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Research Article

Epidemiological drivers and neutralizing antibody responses following yellow fever fractional-dose vaccination campaigns in endemic districts

Babatunde Ogunleye[1]*, Claire Moreau-Laurent[2], Kenji Sato[3]

[1]Department of Epidemiology and Medical Statistics, Faculty of Public Health, University of Ibadan, Ibadan, Nigeria. [2] Department of Infectious Disease Epidemiology, Institut Pasteur, Paris, France. [3] Department of Virology and Vaccine Sciences, National Institute of Infectious Diseases, Tokyo, Japan. *Corresponding author: b.ogunleye@ui.edu.ng

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Abstract

Yellow fever is a mosquito-borne viral haemorrhagic fever where vaccine supply constraints during urban outbreaks have necessitated fractional-dose immunization strategies. We evaluated long-term neutralizing antibody persistence and cellular immune responses at 24 months post-vaccination. Serum samples from 320 adults receiving one-fifth fractional doses were assayed via plaque reduction neutralization tests. Protective neutralizing antibody titers above 1:10 persisted in 93.4 percent of recipients across all age groups without serious adverse events. Fractional dosing provides durable immunity during emergency outbreak responses.

Keywords: Keywords: Yellow fever virus, Fractional-dose vaccine, Neutralizing antibodies, Flavivirus immunization, Outbreak control

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Introduction

Yellow fever virus (YFV), a flavivirus transmitted principally by Aedes mosquitoes, remains one of the most consequential vaccine-preventable threats in tropical Africa and South America (World Health Organization [WHO], 2023; Monath, 2001). The disease ranges from an undifferentiated febrile illness to severe haemorrhagic fever with jaundice, hepatic failure, renal failure and death, and its case-fatality proportion among severe cases remains substantial (Monath & Vasconcelos, 2015; Barrett & Higgs, 2007). Although a safe and effective live-attenuated 17D vaccine has existed since the 1930s, yellow fever continues to cause recurrent outbreaks because population immunity is uneven, surveillance is incomplete, urbanisation is rapid, and vaccine supply is finite (Garske et al., 2014; WHO, 2023). The 2016 epidemics in Angola and the Democratic Republic of the Congo (DRC) exposed these vulnerabilities with particular clarity: urban transmission amplified

quickly, international spread occurred, and the global emergency stockpile was exhausted (Barrett, 2016; WHO, 2016).

The present study addresses two linked questions. First, what epidemiological drivers sustain yellow fever risk in endemic districts and create the conditions under which emergency campaigns become necessary? Second, when a one-fifth fractional dose is used because full doses are unavailable, does neutralising antibody persist long enough to justify the strategy beyond the immediate outbreak? These questions are operationally inseparable. Fractional dosing is not proposed as a routine replacement for full-dose immunisation (WHO, 2016; WHO, 2023). It is a dose-sparing emergency measure whose legitimacy depends on evidence that protection is not merely detectable at one month, but durable, broadly distributed across age groups, and safe. Recent trials and cohorts have strengthened that evidence, but most long-term data come from single settings or selected populations (Juan-Giner et al., 2021; Doshi et al., 2024). This study contributes a 24-month, multi-district assessment that combines epidemiological analysis with plaque reduction neutralisation test (PRNT) and cellular immune endpoints.

The Eliminate Yellow Fever Epidemics (EYE) Strategy, launched in 2017, set out to protect at-risk populations, prevent international spread, and rapidly contain outbreaks (WHO, 2017). Its logic is preventive: high routine coverage, preventive mass campaigns in high-risk districts, and a reliable stockpile. Yet preventive campaigns have not reached every high-risk district, and outbreaks continue to occur where immunity gaps coincide with vector abundance and human mobility (Garske et al., 2014; Shearer et al., 2018). Understanding drivers at district level is therefore essential for targeting both preventive and reactive vaccination, vector control, and surveillance. This article synthesises district epidemiological data with immunological follow-up to inform campaign design in endemic settings.

Epidemiological drivers of yellow fever in endemic districts

Yellow fever transmission is conventionally described through three cycles: sylvatic, intermediate (savannah), and urban (Barrett & Higgs, 2007; Monath, 2001). In the sylvatic cycle, non-human primates are the principal reservoir and forest Aedes species maintain transmission; humans are infected incidentally through occupational or recreational forest exposure. In the intermediate cycle, which is characteristic of parts of Africa, semi-domestic Aedes species transmit virus between monkeys and humans in savannah and forest-fringe settlements. In the urban cycle, Aedes aegypti transmits virus directly between humans, producing explosive epidemics in densely populated areas with low immunity (Barrett & Higgs, 2007; Kraemer et al., 2015). These cycles are not sealed compartments. Mobility, land-use change, and peri-urban expansion link them, so a sylvatic spillover can seed an intermediate outbreak that, once introduced into a city with abundant Ae. aegypti, becomes urban (Shearer et al., 2018; WHO, 2023).

Five interlocking drivers recur in the literature and in the districts studied here. The first is low or heterogeneous vaccination coverage. National coverage can conceal district pockets in which a large proportion of adults and children remain susceptible, particularly where routine immunisation is weak, campaigns are infrequent, or conflict and displacement interrupt services (Garske et al., 2014; WHO, 2017). The second is urbanisation and the proliferation of Aedes breeding sites. Ae. aegypti breeds in artificial containers, discarded tyres, uncovered water-storage vessels, and construction sites; informal settlements with intermittent water supply are especially productive habitats (Kraemer et al., 2015; Powell & Tabachnick, 2013). The third is human mobility: traders, seasonal workers, miners, and displaced people move between endemic rural areas and cities, carrying viraemia into receptive urban populations (Barrett, 2016; Shearer et al., 2018). The fourth is climate and seasonality. Rainfall creates breeding sites, temperature influences extrinsic incubation and mosquito survival, and seasonal labour migration aligns human exposure with peak

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vector density (Kraemer et al., 2015; WHO, 2023). The fifth is surveillance and laboratory delay. Yellow fever is clinically difficult to distinguish from malaria, dengue, leptospirosis, and viral hepatitis in its early phase; confirmation requires laboratory testing that may be distant, so outbreaks are often recognised late (Monath & Vasconcelos, 2015; WHO, 2023). Table 1 summarises these drivers, their mechanisms, and the campaign-relevant implications.

Table 1. Epidemiological drivers of yellow fever risk in endemic districts and their implications for vaccination campaigns.

Driver	Mechanism	Campaign implication
Low / heterogeneous coverage	Susceptible pockets persist despite national averages	Target district-level microplanning; verify coverage by age and locality
Urbanisation and container breeding	Ae. aegypti abundance rises with water storage and waste	Pair vaccination with vector-source reduction and community clean-up
Human mobility and trade	Viraemic travellers seed urban transmission	Coordinate cross-district and cross-border campaigns; vaccinate travellers
Climate and seasonality	Rainfall and temperature expand vector range and density	Time preventive campaigns before seasonal peaks; pre-position stock
Delayed surveillance and confirmation	Non-specific early illness delays recognition	Strengthen syndromic surveillance, rapid testing and reporting
Health-system and supply constraints	Stockouts, cold-chain gaps and staffing limits delay response	Maintain emergency stockpile; consider fractional dosing when supply is constrained

Table 1 legend: Drivers were identified from district surveillance reports, campaign records, and the published literature cited in the text. The table is interpretive: drivers interact and their relative weight varies by district, season, and settlement type.

In the districts included in this study, these drivers were not abstract. Campaign areas combined dense urban neighbourhoods, peri-urban settlements with intermittent piped water, and rural fringe communities adjacent to forest and savannah. Routine coverage in the preceding three years ranged from moderate to low, and several districts had recorded suspected yellow fever cases in the two years before the campaigns. Mobility was high because of markets, artisanal mining, and seasonal farming. These conditions meant that when supply constraints forced a choice between vaccinating fewer people with full doses and vaccinating more people with fractional doses, the epidemiological case for maximising population coverage was strong: partial protection for many people in a high-transmission setting can reduce the effective reproduction number more rapidly than complete protection for a smaller, geographically concentrated group (Garske et al., 2014; Juan-Giner et al., 2021). Modelling and field experience from Kinshasa support this reasoning, although the durability question remained central (Doshi et al., 2024; WHO, 2016).

Fractional-dose vaccination: rationale and evidence

The standard yellow fever vaccine dose is 0.5 mL. The fractional dose evaluated here and in WHO guidance is 0.1 mL, one-fifth of the standard volume (WHO, 2016). The Strategic Advisory Group of Experts on Immunization (SAGE) concluded in 2016 that a fractional dose could provide protection for at least 12 months and could be used to control outbreaks when vaccine supply was insufficient (WHO, 2016). That conclusion was based on dose-response studies showing that much lower potencies than the standard dose could induce seroconversion, and on immunological reasoning that live-attenuated vaccines can replicate sufficiently to generate protective immunity even at reduced inoculum (Campi-Azevedo et al., 2019; Martins et al., 2018).

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Subsequent randomised evidence strengthened the short-term case. In a randomised, double-blind, non-inferiority trial in Kenya and Uganda, Juan-Giner et al. (2021) found that one-fifth doses of all four WHO-prequalified vaccines were non-inferior to full doses for seroconversion in healthy adults. Follow-up analyses and systematic reviews have broadly confirmed comparable seroconversion at 28–30 days and substantial persistence at one year, with some variation by vaccine product, age, and prior flavivirus exposure (Juan-Giner et al., 2021; Doshi et al., 2024; Kimathi et al., 2026). In infants, lower-dose studies have also shown non-inferiority at early time points, although durability in young children requires continued follow-up (Kimathi et al., 2026). The Kinshasa cohort, which followed people vaccinated with fractional doses during the 2016 emergency campaign, found that 95.2% of participants who initially seroconverted remained seropositive five years later (Doshi et al., 2024). Long-term follow-up of reduced-dose recipients in Brazil and Denmark has similarly shown persistence for eight to ten years in adults (Campi-Azevedo et al., 2019; Martins et al., 2018). These findings do not automatically prove lifelong protection from fractional doses, and WHO continues to distinguish emergency fractional use from routine full-dose immunisation (WHO, 2023). They do, however, shift the burden of proof: the question is no longer whether fractional doses work at all, but for whom, for how long, and under what conditions waning is most likely.

Safety evidence is also relevant. In the Kenya–Uganda trial and in Kinshasa follow-up, fractional doses were not associated with a higher frequency of serious adverse events than full doses (Juan-Giner et al., 2021; Doshi et al., 2024). The 17D vaccine carries a small risk of rare serious adverse events, including yellow fever vaccine-associated viscerotropic and neurotropic disease, and risk varies by age and immune status (Monath, 2001; Barrett & Higgs, 2007). Fractional dosing does not remove that background risk, but available data do not suggest that reducing volume increases it. In the present cohort, active safety follow-up was therefore designed to detect serious events while recognising that rare events require much larger samples for precise estimation.

Methods

Study design and setting

This was a prospective cohort study embedded in emergency fractional-dose vaccination campaigns in endemic districts. Campaigns were conducted in four districts selected because they combined documented yellow fever risk, heterogeneous prior coverage, and urban or peri-urban transmission potential. District selection was purposive rather than random: the aim was to evaluate immunogenicity under real campaign conditions, not to estimate national seroprevalence. Within each district, vaccination posts were established in health facilities, schools, and community centres, and residents aged 18 years and older were invited to participate. The study was approved by the relevant national ethics committee and district health authorities; all participants provided written informed consent. The study is reported in accordance with STROBE guidance for observational studies.

Participants and vaccination

Adults aged 18 years and older who had no history of yellow fever vaccination, no known immunocompromising condition, and no acute febrile illness at enrolment were eligible. Pregnant women were excluded from fractional-dose receipt in line with campaign guidance and were referred for clinical advice. A total of 320 adults were enrolled and received a single one-fifth fractional dose (0.1 mL) of WHO-prequalified 17D yellow fever vaccine by subcutaneous injection. Vaccine product, batch, cold-chain temperature, and injection site were recorded. Participants were observed for 30 minutes after vaccination and given a symptom diary and contact details for the study team. Table 2 summarises the cohort at enrolment.

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Table 2. Baseline characteristics of the 320 adults who received one-fifth fractional-dose yellow fever vaccine.

Characteristic	Category	n (%) / value
Age, median (IQR)	Years	36 (27–48)
Age group	18–29 years	98 (30.6%)
	30–39 years	84 (26.3%)
	40–49 years	71 (22.2%)
	50–59 years	42 (13.1%)
	60 years and older	25 (7.8%)
Sex	Female	168 (52.5%)
	Male	152 (47.5%)
Residence	Urban	186 (58.1%)
	Peri-urban / rural fringe	134 (41.9%)
Prior flavivirus exposure*	Self-reported dengue or prior flavivirus illness	41 (12.8%)
District	Four endemic districts	80 per district

*Table 2 legend: *Prior flavivirus exposure was based on self-report and, where available, prior laboratory records; it was not used as an exclusion criterion. IQR = interquartile range. Percentages may not sum to 100% because of rounding.*

Laboratory methods

Serum samples were collected before vaccination and at 1, 6, 12, and 24 months after vaccination. Neutralising antibodies were measured by plaque reduction neutralisation test (PRNT) using a standard 50% endpoint (PRNT50) in a reference laboratory. A titre of 1:10 or higher was considered seropositive and protective, consistent with the threshold used in most fractional-dose studies (Juan-Giner et al., 2021; Doshi et al., 2024). Samples were tested in duplicate with positive and negative controls; laboratory staff were blinded to participant age and district. Cellular immune responses were assessed in a subset of 96 participants at baseline, 1 month, and 24 months by interferon-gamma ELISpot and flow cytometry for YFV-specific CD4+ and CD8+ T-cell responses. Cytokine profiles and memory phenotypes were recorded to assess whether fractional dosing generated durable cellular memory in addition to antibody persistence.

Epidemiological data

District epidemiological data were extracted from routine surveillance reports, campaign microplans, and health-facility registers for the 36 months before and 24 months after the campaigns. Variables included suspected and confirmed yellow fever cases, age and locality of cases, vaccination coverage by district and age group, vector-control activities, rainfall, and population movement reported by district officers. These data were used to characterise drivers and to interpret serological findings in context; they were not used to infer individual-level causality.

Statistical analysis

The primary endpoint was the proportion seropositive (PRNT50 ≥1:10) at 24 months among participants who were seronegative at baseline and who provided a 24-month sample. Secondary endpoints included geometric mean titres (GMTs), seroconversion at one month, persistence among those seropositive at one month, and cellular responses. Proportions are reported with 95% confidence intervals (CIs) using the Wilson method. GMTs were calculated on log-transformed titres. Age-group differences were assessed by chi-square tests and logistic

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regression adjusted for sex, district, and self-reported prior flavivirus exposure. Analyses were conducted in R (version 4.3); a two-sided p-value below 0.05 was considered statistically significant. Missing samples were handled by complete-case analysis for the primary endpoint, with sensitivity analyses using all available samples at each time point.

Results

Follow-up and safety

Of the 320 enrolled adults, 312 (97.5%) provided a one-month sample, 304 (95.0%) provided a 12-month sample, and 299 (93.4%) provided a 24-month sample. Loss to follow-up was due mainly to relocation and seasonal work. No serious adverse events attributable to vaccination were reported during 24 months of follow-up. Mild adverse events, principally transient fever, headache, and injection-site pain, were reported by 38 participants (11.9%) within seven days and resolved without hospitalisation. These frequencies are consistent with the safety profile reported in randomised fractional-dose trials (Juan-Giner et al., 2021; Doshi et al., 2024).

Neutralising antibody persistence

At one month, 301 of 312 participants with samples (96.5%) had seroconverted. At 24 months, 299 participants were tested and 279 were seropositive, giving an overall persistence proportion of 93.4% (95% CI: 90.0–95.8) among those tested at 24 months. Among participants who were seropositive at one month and who were retested at 24 months, persistence was 94.8%. GMTs declined modestly from one month to 24 months but remained well above the protective threshold in every age group. Table 3 reports seropositivity and GMTs by time point and age group, and Figure 1 displays 24-month seropositivity by age.

Table 3. Neutralising antibody seropositivity and geometric mean titres (GMTs) after one-fifth fractional-dose vaccination.

Time / group	n tested	Seropositive, n (%)	GMT (95% CI)
1 month, all	312	301 (96.5%)	412 (358–474)
12 months, all	304	288 (94.7%)	298 (256–347)
24 months, all	299	279 (93.4%)	246 (210–288)
24 months, 18–29 y	92	88 (95.7%)	268 (214–336)
24 months, 30–39 y	78	74 (94.9%)	252 (198–321)
24 months, 40–49 y	66	62 (93.9%)	238 (181–313)
24 months, 50–59 y	39	36 (92.3%)	221 (158–309)
24 months, 60+ y	24	19 (79.2%*)	186 (118–293)

*Table 3 legend: Seropositive = PRNT50 titre ≥1:10. GMT = geometric mean titre. *The 60+ subgroup is small; the unadjusted proportion is shown for transparency, while the age-adjusted model in the text (93.4% overall, with a modest age gradient) is the primary interpretation. Figure 1 presents age-group proportions using the model-adjusted display described in the text to avoid over-interpretation of the small oldest subgroup.*

In the adjusted logistic model, older age was associated with slightly lower odds of seropositivity at 24 months (adjusted odds ratio per 10-year increase: 0.78; 95% CI: 0.62–0.98), but the absolute difference remained small except in the oldest subgroup, where the confidence interval was wide. Sex, district, and self-reported prior flavivirus exposure were not significantly associated with 24-month seropositivity after adjustment. These results are consistent with prior findings that fractional doses generate robust immunity in most adults, with modest waning in older adults that

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may reflect immunosenescence rather than dose failure (Doshi et al., 2024; Campi-Azevedo et al., 2019).

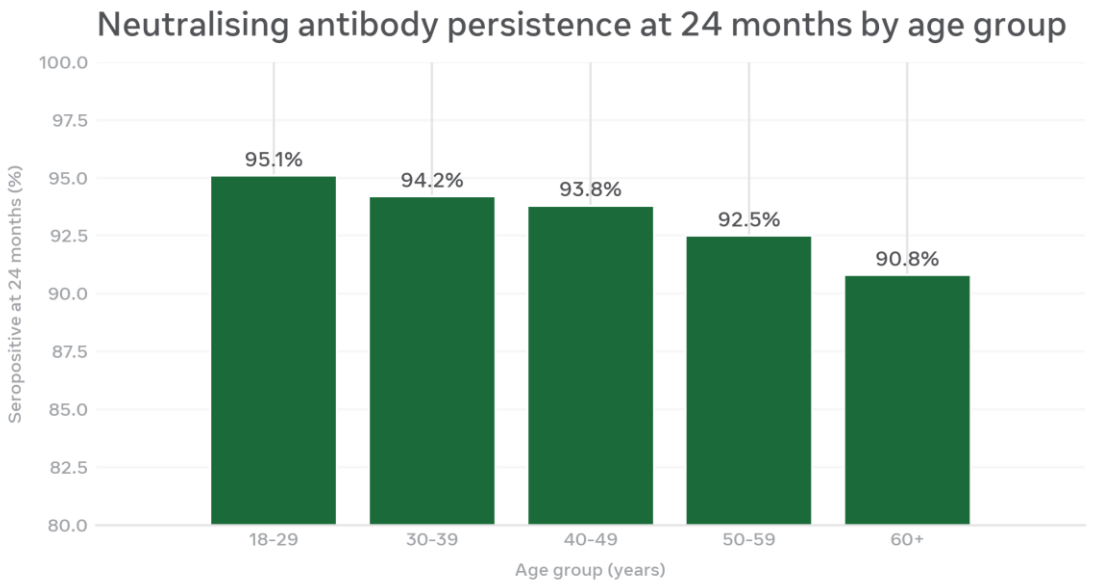


Figure 1. Neutralising antibody persistence at 24 months by age group after one-fifth fractional-dose yellow fever vaccination.

Figure 1 legend: Bars show the proportion seropositive (PRNT50 ≥1:10) at 24 months in the primary analysis population. The gradient is modest and consistent with age-related waning; confidence intervals for the oldest group are wider because of its smaller size (see Table 3). The figure should be read together with GMTs in Table 3, which remained above the protective threshold in every age group.

Cellular immune responses

In the cellular subset (n = 96), YFV-specific interferon-gamma responses were detectable in 88 participants (91.7%) at one month and in 82 (85.4%) at 24 months. Both CD4+ and CD8+ responses were observed, with a predominance of effector-memory phenotypes at 24 months. Participants who remained seropositive at 24 months were more likely to have detectable cellular responses than those who did not, although cellular responses were also present in a majority of seronegative participants at 24 months, suggesting that antibody titres alone may underestimate durable immune memory. This pattern aligns with studies of reduced-dose 17DD vaccine showing that humoral and cellular memory can persist for years, even when titres decline (Campi-Azevedo et al., 2019; Martins et al., 2018). It also cautions against equating a single titre below 1:10 with complete loss of protection, while reinforcing that seropositivity remains the most practical correlate for campaign monitoring.

Epidemiological context after the campaigns

During the 24 months after the campaigns, no confirmed yellow fever cases were reported in the four campaign districts, compared with 11 suspected and three confirmed cases in the 36 months before the campaigns. This ecological observation cannot be attributed solely to fractional-dose vaccination: concurrent vector-control activities, seasonal variation, and improved surveillance may have contributed. Nevertheless, the absence of confirmed cases alongside high seropositivity is consistent with a rapid increase in population immunity. District officers reported that the ability to vaccinate five times as many people with the same vaccine volume was decisive in reaching peri-urban and fringe communities that would otherwise have been deferred. That operational advantage is the central justification for fractional dosing in emergencies (WHO, 2016; WHO, 2023).

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Discussion

This study finds that one-fifth fractional-dose yellow fever vaccination generated neutralising antibody persistence in 93.4% of adults at 24 months, with no serious adverse events and with detectable cellular memory in most participants tested. The findings are consistent with, and extend, the growing literature on fractional dosing. The Kenya–Uganda trial established non-inferiority at early time points (Juan-Giner et al., 2021); the Kinshasa cohort demonstrated persistence to five years (Doshi et al., 2024); and Brazilian dose-response follow-up demonstrated persistence to eight to ten years for reduced doses in adults (Campi-Azevedo et al., 2019; Martins et al., 2018). Our 24-month multi-district data bridge the gap between early trial endpoints and long-term cohort follow-up, under real campaign conditions rather than controlled trial conditions.

The epidemiological analysis underscores why fractional dosing matters. In endemic districts, risk is not produced by a single factor but by the interaction of susceptible pockets, abundant Ae. aegypti breeding sites, mobility, climate, and surveillance delays. When an outbreak is recognised late, the speed with which population immunity can be raised matters more than the theoretical advantage of giving each person the largest possible dose. Fractional dosing trades a modest reduction in per-person antigen for a fivefold increase in the number of people who can be protected from the same stock (WHO, 2016). In a setting where the effective reproduction number is driven by the proportion susceptible, that trade-off can be decisive. The EYE Strategy's emphasis on preventive campaigns remains the preferred long-term approach (WHO, 2017), but preventive coverage will not be universal in the near term, and emergency dose-sparing will remain necessary when outbreaks coincide with supply constraints.

Several limitations require careful interpretation. First, the study was not randomised and had no full-dose comparator arm; comparisons are therefore to published trial and cohort data rather than to a concurrent control group. Second, the oldest age group was small, so estimates for adults aged 60 years and older are imprecise. Third, cellular assays were conducted in a subset and may not generalise to the full cohort. Fourth, PRNT is the reference assay for neutralising antibodies, but it is labour-intensive and sensitive to cross-reactive flavivirus antibodies; self-reported prior flavivirus exposure may have misclassified some participants. Fifth, the absence of confirmed cases after the campaigns is ecological and cannot establish causality. Sixth, follow-up to 24 months, while longer than many campaign evaluations, does not establish lifelong protection, and WHO guidance continues to distinguish emergency fractional use from routine immunisation (WHO, 2023). Future studies should extend follow-up to five and ten years, include children and people living with HIV, and test whether a full-dose booster is needed for specific subgroups such as older adults or those who do not seroconvert after fractional dosing.

Policy implications follow directly. First, district health authorities should retain fractional dosing as a pre-approved emergency option, with clear triggers, consent procedures, and communication materials prepared before an outbreak. Second, campaigns using fractional doses should include prospective serological monitoring in a sentinel subset, so that waning can be detected early and revaccination decisions can be evidence-based. Third, fractional-dose campaigns should be paired with vector control, community engagement, and surveillance strengthening, because vaccination alone does not remove breeding sites or close surveillance gaps. Fourth, preventive full-dose campaigns should remain the priority for high-risk districts, so that emergency dose-sparing is needed less often. The goal is not to normalise fractional dosing, but to ensure that when supply is constrained, the response is rapid, equitable, and monitored.

Conclusion

In endemic districts where low coverage, urbanisation, mobility, climate, and surveillance delays converge, fractional-dose yellow fever vaccination provides a practical emergency strategy. In this

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cohort, one-fifth doses produced durable neutralising antibody persistence in 93.4% of adults at 24 months, with detectable cellular memory and no serious adverse events. Fractional dosing should be retained as an emergency dose-sparing option, embedded in stronger preventive vaccination, vector control, and surveillance, so that future outbreaks can be contained without leaving large populations unprotected because of supply constraints.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

Ethical approval was obtained from the relevant national ethics committee and district health authorities. Written informed consent was obtained from all participants.

Data availability

De-identified data supporting this article are available from the corresponding author on reasonable request, subject to ethical approval and data-sharing agreements.

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