Assessment of barriers to the diagnosis and initiation of ART for children exposed to HIV in three rural districts in Zambézia Province, Mozambique

Final Evaluation Report

(Evaluation completed within the scope of obtaining a Master's degree in Public Health by the first author)

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Executive Summary

Introduction: Low national coverage of the initiation of pediatric antiretroviral treatment (ART) (43%) is of concern to Mozambican health authorities, as it is known that early initiation potentially saves lives. This coverage is lower in Zambézia Province (33%). The aim of this evaluation was to assess the barriers that may have contributed to the late initiation of ART in children under nine months of age.

Methodology: This is an observational, cross-sectional study, which was conducted in nine health facilities (HF), in Zambézia Province, distributed among the districts of Quelimane, Namacurra and Maganja da Costa. Secondary data from children enrolled in the at-risk child consultation between April 2016 and March 2017 were used, with systematic probabilistic sampling. The indicators of early childhood diagnosis were analyzed using descriptive statistics and chi-square and student t tests.

Results: Of the 969 children included, 949 were tested, of which 74% (95% CI: 71.2 - 76.8) before eight weeks of age. Early testing was a protective factor (OR=0.36 [95% CI: 0.23 - 0.57]; p <0.001). About 5% (95% CI: 3.1 - 6.4) of the PCR-DNA HIV results did not return to the HF. The average time to return the results from the HF reference laboratory was 42 days (95% CI: 39 - 45). About 81% (95% CI: 78.4 - 83.6) of the mothers returned to pick up the result, with an average time of 33 days (95% CI: 30 - 35). The PCR-DNA HIV positivity rate was 9% (95% CI: 7.1 - 10.9). Of the 82 positive children, the results were delivered to 76% (n = 62), and all initiated ART. There was no association between the distance from home to the HF and the initiation of ART (OR=0.8 [95% CI: 0.28 - 2.28]; p = 0.671); there was an association between the mother being on pre-natal ART with the initiation of ART (OR=4.16 [95% CI: 1.42 - 12.26]; p = 0.007).

Conclusions: In this analysis, about ¾ of the children were tested within the first two months. Time for return of results is lengthy, and almost a fifth of the mothers did not return to pick up the result. Expansion of early infant diagnosis point-of-care testing is recommended to minimize delivery time and immediately initiate the HIV-positive child on ART. The identification of HIV and early initiation of ART in women of reproductive age is crucial to prevent vertical transmission.

Background

Globally, the first cases of AIDS in children were described in 1982, acquired by blood transfusions (1) and, in 1983, cases were described by vertical transmission from mother to child (2,3).

In 2016, around 36.7 million people worldwide were living with HIV infection; of these 1.8 million were new infections (4). Nearly 19.5 million people living with HIV/AIDS (PLHIV) had access to antiretroviral treatment (ART) and there was an estimated annual HIV/AIDS-related mortality of 1.0 million people (4). It was estimated that, globally, 2.1 million children aged 0 to 14 lived with HIV infection and of these 160,000 were new infections, the majority located in sub-Saharan Africa, from HIV-positive mothers during pregnancy, intra-delivery and postpartum. The estimated mortality was approximately 120,000 children (4). In East and Sub-Saharan Africa, about 1.3 million children aged 0 to 14 were living with HIV/AIDS and, of these, 77,000 were new infections; HIV mortality was around 58,000 (4). It was estimated that only 51% of children aged 0 to 14 years living with HIV had access to ART (4).

According to the Immunization, Malaria and HIV/AIDS Indicators Survey (IMASIDA) conducted in 2015, HIV prevalence in Mozambique is 13.2% in adults aged 15-49 years. Compared to data from the National Survey on Prevalence, Behavioral Risks and Information on HIV and AIDS in Mozambique (INSIDA), conducted in 2009, HIV prevalence increased from 11.5% to 13.2% in 2015 (5). In Zambézia Province, IMASIDA data shows that the prevalence of HIV in adults aged 15 to 49 in 2015 was 15.1% (16.8% for females and 12.5% for males) having increased by 2.5% when compared to 2009 data reported in INSIDA which was 12.6% (15.3% for females and 8.9% for males).

According to IMASIDA data, the prevalence of HIV in children aged 6-23 months was 2.0%, being 1.6% male and 2.3% female. Disaggregating by age group, one concludes that the prevalence is high in the age group of 12-17 months with 2.6% and is lower in children 18-23 months of age with 1.1% (5). HIV prevalence of children whose mothers were HIV-positive was 12.6% (5).

The Ministry of Health (MoH) adopted the Vertical Transmission Prevention Program (PMTCT) in order to reduce new pediatric HIV infections. This program was implemented in 2002, initially in eight health facilities (HFs) in the country, then became a national program in 2004, and was integrated in the Maternal and Child Health (MCH) Services in 2006. From June 2013, the Option B+ strategy was introduced, which consists of access to treatment for HIV-positive pregnant women, regardless of their immune status. By the end of 2017, 1,513 HFs were already offering PMTCT (1,513 implementing Option B+), which corresponds to 93% of the country's HFs (6).

The early infant HIV diagnosis program based on PCR-DNA was introduced in Mozambique in 2006, expanded rapidly to 475 health facilities in 2012, and has four national laboratories

processing PCR-DNA HIV samples in the cities of Maputo, Beira, Nampula and Quelimane. In Mozambique, the diagnosis of HIV in exposed children is based on PCR-DNA virological test, which is performed between the first and ninth months of life. This test is recommended at the first month of life or at the first opportunity that the exposed child is present at a HF. All exposed children with a positive PCR-DNA test must have a second PCR-DNA test at the time the first results are delivered in order to confirm the infection and start ART without waiting for the result of the confirmation test (7–9). All HIV-exposed infants less than nine months of age who did not have a PCR-DNA HIV test or who have had a negative PCR-DNA HIV test, should have a rapid HIV test, and those whose HIV test result is positive, must have a PCR-DNA HIV test done to confirm the diagnosis (10).

Until 2017, the positivity rate in PMTCT services in Mozambique was 13% in children whose testing was done after eight weeks of life and 8% for those who were tested before eight weeks (6). In terms of sample collection for HIV diagnosis by PCR-DNA, it should be noted that, in Mozambique, 72% of exposed children were tested before reaching eight weeks of age, while in Zambézia Province only 64% were so (6).

The early initiation of ART among children, particularly before 12 weeks of age, increases survival in this group by 76%, reduces morbidity and provides immunological benefits (11).

The World Health Organization (WHO) recommends that (12) regardless of the WHO clinical stage or of the CD4 count, ART should be initiated in all children living with HIV;

- For children diagnosed in the first year of life: strong recommendation, with evidence of moderate quality;
- For children ≥1 year and <10: conditional recommendation, with low quality evidence.

In 2017, ART coverage in Mozambique was 38% (4). In 2017, in Zambézia Province, pediatric ART coverage was 36% (6).

Purpose

This evaluation was proposed to assess the barriers to diagnosis and late initiation of pediatric ART in the follow-up of exposed children and children younger than nine months, looking at the details of aspects such as missed opportunities for testing, sample collection flow, as well as the receipt of results, which can influence the return of the caregiver to receive the results and start pediatric ART.

Research Question

What are the potential barriers that may have contributed to the late diagnosis and initiation of pediatric ART in children exposed to HIV less than nine months of age, in three districts of Zambézia Province?

Design/Methods/Limitations

Type

An observational, cross-sectional study was carried out between April 2016 and March 2017 using a quantitative approach.

Summary of stakeholder engagement

FGH technical teams have ongoing collaborations with key stakeholders working in the health facilities and communities in which we are supporting and engaged. The evaluation was developed in collaboration with our partners at the Zambézia Provincial Health Directorate (DPS-Z), one of whom is a co-author for this evaluation. The concept proposal and plan for secondary data analysis evaluation was approved by our sponsoring institution, namely CDC-MZ.

Population

The study population was that of children exposed to HIV less than nine months of age, that is, children born to mothers with known HIV-positive status, and their mothers. Data from children and their mothers were used and analyzed.

Inclusion Criteria

All exposed children less than nine months of age born to HIV-positive mothers enrolled in the Clinic for Children at Risk (CCR) between March 21, 2016 and March 20, 2017, at the rural Hospital of Maganja da Costa, Nante Health Facility, Alto Mutola Health Facility, Namacurra Health Facility, Macuse Health Facility, Malei Health Facility, 17 de Setembro Health Facility, Chabeco Health Facility, and 24 de Julho Health Facility.

Also included were all women with positive serology, who had at least one child enrolled between March 21, 2016 and March 20, 2017 in CCR at the rural Hospital of Maganja da Costa, Nante Health Facility, Alto Mutola Health Facility, Namacurra Health Facility, Macuse Health Facility, Malei Health Facility, 17 de Setembro Health Facility, Chabeco Health Facility, and 24 de Julho Health Facility in the 2016 period.

Exclusion Criteria

Not included in the study were exposed children nine months of age and older; or exposed children less than nine months of age from neighboring districts. Also excluded were children less than nine months of age exposed to HIV whose enrollment information was missing in the record book.

Sampling Strategy and Sample Size

Those whose data were included in the evaluation sample were selected based on a systematic probabilistic sampling in which children exposed to HIV less than nine months of age were selected at regular or systematic intervals from the list of all exposed children enrolled in the CCR. The following steps were followed: 1) listing in the CCR record book of all children less than nine months of age exposed to HIV during the evaluation period; 2) assigning a number to each enrolled child; 3) calculation of the sample interval (i.e., number of children enrolled, divided by the size of the sample); 4) choosing a random number using the draw method without replacement; 5) starting with the random number, the sample interval (step 3) was used to identify the children until the desired sample size was completed.

The sample size was calculated with the total number of children enrolled in the CCR in 2016 (considered population or universe) and the percentage of initiation to pediatric ART was considered the proportion the source of which was the MCH annual report of the DPS-Z. The sample calculation was made for each HF in the three selected districts.

$$n = Z2.p.(1-p) \,.\, N / d2 \,.(N-1) + Z2 \,.\, p\,(1-p)$$

Z = 1.96 when the Confidence Level is 95%

d = risk or error is 0.05

p = proportion found in other studies is 0.21

N = Population

n = Sample size

For the calculation of the sample, a 95% confidence level was assumed, with a risk or error of 0.05; the proportion found in other studies was 0.21 corresponding to the initiation of ART. The population was the exposed children observed at the HFs' CCR in 2016, following the data source of the basic module of the DPS-Z.

Table 1. Size of the study sample and sample interval for selection of study participants

Health Facility	Population (N)	Sample (n)	Sample Interval
17 de Setembro	343	148	2
24 de Julho	189	110	2
Chabeco	150	96	2
Namacura	557	176	3
Macuse	140	92	2
CS Malei	76	60	1
Manganja da Costa	387	154	3
Nante	97	70	1
Alto Mutola	49	42	1
Total	1988	948	2

Data source from the universal Basic Children's Module attended for first consultation with HIV exposure.

Methods

Data collection was carried out in the three districts for three weeks, by a team made up of three elements and routine program data was collected. Collection of secondary data was done by extraction from the CCR record books, PCR-DNA record book, CCR records, ART service record book, and mother and child medical records. A structured data collection instrument (see annex) was used with later compilation and aggregation in Microsoft Excel 2016.

Variables

The study variables were: sex and age of the child exposed to HIV, age of the mother of the child exposed to HIV, distance between the home of the mother/caregiver of the child exposed to HIV and the HF, lactating woman on ART (in this case, the mother of the child exposed to HIV is receiving ART), PCR-DNA testing of the child exposed to HIV, age of the child exposed to HIV when blood sample was taken for the PCR-DNA test, return of the test result to the HF, time taken

to return the PCR-DNA test result to the HF, PCR-DNA test result, enrollment of the child exposed to HIV with a positive PCR-DNA result in pediatric ART services with enrollment date, initiation of ART by the child exposed to HIV with a positive result and the initiation date, return to HF of the mother/caregiver of the child exposed to HIV.

Ethical Considerations

This analysis of secondary data is covered by the VUMC/FGH "umbrella" protocol approved by the CDC, entitled "*Quality Improvement for HIV Care and Treatment in Zambézia Province of the Republic of Mozambique under the President´s Emergency Plan for AIDS Relief (PEPFAR)*" (CGH HSR #: 2016-163a), also approved by Mozambique and VUMC IRB ethics committees. The specific protocol of this evaluation was approved by the Institutional Committee on Bioethics of the Faculty of Medicine of the Eduardo Mondlane University.

Patient Confidentiality

All data included in this analysis were deidentified programmatic data.

Informed Consent

For this evaluation, obtaining informed consent was waived, as the source of data collection is secondary and there was no contact with the patients whose data were included in the analysis. Instead, a Data Usage Agreement was made with the institution where the evaluation was developed as a way of describing and agreeing to the strictly limited access to deidentified individual-level data on the data managers' computers or in the databases located in the FGH's secure servers.

Benefits

With the results of this evaluation, it will be possible to design contextualized strategic approaches for the health facilities of the districts of Namacurra, Maganja da Costa and Quelimane, in order to mitigate the barriers to early infant diagnosis and pediatric ART initation; thus leading to lower rates of morbidity and mortality among patients treated at that health facility or other patients with similar characteristics.

Risks

There are no foreseen risks for patients whose data have been included in these analyses since there were no interventions that could cause physical, psychological and social damage, as only secondary data have been collected and included.

Deviations from Protocol

There was no record of any deviation from the previously designed concept note for this evaluation.

Data Quality

The data in the database that was used were verified to ensure that they were consistent, and adequate and in line with the inclusion and exclusion criteria of the evaluation.

Analysis Plan

Secondary data collected from clinical records were entered, processed and analyzed using Microsoft Excel 2016 and SPSS version 20.0.

An initial descriptive analysis of all variables (univariate analysis) was performed, presented in graphs and tables. Descriptive statistics procedures (absolute and relative frequency, mean, median, standard deviation, variation coefficient, amplitude, minimum, maximum) were used for numerical variables, and the calculation of absolute and relative frequencies for categorical variables. Confidence intervals of 95% were calculated for the following variables: demographic profile of the mothers/caregivers and children exposed to HIV, the mother's ART status (lactating women who started ART pre-delivery), distance from the mother/caregiver's home to HF, PCR-DNA testing, age at PCR-DNA test collection, PCR-DNA result return, PCR-DNA positivity rate, mother/caregiver's return to HF.

The ratio between the average time for return of the PCR-DNA and the average time for return of the mother/caregiver was calculated as a determinant measure of additional barrier to the diagnosis and timely initiation of ART, considering the following categories:

- Ratio = 1 – ideal situation, in which the mother returns, and the result is available;
- Ratio > 1 – result available before the mother/caregiver returns;
- Ratio < 1 – result unavailable when the mother/caregiver returns to the HF.

The bivariate analysis of possible significance of the associations between the category ART initiation (outcome variable), with the other categories of qualitative variables (return of mother/caregiver to pick up the PCR-DNA result, distance HF/home and mother/caregiver on ART), and between the result of the PCR-DNA test (binary dependent variable) and the age of collection of the PCR-DNA (binary independent variable), was performed using the chi-square

test, and the odds ratios were calculated for each category analyzed. The bivariate analysis for possible significance of the associations between the category ART initiation (outcome variable), with the time for the return of the PCR-DNA result (quantitative variable) was performed using the independent, two-tailed t test and assuming the principle heteroscedasticity, that is, use of sample standard deviation since the standard deviation in the population is not known. For all statistical procedures, a significance level of 0.05 was adopted to reject the null hypothesis.

Definitions considered for the present study:

- Infected child (i.e., HIV-positive child) was defined as an exposed child less than nine months of age who had a positive result for the first PCR-DNA test.
- Time of return of the PCR-DNA test result to the HF was calculated by the difference between the date of collection of the PCR-DNA blood sample and the date of return of results from the HF.
- Time of delivery of the PCR-DNA test result to mother/caregiver was calculated as the difference between the date of arrival of the result to the HF and the date of delivery of the result to the mother/caregiver.
- Time of ART initiation for the infected child (exposed to HIV) was calculated as the difference between the date of ART initiation for the exposed child and the date of collection of the PCR-DNA sample.
- Proportion of children exposed to HIV less than nine months of age tested were those children exposed to HIV less than nine months who underwent the PCR-DNA test divided by the total number of children exposed to HIV less than nine months enrolled in the CCR.
- Proportion of children exposed to HIV less than nine months of age infected (i.e., HIV-positive) were children exposed to HIV less than nine months of age with a positive result divided by the number of children exposed to HIV less than nine months of age tested.
- Proportion of infected exposed children enrolled in the ART service were children exposed to HIV with a positive result enrolled in the ART service divided by the total number of children exposed to HIV with a positive result.
- Proportion of children exposed to HIV infected who initiated ART were children exposed to HIV with a positive result divided by the total number of children exposed to HIV with a positive result enrolled in the ART service.

Limitations of design

Due to the absence, state of degradation and/or lack of location of the CCR record books for the years 2013, 2014, and 2015, the study period was limited to the year 2016.

The results of the study cannot be generalized for Zambézia Province and/or the country because population-based sampling was not done with enumeration areas (sample units) for each province district or for other provinces in the country. However, the sample representativeness of the

districts in question allows for a generalization to be made for the districts included in the evaluation.

Results

During the preparation of the research protocol, a sample of 948 children exposed to HIV was predicted; however, 969 children were included, having exceeded the expected sample by 2%, as shown in Table 2. The sample was altered because it was not possible to fulfill the sample size in the districts of Maganja da Costa and Namacurra, thus, the sample size in Quelimane District was increased in order to compensate and guarantee a representative sample.

Table 2: Sample in the three districts included in the evaluation

District	Health Facility	Population* (N)	Sample (n)	Sample Found (n)	% Achieved or reached
Quelimane	17 de Setembro	343	148	148	100%
	24 de Julho	189	110	132	120%
	Chabeco	150	96	100	104%
Namacurra	Namacura	557	176	175	99%
	Macuse	140	92	92	100%
	Malei	76	60	60	100%
Maganja da Costa	Manganja da Costa	387	154	154	100%
	Nante	97	70	70	100%
	Alto Mutola	49	42	38	90%
	Total	1.988	948	969	102%

Demographic profile of the mothers of children exposed to HIV

The mean age of mothers of children exposed to HIV in the three districts was 29.03 years (95% CI: 28.9 - 29.1), being 28.19 years (95% CI: 28.0 - 28, 4) in Quelimane, 28.26 years (95% CI: 28.1 - 28.4) in Namacurra and 28.88 years (95% CI: 28.7 - 29.1) in Maganja da Costa. In the three districts, the median was 29 years (range: 27 - 29 years). The age range in the three districts was 37 years (min: 15 years; max: 52 years), with no great dispersion in each of the districts (standard

deviation was 6.50 years in Quelimane, 7.06 years in Namacurra and 7.41 years in Maganja da Costa). Since the age confidence intervals overlap in the three districts, they are considered to have no statistically significant differences.

The sample was downsized to 876 because 64 records of mothers whose ages were not recorded were excluded from this analysis.

Demographic profile of children exposed to HIV

The average age of children exposed to HIV in the three districts was 1.79 months (95% CI: 1.7 - 1.9), being 1.64 months (95% CI: 1.5 - 1.8) in Quelimane, 1.81 months (95% CI: 1.6 - 2.0) in Namacurra and 1.98 months (95% CI: 1.8 - 2.2) in Maganja da Costa. In all districts, the median and mode were 1 month, and the range was 8 months (min: 1 month; max: 9 months). No dispersive age variability was found. Since the average age confidence intervals overlap in the three districts, they are considered to have no significant differences between the districts.

Approximately 80% of the children exposed to HIV in follow-up at CCR were 1 month (more than 60%) and 2 months (more than 15%) of age, as shown in Table 3.

Table 3: Frequencies by age (absolute and relative frequency) of children exposed to HIV in the three districts included in the evaluation.

	Districts							
	Quelimane		**Namacurra**		**Maganja da Costa**		**Total**	
Age (months)	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
1	261	68.7	213	65.1	155	59.2	629	64.9
2	68	17.9	55	16.8	54	20.6	177	18.3
3	16	4.2	24	7.3	19	7.3	59	6.1
4	17	4.5	9	2.8	6	2.3	32	3.3
5	6	1.6	11	3.4	11	4.2	28	2.9
6	7	1.8	5	1.5	7	2.7	19	2
7	2	0.5	6	1.8	6	2.3	14	1.4
8	1	0.3	1	0.3	1	0.4	3	0.3
9	2	0.5	3	0.9	3	1.1	8	0.8
Total	380	100	327	100	262	100	969	100

Of the 969 children exposed to HIV evaluated in the three districts, 51.3% (497/969) are female. The percentage of female children exposed to HIV by district was 57.6% (151/262) in Quelimane, 50.5% (165/327) in Namacurra, and 47.6% (181/380) in Maganja da Costa.

Pre and postpartum ART of children's mothers

Of the 969 caregiving mothers evaluated in the three districts, 96% (931) initiated ART before delivery. The percentage of caregiving mothers in pre-delivery ART was 97% (317) in Namacurra, 96% (363) in Quelimane and 96% (251) in Maganja da Costa.

Table 4. Proportion of mothers of exposed children who initiated pre-delivery ART in the three districts included in the evaluation.

Districts	n	%	CI 95%
Quelimane	363	96	94.0 - 98.0
Namacurra	317	97	95.2 - 98.8
Maganja da Costa	251	96	93.6 - 98.4
Total	931	96	94.8 - 97.2

Distance between HF and the home of the child exposed to HIV

The average distance between the children's homes and the HF in the three districts was 6.27 km (95% CI: 5.7 - 6.9), with 3.37 km (95% CI: 3.2 - 3.2) in Quelimane, 9.41 km (95% CI: 8.3 - 10.5) in Namacurra and 6.55 km (95% CI: 5.9 - 7.2) in Maganja da Costa. In the three districts, the median was 4 km, with 3 km in Quelimane, 7 km in Namacurra, and 6 km in Maganja da costa. The distance presented dispersive variability, as in Namacurra and Maganja da Costa the residences were on average 1.6 times and 1.4 times more distant from the HF than in Quelimane, respectively. Since there is no overlap in the confidence intervals for the distances between Quelimane and the other two districts (Namacurra and Maganja da Costa), they are considered to be significant (Quelimane vs Namacurra and Maganja da Costa).

PCR-DNA testing of children exposed to HIV

In the three study districts, the percentage of children exposed to HIV and under the age of nine months who underwent PCR-DNA testing was 98% (949) [95% CI: 97.1 - 98.9]; being that in Quelimane, PCR-DNA testing was 99.7% (379) [95% CI: 99.2 - 100.2]; in Namacurra it was 95.4% (312) [95% CI: 93.1 - 97.7] and in Maganja da Costa it was 98.5% (258) [95% CI: 97.0 - 100]. The mean age of the PCR-DNA collection was 7.11 weeks (95% CI: 6.7 - 7.5); 6.59 weeks (95% CI: 6.1 - 7.1) in Quelimane, 7.26 weeks (95% CI: 6.6 - 7.9) in Namacurra and 7.72 weeks (95% CI: 6.9 - 8.5) in Maganja da Costa. In all districts, the median was four weeks, with a span

of up to 32 weeks (min: 4 weeks; max: 32 weeks). There was no dispersion of the average age for PCR-DNA testing between the districts.

Return of PCR-DNA result to HF

In the three districts, 5% (44/949) [95% CI: 3.1 - 6.4] of PCR-DNA collections had no return, with 3.4% (10/379) [95% CI: 1.6 - 5.2] in Quelimane, 7.7% (24/312) [95% CI: 4.7 - 10.7] in Namacurra and 3.9% (10/258) [95% CI: 1.5 - 6.3] in Maganja da Costa. The average return time in the three districts was 42.26 days (95% CI: 39.6 - 45.0), with 32.76 days (95% CI: 30.7 - 34.8) in Quelimane, 46.96 days (95% CI: 41.4 - 52.6) in Namacurra, and 50.93 days (95% CI: 44.5 - 57.4) in Maganja da Costa. In all districts, the median was 30 days, the mode was 33 days, but the range was up to 386 days (min: 0 days; max: 386 days). The average time for the PCR-DNA result to return to the HF took about 1.4 times longer in the districts of Namacurra and Maganja da Costa, compared to Quelimane District.

Positivity among children

In the three districts, the positivity rate among exposed children, tested with PCR-DNA result returned to the HF was 9% (82/905) [95% CI: 7.1 - 10.9]; 8% (31/369) [95% CI: 5.2 - 10.8] in Quelimane, 9% (26/288) [95% CI: 5.7 - 12.3] in Namacurra, and 10% (25/248) [95% CI: 6.3 - 13.7] in Maganja da Costa.

Early diagnosis before 8 weeks

In the three districts, of children exposed to HIV tested with PCR-DNA, 74% (698) [95% CI: 71.2 - 76.8] were tested before eight weeks of age, with 70% (265/379) [CI 95%: 65.4 - 74.6] in Quelimane, 88% (275/312) [95% CI: 84.1 - 91.6] in Namacurra, and 61% (158/258) [95% CI: 55.0 - 67.0] in Maganja da Costa.

A difference in positivity between children tested before and after eight weeks of age and with statistical significance was verified in each of the study districts (Quelimane: 5% and 15% - *p value* = 0.002; Namacurra: 7% and 19% - *p value* = 0.014 ; Maganja da Costa: 6% and 15% - *p value* = 0.026).

Table 5. Association between PCR-DNA collection age and PCR-DNA test result, in the three districts included in the evaluation

District	Characteristics	PCR-DNA test result	Total	OR	p-Value
	Age of PCR-DNA test sample	Positive (n, %)			
Quelimane	Less than 8 weeks More or equal to 8 weeks	14 (5%) 17 (15%)	258 111	0.28	0.002
Namacurra	Less than 8 weeks More or equal to 8 weeks	18 (7%) 8 (19%)	246 42	0.33	0.031*
Maganja da Costa	Less than 8 weeks More or equal to 8 weeks	9 (6%) 16 (15%)	141 107	0.38	0.026
Total	Less than 8 weeks More or equal to 8 weeks	41 (6%) 41 (16%)	645 260	0.36	<0.001

*Use of chi square test com Yates correction

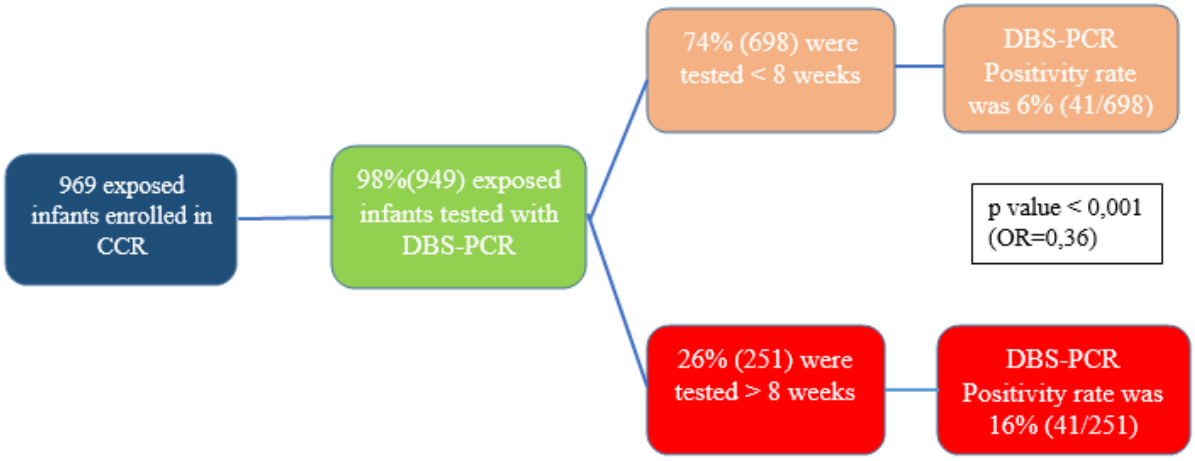

Figure 1. Testing of exposed children (EC) by age of PCR-DNA collection and their seropositivity, in the three districts included in the evaluation

There is less risk of testing positive when the test is done before eight weeks of age, being thus a protective factor with OR = 0.36.

Return of the Mother/Caregiver to the HF to pick up the PCR-DNA results

In the three districts, 81.0% (733) of the mothers/caregivers returned to the HF to pick up the PCR-DNA test result of the child exposed to HIV; 88.6% (327) in Quelimane, 76.7% (221) in Namacurra, and 74.6% (185) in Maganja da Costa.

The average time of return of the mother/caregiver to the HF to pick up the results was 33.11 days (95% CI: 30.3 - 35.9) in the three districts; 17.32 days (95% CI: 15.1 - 19.6) in Quelimane, 46.87 days (95% CI: 42.3 - 51.4) in Namacurra, and 44.58 days (95% CI: 36.5 - 52.6) in Maganja da Costa. In all districts, the median was 23 days, with 13 days in Quelimane, 44 days in Namacurra and 28 days in Maganja da Costa. The average time of return of the mother/caregiver to pick up the result was about twice as long in the districts of Namacurra and Maganja da Costa, in relation to Quelimane District.

Loss of follow-up at each stage of follow-up of children exposed to HIV under 9 months of age enrolled in the child at risk consultation

During the evaluation period, 969 children exposed to HIV less than nine months of age were enrolled in the CCR services in the three districts (380 in Quelimane, 327 in Namacurra, and 262 in Maganja da Costa). Of these, 98% (949) [95% CI: 97.1 - 98.9] were tested with PCR-DNA (99.7% in Quelimane, 95.4% in Namacurra and 98.5 in Maganja da Costa), with samples being sent to the reference laboratory (in Quelimane). About 95% (905) [95% CI: 93.6 - 96.4] of the results were returned to the HF (97.4% in Quelimane, 92.3% in Namacurra, and 96.1% in Maganja da Costa). About 81% (733) [95% CI: 78.4 - 83.6] of the mothers whose infants' results returned to the HFs picked them up (88.6% in Quelimane, 76.7% in Namacurra, and 74.6% in Maganja da Costa). Of the results that were returned to the HFs, 9% (82) [95% CI: 7.1 - 10.9] were positive (HIV-infected) – 8.4% in Quelimane, 9.0% in Namacurra, and 10.1% in Maganja da Costa. Of those who had positive results, 76% (62) [95% CI: 66.8 - 85.2] were enrolled and initiated ART (71% in Quelimane, 84.6% in Namacurra, and 72.0% in Maganja da Costa).

In general, mothers/caregivers returned to the HF before the PCR-DNA test results were returned. This situation is verified in Quelimane and Maganja da Costa, being more considerable in Quelimane. In Namacurra district, on the other hand, the average time of return of the mother/caregiver to the HF overlaps with the average time of return of the PCR-DNA results.

The return of the mother/caregiver to the HF to pick up the PCR-DNA result of the child exposed to HIV and the status of the lactating woman in relation to ART were strongly associated with

ART initiation for the child in pediatric services (87.3% and 83, 3%, p <0.001 and p = 0.007, respectively). On the other hand, the distances between the mother/child's home and the HF did not influence the initiation of ART.

Discussion

The results of the research show that the barriers that contribute to the late initiation of pediatric ART in the follow-up of exposed children less than nine months in the districts of Quelimane, Namacurra, Maganja da Costa were:

i) the prolonged average time to return the results from the laboratory to the HFs (42.26 days);
ii) the prolonged average time of delivery of the PCR-DNA results to mothers/caregivers (33.11 days);
iii) failure of the mother/caregiver to return to the HF to pick up the PCR-DNA results (19%);
iv) age at testing greater than eight weeks (26%);
v) non-return of the PCR-DNA result from the laboratory to the HF (5%); and
vi) not initiating ART even with a positive test result (24%).

The prolonged average time for the return of PCR-DNA results to HF (42 days, exceeding the recommended time of 28 days) was similar to the findings of a study carried out in the city of Maputo, where 40% of the cases had an average return time of 41 days (13). Other studies, using the same type of PCR-DNA delivery system, reported an average return time of 27 days (Zambia) (14), 38 days in Uganda (15) and Kenya (16), which is similar to the mean time of 30 days also found in this study. This prolonged time for the PCR-DNA results to return to HF is probably due to the overburden of the central laboratory as there is only one laboratory for the whole province, which receives and processes all kinds of biological samples (from the 22 provincial districts), with no PCR-DNA sample prioritization flow. Other possible contributing factors were: i) the absence of a priority workflow for dried blood spot (DBS) samples for HIV PCR-DNA, ii) lack of personnel (in terms of number and technical capacity) and iii) lack of materials for sample processing of DBS for HIV PCR-DNA.

Other studies show the median return time of the PCR-DNA result much higher than that found in this study, most likely due to the mechanisms for sending the results from the central laboratory to the HF, which are physically (in-person) and by vehicle. These are the cases of Lesotho (50.6 days) (17), Zambia (54 days) (14) and the city of Maputo (68 days) (18).

The delivery time of the PCR-DNA results to mothers/caregivers revealed statistically significant differences in relation to the outcome (the child's ART initiation in the chronic diseases consultation) with a *p-value* of 0.019. Thus, children whose mothers/caregivers had a shorter

average return time to the HF to collect the results, were more likely to initiate ART compared to those whose mothers/caregivers had a longer average return time to collect the results.

The WHO recommends that the return of parents/caregivers to receive the result of the virological test should be done as soon as possible or within four weeks (28 days) after the sample is collected. The delivery of the result to the mother/caregiver may have been influenced by the first barrier, that is, by the prolonged time taken to return the result from the central laboratory to the HF. Other factors that may have influenced could have been the absence of mother/caregiver on the day of the scheduled appointment (in 19% of cases the mother/caregiver did not return to the HF to pick up the result), absence of results on the day that the mother/caregiver returns to HF (mother return/result ratio = 0.79), as well as the non-return of the laboratory result to the HF (5%).

The study revealed a strong association between the mothers' return to pick up the PCR-DNA result and the child's initiation of ART with *p value* <0.001.

In this study, 19% of mothers/caregivers did not return to pick up the results at the HF. Smaller proportions have been found, with figures of 2.2% in Burkina Faso (19), 5% in Tanzania (20) and 13% in Zambia (14,21). However, higher figures have been reported, namely: 41% in Uganda (15), 40% in Malawi (16) and 33% in Myanmar (22).

Another barrier that is striking in the evaluation is the age of testing. In this study, 26% of children exposed to HIV were tested after eight weeks of age with a positivity rate of 16%; however, the majority (74%) of exposed children were tested before eight weeks of age (as recommended by the WHO), and had a lower positivity rate (6%). The difference in positivity between tested and positive children before and after eight weeks was statistically significant (p <0.001). This high testing positivity after eight weeks may be explained by the longer time of exposure to HIV.

The positivity of 6% of children exposed to HIV tested with PCR-DNA before eight weeks of age can be attributed to a good coverage of the prenatal consultation and the initiation of pre-delivery ART; 96.0% of mothers/caregivers had initiated ART before delivery. A similar result was found in Lesotho, which had 98.0% of mothers who had initiated ART before delivery with 5% positivity for those tested before eight weeks (17).

In this study, the average test age of the exposed child using PCR-DNA was 7.11 weeks of age (4 to 36 weeks range) and a median of 4 weeks. The WHO recommends the collection of HIV PCR-DNA test between 4 to 6 weeks of age (23). The breadth of the results demonstrates that there are still weaknesses and gaps in completing early infant diagnosis process in a timely manner, and the contributing factors previously mentioned may be leading to this situation.

In the present study, about 5% of the PCR-DNA results did not return to the HF. It is likely that HIV PCR-DNA results that did not return to the HF were rejected samples at the central laboratory. On the other hand, 24% of children with a positive result (results that were returned to the HF) did not initiate ART. Contrary to expectations, distance and place of residence did not reveal an

association with the initiation of ART. Such lack of association was also found in the study in Myanmar (22).

Conclusions and Recommendations

The above analyses lead us to conclude that nearly ¾ of children were tested within the first two months of life. The time for result return is prolonged, and almost a fifth of the mothers did not return to pick up the result. Expansion of early infant Point-of-Care diagnosis is recommended to minimize result delivery time and for immediate ART initiation for HIV-positive children. The identification of HIV and early initiation of ART in women of reproductive age is crucial to prevent vertical transmission.

Based on the conclusions, the following is recommended:

BARRIER	**RECOMMENDED STRATEGY**
Prolonged average time to return laboratory results to health facilities	Implementation of *Point-of-Care* (POC) in early childhood diagnosis associated with the conventional testing model in the central laboratory - **Central Level**
Prolonged average time for delivery of PCR-DNA results to mothers/ caregivers; Non-return of mother/caregiver to the HF to collect HIV PCR-DNA results	Implementation of *Point-of-Care* (POC) in early childhood diagnosis associated with the conventional testing model in the central laboratory) - **Central Level** SMS notification to mothers to inform that HIV PCR-DNA result is available at HF - **Provincial Level and District Level** In places without cell phone access/poor or non-existent network coverage, community health workers can be notified to contact mothers/caregivers - **Provincial and District Level** Active search: direct communication to mothers/caregivers about the availability of the result at the HF - **Provincial and District Level**
Non-initiation of ART among children with HIV-positive DNA PCR	Implementation of the 'Test and Start' strategy recommended by the WHO - **Provincial and District Level**
Testing after eight weeks of age	Strengthen communication to raise awareness about the importance of PCR-DNA testing before eight weeks of age - **Central, Provincial and District Levels**

	Expansion and optimization of HIV PCR-DNA testing at all entry points at the first contact of children at the HF - **Central, Provincial and District Levels**
Failure to return the HIV PCR-DNA result from the processing laboratory to the HF	Establishment of an organizational flow and prioritization of HIV PCR-DNA samples in central laboratories - **Provincial Level** Creation of a notification system, by SMS, of rejected samples - **Provincial and District Level** Provide training in collecting DBS samples for HIV PCR-DNA, recording information in routine books - **Central, Provincial and District Levels**
Mother not being on pre-delivery ART	Strengthening of the Option B+ and universal ART strategy - **Central and Provincial Level** Implementation of the Male Champions strategy, traditional medicine practitioners, traditional midwives for referral of pregnant women for prenatal consultation and delivery at the maternity, lactating women - **Central, Provincial and District Levels**.

Dissemination Plan

In an effort to share best practices and lessons learned from this evaluation, a summary approved by CDC-Mozambique with these findings was presented in an *oral presentation* format at the Provincial Health Meeting, which took place in August 2019 in Quelimane, and in *poster* format at the International Conference on AIDS and Sexually Transmitted Diseases (STDs) in Africa (ICASA) in Kigali, Rwanda, in December 2019. In addition, this report will be translated and shared with MoH and stakeholders at the local level, where results will be discussed to improve strategies to increase uptake of services.

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Appendices

Approved Protocol

This evaluation is covered and has been approved by the CDC-Mozambique Associate Director of Sciences (ADS) under the general protocol VUMC/FGH for secondary data analysis to evaluate and improve program results using HIV Care and Treatment data routinely collected (CGH HSR #: 2016-163a).

Instruments

Not applicable.

Informed Consent

Informed consent was not necessary for the use of the data in this evaluation as it was a secondary analysis of programmatic data collected routinely and anonymized. A waiver of informed consent was approved as the evaluation involved no more than minimal risk.

Bio-sketches

Not applicable.

Conflict of interest statement

The collaborators of this evaluation have no conflicts of interest to declare.

Evaluation costs

Not applicable.

Results or logical framework

Not applicable.