

HIV SECOND GENERATION SURVEILLANCE IN PAKISTAN



Antenatal Sero-Surveillance for HIV/AIDS in Pakistan 2012

NATIONAL AIDS CONTROL PROGRAM
BALOCHISTAN AIDS CONTROL PROGRAM
KHYBER PUKHTUNKHWA AIDS CONTROL PROGRAM
PUNJAB AIDS CONTROL PROGRAM
SINDH AIDS CONTROL PROGRAM
CANADA PAKISTAN HIV/AIDS SURVEILLANCE PROJECT



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ABBREVIATIONS AND ACCRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
CBC	Complete Blood Count
CDCU	Central Data Coordinating Unit
CIDA	Canadian International Development Agency
DBS	Dried Blood spot
ELISA	Enzyme-Linked Immunosorbent Assay
FSW	Female Sex worker
HASP	HIV/AIDS Surveillance Project
HIV	Human Immunodeficiency Virus
HSW	Hijra Sex Worker
IBBS	Integrated Behavioral and Biological Surveillance
IDU	Injecting Drug User
KP	Khyber Pukhtunkhwa
NACP	National AIDS Control Program
NGO	Non Governmental Organization
NRL	National Reference Laboratory
PACP	Provincial AIDS Control Program
PDCU	Provincial Data Coordinating Unit
PPTCT	Prevention of Parent to Child Transmission
SGS	Second Generation Surveillance
UAT	Unlinked Anonymous Testing
UNICEF	United Nations Children's Fund
VCT	Voluntary Counselling and Testing
WHO	World Health Organization





ACKNOWLEDGMENTS

The Canada-Pakistan HIV/AIDS Surveillance Project (HASP) would like to thank all the health facilities across the nine districts which participated as sentinel sites in this study and would especially like to acknowledge the contribution and dedication of the health facility staff, whose efforts ensured completion of the study on time. In addition, the women who attended antenatal care although silent and anonymous, are acknowledged for their contribution. HASP is grateful to the contracted NGOs (Human Resource Development Center, Research Associates, Al-Nijat Welfare Society, Bridge Consulting Foundation, Metro Consulting Ltd) and their field staff for their tireless efforts and constant diligence in carrying out this study.

The samples from the antenatal care study were tested at the National Reference Laboratory at National Aids Control Program. We would like to thank Dr. Nadeem Ikram for his supervision; Mr. Mahmood ul Hassan and Mr. Tariq Hayat Mir for testing of the dried blood spot samples promptly without compromising on quality and Miss Nosheen Khalid for her assistance with laboratory coordination.

We are grateful to the National AIDS Program Manager, Dr. Sajid Ahmad for his strong leadership and constant support to HASP. We particularly appreciate the role of the Provincial Program Managers, Dr. Salman Shahid (Punjab), Dr. Nasir Khan (Balochistan), Dr. Sher Mohammad (Khyber Pukhtunkhwa), Dr. Abdul Jabbar Sheikh (Sindh) and Dr. Qamar Abbas (Deputy Manager Sindh) for their continued support and critical inputs whenever required.

We are thankful to Ms. Michelle Monro, Canadian Project Director HASP for her constant support and guidance throughout the study as well as Dr. Simon Azariah, HASP Country Director, who led all of HASP's efforts in 2011 with sound and supportive management.

We would also like to thank our consortium partners: University of Manitoba, ProAction: Partners for Community Health, and Public Health Agency of Canada represented respectively by Dr. James Blanchard, Dr. Alix Adrien, Dr. Chris Archibald and Dr. Paul Sandstrom who continued to provide valuable guidance and support for this study at each step.

We would like to acknowledge the effort put in by the HASP field teams and Central and Provincial Data Coordinating Unit members, who ensured correct implementation, as well as the constant guidance from the technical team in Islamabad. Mr. Suleman Azariah, the National Antenatal Field Coordinator devoted many long hours to the study and deserves particular mention.

HASP would also like to thank CIDA for enabling us to conduct this study and UNICEF for providing funds to enable us to include Larkana.

Dr. Nosheen Dar
Principal Investigator





FOREWORD

The past three Rounds of HIV Surveillance in Pakistan conducted by HASP have focused on key populations at risk including Injecting Drug Users, Hijra Sex Workers, Male Sex Workers and Female Sex Workers. Data from these studies show that over the years the HIV epidemic has firmly established itself in these groups and is increasing particularly in Injecting Drug Users and Hijra Sex Workers. As noted in the report of the last round of HIV Surveillance, the potential threat of HIV spreading into the general population cannot be ignored and the National AIDS Control Program expressed the need to survey antenatal clients in order to ascertain the prevalence of HIV in the general population.

The Government of Pakistan is fully aware and sensitive to the gravity of the HIV situation in Pakistan. Over the years HASP has been providing valuable information, which has enabled the Government and its partners to plan a targeted and multi-sectoral response. It is important that studies required to ascertain HIV prevalence in the general population are conducted on a regular basis. For this purpose the study of HIV prevalence among women attending antenatal clinics, which serves as a proxy for the general population was considered to be of great benefit. Consequently HASP undertook the first national 'Antenatal Sero-Surveillance for HIV/AIDS' study in nine districts of Pakistan: originally eight districts were planned as per HASP study design and later Larkana district was added with UNICEF's support.

This report is the result of dedication, commitment and hard work shown by the people involved in this study. I would like to acknowledge the efforts of the HASP team, the Provincial AIDS Control Program managers and their teams, the implementing organizations as well as all the health facilities which agreed to participate. This study would not have been possible without them.

I also like to appreciate the guidance and hard work of Ms. Michelle Munro, Canadian Project Director HASP, Dr. Simon Azariah, Country Director HASP and the technical team of HASP.

Dr. Sajid Ahmad
National Program Manager
National AIDS Control Program





EXECUTIVE SUMMARY

The report holds an account of the first ever national antenatal sero-surveillance for HIV/AIDS in Pakistan. Since HASP's inception in 2004, it has conducted four rounds of Integrated Biological & Behavioral Surveillance in key populations at risk. The primary objective of this particular study was to devise an applicable, functional methodology to conduct surveillance and gather biological and demographic data from pregnant women in order to estimate the level of the HIV epidemic in the general population. A sample size of more than 150,000 was required to estimate prevalence but the primordial focus of the study was to devise a method; hence 27,000 women were screened. Sentinel sites were set up in 42 selected health facilities across 9 districts. Selection and training of personnel from health facilities was followed by active data collection.

Only 12 positive cases were reported out of 26510 samples tested. Seven of the 12 cases were reported from Peshawar. The report holds descriptive analyses on the socio demographic data characteristics recorded. It does not have any inferential and comparative statistical analyses due to small number of positive cases, small sample size and being first ever national surveillance round.





1 INTRODUCTION

The HIV pandemic is one of the greatest challenges that the world faces today. At the end of 2010, 34 million people were estimated to be living with the virus. There were 390,000 new infections among children under the age of 15. Most of these new infections are believed to stem from transmission *in utero*, during delivery (vertical transmission) or as a result of breast feeding.

In Pakistan, the National AIDS Control Program's (NACP) figures show that over 4,000 HIV cases have been reported since 1987 but UN AIDS estimated that in 2009 there were more than 98,000 HIV positive cases in the country. However, a combination of factors are increasing the risk that Pakistan could transition from a concentrated to a generalized epidemic through transmission from key populations at higher risk to groups seen as being at lower risk. A recent surveillance round of Integrated Biological and Behavioral Surveillance (IBBS) in key populations at higher risk shows an overall prevalence of 37.8% among injecting drug users (IDUs), 5.2% among hijra (transgender) sex workers (HSW) and 0.8% in female sex workers (FSW). These factors include the widespread presence and interlinking of those who engage in injecting drug use and high risk sexual networks, as is the pattern in several other Asian countries. Organized and focused prevention efforts are therefore required to minimize the size of this impact and contain the epidemic at an early phase.

Pregnant women are a population vulnerable to HIV because of potential infection from their regular sexual partners (husbands) who may have been infected by a sex worker or other key population at higher risk. The World Health Organization (WHO) suggests that the general population (pregnant women or blood donors) should be screened for HIV once there is a concentrated epidemic (about 5% or more) among at least one key population at higher risk. Population based studies and the screening of pregnant women who come for routine antenatal care (ANC) sero-surveillance concurrently conducted in the same area in several Sub-Saharan African countries have provided similar prevalence data supporting the use of ANC attendees as good proxies for the general population.

Over the past years a few surveys have been conducted to collect data from women attending ANC services in Pakistan. Screening of antenatal clinic attendees coming to Prevention of Parent to Child Transmission (PPTCT) centers showed that only 21 out of the 8000 (0.003%) were HIV positive. Nearly all of them were wives of known HIV patients. More recently a study, "Prevalence of HIV in pregnant women identified with a risk factor at a tertiary care hospital", was conducted by the NACP with the help of UNICEF. It reported 2 HIV positive women out of 779.

The data presented in this report is derived from sero-surveillance among pregnant women attending health facilities across Pakistan and was developed with the following objectives:

- 1) To determine an appropriate methodology for HIV surveillance among antenatal women in Pakistan;
- 2) To estimate HIV prevalence among antenatal women;
- 3) To determine demographic characteristics of HIV positive antenatal women;
- 4) To generate data for use in National HIV estimations and projections; and
- 5) To promote appropriate policy and program response through dissemination and knowledge translation

This survey is part of wider Second Generation Surveillance (SGS) system. First generation or routine surveillance systems collect and monitor data for disease trends and/or outbreaks. These systems largely rely on the passive collection and analysis of data from cases of disease diagnosed by the health care system, usually based on analysis of a biological sample (e.g. blood). In contrast, SGS systems include the active collection of both biological and behavioral data. An effective SGS system: 1) contributes to understanding the dynamics of HIV in the country context (e.g., who is at risk for or vulnerable to HIV infection); 2) provides basic information for focusing and designing interventions proposed within a National strategic plan such as levels and trends in HIV infection; and, 3) provides information for decision makers to help them understand the impact



of prevention activities in different populations leading to informed policies and program development.

1
SGS in Pakistan was introduced by the HIV/AIDS Surveillance Project (HASP) (2004-2011). With support from the Canadian International Development Agency (CIDA) and the Government of Pakistan, HASP has been instrumental in establishing and streamlining key aspects of a SGS system for HIV in Pakistan. Key Pakistani partners are the NACP, and Provincial AIDS Control Programs (PACPs) in Punjab, Sindh, Khyber Pukhtunkhwa (KP) and Balochistan, as well as local non-governmental organizations (NGOs), research organizations and other stakeholders. The project has been implemented by a consortium consisting of Agriteam Canada Consulting Ltd, University of Manitoba and Pro Action: Partners for Community Health, Inc. This report presents results from Pakistan's first round of antenatal HIV sero-surveillance. It is anticipated that this report would be useful in laying the groundwork for building an efficient antenatal HIV sero-surveillance program in Pakistan.





2 METHODOLOGY

A cross sectional design using the WHO's unlinked anonymous methodology was used to conduct this survey. Briefly, residual blood from samples collected from pregnant women attending health facilities for their first blood test was used for HIV rapid testing. In addition socio-demographic data was collected from antenatal client records. The survey protocol was approved by Research and Ethics Board of the Public Health Agency of Canada and Health Oriented Preventive Education (HOPE) ethical review board in Pakistan.

2.1 Study Period

The study was conducted over a span of four months. Sentinel sites were selected in the first month following which data was collected over a period of three months i.e. July, August and September 2011.

2.2 District Selection

The study population comprised of pregnant women attending health facilities in four provinces - KP, Punjab, Sindh, and Balochistan (Table 2.2). Within each province, one high and one low HIV prevalence district was selected. Due to the high prevalence of HIV in IDUs in Larkana and at the request of UNICEF, this district was also included in the sampling framework.

Table 2.2 Provinces and Districts selected for antenatal surveillance of HIV infection

Province	District
Punjab	Lahore (High Prevalence)
	Multan (Low Prevalence)
Sindh	Karachi (High Prevalence)
	Thatta (Low Prevalence)
	Larkana (High Prevalence)
KP	Peshawar (High Prevalence)
	Abbottabad (Low Prevalence)
Balochistan	Quetta (High Prevalence)
	Gawadar (Low Prevalence)

2.3 Sampling Framework

The representative sample size for the estimation of HIV prevalence in the general population in Pakistan was calculated to be 150,000. However, given the main

objectives of this study, to test the feasibility of antenatal surveillance within Pakistan and to determine the factors associated with HIV infection among pregnant women, a sample size of 27,000 pregnant women was decided upon. The number of pregnant women clients attending health facilities in each district during a three-month time period was estimated and this estimation became the sample size for each district (Table 2.3).

Inclusion criteria:

“Any pregnant women attending regular antenatal services in one of the selected ANC clinics and having her first haematological screening at the ANC facility during the study period was eligible for testing.”

Table 2.3 Sample size for selected districts.

District	Sample size
Karachi	6,300
Thatta	1,200
Lahore	6,000
Multan	3,000
Quetta	2,500
Gawadar	500
Peshawar	3,000
Abbottabad	1,500
Larkana	3,000
Total	27,000

2.4 Sentinel Site Selection

The sample size calculated for each district was distributed among selected health facilities from that particular district based upon the daily turnover of antenatal clients. Sentinel sites (i.e. health facilities) within each city were selected using a three step process.

1. A list of maternity homes and health facilities within each district that provide maternity services was prepared. These health facilities had to meet the following criteria.
 - a. The health facility must be public or private.
 - b. The facility must be located within the selected district and must offer regular outpatient antenatal care. The list prepared should be separate for public



achievement of sample collection as per designated sample size within the allotted time.

2. These health facilities were evaluated and assessed on their ability to participate. A site assessment form (Annex 1) was used to evaluate these health facilities. Assessment was based on three criteria:
 - a. The health facility should have a separate laboratory/pathology laboratory.
 - b. The hospital administration must be willing to cooperate.
 - c. Justifiable daily turnover of the ANC clients.
These assessments were verified by HASP's technical team.
3. The list of health facilities found to be eligible were selected through weighted random selection using SPSS™.

2.5 Training of Personnel

A two day master training was held in Lahore for the field staff and the participating NGOs from each province. The core modules of the training were as follows:

1. Understanding surveillance
2. Study methodology
3. Gender and rights sensitization
4. Rapid testing
5. Dried Blood Spot (DBS) cards
6. Specimen handling and personal safety

Separate training workshops were also held in each district for the staff contracted i.e. (nurses, phlebotomists) from the selected health facilities.

2.6 Data Collection Instrument

An antenatal sero-surveillance form (Annex 2) was designed by the HASP technical team to collect demographic information and record rapid test result for each study participant. The socio-demographic variables included in the form included the following:

1. Age as recorded in hospital records
2. Woman's education
3. Husband's occupation
4. Pregnancy history

5. Status of last child

Rapid test results i.e. reactive, negative and indeterminate were also recorded on the sero-surveillance form.

2.7 HIV Testing

HIV rapid testing was performed on whole blood in the pathological laboratories of the selected sentinel sites/health facilities, using M/S *Determine™ HIV1/2* kit. *Determine* is one of the WHO recommended kits for HIV rapid testing and has been extensively used in Voluntary Counselling and Testing (VCT) studies and in VCT centres in Pakistan. This kit has 100% sensitivity on serum and greater than 98% on whole blood. In case of a reactive or weak positive rapid test result a DBS card was made and sent to the National Reference Laboratory (NRL) where it was screened for HIV using the *Avioq HIV 2 Micro ELISA* system (Avioq Inc USA). If the ELISA result was negative the sample was confirmed negative. If the ELISA result was positive then it was further confirmed by testing with M/S Biomerieux *Vironostika® HIV uniform II plus O* kit. If the second ELISA was also positive the sample was confirmed HIV positive. In case a sample tested indeterminate i.e. positive on screening but negative on second ELISA, M/S Biorad GS HIV 1 western blot kit was used to confirm the status of the sample. DBS cards made for quality assurance from non-reactive samples were also tested with ELISA.

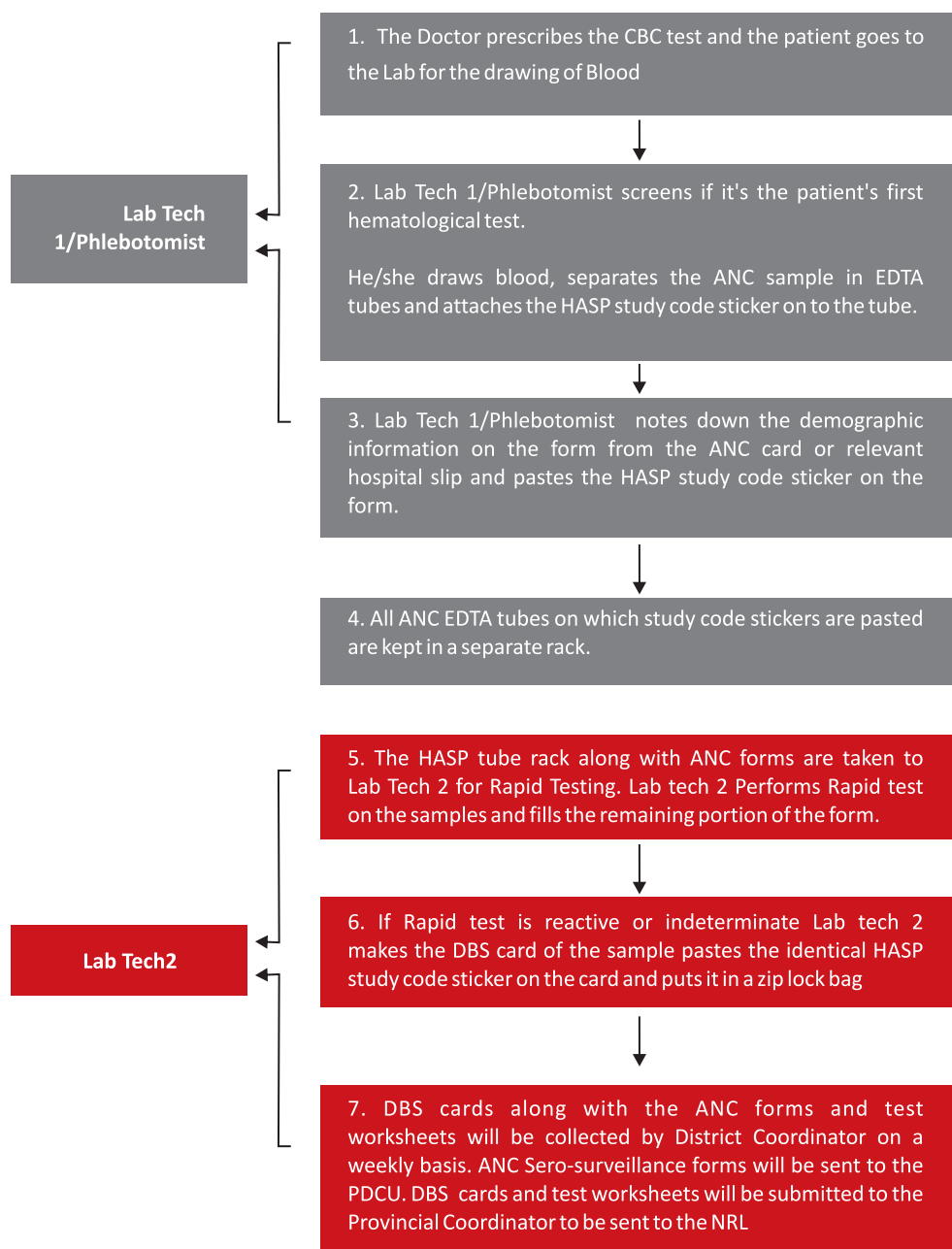
2.8 Data Collection Process

Two types of data (i.e. socio-demographic and biological data) were collected for this study. However, different health facilities had different procedures for prenatal blood testing of patients. To account for these differences, two applicable models were devised (Figures 2.8a and 2.8b). Briefly, on prescription of a blood test by the physician/gynaecologist, the women were screened by an attending staff member (nurse or phlebotomist) to determine their eligibility to participate in the study (Figure 2.8c). The staff member also extracted required socio-demographic information from the patient's antenatal card or medical records. The



staff member was instructed not to ask the patient any questions other than those required for screening purposes to determine eligibility. Therefore, with the exception of age, if the information was not available in the patient's antenatal card or medical record, these fields were left uncompleted on the sero-surveillance form.

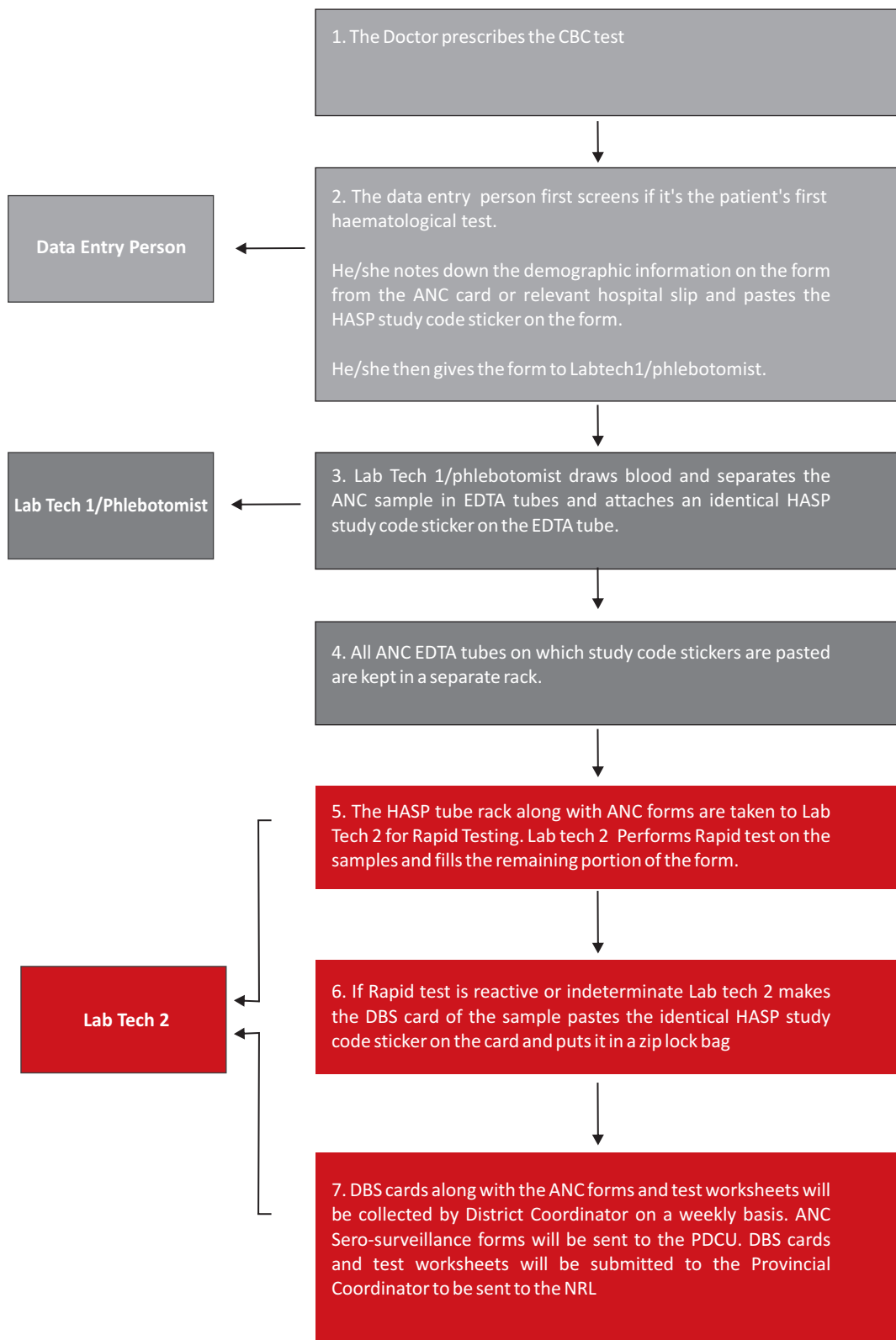
Fig 2.8a Model 1 for data collection



2



Fig 2.8b Model 2 for data collection

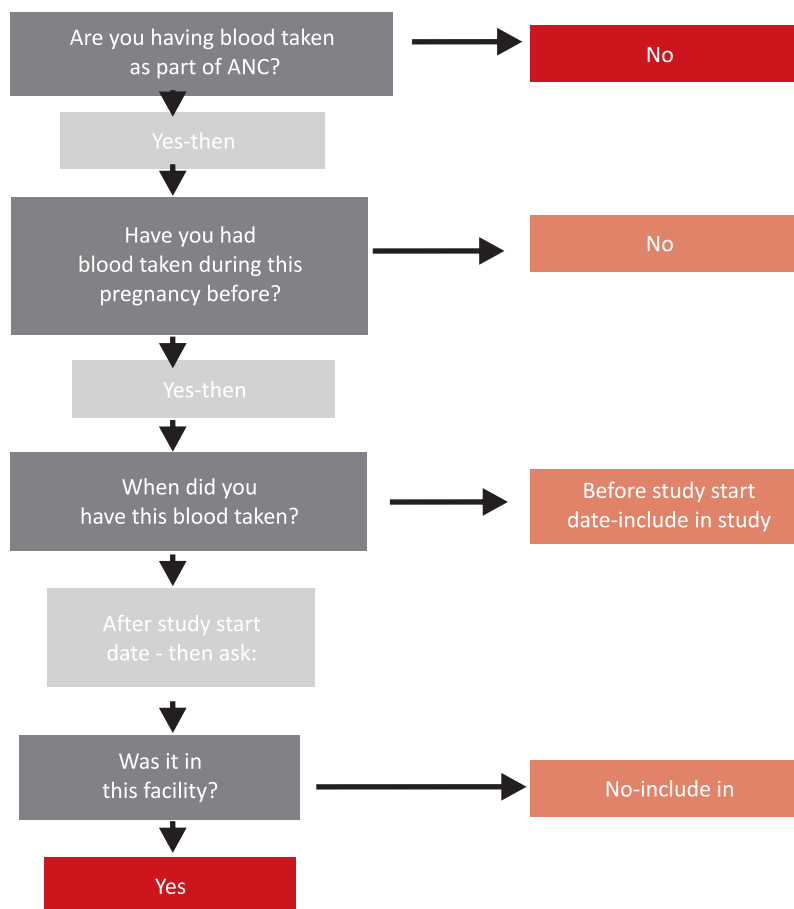


2





Fig 2.8c Screening questions to determine study eligibility



2

Blood was taken by the staff member collecting the demographic data or by another staff member specifically designated for this purpose. Once the blood sample was extracted from the patient for routine testing a few millilitres of left over blood were poured in the EDTA tube to facilitate HIV screening; this particular amount of blood would not have affected the patient's normal blood testing. 50 µl of blood was taken by a micro pipette and poured on the strip. It was immediately followed by pouring a drop of chase buffer (according to the manufacturer's instructions). If the sample was tested reactive or indeterminate a DBS card was immediately made. Results of each sample were recorded on the corresponding antenatal sero-surveillance form. DBS cards were dried overnight and then packed in zip lock bags along with desiccants and humidity indicator cards. Lab work sheets (Annex 3) were specifically filled by the person performing the

testing. DBS cards, lab worksheets and the antenatal sero-surveillance were collected by the respective field staff from each facility on a weekly basis. DBS cards along with lab worksheets were transported to Islamabad while the antenatal sero-surveillance forms were provided to Provincial Data Coordinating Units (PDCUs) for data entry.

2.9 Ethical Review and Considerations

The study protocol was reviewed and approved by the Ethical Review Board of the Public Health Agency of Canada, as well as in Pakistan by HOPE International's Ethical Review Board. This survey was designed to meet international ethical guidelines, specifically addressing the following ethical issues:



Confidentiality: To protect participant identities, the only information collected from women was that available on client records, as recommended by the WHO and UNAIDS Guidelines for Conducting HIV

Sentinel Serosurveys among Pregnant Women and Other Groups. Moreover all health facilities signed confidentiality agreements. The key operational measure to ensure confidentiality was that the person taking the blood and person performing the tests were always different, regardless which data collection model was used. Furthermore, a strip of bar code stickers was provided to each health facility. Each strip had an array of two stickers with identical specimen code numbers. Each specimen code was comprised of 10 digits. The first four digits represented the district, the next two digits represented the site number or health facility where as the last four digits represented the serial number. No identifying information such as the name of patient, or lab number was allowed to be written on the antenatal sero-surveillance form. One sticker from each pair of stickers was pasted on the antenatal sero-surveillance form while the other sticker was pasted on an EDTA treated, anti-coagulant tube (provided separately) to facilitate data linkage.

Notifications on the study: Posters were posted in all participating health facilities to notify ANC clients and the public that the study was ongoing, the rationale in terms of HIV prevention, that blood of some women would be tested, that the results would not be provided and that HIV counseling and testing was also available and where.

HIV test results: HIV test results were kept confidential from study personnel and were not provided to participants. However all health facility staff received training on HIV and vertical transmission, as well as on gender and HIV, and were advised to include messages on HIV as part of routine ANC, as well as to advise women regarding where they could be tested for HIV.

2.10 Quality Assurance and Monitoring

Two percent (2%) of DBS cards were made of negative samples from each health facility, to ensure that all procedures were being done according to protocol and the rapid test kits were functioning properly. These DBS cards were also tested with ELISA. Each district had a

designated HASP District Monitor and also a District In-charge appointed by the contracted NGO, to ensure quality in data collection. Vigilant monitoring was done by the District Monitor to ensure that the standard operating lab procedures were being followed and confidentiality was being maintained. A Provincial Coordinator in each province was appointed to coordinate with the National Office and provide support and direction to the District Monitors. The Provincial Coordinator was also responsible for conducting weekly monitoring visits to each sentinel site. The National Office also conducted active monitoring in each district to ensure the quality of work and resolve any issues regarding data collection. A separate “Gender and Rights Monitoring” visit was conducted in each district to ensure that ethical recommendations were being met properly. As a monitoring directive, District Monitors were required to complete monitoring forms (Annex 4) upon the completion of each monitoring visit. Along with technical monitoring aspects this form also had a separate gender and rights section, which was a clear indicator on how ethical recommendations were being met.

2.11 Data Management

Data entry was done at the PDCU's in each province. To ensure the quality of data, a separate data entry personnel was hired. The data was entered in to an MS Access database especially designed for HASP antenatal sero-surveillance project. Laboratory results were linked to the corresponding socio-demographic data using identical site codes on the antenatal sero-surveillance form and DBS cards; no personal information or identifying markers accompanied the record.



3 RESULTS

A total of 26,510 pregnant women were screened for HIV in 42 sentinel sites spread across nine districts. This sample size is slightly lower than the required 27,000 to estimate HIV prevalence and the small absolute number of HIV positive cases do not allow for extrapolation or epidemic modeling. Nevertheless, the results provide the first-ever National picture of HIV among women attending antenatal clinics and related health facilities and could serve as a baseline for subsequent rounds of data collection.

3.1 Sample Distribution

Figure 3.1a shows the distribution of study participants across the nine selected districts for this survey and figure 3.1b shows the number of sentinel sites selected per district. The largest numbers of study participants were recruited from Karachi and Lahore (6,080 from 7 sites in Karachi and 5,986 from 9 sites in Lahore) followed by Multan, Peshawar, Larkana, and Quetta with 3,073, 3,048, 2,990, and 2,425 pregnant women recruited respectively. Gawadar had the fewest absolute number of women recruited at 607 from two facilities (Figure 3.1a and Figure 3.1b).

Fig 3.1a Sample distribution per district

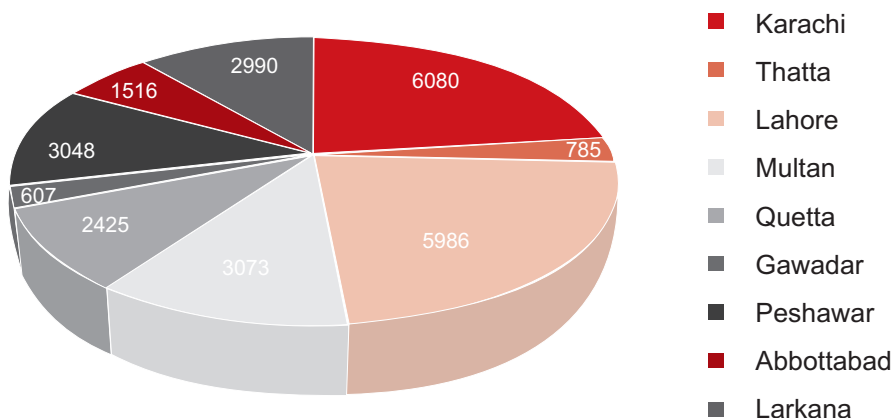
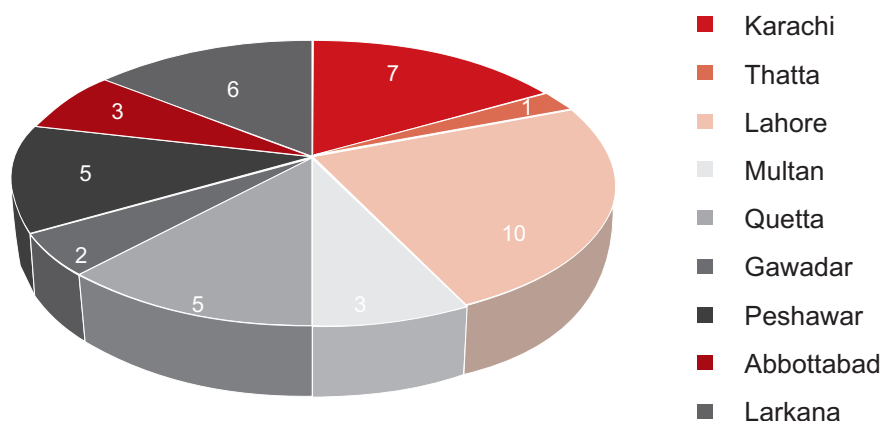


Fig 3.1b Number of sentinel sites/health facilities selected per district



3

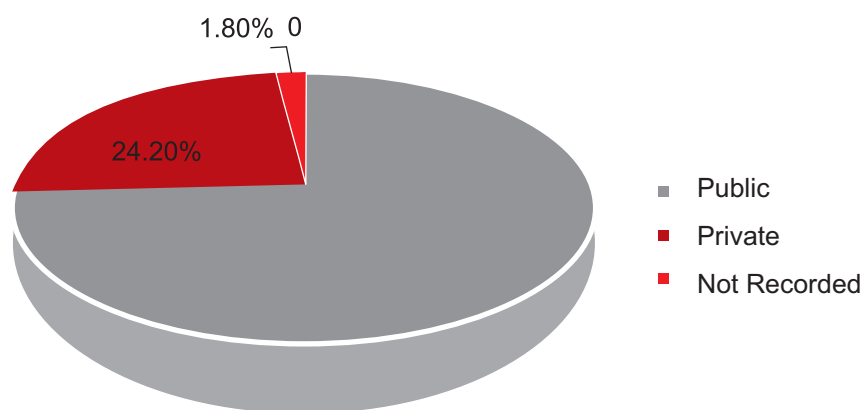


3.2 Type of Health Facility

As depicted in Figure 3.2, 74% of the sampled participants were screened in public health facilities whereas 24.2 % of sampled participants attended private health facilities. The type of facility was missing for 1.8% of the cases.

surveillance forms from Sindh didn't have the mention of settlement. In KP, all study participants were screened in urban health facilities.

Fig 3.2 Distribution of participants by type of facility (public, private)

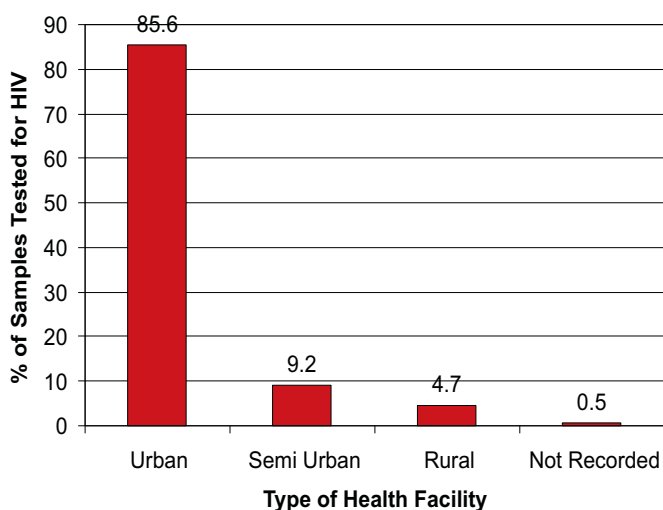


3

3.3 Location of Health Facility

Approximately 85% of study participants were recruited from health facilities located in urban settings whereas 9.2% and 4.7% were recruited from health facilities in semi-urban and rural settings, respectively. Data was missing for 0.5% of the study participants with respect to health facility location (Figure 3.3a).

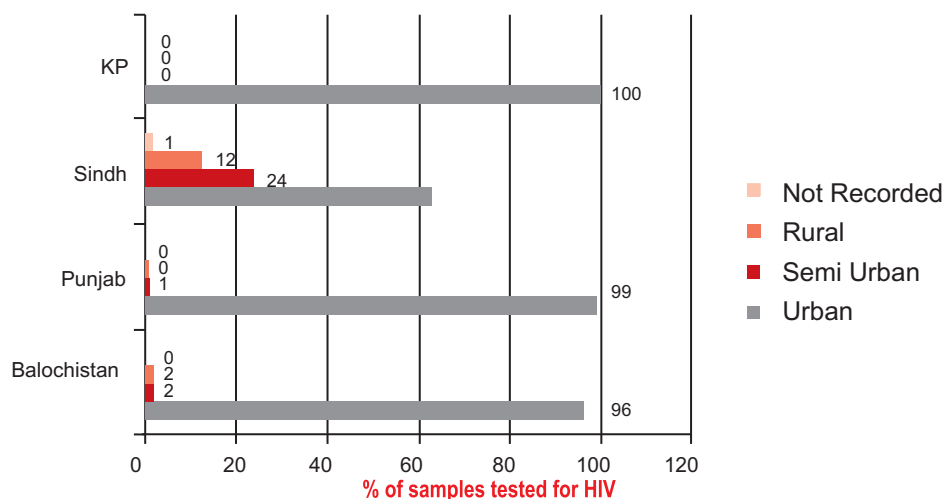
Fig 3.3a Distribution of samples tested by location of health facility (urban, semi-urban, rural)



Further analyses by province indicated that in Balochistan, 96% of the study participants were screened in health facilities located in urban setting, 2% were screened in health facilities located in semi urban setting and 2% were screened in rural health facilities (Figure 3.3b). In Punjab, 99% pregnant women were screened in urban health facilities and 1% were screened in semi-urban health facilities (Figure 3.3b). In Sindh, 63% pregnant women were screened in urban health facilities, 24% pregnant women were screened in semi urban health facilities and 12% pregnant women were screened in rural health facilities and 1% of sero-



Fig 3.3b Distribution of samples tested according to location of health facility, by province.



3.4 Age Distribution of Study Participants

Table 3.4a shows that majority of study participants (35.7%) were between 25 and 29 years of age. Women aged 20 to 24 years formed the second largest group (29.2%) followed by those between 30 and 34 years of age. Approximately 6.9%, 5.1%, and 1.2% of women were between 35-39, 15-19, and 45-49 years old, respectively. Information on age was missing for 1.1% of the study participants (Table 3.4a).

Table 3.4a Age distribution of study

Age	Participants	Percentage
15 – 19	1346	5.08%
20 – 24	7749	29.2%
25 – 29	9467	35.7%
30 – 34	5460	20.5%
35 – 39	1841	6.94%
40 – 44	321	1.21%
45 – 49	31	0.11%
Not recorded	295	1.11%

There were no significant differences in the mean age of study participants by province as shown in Table 3.4b.

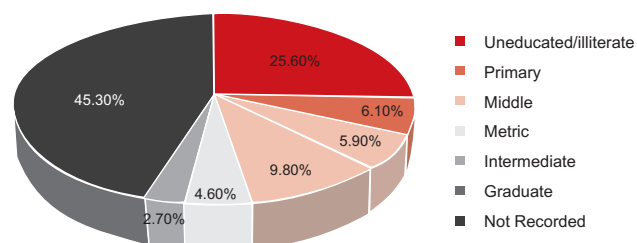
Table 3.4b Mean age of study participants by province

Province	Mean Age	± SD
Balochistan	28	6
Punjab	27	5
KP	28	4
Sindh	27	5

3.5 Education Levels of Study Participants

Figure 3.5 shows the distribution in the level of education among study participants. Information on education levels were missing for 45.3% of study participants. Approximately one quarter of the women (25.6%) were illiterate, 9.8% had completed matriculation, 6.1% had completed primary education and 5.9% had completed middle school, and 4.6% had completed their intermediate studies. Approximately 2.7% of the sampled participants had a Bachelor's degree (Figure 3.5).

Fig 3.5 Education levels of study participants



Further analyses by province indicated that the highest illiteracy rate was in Balochistan (60.5%) followed by KP (33.5%), Sindh (30.3%) and Punjab (4.6%) Table 3.5. However, Punjab had the highest number of missing data (38.8%).



Table 3.5 Education levels among study participants by province

Level of Education	Province			
	Balochistan n(%)	KP n(%)	Punjab n(%)	Sindh n(%)
Illiterate	1835 (60.5%)	1531 (33.5%)	421 (4.6%)	2988 (30.3%)
Primary	240 (7.9%)	389 (8.5%)	213 (2.4%)	764 (7.8%)
Middle	166 (5.5%)	451 (9.9%)	284 (3.1%)	685 (7.0%)
Metric	243 (8.0%)	844 (18.5%)	516 (5.7%)	1008 (10.2%)
Intermediate	91 (3.0%)	306 (6.7%)	448 (4.9%)	366 (3.7%)
Graduate	89 (2.9%)	209 (4.6%)	209 (2.3%)	221 (2.2%)
Not Recorded	368 (12.1%)	834 (18.3%)	6968 (76.9%)	3823 (38.8%)

3.6 HIV Status of Study Participants

Overall, 12 out of 26,510 study participants were confirmed positive for HIV infection corresponding to a prevalence of 0.05% (Table 3.6a). Peshawar had the highest prevalence of HIV infection; 13 out of 3,048 women screened positive on the rapid test and of these, seven (0.22%) were confirmed positive for HIV infection (Table 3.6a). Multan followed with a prevalence of 0.07% since 3,073 women were screened for HIV infection out of which two were confirmed positive. In Karachi out of 6,080 participants who were screened for HIV infection,

two (0.03%) were confirmed positive for HIV infection. In Larkana, out of 2,990 participants screened, one (0.03%) was confirmed HIV positive. In Lahore, out of 5,986 women screened six were reactive on the rapid test but were confirmed negative after further testing. No HIV positive infections were identified in Thatta, Quetta, Gawadar, and Abbottabad (Table 3.6a). The percent of false positive results using HIV rapid tests was less than 0.2% in any district.

Table 3.6a HIV reactive and confirmed positive cases by district

Province	District	No of Rapid Tests Performed	No of HIV Reactive Samples	No of Confirmed HIV Positive Samples
Sindh	Karachi	6080	2	2
	Thatta	785	0	0
	Larkana	2990	1	1
	Total	9850	3	3
Punjab	Lahore	5986	6	0
	Multan	3073	2	2
	Total	9049	8	2
KPK	Peshawar	3048	13	7
	Abbottabad	1516	0	0
	Total	4536	13	7
Balochistan	Quetta	2425	0	0
	Gawadar	607	0	0
	Total	3032	0	0
Total		26,510	24	12



Further analyses of the HIV cases by age indicated that out of the 12 positive infections, four were among women between 20 and 24 years old and the same number (4) were among women between 30 and 34 years of age (Table 3.6b). Three HIV positive women were between 25 and 29 years old and one woman was between 35 and 39 years of age.

Table 3.6b HIV status by age

Age	No of Participants	Percentage of Participants	Percentage of HIV Positive
15 – 19	1346	5.08	0
20 – 24	7749	29.2	0.05
25 – 29	9467	35.7	0.03
30 – 34	5460	20.5	0.07
35 – 39	1841	6.94	0.05
40 – 44	321	1.21	0
45 – 49	31	0.11	0
Not recorded	295	1.11	0

Out of the 12 positive HIV cases, nine (75%) were illiterate (Table 3.6c). The education level of one case was unknown and the remaining two cases had completed middle school and intermediate school, respectively.

Table 3.6c HIV status by education of study participants

Education Level	Participants	No of HIV Positive
Illiterate	6775	9
Primary	1606	0
Middle	1586	1
Metric	2611	0
Intermediate	1211	1
Graduate	728	0
Not Recorded	11993	1

Husbands' occupations of the HIV positive study participants were as follows: two unskilled labourers, three skilled labourers, three small scale traders, one fisherman, one professional and two not recorded.



4 DISCUSSION

The main purpose of antenatal sero-surveillance is to monitor HIV trends and to understand the extent of HIV spread in the general population. The major assumption when extrapolating to the general population is that HIV prevalence among pregnant women serves as a proxy to estimate prevalence among the adult population of men and women 15-49 years. In countries with a concentrated epidemic, as is the case with Pakistan, antenatal surveillance is recommended every 2 years. However, Pakistan has lacked a national antenatal surveillance system. Although studies have been conducted in the past among pregnant women in Pakistan, these were limited to a few cities and were not representative of the general population.^{11, 12} HASP has played a key role in identifying this gap and addressing it in terms of developing a system and methodology to determine the feasibility of screening pregnant women for HIV in Pakistan and to record important socio-demographic variables associated with HIV infection in this population.

A total of 26,510 pregnant women who were screened for HIV in 42 sentinel sites spread across nine districts. The largest numbers of study participants were recruited from Karachi and Lahore followed by Multan, Peshawar, Larkana, and Quetta. Gawadar had the fewest number of women recruited (Figure 3.1a). While the final sample recruited for this study is lower than the 150,000 required to estimate HIV prevalence among the general population and the small absolute number of HIV positive cases do not allow for extrapolation or epidemic modeling, the results provide the first-ever national picture of HIV among women attending antenatal clinics and related health facilities and could serve as a baseline for subsequent rounds of data collection.

Overall, 12 out of 26,510 study participants were confirmed positive for HIV infection corresponding to a prevalence of 0.05% (Table 3.6a). Peshawar had the highest prevalence of HIV infection (0.22%), followed by Multan (0.07%), Karachi (0.03%), Larkana (0.03%). No confirmed HIV positive infections were identified in Lahore, Thatta, Quetta, Gawadar, and Abbottabad. It is unclear whether HIV infection was related to migration or was locally acquired as such information is not

collected on the ANC card or medical records. Of note, there are a large number of Afghan refugees in the border areas of Pakistan seeking medical care and this should be taken in to account while planning the next data collection cycle. Peshawar also has a migrant population comprising of people who have worked abroad. Regardless, the results suggest that Peshawar may require special attention in terms of establishing PPTCT centers to prevent vertical transmission. Although initially it was suspected that districts with a high HIV prevalence among Key population at higher risk might exhibit more positive cases this was not the case. It is possible that HIV has not been established in “bridge” populations such as FSWs; more data is needed to verify this hypothesis. It is also worth reiterating that the results need to be interpreted with caution given the sample size considerations as have been previously discussed.

HIV positivity rates corresponded with the age distribution of pregnant women in Pakistan; out of the 12 positive infections, four were among women between 20 and 24 years old and the same number, four, of positive cases were identified among women between 30 and 34 years of age (Table 3.6b).

Among the 12 HIV positive cases, 9 (75%) were illiterate (Table 3.6c) and reliant on low or daily wage income. The highest illiteracy rates were reported in Balochistan while Punjab has the lowest reported illiteracy levels among the sampled women. However, Punjab also had a very high percentage of missing data on this variable. Given the high illiteracy rates, raising awareness using innovative approaches that reach all members of the population are required and could be supported by the National AIDS Control Program.

As one of the key objectives of this cycle was to determine the feasibility of antenatal sero-surveillance in Pakistan, several lessons learnt from this inaugural cycle bear mention. The model used in this survey was to conduct surveillance in antenatal clinics where blood is drawn. In Pakistan this may have excluded more rural and poorer women.

The inclusion of outreach partners from both public and



private sectors to conduct the survey and as partner antenatal facilities was extremely valuable. HASP extended its outreach by contracting various local NGO's in the provinces. These NGO's were provided a separate organogram in the protocol to designate personnel dedicated to the antenatal sero-surveillance study. HASP also hired District Monitors in each district headed by a Provincial Coordinator for the province. These individuals were responsible to keep the National Office abreast of project progress and ensuring the quality of data collection and no breaches in confidentiality. Another very important accomplishment was the focused effort made to sensitizing personnel on issues related to gender and rights. This was facilitated via special sessions during the training workshop and then further supported via advocacy and raising awareness throughout the project implementation cycle. Admittedly, this area is still a work in progress.

Hospitals selected to participate were generally comfortable with their participation and able to use participation to contribute to organizational learning. However, within these facilities and in all Pakistan facilities that provide antenatal care there is little standardisation of screening and antenatal routines. Therefore, the HASP ANC team developed two models (Figures 2.8a and 2.8b) for antenatal screening and testing which turned out to be applicable both in terms of quality data collection, acceptability, and ensuring patient confidentiality.

With respect to patient confidentiality, there was justifiable concern that it could be compromised given the requirement to conduct HIV testing on left over blood; almost all facilities had a laboratory number on the blood collection tubes through which patients could be identified so as to provide them with their test results. This issue was addressed by collecting the left over blood in an EDTA treated vacutainer tube and pasting a sticker with a unique sample code which could be linked to the sero-surveillance questionnaire but not to the patient. The procedures were actively monitored by HASP's monitoring staff from the district, province and national offices.

Unlinked anonymous testing (UAT) has been used globally in surveillance activities. However, there is still a

debate about the ethics of not providing test results to study participants. To address the concerns expressed by field staff, particularly clinicians, on this issue, separate sessions were added to training workshops and additional meetings were set up to facilitate a better understanding of UAT in the antenatal surveillance context. Furthermore, following recommendations to conduct UAT in partnership with program delivery systems, study participants were provided information through posters on HIV/AIDS, the antenatal sero surveillance study and voluntary counselling and testing centers. In this regard, linking with service providers and the inclusion of the administrative staff and management within each health facility in project design and implementation was imperative. The cooperation of staff in public health facilities was noteworthy particularly given the daily turnover of pregnant women in these facilities. Figures 3.2, 3.3a, and 3.3b illustrate the distribution of health facilities by sector during the project cycle. With the exception of Sindh province, the over-representation of urban health facilities is indicative of the dearth of facilities with antenatal care in semi-urban and rural areas. Even when available, these facilities lacked separate laboratories and the capacity to maintaining testing equipment and samples.

Unfortunately, target sample sizes were not achieved in certain districts (Figure 3.1). This was sometimes due to factors beyond the control of HASP. For example, one of the health facilities in Karachi was submerged under water due to flooding. A similar occurrence took place in Thatta. Political instability in certain areas also resulted in the premature termination of data collection or inability for regular project monitoring. In other cases, logistical issues impacted data collection. For example, bar code stickers (which are facility specific) were inadvertently swapped resulting in a mismatch between the biological sample and sero-surveillance form. Data collection needed to be suspended until the problem was rectified. These experiences informed the need for constant monitoring to ensure data quality as well as the need to be flexible when addressing unforeseen issues.

In conclusion, antenatal sero-surveillance for HIV infection is feasible within the Pakistani context, albeit in urban areas. As the number of HIV positive cases was low (n=12), approximate prevalence 0.05 %, subsequent

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the population are needed to confirm this result. Factors associated with HIV infection among pregnant women also need to be further explored but initial results suggest differences with respect to literacy, location (urban vs. rural), and age.



5 RECOMMENDATIONS

The following are recommended.

1. In order to more accurately estimate HIV prevalence among pregnant in the general population, the next round of data collection should be larger.
2. Sentinel sites should be derived from both public and private sectors and from urban/semi-urban/ areas to ensure a representative sampling of pregnant women across the country. Rural data collection would need to use another methodology.
3. The new round of data collection should include the facilities that have in this data collection cycle so that surveillance data (socio-demographic and biological) could be temporally compared.
4. In order to compare data spatially new districts and health facilities should be included in the next rounds of data collection.
5. A strategy for conducting antenatal surveillance among rural women needs to be explored.
6. Given current challenges associated with incorporating HIV screening as a component of routine antenatal care, breaches of confidentiality remain a concern. Therefore, for surveillance purposes, Unlinked Anonymous Testing remains a viable option as long as participants are linked with service delivery programs for appropriate HIV counseling and testing.
7. The National AIDS Control Program along with Provincial AIDS Control Programs needs to launch awareness campaigns and extend outreach to semi-urban and rural areas.
8. Any HIV awareness and prevention campaign should also be able to connect all sectors of the population and should be comprehensive in scope.
9. The National AIDS Control Program and its provincial counterparts need to continue and expand HIV prevention efforts and promote strong prevention strategies for all women, but particularly those in the lower socioeconomic quintiles.
10. The impacts of migration (refugees from Afghanistan and pregnant women crossing the border to seek seeking medical attention) on HIV transmission should be estimated.



Annex 1: Site Assessment Form

Site Assessment Form	
Information to be collected	Explanation
1- Date of entry :	Refers to the date on which the ANC was visited and assessed. Date should be filled consistently as DD/MM/YY
2- District:	Refers to the pre-selected district from where sample will be drawn
3- Tehsil:	Tehsil in which the health facility is located
4- Name of UC:	Name of Union Council in which the health facility is located.
5- Type of facility:	Identify whether the health facility is Public (run and staffed by Govt.) or Private (run by individual or a private organization, trust, board etc.)
6- Address of health facility (please also submit a district/city map, and mark each facility proposed on the a district/city map):	The health facility name and location along with name of the town/village. (Postal Address)
7- A-Contact Information of focal person: Name: _____ Position /Designation: _____ E mail (if available): _____ Cell Phone: _____ Work Phone: _____ B- Number of Obstetrics and Gynecology Units in the health facility: _____	Name and contact details of the person who will be the nominated focal person at the health facility. It could be the registrar, head of obstetrics and gynecology department or the doctor who owns the health facility.
Information to be collected	Explanation
8- Willingness to cooperate (on a scale of 1 to 10) _____	How willing the management is for participation in study.
9- Number of dedicated ANC staff: Doctors: _____ Nurses: _____ LHVs: _____ LHWs: _____	Refers to the total number of staff dedicated for the ANC services at the health facility on the days that ANC is provided



Nurses: _____ LHV's: _____ LHW's: _____	faculty on the days that ANC is provided
10- Working days of ANC *tick): <input type="checkbox"/> Mon <input type="checkbox"/> Tue <input type="checkbox"/> Wed <input type="checkbox"/> Thu <input type="checkbox"/> Fri <input type="checkbox"/> Sat	The days on which the ANC services are offered/available at the health facility
11- Hours of ANC: Starts at: _____ Closes at: _____	The hours of ANC service provision at the health facility on the days specified above
12- A- Average number of clients receiving Antenatal Care per day: _____ B- Average number of clients coming for their 1st ANC check-up per day: _____	Average daily number of clients attending the ANC service. Please note that this number is for the days in which ANC services are provided. For example if services are provided for 3 days/week and the total clients for 3 days is 126 then the average daily number will be $126 \div 3 = 42$ The average number of first visit ANC clients/day. For example if services are provided for 3 days/week and the total first visit ANC clients is 60 then average daily number will be $60 \div 3 = 20$
13- Lab available at the health facility (tick): <input type="checkbox"/> Yes <input type="checkbox"/> No	
14- is the Lab facility in the same premises <input type="checkbox"/> Yes <input type="checkbox"/> No If No please note the address of the Lab facility:	Some health facilities may have lab services sourced in/out. Therefore for such case this information is required.
15- Is routine blood testing done for ANC Clients (tick): <input type="checkbox"/> Yes <input type="checkbox"/> No	
16- Is blood testing routinely done at 1st ANC check-ups (tick): <input type="checkbox"/> Yes <input type="checkbox"/> No:	
17- Type of routine tests done at the lab	What are the routine tests conducted





<p>(tick):</p> <p><input type="checkbox"/> 1. FBC <input type="checkbox"/> 2. Urine R/E</p> <p><input type="checkbox"/> 3. Blood sugar <input type="checkbox"/> 4. Blood group</p> <p><input type="checkbox"/> 5. Other (e.g. syphilis, HIV...please specify)</p> <p>_____</p>	by the clinic
<p>18- Are personnel who are qualified to undertake HIV rapid tests (Lab Technician's) available at the lab (tick)?</p> <p><input type="checkbox"/> Yes <input type="checkbox"/> No</p> <p>Note: This may need to be explained with the lab management if the lab is under a contractual arrangement and not part of the health facility.</p>	Identify the staff, which will be responsible for HIV testing.
<p>19- Can a space/room be provided for this study (tick)?</p> <p><input type="checkbox"/> Yes <input type="checkbox"/> No</p>	Please assess whether the facility has sufficient space which could be provided to the study staff for keeping their supplies and data.
<p>20- If not, how will confidentiality be maintained? Please explain arrangements:</p>	Refers to the alternative options available or not.





Annex 2: Data Collection Form

CANADA - PAKISTAN HIV / AIDS SURVEILLANCE PROJECT WITH NATIONAL/PROVINCIAL AIDS CONTROL PROGRAMME

ANC SEROSURVEILLANCE SURVEY

Study Code									
	District	City		ANC site			Serial No.		

QUESTIONNAIRE FOR ANC RECEIEVER

Name of the District: _____ Name of ANC: _____

Settlement: 1. Urban 2. Rural 3. Semi-urban Type of ANC Facility: 1. Public Sector 2. Private

1-Age as recorded in hospital record: _____ (in completed years)

2-Maternal education:

Uneducated / Illiterate 1 Primary 2 Middle 3 Metric 4
Intermediate 5 Graduate 6 Not recorded 98

3-Husband's occupation: _____

4-Pregnancy history: Gravida: _____
Para: _____
Abortion: _____

5-Status of last child: Alive 1 Dead 2

Rapid Test Result: 1.Reactive 2.Indetermine 3.Nonreactive/Negative:

(Mark ✓ for one only)

DBS Card obtained 1.Yes 2.No

Signature of:

Lab Technician II: _____

Date: ____/____/____
dd mm yy

Signature of Nurse: _____

Date: ____/____/____
dd mm yy

Checked by: District ANC Coordinator Name/sig: _____

Date: ____/____/____
dd mm yy

Data Entry: Date: ____/____/____

dd mm yy





Annex 3: Lab Work Sheet

ورک شیٹ (سپیل)		
اے این سی ورک شیٹ		
1	تاریخ	
2	نام ٹیکنیشن	
3	سٹڈی کوڈ	
4	ٹیسٹ کٹ لائٹ نمبر	
5a	کیا ریپڈ ٹیسٹ کسی کی نگرانی میں کیا گیا؟	ہاں
5b	اگر ہاں، سپروائزر کا نام	نہیں
6	ری ایکٹیو	ری ایکٹیو
7a	کیا ٹیسٹنگ کے دوران ریپڈ سٹریپ کنٹرول لائن نمودار ہوئی تھی؟	ہاں
7b	اگر نہیں، تو کیا آپ نے دوبارہ ٹیسٹ کیا	نہیں
8	کیا آپ نے ایک ڈی بی ایس کارڈ بنایا؟	ہاں
9	کیا ڈی بی ایس کارڈ پر سٹڈی کوڈ تھا؟	نہیں
10	کیا ڈی بی ایس کارڈ اور ورک شیٹ پر موجود سٹڈی کوڈ ایک جیسا تھا؟	ہاں
11a	کیا ڈی بی ایس کارڈ نگرانی میں تیار کیا گیا؟	نہیں
11b	اگر ہاں تو سپروائزر کا نام	ہاں
12a	کیا ڈی بی ایس کارڈ پہلی کوشش میں بنایا گیا؟	نہیں
12b	اگر نہیں تو کتنے کارڈ استعمال کیے گئے؟	
13	ریپڈ ٹیسٹ یا ڈی بی ایس کارڈ بنانے کے دوران کوئی مسئلہ پیش آیا؟	



Annex 4: ANC Monitoring Form

Name of Monitor					
ANC Site Monitored					
Date					
Checklist for Monitoring of ANC Study					
	Checklist		Yes	No	Observations
1	Are the rapids test kits stored appropriately				
		In a clean dry place			
		Away from sunlight			
		In the refrigerator if available			
2	Are enough rapid tests available				
		For a week			
		For a month			
3	Are the following lab supplies provided by HASP available				
		Powder-free disposable gloves			
		Micropipette			
		Micropipette tips			
		Hand disinfectant			



		Biohazard Bags			
4	Are DBS cards available				
5	Are the unused DBS cards stored				
		In a clean dry place			
		Away from sunlight			
6	Are the following items available				
		Desiccant			
		Humidity Indicator			
		Ziploc bag			
		Permanent marker			
7	Are the Rapid tests being performed according to SOP provided?				
8	Are DBS cards made, packaged and stored according to SOP provided?				
9	Are the cards and demographic data being coded according to SOP?				
10	Are the ANC forms complete and signed by the person taking demographic data and technician performing the tests.				
11	Is the log sheet maintained				
12	Is blood being taken from the patient and, Rapid tests performed/DBS cards made by two different people?				



13	Is the person asking screening questions asking the patient other questions?			
14	Are the blood collection tubes being labeled properly with the HASP study code?			
15	Is the HASP study code pasted on the Blood collection tube and the HASP study code pasted on the ANC sero-Surveillance form the same?			
16	Who takes the Blood samples and ANC Sero-Surveillance forms to the person who performs Rapid Test and makes DBS cards?			
17	Where are the prepared DBS cards stored?			
18	Are the DBS cards being packed in the zip lock bags with desiccants and a humidity indicator and are they properly sealed			
19	Is the sealed zip lock bag properly labeled with ANC Site Name, District Name and Date?			
20	Additional comments/observations			



Checklist for Gender & Rights Based Monitoring of ANC Study

For the person who is asking screening questions Kindly tick (✓)

#	Checklist	Yes	No	Observation
1	kept ethical considerations in view while screening			
2	Screeener's body language was appropriate			
3	Gender of the screener is Female			
4	Is screener maintaining the confidentiality of patients			
5	Using any term that indicates labeling, stigma, discrimination or marginalization of the patient.			

For the person (lab technician) who is taking blood samples questions Kindly tick (✓)

#	Checklist	Yes	No	Observation
1	appropriately dressed according to situation & local norms			
2	Lab technician's body language was appropriate			
3	Before drawing the blood lab technician, explained to the women regarding the procedure, prick and pain.			
4	Maintaining appropriate distance from patients			



5	Communications with the patient are respectful			
6	Using any term that indicates labeling, stigma, discrimination or marginalization of the patient.			
7	Clearly and patiently answering any questions about risks and fears from the patients			
8	maintaining the confidentiality of patients			
9	Additional comments/observations			

For the person(lab technician who is performing rapid test questions Kindly tick (✓)

#	Checklist	Yes	No	Observation
1	Maintaining confidentiality			
2	Seals envelop and results before dispatching or handing over to the relevant staff			

For the person who is recording demographic information Kindly tick (✓)

#	Checklist	Yes	No	Observation
1	Correctly noting the information provided from the patients ANC hospital cards			
2	Interpreting some information from the patients appearance and noting down on HASp demographic form			
3	Additional comments/observations			

