721 volunteers, 691 (95.8%) met all eligibility criteria and were included in the analysis (Fig. 1). The eligible volunteers were predominantly male (73.4%), aged 18–83 years, and the time since vaccination ranged from 30 days to 18 years. In the newly vaccinated subgroup all subjects were male, aged 18–30 years, and the time since vaccination ranged from 30 to 45 days (data not shown). Subjects aged 31–59 years had that highest proportion with 12 years or more of vaccination, whereas most volunteers 60 years and older had been vaccinated 5–9 years before (Table 1).

In the newly vaccinated subgroup, the levels of neutralising antibodies prior to vaccination indicated that 10 subjects were
The percentage of subjects with anti-dengue IgG titres $>1:40$ was 61.9%, overall, and 89.0% among subjects from Rio de Janeiro and 13.7% for Alfenas residents. There was no apparent correlation between the immunological statuses for dengue and yellow fever, as the rate of yellow fever seropositives by PRNT was similar to that of seropositives and seronegatives (IgG) for dengue (Table 4). The distribution of post-vaccination titres was somewhat skewed for higher values in dengue-IgG positive subjects, whose yellow fever antibody GMT was 3118 IU/mL (95%C.I.: 2756–3527), whereas dengue IgG negative subjects had a GMT 2445 IU/mL (95% C.I.: 2094–2860). However, the comparability of dengue IgG positive and negative subgroups was confounded by age and time since vaccination.

In the multivariate analysis, only the elapsed time since vaccination had a significant correlation with the antibody titres (using the multiple regression model) and with positive serology for...
yellow fever (using the logistic regression model). Consistent with the effects of the elapsed time since vaccination and age on antibody titres shown in Fig. 3, the interaction term of those two independent variables in the multiple regression model was statistically significant ($p < 0.001$).

### 4. Discussion

The duration of immunity after yellow fever vaccination is the main parameter to determine the need and timing for revaccinations, which have important implications in immunisation programmes for residents and travellers to and from endemic areas, and for laboratory personnel who handle vaccine or sylvatic virus strains. The yellow fever vaccine is the only attenuated virus vaccine in which the recommendation for revaccination is every 10 years, indefinitely, without sound scientific basis. The recommendation of a single vaccine dose for life is still controversial, and should probably await more convincing scientific evidence [13,14] before implementation. An alternative to consider is that, similarly to other vaccines, primary and secondary yellow fever vaccine failures might occur and should discourage both the recommendation of a single dose for life and the need to wait 10 years for revaccination. In this study, the percentage of seropositive subjects and the GMTs of anti-yellow fever antibodies were substantially lower at 5 years post-vaccination when compared with the newly vaccinated subjects (up to 45 days), and continued decreasing, albeit slightly, up to 10–11 years post-vaccination. The rate of seropositivity in the newly vaccinated subjects (93.6% with titres $\geq 2.9\log_{10}$ IU/mL) was slightly lower than in other studies involving adults: 96.0–98.0% [15,16].

A decreasing trend in neutralising antibody titres had been reported in 1948 in Brazilian vaccinees of various age groups, among whom 87% and 72% were reactive (intraperitoneal protection test in adult mice) at 2 and 6 years post-vaccination, respectively [17]. A pronounced decrease in the first 5 years post-vaccination was also shown in 1999 in German vaccinees 10–79 years old [18]. Among those volunteers vaccinated for 11–38 years, 25.5% had neutralising antibodies (PRNT) $\leq 1:10$.

### Table 4

<table>
<thead>
<tr>
<th>Antibody titres against yellow fever ($\log_{10}$ IU/mL)</th>
<th>Anti-dengue virus IgG antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inconclusive</td>
<td>Negative</td>
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<tr>
<td>$N$</td>
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<td>$\geq 2.90$</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
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2008, Colombian volunteers aged 1–76 years were shown to have their seropositivity rates (titres > 1:10, PRNT) decreased from 97.1% among subjects that had been vaccinated for less than 1 year to 68.4% with 4 or more years post-vaccination [16]. Conversely, 95% of subjects vaccinated at the Pasteur Institute for over 10 years had antibody titres detected by PRNT [19]. Volunteers were over 60 years of age and vaccination time was inferred for some of them, without mention of the number of doses.

A study performed in a randomly selected population 16–83 years old, based on travel vaccination records of residents in Recife, Brazil, where there is no yellow fever transmission, reported that the mean neutralising antibody titres by PRNT were higher in 20 subjects vaccinated for 5 years than in 20 subjects vaccinated for 10 years. All subjects were seropositive (PRNT), whereas 60% and 55%, respectively, were IgG positive [20]. However, it was not mentioned that the subjects might have travelled to regions susceptible to disease transmission (with potential for natural boosting) or might have received more than a single vaccine dose.

The higher seropositivity rate in the group immunised for ≥12 years, compared to the group vaccinated for 10–11 years could possibly be due to differences in the vaccine and to non-recorded or non-reported multiple vaccination, as it is plausible that revaccination is more likely in individuals eligible after 10 years of the previous dose. Most likely, these unreported vaccinations also occurred in the 10–11-year vaccination subgroup ceasing antibody decay in some individuals and leading to overestimated seropositive rates attributable to a single dose. That observation disclosed a limitation of this study and illustrated the challenge of ascertaining the number of vaccine doses and time since immunisation in adults. Even more challenging was the characterisation of potential for exposure to natural infection, which led to exclusion of volunteers.

In addition to selecting subjects not likely to be exposed to natural infections, to ensure that yellow fever seropositivity was explained by a single reported dose of the vaccine was a major challenge in this study. In a study used as reference for the single vaccination recommendation by the WHO [21], 9 of 24 volunteers were revaccinated. However, other reference studies have not clarified whether revaccination was considered when assessing the duration of immunity [7].

Methodological differences across studies, such as, the vaccine itself, different sub-strains of vaccine virus, vaccination procedures, volunteer profile, serological test methods and seropositivity criteria, are important factors that may have contributed to the discrepancy of results previously reported. In general, these studies were cross-sectional and the comparison across subgroups with distinct elapsed times since vaccination disregarded variations in immunisation procedures and in the vaccine potency over time. In Brazil, vaccination against yellow fever in routine health care has used the same vaccine and similar procedures for several decades, thus favouring the comparability of results from the different cohorts represented in the present study. On the other hand, the representativeness of non-randomly selected volunteers may be limited.

The selection of volunteers for this study entailed the exclusion of those who resided or remained in geographical areas susceptible to yellow fever transmission so that natural booster infections would not confound the experimental results. Even in areas, such as Alfenas, where vaccination is recommended for residents, yellow fever cases have not occurred in humans for many decades. In addition, epidemiological surveillance data have indicated the lack of circulation of sylvatic virus strains in non-human primates (unpublished data available in worksheets from Minas Gerais State Health Secretary).

In this as in other studies [20,22], yellow fever seropositivity assessed by PRNT did not appear to have been inflated by prior exposure to dengue infection. It seemed more likely that dengue IgG seropositivity (Elisa) could be partly attributed to persisting neutralising antibodies against yellow fever. In contrast, higher neutralising capacity for the yellow fever virus in subjects with anti-dengue IgG antibodies has been reported, and hypothesised that subgroups with positive serology for dengue could develop cross-reactions with anti-yellow fever antibodies [16].

In 2013, the WHO Strategic Advisory Group of Experts (SAGE) announced that a single dose of the yellow fever vaccine provides life-long immunity and that revaccination every 10 years is not necessary for people who live in or travel to risk areas [4]. This new guideline was based on surveillance data indicating that vaccination failures are extremely rare and do not cluster as time increases after immunisations [4]. However, the known limitations in the surveillance of yellow fever cases and in the management of vaccination records, particularly in adults, suggest that data on vaccination failure are underestimated [14]. The rarity of vaccination failure could also be partly explained by the revaccination requirement in immunisation programmes and prior travel to endemic areas. However, the absence of yellow fever cases in vaccinated travellers does not appear to be a good indicator of the duration of immunity, considering that potential natural exposures, which warrant recommendation for vaccination, can impair the assessment of the long-term effects of vaccination.

WHO’s recent recommendations have also generated controversies because the serological methods used have varied over the many decades during which the studies that served as the basis for the recommendations were performed [14]. In addition, the PRNT method that determines neutralising antibody titres, which is considered the best available measure of seroprotection following vaccination, has exhibited considerable heterogeneity and allows only limited comparability between results [14].

A review exploring the scientific evidence for a change in the vaccination recommendation proposed by the WHO [7] appears to disregard the possibility that seronegative subjects may have been a result of primary or secondary failures of the vaccine. In fact, the high levels of vaccine immunogenicity in clinical studies under controlled immunisation conditions in selected groups may not be reproduced in routine immunisation programmes. These are generally affected by problems related to vaccine conservation and application, and may include subjects with clinical complications that could compromise their immune response. Accordingly, the rate of seroconversion following routine vaccination in military personnel in this study has been reported to be slightly lower than that in healthy volunteers in controlled studies [15]. In addition, a weaker immune response can result in shorter immunity duration. Cut-off values correlating with protection are not available for antibody titres measured by serum-dilution plaque-reduction tests. As in several previous studies, we analysed seropositivity as a convenient, but possibly exaggerated proxy of protection. On the other hand, antibody titres are important indicators of the occurrence of immunological memory and may indicate a direct association between positive serology and immune protection. Therefore, a complete assessment of the immunological memory must include components of the cellular immune system, which are crucial for cytotoxic responses and the effective production of neutralising antibodies [11].

Considering the absence of herd immunity during the sylvatic cycle of yellow fever, immunisation programmes need to effectively reach all individuals at risk because viral circulation occurs independently of human hosts. In sub-Saharan Africa, where yellow fever outbreaks result from the urban transmission cycle, herd immunity assumes that the vaccination coverage should be homogeneous to avoid the occurrence of outbreaks in susceptible population groups. SAGE also indicated in the position paper [4] that surveillance data and clinical studies can identify specific risk
groups, such as infants and HIV-infected individuals, who could benefit from a second immunisation or a booster dose. In South American countries, where yellow fever vaccination is routinely administered during the first year of life, and in African countries, where the risk of yellow fever and the high prevalence of HIV infection coexist, a second immunisation or a booster dose might therefore be indicated, consistent with evidence suggesting that those subgroups appear to mount less intense responses after vaccination [7].

In conclusion, serological data from this and other studies may indicate the need to anticipate revaccination, considering that the percentage of seronegative subjects is high at 5 years post-vaccination, and the performance of serological tests to select subjects in need of revaccination is not recommended as a public health measure. The recommendation to abolish subsequent vaccination every 10 years would appear safer if the administration of 2 doses is adopted in endemic areas, particularly those where primovaccination is routinely performed in children under 2 years old.

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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 ascertain- ment 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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