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HIV positivity, Coverage and adherence to the Early Infant Diagnosis testing algorithm, among HIV-exposed infants in Uganda: a seven-year national study, 2017–2023

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Research Article

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Abstract

Background

Uganda has made substantial progress in preventing mother-to-child transmission (PMTCT) of HIV; however, the performance and contribution of the Early Infant Diagnosis (EID) program has not been comprehensively evaluated at a national level. This study assessed trends in EID HIV positivity rates, coverage, adherence to the national testing algorithm, and associated risk factors among HIV-exposed infants (HEI) in Uganda from 2017 to 2023.

Methods

We conducted a retrospective secondary data analysis of routinely archived national EID program data from 2017–2023. Key outcomes included EID coverage within two months of birth, adherence to the national EID testing algorithm, HIV positivity rates among HEI among 18 months old infants. Trends over the years, regional variations, and the impact of the COVID-19 period were evaluated. Multilevel mixed effect logistic regression was used to identify associated factors to HIV positivity.

Results

A total of 884,208 eligible records were analyzed. HIV positivity among HEI declined significantly from 3.0% in 2017 to 1.4% in 2023 ($p < 0.01$), reflecting important progress toward elimination of mother-to-child transmission (eMTCT). Coverage of first PCR testing within two months of birth and adherence to national EID testing algorithm varied across the years with no significant increase ($P > 0.05$). Consequently, there was marked decline in adherence after the first PCR tests. Regional disparities were evident in coverage, adherence, and HIV positivity rates. Absence of maternal postnatal antiretroviral therapy (OR = 5.1) and delivery outside a health facility (OR = 3.5) were the strongest associated factors to infant HIV positivity.

Discussion

Uganda's EID program has contributed to the reduction of vertical HIV transmission, by providing rapid identification of HEI infection status and supporting linkage into care for the positive ones. Despite these achievements, testing coverage and adherence to the EID testing algorithm remain suboptimal, and significant regional inequities persist. Strengthening retention across the 18-month testing continuum by targeting vulnerable mothers, as well as focusing on poor performing regions will be critical to achieving and sustaining eMTCT goals.

Trial registration

: Not applicable.

Background

Mother-to-child transmission (MTCT) of HIV remains a major source of new pediatric HIV infections despite significant global declines in incidence. In 2023, an estimated 120,000 children acquired HIV through vertical transmission, of which over 80% were from sub-Saharan Africa(1). Vertical transmission can occur during pregnancy, labor and delivery, or breastfeeding. The scale-up of maternal antiretroviral therapy (ART) and infant prophylaxis has dramatically reduced the risk of transmission from approximately 30% (absence of interventions) to less than 5%, and to below 2% in settings where breastfeeding is not practiced (2).

Uganda has prioritized prevention of mother-to-child transmission (PMTCT) as a core component of its national HIV response, in alignment with global targets for the elimination of mother-to-child transmission (eMTCT) by 2030. Since the introduction and scale-up of lifelong antiretroviral therapy, the national PMTCT program has provided HIV testing, treatment, and follow-up care for pregnant and breastfeeding women and their infants. Recent evaluations of the program have demonstrated important progress, with an overall MTCT rate of 2.7%, below the 5% threshold for elimination(3). These gains have been largely attributed to increased maternal ART coverage, viral suppression, and higher rates of facility-based deliveries attended by skilled health workers (3).

Complementing maternal PMTCT interventions, the Early Infant Diagnosis (EID) program provides a critical mechanism for monitoring HIV infection outcomes among HIV-exposed infants (HEI) and ensuring timely linkage to care. Established in Uganda in 2006, the program facilitates virological testing and follow-up of HEI throughout the breastfeeding period (4). The national EID testing algorithm initially consisted of two routine polymerase chain reaction (PCR) tests: the first at six weeks of age (within 2 months) and the second six weeks after cessation of breastfeeding. In 2019, the algorithm was revised to include an additional PCR test at the nineth month of age, aligned with routine measles immunization visits, to facilitate earlier identification of postnatal HIV transmission, and to strengthen longitudinal follow-up (5).

To improve access and efficiency, Uganda has implemented several EID system-strengthening interventions within the study period including; the National Sample Transportation Network; integration of chronic care models; the Electronic Results Dispatch System; and point-of-care testing (POCT) (6–8). Introduced in 2019, POCT complements centralized laboratory testing and expanded to 130 facilities by 2020(9). These innovations are intended to improve timely diagnosis, linkage to treatment, and retention across the infant testing continuum. Despite these investments, facility-level studies continued to report important operational challenges, including poor adherence to the national EID testing algorithm, delayed first PCR testing beyond the recommended two-month window, and incomplete progression through the testing cascade, with only about half of HIV-exposed infants completing recommended testing (10, 11).

Despite considerable investments in EID service delivery and system strengthening, evidence on the long-term national performance of the program remains limited. Previous studies have largely been facility-based or focused on specific components of the EID cascade, providing limited insight into national trends in testing coverage, adherence to the testing algorithm, infant HIV positivity, and regional disparities. A comprehensive evaluation of routinely archived program data is therefore needed to assess progress, identify persistent gaps, and inform strategies to achieve and sustain elimination of mother-to-child transmission of HIV. This study evaluated national trends in EID coverage, adherence to the national testing algorithm, HIV positivity, and factors associated with HIV infection among HIV-exposed infants in Uganda from 2017 to 2023.

Methods

Study aim

This study was aimed at evaluating national trends in EID coverage, adherence to the national testing algorithm, HIV positivity, and factors associated with HIV infection among HIV-exposed infants in Uganda from 2017 to 2023.

Design and setting

We conducted a retrospective secondary analysis of patient level data between 2017 to 2023. This data is routinely archived in the national EID and POCT testing programs, housed at National Health laboratory and Diagnostics Services (NHLDS)- Ministry of Health Uganda. The data is from samples collected from HEI from facilities in 15 health regions across the country through the national laboratory transport system (7) and tested at Central Public Health Laboratories (CPHL) also housed at NHLDS. The 15 health regions include; Busoga, Bugisu, Bukedi, Karamoja, Lango, Teso, Acholi, Tooro, North Central, South Central, Kigezi, Ankole, Bunyoro, Kampala, and West Nile. The facilities within these regions are either government, private-for-profit, or private not-for-profit owned.

The national EID program is integrated within Uganda's national prevention of mother-to-child transmission (PMTCT) framework with the primary role of early identification of HIV infection among HEI and timely linkage to treatment and care. Annual aggregated PMTCT records of- HIV-positive women enrolled in antenatal care (ANC) and live births to HIV-positive mothers between 2017–2022 were extracted from the District Heath Information System 2 (DHIS2). POCT is conducted by 130 facilities across the 15 health regions through the decentralization process.

Participants

This study used a census approach where all eligible patient level records were considered. HIV-exposed infants aged 0–18 months whose records existed in the national EID database between 2017 and 2023

were included in the study. We therefore did not consider records in the database for children older than 18 months of age.

Data abstraction

On average the EID program records 50,000 tests annually. Therefore, all eligible records were included to maximize precision, geographical representation, and the ability to evaluate temporal trends and subgroup differences. The patient level data was extracted from the laboratory information management system tables for EID program for each year by the designated database manager trained in structured query language and secure data handling exported into CSV files, under the supervision of the study coordinator. Demographic variables extracted included; sample ID, age, gender, facility name, district, region, ownership type. Clinical variables included; facility level, dates for sample collection, reception and testing, result, Maternal antenatal and postnatal ART status, infant prophylaxis, infant feeding, PCR type, and facility delivery status. Similarly, the POCT data was abstracted.

Additionally, annual district level aggregated ANC records program between 2017 to 2022 including live births were extracted MoH DHIS2 into excel workbook by the DHIS2 officer. Extracted variables included; District name, year, HIV positive women at ANC, and live births to these women.

Data safety and security was ensured by controlled storage in password protected computers, following procedures that aligned with national and international ethical standards, including data protection regulations.

Outcomes

Coverage

Coverage was defined as the proportion of HIV-exposed infants (HEI) who received a first PCR test by two months of age between 2017 and 2022. The denominator comprised annual regional ANC records of HIV-positive women enrolled in antenatal care, while the numerator included first PCR tests performed through both centralized laboratory (CPHL) and point-of-care testing (POCT) platforms.

Adherence to the EID testing algorithm

Adherence was defined as the proportion of eligible HIV-exposed infants who received HIV testing within the recommended age interval for a given PCR type (first, second and third) allowing a ± 6-week window around each scheduled test.

- First PCR test: 0–2 months of age;
- Second PCR test: 12–18 months of age (2017–2018 algorithm);
- Second PCR test: 8–11 months of age (2019–2023 algorithm); and

- Third PCR test: 12–15 months of age (2019–2023 algorithm).

The denominator consisted of annual live births to HIV-positive mothers between 2017 to 2022 from ANC records. Overall adherence was calculated using the aggregated number of infants who received testing within the recommended age window for a given PCR type during the study period.

HIV positivity

HIV positivity was defined as the proportion of HIV-exposed infants with at least one positive PCR result by 18 months of age during the study period (2017–2023).

Data management and analysis

Data cleaning managed in excel involved; (a) identification of duplicated records using sample identifiers and test dates. These were resolved by retaining the most complete record, (b) Fixing missing values through harmonization of administrative boundaries across facilities, and (c) Correcting inconsistencies like typos. Lastly, patient identifiers like names, caregiver telephone numbers were removed to facilitate analysis.

Descriptive analysis; Continuous variables were summarized using medians and interquartile ranges (IQRs), while categorical variables were summarized using percentages. Infant age was categorized into five algorithm-aligned groups (0–2 months, 3–8 months, 9–12 months, 13–15 months, and > 15 months).

For coverage, adherence, and positivity rate proportions and their 95% confidence intervals were calculated. Additionally, trends were evaluated using the Mann-Kendall test for monotonic trends. Facility regions were used to generate maps for coverage, adherence, and positivity rates using QGIS desktop version 3.44.6 to identify regional disparities and hotspots. Consequently, for comparative analyses, testing periods were categorized according to the COVID-19 pandemic [pre-COVID (2017–2019), COVID-era (2020–2022), and post-COVID (2023)], as well as according to the national EID algorithm in use. Pre-algorithm update (2017–2018) only two PCR tests were recommended 1st at 0-2months and 2nd at 12–18 months of age. Post-algorithm update (2019–2023) three PCR tests are recommended- PCR tests; 1st, 2nd and 3rd at 0-2months, 8–11 months, and 12–15 months of age respectively.

Analysis for associated factors. Multilevel mixed-effects logistic regression model was used to account for clustering at facility and regional levels with independent covariances and random effects. Independent variables included demographic, clinical, and years. Variables with $p < 0.2$ were included in the adjusted model. Multicollinearity was assessed using variance inflation factors (VIF) and the model assessed for goodness of fit using the likelihood ratio test. Results were reported as adjusted odds ratios (OR) with 95% confidence intervals (CI).

Geospatial heat maps were generated using QGIS (version QGIS-OSGeo4W-3.44.6-1; https://qgis.org/). All analyses were conducted using Stata version 17.0 (StataCorp LLC, College Station, TX, USA).

Results

Characteristics of study population

Of the 1,120,973 records screened, 884,208 (78.9%) met the eligibility criteria and were included in the analysis (Fig. 1). Infants aged ≤ 2 months constituted the largest age group (36.8%), and the sex distribution was balanced. Health Centre III facilities contributed nearly half of all records (49.7%), while the South-Central region accounted for the largest proportion (19.0%). The highest number of records was reported in 2019 (19.3%), and first PCR tests comprised 47.8% of all observations. Detailed demographic, geographic, facility, and testing characteristics are presented in Tables 1 and 2.

Records identified in the national EID database (2017–2023)

n = 1,130,945

Figure 1. Selection of HIV-exposed infant records from the national EID database, Uganda 2017–2023.

Table 1
Characteristics of HIV-exposed infants included in the analysis, Uganda, 2017–2023

Demographic Factors	Number of HEI (%)
Gender	
Males	433,858 (49.1)
Females	440,109 (49.8)
Unknown	10,241 (1.2)
Age groups	
≤ 2 months	325,491 (36.8)
3–8 months	106,146 (12.0)
9–12 months	229,027 (25.9)
13–15 months	204545 (23.1)
> 15 months	18,999 (2.2)
Years	
2017	61,401 (6.9)
2018	142,889 (16.2)
2019	170,852 (19.3)
2020	157,681 (17.8)
2021	149,692 (16.9)
2022	116,274 (13.2)
2023	85,419 (9.7)
Health Regions	
Tooro	82,203 (9.3)
Acholi	41726 (4.7)
Ankole	83,399 (9.4)
Bugisu	19,353 (2.2)
Bukedi	20,176 (2.3)
Bunyoro	62,583 (7.1)
Busoga	52,123 (5.9)

Demographic Factors	Number of HEI (%)
Gender	
Kampala	111,845 (12.7)
Karamoja	3,180 (0.4)
Kigezi	27,027 (3.1)
Lango	44,715 (5.1)
North Central	113,577 (12.9)
South Central	168,134 (19.0)
Teso	32,315 (3.7)
West Nile	21,846 (2.5)
Ownership	
Private not-for-profit (PNFP)	201,095 (22.7)
Private for- profit (PFP)	15,944 (1.8)
Government	626,737 (70.9)
Unknown	40,432 (4.6

HEI-HIV exposed infants. Unknown-status could not be determined due to missing entries

Table 2
Distribution of HEI by clinical factors, Uganda 2017–2023

Clinical Factors	Number of HEI (%)
Facility level	
HC III	439,653 (49.7)
Clinic	26,061 (3.0)
HC II	58,279 (6.6)
HC IV	164,702 (18.6)
Hospital	109,551 (12.4)
NRH	652 (0.1)
RRH	31,532 (3.6)
COE	53,778 (6.1)
Maternal antenatal ART status	
On ART	141,182 (16.0)
Unknown	743,026 (84.0)
Infant prophylaxis	
Given ART	345,196 (39.0)
Unknown	539,012 (61.0)
PCR	
First	422,275 (47.8)
Second	287,408 (32.5)
Third	111,529 (12.6)
Unknown	62,996 (7.1)
Maternal postnatal ART status	
On ART	357,096 (40.4)
Not on ART	11,955 (1.4)
Unknown	515,157 (58.3)
Infant feeding status at test	
Breast feeding	141,470 (16.0)

Clinical Factors	Number of HEI (%)
Facility level	
Not breast feeding	59,274 (6.7)
Unknown	683,464 (77.3)
Facility delivery	
Yes	83,396 (9.4)
No	5,350 (0.6)
Unknown	795,462 (90.0)

HEI-HIV exposed infants. Unknown-status could not be determined due to missing entries. HC- Health Centre. NRH- National referral hospital. RRH- Regional referral hospital. COE- Centers of excellence which are specialized centers for HIV care and management like the AIDS support organizations. ART-antiretroviral therapy. PCR-Polymerase chain reaction

First PCR coverage among HEI ≤ 2 months of age

Across the study period, average coverage was 55.7% (95% CI: 55.6–55.9%). Coverage of first PCR testing within two months of birth increased over the study period, peaking at 65.0% in 2022, although the overall trend was not statistically significant (P = 0.13). Following its introduction, POCT contributed 8.5% to overall testing volumes, and therefore, centralized testing continued to account for the majority of first PCR tests performed (Fig. 2 **and supplementary Table 1**).

Regional coverage increased from 50.2% during the pre-COVID period (2017–2019) to 61.9% during the COVID-era (2020–2022). Tooro and Kampala consistently recorded the highest coverage levels, whereas lower coverage was observed in Karamoja and West Nile. Substantial geographic variation persisted throughout the study period, indicating regional inequities in access to timely infant HIV testing (Fig. 3 **and supplementary Table 2**).

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Figure 2. *Trends in first PCR testing volumes and coverage among HIV-exposed infants (HEI) aged ≤ 2 months, 2017–2022. (A) Comparison of annual testing volumes by platforms: centralized testing (green) versus point-of-care testing (POCT) testing (orange). (B–D) Annual per* **3**. **Trends in first PCR testing volumes and coverage among HIV-exposed infants (HEI) aged ≤ 2 months, 2017–2022. (A)** *Comparison of annual testing volumes by platforms: centralized testing (green) versus point-of-care (POCT) testing (orange).* **(B–D)** *Annual percentage coverage, shown as black dots with 95% confidence intervals (shaded areas) for*: **(B)** *overall coverage that combined centralised and POCT testing;* **(C)** *centralized testing coverage using HEI tested at Central Public Health Laboratories (CPHL); and* **(D)** *POCT testing coverage using HEI tested at peripheral facilities.*

Adherence to EID testing algorithm among HEI ≤ 18 months of age

Overall adherence was 60.8% (577,826/951,156; 95% CI: 60.75–60.94) across the years. This varied across the years (P = 0.70) starting at 41.1% 2017 peaking at 85.0% 2018 and dropping to 55.9% in 2022. Adherence was highest for the first PCR tests at ≤ 2 months of age (80.6%), but declined substantially for subsequent tests 58.8% and 33.3% for second and third PCR respectively. (Fig. 4 **and supplementary Table 3**).

Marked regional variation was observed in adherence to the first PCR test at ≤ 2 months of age. Adherence exceeded 85% in South Central, Bunyoro, Kampala, Teso, Busoga, and North Central, but was substantially lower in Karamoja (46.9%) and Bugisu (30.5%) (Fig. 5 **and supplementary Table 4**).

HIV positivity rates among HEI ≤ 18 months of age

HIV positivity among HEI declined significantly over the study period, from 3.0% in 2017 to 1.4% in 2023 (P < 0.01). The overall positivity rate was 2.0% (16,948/857,418; 95% CI: 1.95–2.01), indicating substantial progress toward reducing mother-to-child HIV transmission (Fig. 6 **and supplementary Table 5**).

Positivity rates differed by adherence status and geographic region. Among infants who adhered to the national EID testing algorithm, HIV positivity declined significantly over time (P = 0.01), whereas no significant decline was observed among non-adherent infants (P = 0.06) (Table 3).

Regional variation persisted throughout the study period, with higher positivity rates observed in Bugisu, Busoga, West Nile, Bukedi, and North Central compared with other regions (Fig. 7 **and supplementary Table 6**).

Table 3
HIV positivity among HIV-exposed infants (HEI) by adherence status and PCR stage, Uganda, 2017–2023

Adherence status	PCR level	Total tested	HEI Positivity n (%, 95%CI)	P value
Adherent	**Overall**	**577,826**	**6,237 (1.1, 1.05–1.11)**	**0.01**
	1st PCR (≤ 2 months)	292,359	4,671 (1.6, 1.55–1.64)	
	*2nd PCR (12–18 or 8–11 months)	206,402	1,382 (0.7, 0.63–0.71)	
	3rd PCR (12–15 months)	79,065	184 (0.2, 0.19–0.27)	
Non-adherent	**Overall**	**200,280**	**9,543 (4.8, 4.67–4.86)**	**0.06**
	1st PCR (≤ 2 months)	91,011	6,908 (7.6, 7.42–7.76)	
	*2nd PCR (12–18 or 8–11 months)	61,101	1,126 (1.8, 1.74–1.95)	
	3rd PCR (12–15 months)	14,079	87 (0.6, 0.49–0.75)	
	Unknown	34,089	1,422 (4.2, 3.96–4.38)	

*PCR-polymerase chain reaction. Unknown-status could not be determined due to missing entries. Adherent-number of HEI who tested timely as per the algorithm at 1st PCR (0-2months), *2nd PCR (12-18month between 2017 and 2018, and 8-11months from 2019 onwards), and 3rd PCR (12–15 months from 2019 onwards). Non-adherent did not test on time as per the algorithm. CI-confidence interval.*

Factors associated with HIV positivity rate

Multivariable analysis identified several independent predictors of HIV positivity among HIV-exposed infants (Tables 4 and 5). The strongest risk factor was mothers not on ART postnatal (aOR 5.1, 95% CI: 4.77–5.43), followed by delivery outside a health facility (aOR 3.5, 95% CI: 3.07–4.07), age > 15 months (aOR 3.4, 95% CI: 3.00–3.83), and non-adherence to the EID testing algorithm (aOR 2.5, 95% CI: 2.36–2.75). Significant regional variation persisted after adjustment, indicating geographic disparities in infant HIV outcomes. Lower odds of HIV infection were observed among infants receiving care at Centres of Excellence (COE) and private-for-profit facilities. In addition, infants tested in later years had progressively lower odds of HIV positivity compared with those tested in 2017.

Table 4
Multivariable analysis of demographic factors associated with HIV positivity among HIV-Exposed Infants (HEI) in Uganda, 2017–2023.

Demographic Factors	Number of HEI (n)	Positivity rate %(n)	OR	[95%CI]	P value	aOR	[95%CI]	P value
Years								
2017	59,136	3.0(1797)	Ref			Ref		
2018	136,274	2.8(3848)	0.9	[0.88–0.99]	0.02	0.9	[0.86–0.97]	< 0.01
2019	162,346	2.2(3536)	0.7	[0.68–0.76]	< 0.01	0.8	[0.74–0.84]	< 0.01
2020	153,972	1.8(2747)	0.6	[0.55–0.62]	< 0.01	0.7	[0.65–0.75]	< 0.01
2021	146,919	1.5(2231)	0.5	[0.47–0.54]	< 0.01	0.7	[0.63–0.74]	< 0.01
2022	114,644	1.4(1621)	0.5	[0.44–0.50]	< 0.01	0.7	[0.60–0.71]	< 0.01
2023	84,127	1.4(1168)	0.5	[0.43–0.50]	< 0.01	0.5	[0.48–0.57]	< 0.01
Gender								
Males	420,767	1.9(7939)	Ref			Ref		
Females	426,650	2.1(8748)	1.1	[1.05–1.12]	< 0.01	1.1	[1.05–1.12]	< 0.01
Age groups								
≤ 2 months	318,496	1.6(5066)	Ref			Ref		
3–8 months	101,822	4.7(4752)	3.0	[2.92–3.17]	< 0.01	1.5	[1.33–1.59]	< 0.01
9–12 months	223,173	1.5(3309)	0.9	[0.89–0.98]	0.01	1.6	[1.47–1.75]	< 0.01
13–15 months	196,019	1.5(3000)	1.0	[0.92–1.01]	0.11	1.8	[1.66-2.00]	< 0.01
≥ 15 month	17,908	4.6(821)	2.9	[2.67–3.11]	< 0.01	3.4	[3.02–3.81]	< 0.01
Ownership								
PNFP	195,512	1.8(3428)	Ref			Ref		
PFP	15,350	1.8(275)	1.1	[0.97–1.26]	0.14	0.9	[0.79–1.04]	0.18

Demographic Factors	Number of HEI (n)	Positivity rate %(n)	OR	[95%CI]	P value	aOR	[95%CI]	P value
Years								
GOV	607,357	2.1(12582)	1.1	[1.10–1.16]	< 0.01	1.1	[1.04–1.14]	< 0.01
Unknown	39,199	1.7(663)	1.1	[0.98–1.17]	0.15	1.0	[0.96–1.15]	0.30

HEI-HIV exposed infants. PNFP- private not-for profit. PFP-private for profit. GOV-government. Crude odds ratios (OR) and adjusted odds ratios (aOR) with 95% confidence intervals (CI). Adjusted odds ratios (aOR) account for relevant covariates. HEI- HIV exposed infants

Table 5
Multivariable analysis of clinical and facility-level factors associated with HIV positivity among HIV-Exposed Infants (HEI) in Uganda, 2017–2023.

Clinical Factors	Number of HEI (n)	Positivity rate (%)	OR	[95%CI]	P value	aOR	[95%CI]	P value
Maternal antenatal ART status								
On ART	133806	2.3(3122)	Ref			Ref		
Unknown	723612	1.9(13826)	0.9	[0.84–0.91]	< 0.01	0.8	[0.79–0.87]	< 0.01
Facility delivery								
Yes	81521	1.3(1026)	Ref			Ref		
No	5207	5.4(282)	4.5	[3.82–5.01]	< 0.01	3.5	[3.06–4.06]	< 0.01
Unknown	770690	2.0(15640)	1.6	[1.47–1.67]	< 0.01	1.3	[1.23–1.44]	< 0.01
PCR								
First	419,597	3.0(12404)	Ref			Ref		
Second	285,705	0.9(2636)	0.3	[0.29–0.32]	< 0.01	0.2	[0.23–0.26]	< 0.01
Third	111,282	0.3(324)	0.1	[0.09–0.11]	< 0.01	0.07	[0.069–0.089]	< 0.01
Unknown	40,834	3.9(1584)	1.3	[1.26–1.4]	< 0.01	0.6	[0.52–0.60]	< 0.01
Maternal postnatal ART status								
On ART	340394	1.9(6475)	Ref			Ref		
No ART	11294	18.5(2087)	11.6	[10.97–12.22]	< 0.01	5.1	[4.77–5.41]	< 0.01

HEI-HIV exposed infants. ART-antiretroviral therapy. PCR-polymerase chain reaction. Pre-update (2017–2018) when only two PCR tests were recommended 1st PCR at 0-2months and 2nd PCR at 12–18 months of age. Post-update (2019–2023) three PCR tests 1st, 2nd and 3rd each at 0-2months, 8–11 months, and 12–15 months of age respectively. Unknown-status could not be determined due to missing entries. HC- Health Centre. Crude odds ratios (OR) and adjusted odds ratios (aOR) with 95% confidence intervals (CI). Adjusted odds ratios (aOR) account for relevant covariates. ART = Antiretroviral Therapy; PCR = Polymerase Chain Reaction. HEI- HIV exposed infants

Clinical Factors	Number of HEI (n)	Positivity rate (%)	OR	[95%CI]	P value	aOR	[95%CI]	P value
Maternal antenatal ART status								
Unknown	505730	1.7(8386)	0.9	[0.88–0.94]	< 0.01	1.5	[1.42–1.59]	< 0.01
Infant feeding								
breast feeding	137010	1.5(2027)	Ref			Ref		
Not breast feeding	57911	0.8(475)	0.6	[0.50–0.62]	< 0.01	1.3	[1.15–1.43]	< 0.01
Unknown	662497	2.2(14446)	1.4	[1.38–1.52]	< 0.01	1.3	[1.26–1.40]	< 0.01
Algorithm status								
Pre- update	195410	2.9(5645)	Ref					
Post-update	662008	1.7(11303)	0.6	[0.57–0.61]	< 0.01			
Adherence status								
Yes	624188	1.1(6718)	Ref			Ref		
No	233230	4.4(10230)	4.2	[4.06–4.32]	< 0.01	2.6	[2.36–2.75]	< 0.01

HEI-HIV exposed infants. ART-antiretroviral therapy. PCR-polymerase chain reaction. Pre-update (2017–2018) when only two PCR tests were recommended 1st PCR at 0-2months and 2nd PCR at 12–18 months of age. Post-update (2019–2023) three PCR tests 1st, 2nd and 3rd each at 0-2months, 8–11 months, and 12–15 months of age respectively. Unknown-status could not be determined due to missing entries. HC- Health Centre. Crude odds ratios (OR) and adjusted odds ratios (aOR) with 95% confidence intervals (CI). Adjusted odds ratios (aOR) account for relevant covariates. ART = Antiretroviral Therapy; PCR = Polymerase Chain Reaction. HEI- HIV exposed infants

Discussion

This seven-year national study of EID program demonstrates substantial progress toward elimination of mother-to-child transmission (eMTCT) of HIV in Uganda. HIV positivity among HIV-exposed infants declined significantly from 3.0% in 2017 to 1.4% in 2023, reflecting the continued impact of PMTCT interventions and expanded maternal ART coverage. Despite these gains, important gaps remain in early testing coverage, adherence to the EID testing algorithm, and equitable access to services across regions.

Overall coverage among HEI who tested within two months peaked at 65.0% by 2022, though the upward trend was not statistically significant (P = 0.13). The average coverage across the study period was 55.7% far below the 95% global target for diagnosis. These finding align with the 2025 UNAIDS report were 58% of HIV-exposed infants received a virological test within 2 months of birth (12). These results also mirror Uganda AIDS Commission progress reports between 2016 and 2023 (13–17). Importantly, regional comparisons revealed substantial gains during the COVID-19 era, with coverage rising from 50.2% pre-COVID to 61.9% during the pandemic. The observed increase in coverage during the COVID-era coincided with continued expansion of POCT services in the country. Therefore, the findings highlight the importance of hybrid testing models that combines centralized laboratory networks with POCT. POCT testing has been shown to improve turnaround time, enabled same-day ART initiation, and support adherence to the testing algorithm (18–20). Lastly, it is important to note persistent regional disparities, particularly the consistently higher performance in Tooro and Kampala compared to other regions, underscoring inequities in service delivery and access that warrants targeted interventions.

Although adherence to the first PCR test was high (80.6%), adherence declined substantially at later testing milestones, falling to 58.8% for the second PCR and only 33.3% for the third PCR. Overall adherence was 60.8%, varying between 41.1% and 56.8% across years, with no significant temporal trend (P = 0.70). These findings suggest that while initial uptake of infant HIV testing is relatively strong, retention of HIV-exposed infants throughout the full testing continuum remains suboptimal. A recent Ugandan study among mothers living with HIV whose infants tested HIV-negative at 4–12 weeks similarly reported that only 53% of HIV-exposed infants completed testing at the recommended time points, with stigma and sexual violence identified as important barriers to retention(11). The substantial decline observed at later testing milestones is also consistent with global evidence showing progressive attrition after the first PCR test (12). The decline in adherence observed across testing stages after 2020 suggests that disruptions associated with the COVID-19 pandemic may have exacerbated existing weaknesses in retention and follow-up systems. However, the absence of a significant long-term trend indicates that challenges with retention predated the pandemic and continue to affect EID program performance. Regional variation in adherence further highlights inequities in access to and utilization of infant HIV services, suggesting differences in health system capacity, caregiver support, and effectiveness of patient follow-up mechanisms.

A significant decline in HIV positivity was observed over the study period, decreasing from 3.0% in 2017 to 1.4% in 2023 ($p < 0.01$). The overall positivity rate of 2.0% reflects substantial progress in PMTCT interventions and maternal ART coverage and is comparable to the 2.7% MTCT rate reported in the most recent national PMTCT impact evaluation (3). Similar declines have been reported globally and are largely attributed to expanded maternal ART coverage, improved viral suppression, and strengthened PMTCT services (12). Despite these gains, important geographic disparities persisted, indicating that improvements have not been experienced uniformly across the country. The persistence of regional variation suggests differences in access to maternal HIV services, retention in care, adherence to infant testing schedules, and broader health system capacity. These findings highlight the need for targeted programmatic interventions to ensure that progress toward eMTCT is equitably distributed.

Risk factor analysis confirms that infant HIV infection is largely preventable. In our study, the strongest risks to positivity were among mothers who were not on ART postnatal ART (OR = 5.1), and infants who were delivered outside health (OR = 3.5), underscoring the protective role of skilled birth attendance and immediate linkage to PMTCT interventions. The recent PMTCT evaluation demonstrated that maternal viral suppression, skilled delivery, and adequate ANC attendance are critical to sustaining eMTCT gains (3). Conversely, lower odds to infection pointed toward solutions: longitudinal confirmatory testing and recent testing years suggesting improved quality of care, counseling, follow-up, and resource availability. Our findings highlight the interplay between maternal treatment, facility delivery, and adherence to EID testing algorithm as key determinants of infant HIV outcomes. Importantly, non-adherence to the EID testing algorithm was independently associated with increased odds of infant HIV infection in this study. While adherence itself may not directly prevent transmission, it likely reflects broader engagement with maternal and child HIV services. Caregivers who remain engaged throughout the testing continuum are more likely to receive counseling, prophylaxis, and timely interventions(21), whereas loss to follow-up may delay identification and management of infants at increased risk of HIV acquisition. Strengthening retention across the full EID cascade should therefore remain a central priority for achieving and sustaining elimination of mother-to-child transmission of HIV in Uganda.

Limitations and strengths

This retrospective analysis of routine program data has inherent constraints. Documentation gaps in maternal ART, infant feeding, and prophylaxis were categorized as ‘unknown,’ but missing data may still bias results. Adherence and coverage calculations relied on routinely collected national program records and may not have captured all infants tested outside the central repository or during facility transfers. In addition, coverage estimates used HIV-positive women enrolled in ANC services as the denominator rather than documented live births to HIV-positive mothers. Consequently, coverage may have been under- or overestimated if pregnancies did not result in live births, infants died before testing, or mother-infant pairs were not successfully linked across reporting systems. COVID-19 disruptions further affected health-seeking behavior and supply chains beyond what adjustments could capture. As with all observational designs, residual confounding from unmeasured factors such as socioeconomic status or caregiver mobility remains possible. Finally, the study reflects infants enrolled in EID; triangulation with PMTCT and ANC indicators helped contextualize coverage but cannot fully account for infants who never accessed services.

This study leverages one of the largest national EID datasets, spanning seven years of implementation. Triangulation with PMTCT and ANC indicators situates infant outcomes within the maternal continuum of care. Stratified analyses across regions, facility types, and time periods strengthen confidence in observed associations. Integration of national PMTCT evaluation results with infant-level findings provides a comprehensive picture of Uganda’s progress toward eMTCT.

Conclusion

Uganda's seven-year EID study confirms major progress toward eMTCT, with HEI positivity declining from 3.0% to 1.4%. Gains reflect rapid identification of HEI status and supporting linkage into care for the positive ones, yet systemic bottlenecks persist. Coverage averaged 55.7%, far below the 95% global target, and adherence declined substantially after the first PCR. Regional disparities in coverage, adherence, and positivity rates underscore inequities in service delivery. Maternal ART non-adherence and non-facility deliveries remained key drivers of infant HIV infection. Achieving elimination will require targeted interventions in high-burden regions, strengthened retention across the infant testing continuum, and improved systems for tracking HIV-exposed infants through 18 months of follow-up.

Abbreviations

ANC Antenatal Care

ART Antiretroviral Therapy

COE Centre(s) of Excellence

CPHL Central Public Health Laboratories

CSV Comma-Separated Values

DHIS2 District Health Information System 2

EID Early Infant Diagnosis

eMTCT Elimination of Mother-to-Child Transmission

GOV Government

HC Health Centre

HEI HIV-Exposed Infant(s)

HIV Human Immunodeficiency Virus

MoH Ministry of Health

MTCT Mother-to-Child Transmission

NHLDS National Health Laboratory and Diagnostic Services

NRH National Referral Hospital

PCR Polymerase Chain Reaction

PFP Private For-Profit

PMTCT	Prevention of Mother-to-Child Transmission
PNFP	Private Not-for-Profit
POCT	Point-of-Care Testing
QGIS	Geographic Information System software used for mapping
RRH	Regional Referral Hospital

Declarations

1. Ethics approval and consent to participate

Ethical approval for this study was obtained from the Uganda National Health Laboratory and Diagnostic Services Research Ethics Committee (UNHLS-REC), approval number UNHL-2026-227. The study was conducted in accordance with the Declaration of Helsinki and relevant national guidelines and regulations. As this was a retrospective secondary analysis of routinely collected, anonymized national Early Infant Diagnosis program data, the requirement for individual informed consent was waived by UNHLS-REC. Administrative clearance to access and analyze the data was obtained from the Ministry of Health, Uganda. All data were anonymized prior to analysis and handled in accordance with applicable data protection and confidentiality requirements.

2. Consent for publication

Not applicable

3. Availability of data

The datasets used and/or analyzed during the study are available from the corresponding author on reasonable request and permission from Ministry of Health Uganda.

4. Competing interest disclosures

The authors declare no conflict of interest.

5. Funding acknowledgments

There is no funding for this study.

6. Authors’ contribution

IS, LKN, CM, FM contributed in the conceptualization of the study including research questions. AS, CK and HS contributed in the design of the study. SPM, JM, CO, FN and PM contributed in the acquisition of the data. CM, AS and AA contributed in the draft of the manuscript. All authors made substantial

contributions to interpretation of the data and study of the manuscript. All authors provided final approval of the manuscript to be published and agreed to be held accountable for all aspects of the work.

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Figures

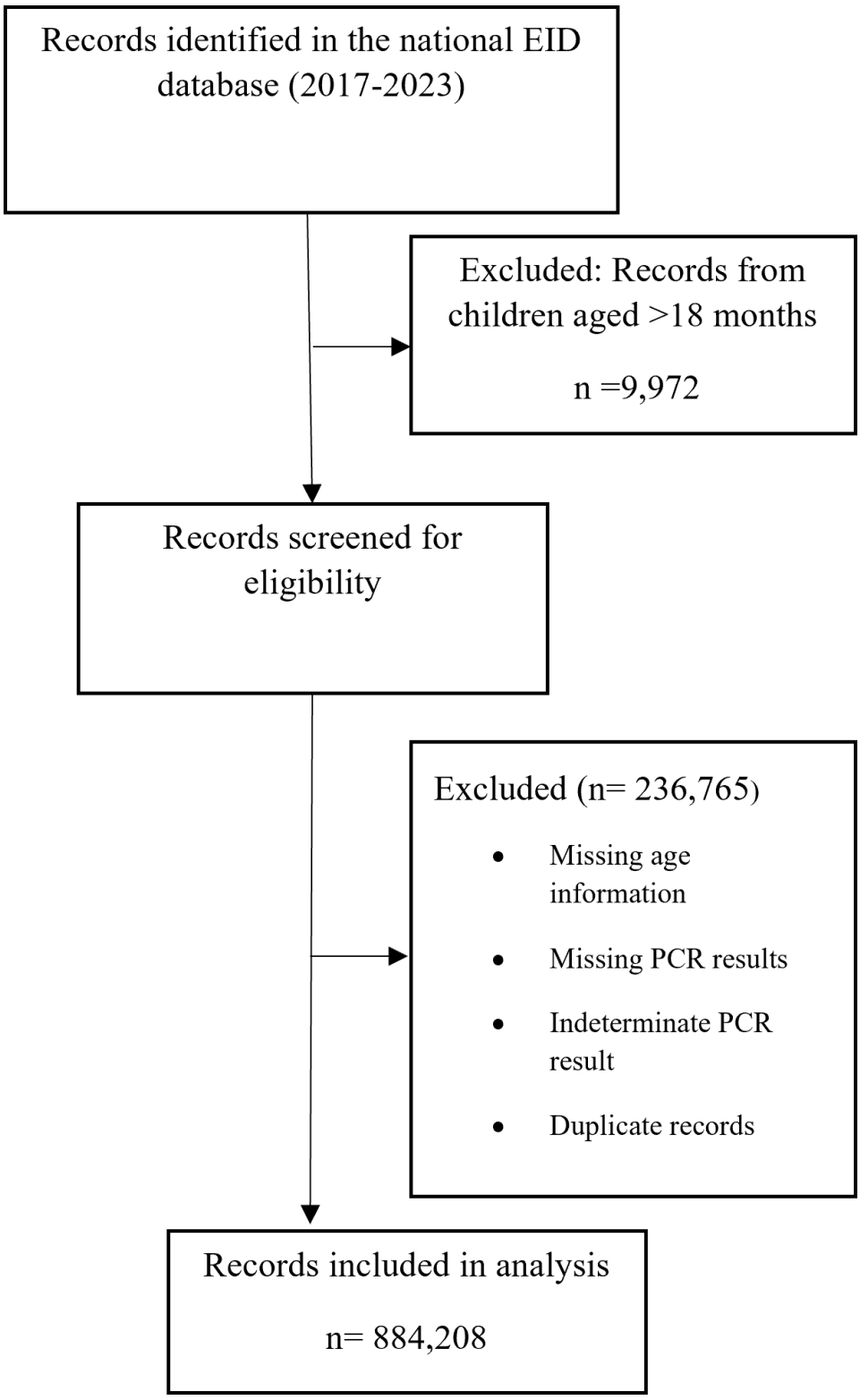


Figure 1

Selection of HIV-exposed infant records from the national EID database, Uganda 2017-2023.

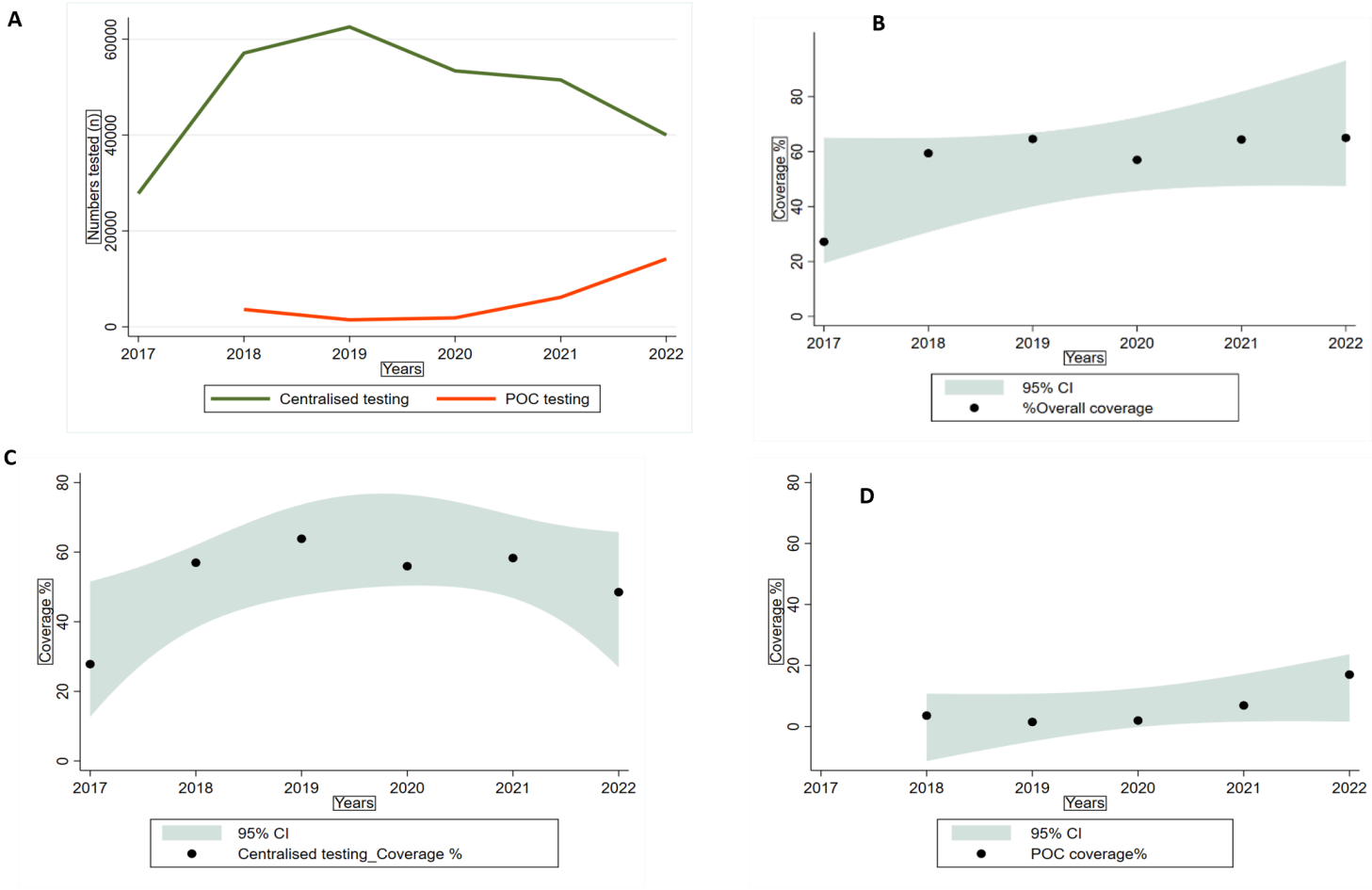


Figure 2

Trends in first PCR testing volumes and coverage among HIV-exposed infants (HEI) aged ≤2 months, 2017–2022. (A) Comparison of annual testing volumes by platforms: centralized testing (green) versus point-of-care testing (POCT) testing (orange). (B–D) Annual per **3**. **Trends in first PCR testing volumes and coverage among HIV-exposed infants (HEI) aged ≤2 months, 2017–2022. (A)** Comparison of annual testing volumes by platforms: centralized testing (green) versus point-of-care (POCT) testing (orange). **(B–D)** Annual percentage coverage, shown as black dots with 95% confidence intervals (shaded areas) for: **(B)** overall coverage that combined centralised and POCT testing; **(C)** centralized testing coverage using HEI tested at Central Public Health Laboratories (CPHL); and **(D)** POCT testing coverage using HEI tested at peripheral facilities.

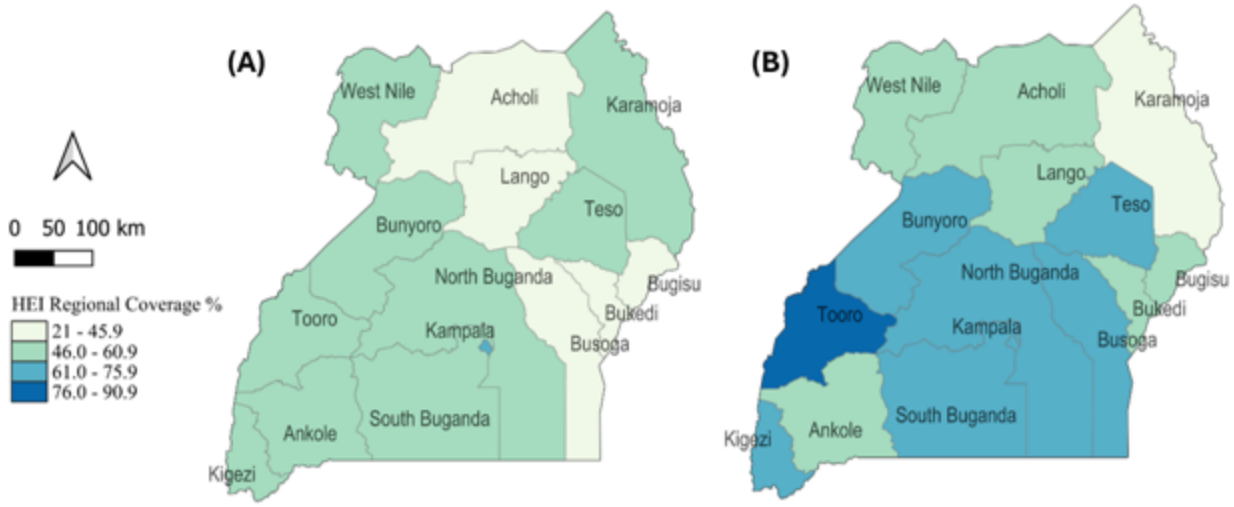


Figure 3

Geospatial distribution of first PCR coverage among HIV-exposed infants (HEI) aged ≤2 months in Uganda. Aggregated regional coverage rates during **(A)** the pre-COVID-19 period (2017–2019) and **(B)** the COVID-19 pandemic period (2020–2022).

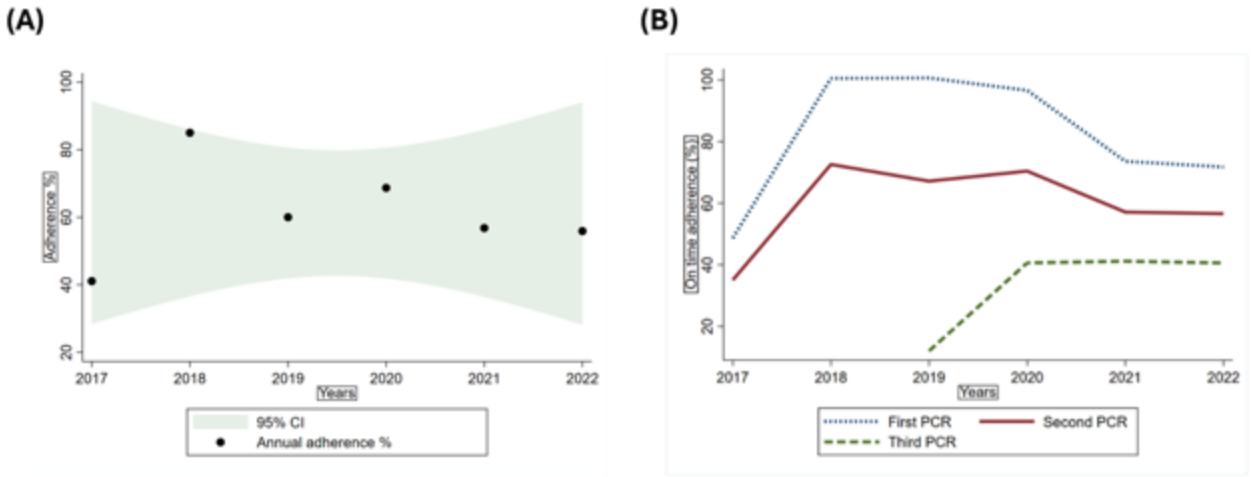


Figure 4

Trends in adherence to the national testing algorithm, 2017–2022. (A) Annual overall adherence shown with 95% confidence intervals **(B)** Annual on-time adherence percentages for First PCR (blue dotted line), Second PCR (red solid line), and Third PCR (green dashed line).

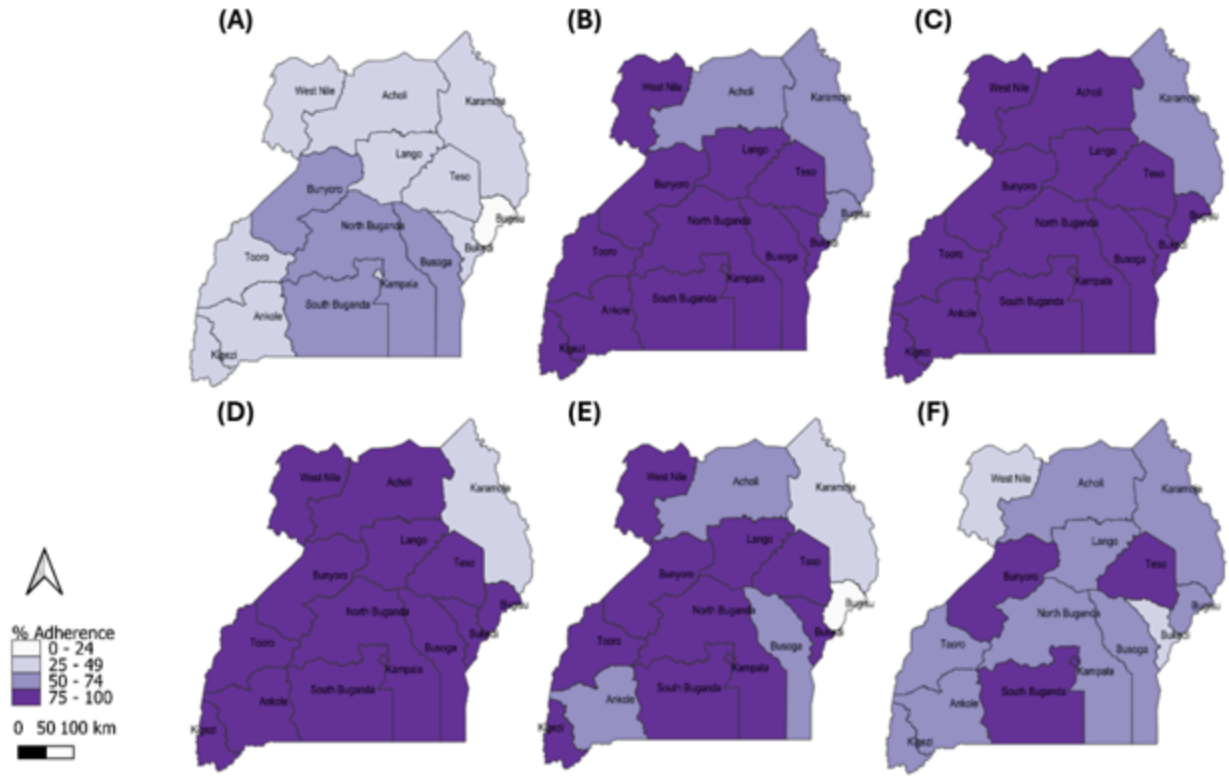


Figure 5

Spatiotemporal trends in regional adherence to first PCR testing among HIV-exposed infants (HEI) aged ≤2 months in Uganda in (**A**) 2017, (**B**) 2018, (**C**) 2019, (**D**) 2020, (**E**) 2021 and (**F**) 2022 with the darker purple shade indicating regions with higher adherence (≥75%) to the national testing guidelines.

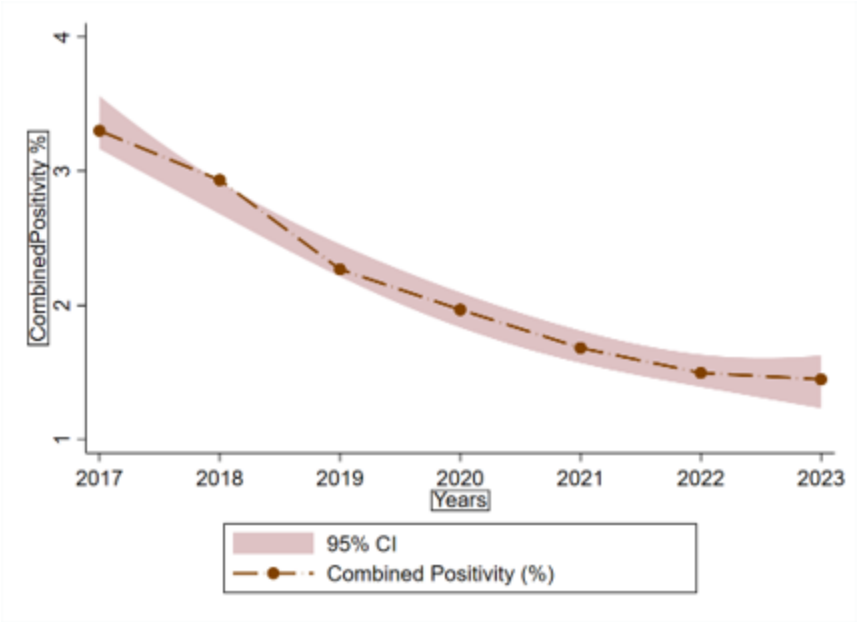


Figure 6

Annual HIV positivity among HIV-exposed infants (HEI) aged ≤18 months (2017–2023). Point proportions for combined positivity are shown with their corresponding 95% confidence intervals (shaded region).

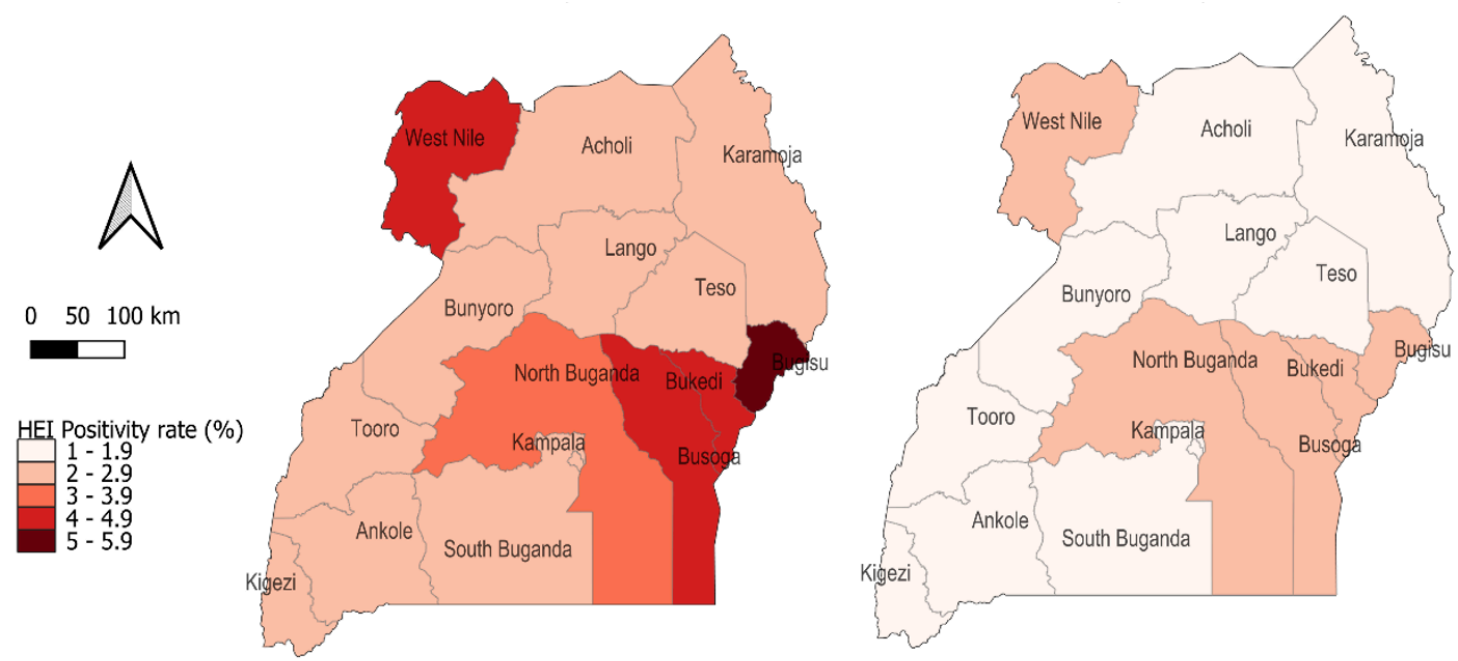


Figure 7

Regional **comparison of HIV positivity rates among HIV-exposed infants (HEI) aged ≤18 months before and after the algorithm update.** Maps display regional positivity rates during **(A)** the pre-update period (2017–2018) when only two PCR tests were recommended 1st PCR at 0-2months and 2nd PCR at 12-18 months of age and **(B)** the post-update period (2019–2023) three PCR tests 1st, 2nd and 3rd each at 0-2months, 8-11 months, and 12-15 months of age respectively. Darker red shading indicates regions with a higher burden of HIV positivity (≥5.0%).

Supplementary Files

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- Additionalfile1.Supplimentarytables.docx