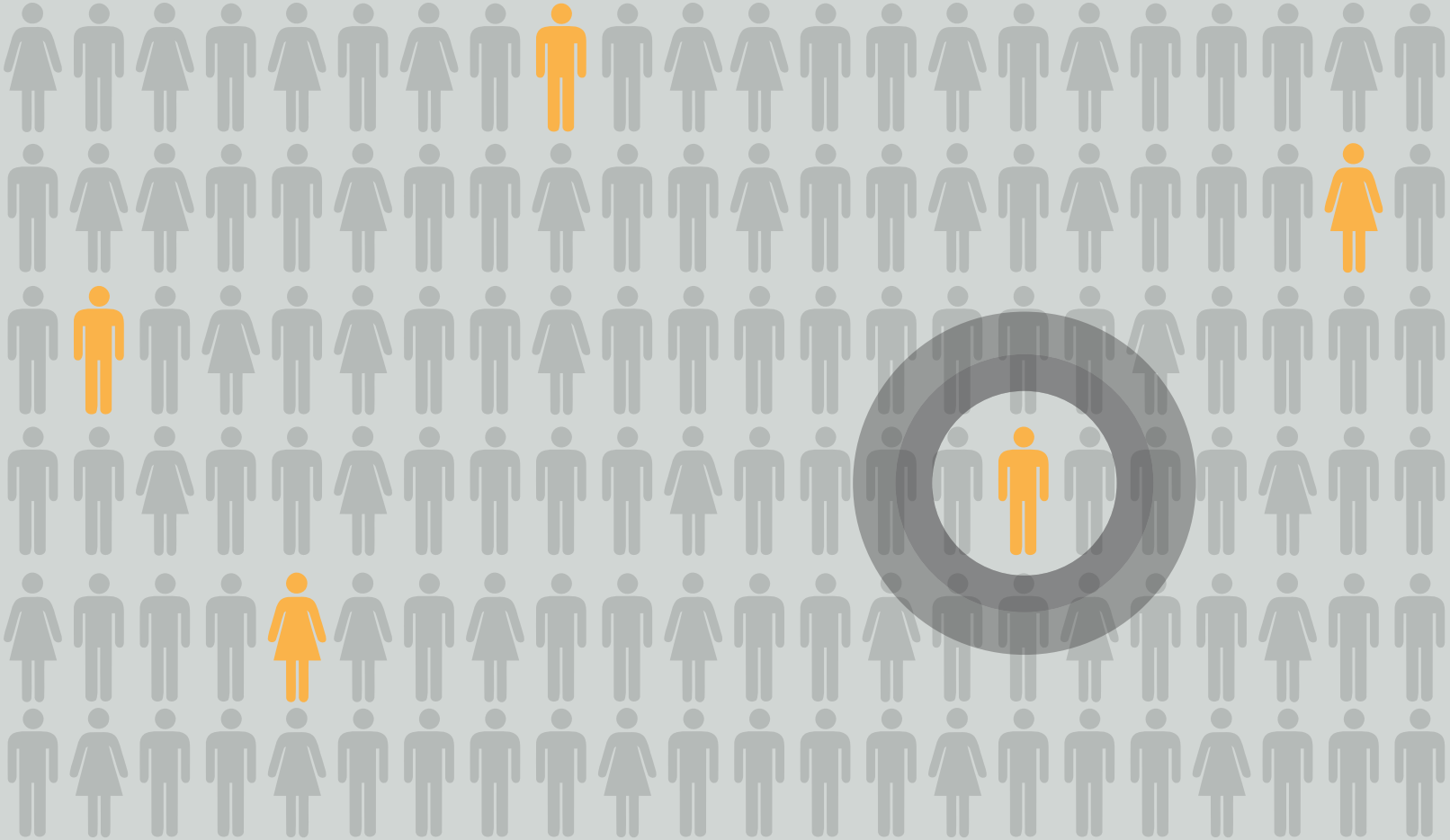

PREVALENCE OF PULMONARY TUBERCULOSIS

AMONG THE ADULT POPULATION OF PAKISTAN

2010-2011

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Preface

Tuberculosis (TB) remains a major threat to public health in Pakistan. The disease contributes 5% to the overall disease burden and is responsible for 10% of disability adjusted life years (DALYs) in Pakistan. The government of Pakistan declared TB a national emergency in 2001 and the National Assembly of Pakistan passed a unanimous resolution to control and eliminate TB as a public health problem.

The TB control activities were geared up in early 2000 after successful piloting of directly observed treatment, short-course (DOTS) in selected districts. The country witnessed the rapid scale up of DOTS services from 2000 to 2005, and as a result all public health facilities in the country adopted DOTS until 2005. National, provincial and district TB control programs were established and they performed their function in an organized fashion. Rapid scale up of TB services and strong collaboration with private sector enabled the program to increase its case notification rate from 5% in 2001 to 69 % in 2011 and maintain the proportion of TB patients successfully treated for TB at more than 85%.

However, the critical question of the true burden of disease remained unanswered, as the prevalence and incidence were based on the last disease prevalence survey conducted in 1987 and WHO estimates thereafter.

There was a need to determine a precise estimate of incidence and prevalence by using state-of-the-art scientific tools and document the progress of the program in terms of morbidity and its socioeconomic impact. The Ministry of Health of Pakistan advised the NTP to consult technical partners to conduct a national TB disease prevalence survey. The World Health Organisation (WHO) recommended Pakistan conducted a TB prevalence survey at the meeting of the impact measurement task force, held in Geneva in 2007.

The survey was conducted in 95 sub-districts (*tehsils*) of the country by using sampling proportional to population size with a sample size of 133,000 adult individuals. This was the second largest disease prevalence survey conducted to date. The execution of the survey was a major undertaking due to the large sample size, widespread geographical coverage, logistic challenges, extreme weather conditions and specific security concerns.

The survey was possible thanks to financial support of USAID through TBCAP and TB CARE I. KNCV Tuberculosis Foundation, as lead technical partner, provided technical support through more than 20 technical missions and backstopping at its headquarters, from protocol development to data analysis and report writing. Other partners include the WHO, the International Union Against Tuberculosis and Lung Disease (The Union), and the supra-national reference laboratory at the Institute of Tropical Medicine in Antwerp, Belgium.

The TB disease prevalence survey is a major landmark in the history of public health in Pakistan. The results of the survey will guide the Ministry of Health and Planning to make more concerted efforts when developing a future strategy for TB control in the country and to allocate adequate resources to achieve local and millennium development goals related to TB.

Acknowledgements

We acknowledge the support of the USAID Country office for both financial contributions and technical inputs. We highly appreciate and acknowledge the efforts of KNCV Tuberculosis Foundation (KNCV) particularly Masja Straetemans and Marieke Van der Werf, Dato Chorgoliani and Edine Tiemersma who technically assisted us in preparing, conducting and analyzing the survey, and Nico Kalisvaart who was of great help in preparing the data management plan, monitoring data management activities, advising on both of these and developing the final database.

We are thankful to the World Health Organization (WHO), especially Dr Amal Bassili (Regional office of Eastern Mediterranean region), for supervision and guidance, for drafting survey protocols, and for sample size calculation with experts from KNCV Tuberculosis Foundation. We are thankful to Sang-Jae Kim from the International Union against Tuberculosis and Lung diseases (The Union) for providing technical support and valuable comments in writing the protocol section on laboratory methods and continuously supervising survey activities at the National Reference Laboratory (NRL).

We extend our compliments and acknowledge the contribution of the impact measurement unit at WHO headquarters. We acknowledge in particular Dr. Ikushi Onozaki, who supported us from the inception phase through to implementation, guiding us and following the progress during international conferences and impact measurement workshops.

We are thankful for the active participation of the team of the Pakistan office of KNCV under leadership of Dr Abdul Ghafoor who continuously supervised and managed the preparation and implementation of the prevalence survey.

We also acknowledge the continuous support and guidance of Provincial Managers including Dr Darakhshan Badar (Punjab), Dr Ismat Ara (Sindh), Dr Munir Raisani (Balochistan) and Dr Ubaid (KPK), who supported the implementation of survey activities in their respective Provinces. We are thankful to staff members of the units Research and technical support at the NTP who have been involved from inception phase until the completion of the survey and the report writing. Our special thanks goes to the National Reference Laboratory and the staff involved in managing the huge workload of sputum samples for microscopy, culture and drug susceptibility testing.

And last but certainly not least, we are particularly grateful to those who worked in the field teams (Annex 1) for the successful implementation of the survey and to those who participated in the prevalence survey, since without participants, there would have been no results.

Dr. Ejaz Qadeer
Pakistan NTP Manager

List of Abbreviations

ACSM Advocacy, Communication and Social Mobilization
AFB Acid Fast Bacilli
CI Confidence Interval
CDR Case Detection Rate
CXRU Central X-ray Unit
DMC Data Monitoring Committee
DMP Data Management Plan
DMU Data Management Unit
DOT Directly Observed Therapy
DOTS Directly Observed Treatment, Short Course
DST Drug Susceptibility Testing
EDOH Executive District Officer for Health
FBS Federal Bureau of Statistics
HFN High False Negative
HIV Human Immunodeficiency Virus
KNCV KNCV Tuberculosis Foundation
LHW Lady Health Worker
LPA Line Probe Assay
MDR Multidrug-resistant
M&E Monitoring and Evaluation
MTB Mycobacterium tuberculosis
NAAT Nucleic Acid Amplification Test
NEPI National Expanded Program for Immunization
NGO Non-Governmental Organization
NIPS National Institute of Population Study
NRL National Reference Laboratory
NTM Non-Tuberculous Mycobacteria
NTP National Tuberculosis Program
NTRL National Tuberculosis Reference Laboratory
PDR Patient Diagnostic Rate
PIN Personal Identification Number
PNB Paranitrobenzoic Acid
PTP Provincial TB Program
SOPS Standard Operating Procedures
TB Tuberculosis
TST Tuberculin Skin Tests
UC Union council (fourth tier in the administrative division of Pakistan)
USAID United States Agency for International Development
WHO World Health Organization
ZN Ziehl-Neelsen

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Mrs. Saira Afzal Tarar

Minister of state for National Health Services, Regulation & Coordination

Tuberculosis still remains a public health challenge in the country accounting for 65% of TB burden in EMRO, accounting for 5.1% of the national disease burden in Pakistan.

Prior to completion of National TB Prevalence survey, TB burden was estimated by WHO since 2001 based on TB case notification data interpreted by epidemiologists every year. Being a high burden country for TB coupled with direct socio-economic and healthcare implications, there was a need to address the issue of disease burden for better planning and effective TB control strategies in the country.

I, on behalf on Ministry of National Health Services, Regulation & Coordination, take this opportunity to acknowledge the valuable contribution of National, Provincial & regional TB control programs and the international partners including KNCV, USAID and TB CARE-I in completing the survey and providing scientific basis to calculate incidence and prevalence of TB in Pakistan thus enabling NTP for evidence based planning and implementation activities in future.

Dr. Jehanzeb Aurakzai

DG
Ministry of NHSR&C, Islamabad

It is matter of great pleasure and satisfaction that National TB Control Program has successfully completed an important milestone to conduct National Prevalence survey on Tuberculosis during 2010-2011.

The survey was largest activity on part of NTP conducted on national scale, providing an opportunity to determine the exact prevalence of TB in the country, hence estimating the disease burden for planning and implementation of activities for TB control in a scientific manner. The surveys will provide a direct measure of disease burden for the first time, and will be used to update estimates of disease burden for strategic planning in the country.

TB is a life-threatening but curable disease and Pakistan ranks 5th among the 22 high TB burden countries globally. The burden of disease caused by TB can be measured in terms of incidence, prevalence and mortality. The TB burden in Pakistan was previously based on estimates from the WHO that use notification data, previous surveys, trends in surrounding countries and expert opinion. In order to get a more precise understanding of the TB burden, there was a need to conduct a new prevalence survey.

In 2007, the WHO Global Task Force on TB Impact Measurement developed a set of criteria to identify countries that can be considered eligible to carry out nationwide surveys of the prevalence of TB disease and Pakistan was among those. The information about registration status of detected cases, health seeking behavior and socioeconomic status of TB patients will enable the Ministry of Health and National program to develop the appropriate future strategy for TB Control.
I extend my gratitude to the contribution and collaboration of partners involved for completion of the survey viz; KNCV, USAID and TB CARE-I.

1 SUMMARY

Pakistan ranks 5th amongst the 22 highest tuberculosis (TB) burden countries. In 2012, the World Health Organization (WHO) estimated the incidence and prevalence rate of all forms of TB at 231/100,000 (95% confidence interval (CI), 190-276) and 350/100,000 (95% CI, 158-618) population, respectively. More than two decades have passed since the last TB prevalence survey was conducted in Pakistan. Therefore in order to get a more precise understanding of the TB burden there was a need to conduct a new prevalence survey. In 2007, the WHO Global Task Force on TB Impact Measurement nominated Pakistan as one of the 22 high TB burden countries eligible for doing a TB prevalence survey. The primary objective of the survey was to estimate the prevalence of bacteriologically confirmed pulmonary TB amongst the adult population (∑15years) in a nationwide representative survey conducted during 2010-2011.

Methods

A household based cross-sectional survey was conducted based on multistage cluster sampling, selecting clusters with a probability proportional to their population size. The survey was conducted in 95 clusters each targeting 1,400 individuals of 15 years and older between August 2010 and December 2011. There were six field teams (one team per cluster, teams visiting consecutive clusters) that simultaneously conducted field work. The fieldwork lasted 14 days per cluster and started with a household census followed by screening for signs and symptoms of TB using a questionnaire and chest X-ray. Those found with signs and/or symptoms of TB were requested to submit two sputum smear specimens (spot and the following morning) for smear microscopy and culture examinations. Persons eligible for sputum examination were those being on TB treatment at the time of the survey, those having a cough for more than two weeks and/or those having abnormal X-ray shadows. Those with a cough of any duration who, for any reason, did not have a chest X-ray or whose chest X-ray image was not interpretable were also request to submit sputum.

Bacteriologically confirmed cases (definite cases) were those with a culture positive with five or more colonies or positive with less than five colonies but having either a positive smear or an abnormal chest X-ray result consistent with TB, and those with a positive smear in combination with a positive nuclear acid amplification test (WHO-endorsed NAAT test). A probable TB case was defined as having at least one positive smear in combination with a chest X-ray abnormal finding in lung BUT not MTB culture- (or NAAT) positive NOR NTM grown on culture; OR two positive smears from two different specimens BUT not MTB culture- (or NAAT) positive nor NTM grown on culture. [Full definitions see section 5.5.] Data were entered in EpiData (EpiData Association, Odense, Denmark). Data analysis was performed in Stata version SE 11.2 (Stata Corporation, College Station, TX, United States of America) using svy prefix commands designed to handle complex survey data.

Results

This was, after China, the second largest prevalence survey ever. The survey had several challenges. These included security issues, floods, severe weather conditions, frequent moving of survey team, transport of staff and equipment, staff turnover, and issues in assigning and linking personal identification numbers (PIN). Appropriate measures were taken to solve these issues and a sensitivity analysis was done which showed the robustness of the estimate.

The total number of eligible adults was 131,331, of whom 105,915 participated in the survey. Eligible females were more likely to participate (88%) than eligible males (72%; p<0.001). The participation rate was fairly similar in the different age categories. Among the 105,915 individuals, screening was conducted by a questionnaire on 104,633 individuals; 102,187 participants were screened by X-ray; and 100,905 were screened by both methods. In total, 10,471 participants were eligible for sputum examination, from whom 8,521 (78,6%) submitted at least one sputum specimen.

The prevalence estimates were calculated in three different ways as recommended by Floyd et al. The final prevalence estimates, produced by applying multiple missing value imputation for participants with missing smear and/or culture results, and inverse probability weighting to represent all eligible individuals in the survey, are given below. The prevalence of definite and probable TB combined for all forms was 396/100,000 (95% CI: 332-458/100,000). This figure is in range with the rate previously estimated by WHO (2011), but with a smaller confidence interval. Of 315 definite TB cases, 7.6 % were on treatment for TB at the time of enrolment in the survey. After extrapolation, using the above mentioned prevalence estimates, with assumptions on the ratio of pulmonary to extrapulmonary TB and the ratio of childhood to adult TB, WHO estimated the prevalence of all forms of TB among all age groups at 348 per 100,000 population and the incidence of all forms of TB at 276 per 100,000 population (of all ages).

Summary of Pulmonary TB Prevalence Estimates

Type of TB	Method 1		Method 2	Method 3
	(svy Stata)	(logit robust cluster)		
Bact +ve	**321**	**328**	**361**	**396**
95% CI	*269-373*	*275-381*	*308-414*	*332-458*
Smear +ve	**219**	**224**	**252**	**270**
95% CI	*175-263*	*179-269*	*205-298*	*217-322*

Prevalence and incidence of all forms of TB for all ages for Pakistan.		
	Point estimate	95% CI
TB prevalence (all forms, all ages), per 100,000 population	*348*	*287-409*
TB incidence (all forms, all ages), per 100,000 population per year	*276*	*158-424*

Program Implications

The bacteriologically confirmed TB prevalence estimate for the population ∑ 15 years of 297 per 100,000 population (95% confidence interval, 248-345) shows that TB is still prevalent in the country and remains a public health problem. The government of Pakistan should continue to keep TB high on the agenda of the Ministry of Health and develop a comprehensive plan to combat TB with more commitment and resources.

The estimated patient diagnostic rate of 41.5% for adults >15 years and an overall case detection rate of 45.4% suggest that a high proportion of cases present in the community are being missed. There is a need to further

strengthen and expand diagnostic and treatment services including augmented and context specific case finding strategies.

The high number of undiagnosed cases detected in the community is alarming. It indicates that people may not be aware of the symptoms of TB and the presence of diagnostic and treatment services. The National Program should develop strategies to enhance the awareness of TB symptoms and the presence of TB services in the community by a comprehensive advocacy, communication and social mobilization (ACSM) strategy and to engage communities effectively.

A higher prevalence of TB cases in males as compared to females (365 versus 246 per 100,000 population) further implies that TB care needs to be expanded to the private sector. The public health care facilities currently offer services only in the morning hours, which is a challenge for the working population.

Similarly, the high prevalence in older age groups and in rural areas demonstrate the need for improved case finding by actively engaging trained community health workers in suspect identification and referral.

68% of all TB cases diagnosed in the survey were smear positive, and out of these more than 85% were reported as 1+ or above and most of them were not on treatment. This important finding indicates that the target of 70% case detection can be achieved by improving access to TB care and that further expansion of microscopy network is needed.

Around 45% of smear positive cases were reported high positive, suggesting significant delays in diagnosis. This may indicate that patients do not seek health care even when their disease has reached an advanced stage or, when they do come forward, are not recognized as having TB symptoms by the health care providers. This finding calls for improving awareness about disease symptoms, availability of free TB care and contact tracing in the community. Further analysis of questions on health seeking behavior and awareness among the health care providers, will help the program to design interventions to improve health seeking behavior.

In 32 of the 95 clusters, the prevalence estimate was found to be more than 500/100,000 population, which included five clusters having a prevalence of more than 1000/100,000 population. This requires the NTP to design and implement context specific interventions for enhanced case finding in these clusters on a priority basis.

The specimen transport system was successfully used for first time in the survey for the transport of specimens in cold chain from field cluster sites to the NTRL. Experience gained in the survey will be implemented in the regular program for the transportation of specimens from lower level laboratories to higher level laboratories for advance testing.

The NTP Pakistan recruited a PhD student who will develop the report into scientific publications and do further detailed analysis of the survey that will give guidance on policies. KNCV has committed to give technical support for this process.

2 INTRODUCTION

2.1. Pakistan's Health System

Pakistan's health care system is a three-tiered health care delivery system which consists of primary, secondary and tertiary care. The health system strengthening mechanism starts at grass roots level through lady health workers (LHWs) who are connected to basic health units with an upward referral pathway to rural health centers, *tehsil* hospitals and district hospitals. There are also well-equipped tertiary care teaching hospitals. Figure 1 shows the health care delivery system of Pakistan[1].

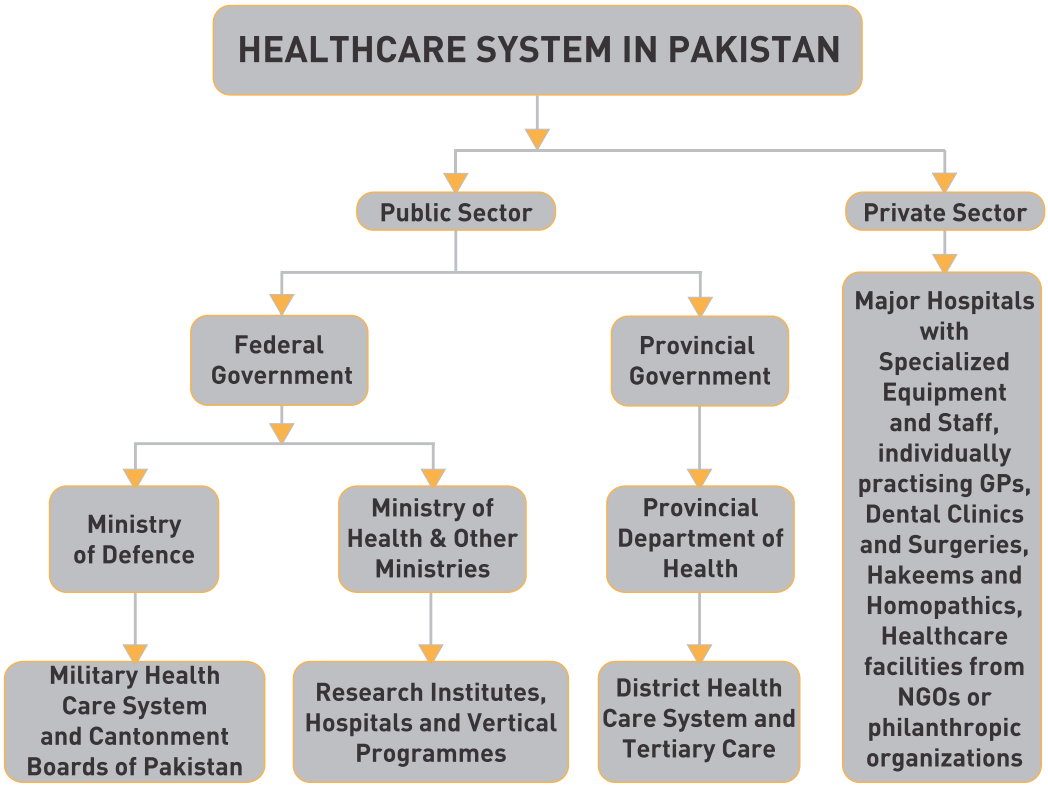


Figure 1. Schematic Organizational Diagram of the Pakistan Health Care System
Source: http://www.slideshare.net/AMCOLIANZ/healthcare-system-in-pakistan.

The healthcare system in Pakistan is part vertical and part, horizontal. Vertical segmentation is reflected in the manner in which separate organizations such as the Federal Ministry of Health (which after devolution was renamed as the Ministry of national health regulation and coordination), the provincial health departments, private sector healthcare providers, non-governmental organizations (NGOs), armed forces health facilities, parastatals which are semi-governmental facilities in nature and the Employees' Social Security institutions, which all raise and allocate their own funds, pay their own providers, and deliver stand-alone services. For

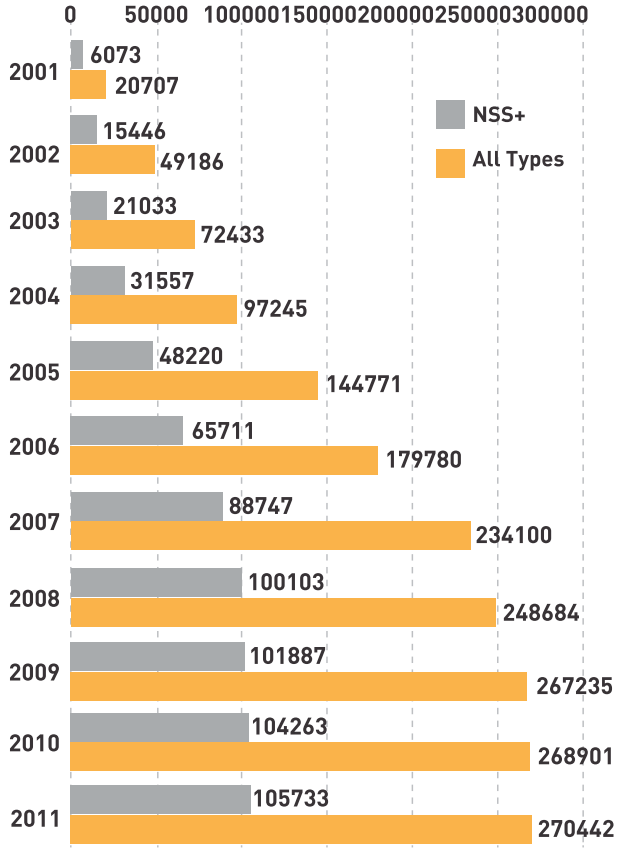


Figure 2a. Case notification of all types of tuberculosis and of new smear positive (NSS+) tuberculosis cases in Pakistan (2001-2010).
Source: NTP, Annual report 2011[2]

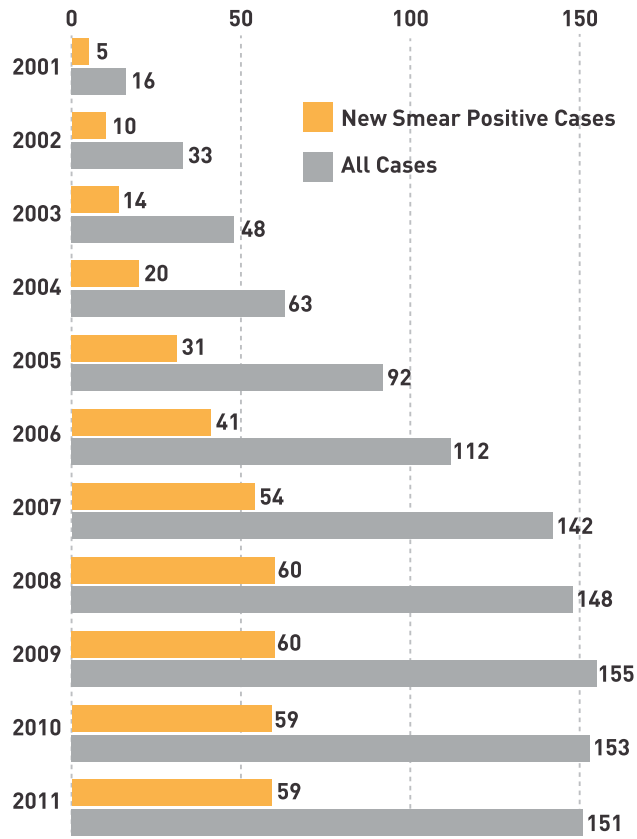


Figure 2b. Case notification rates per 100,000 of all types of tuberculosis and of new smear positive (NSS+) tuberculosis cases in Pakistan (2001-2011).
Source: NTP, Annual report 2011[2]

certain diseases, there are vertical programs, such as the TB program that covers TB managing sites only. Likewise, other programs, e.g. for the control of malaria and HIV, are being implemented separately. Also, there are separate services serving non-overlapping populations as in the case for the armed forces, parastatals and social security staff. This is schematically depicted in Figure 1.

The public sector has so far been the main source of TB directly observed therapy, short course (TB-DOTS) care in the country. The public sector TB care in a district is provided through a network of hospitals, rural health centers, basic health units, and community based LHWs. The district health authorities are responsible for planning, financing, implementing and monitoring TB care in their respective districts. However, the Provincial TB Control Program assist the districts through provision of training, drugs, print materials and supplement

of laboratory supplies. In most of the cases the first point of contact of TB suspect is with private providers who initially manage and sometimes refer these suspects to public health facilities (TB management units) for diagnosis. An unknown proportion of such TB suspects is treated in the private sector, and potential TB cases remain un-notified because of this.

Despite the 100% DOTS coverage reached since 2005 in the public sector[2], the estimated case detection rate (CDR) remained 64%. This means that the case notification rates are missing around 36% of the TB patients. As in most countries with a significant burden of TB, DOTS implementation is limited largely to public sector services under the national tuberculosis programs (NTPs). In reality, however, many patients with symptoms of TB, including the very poor, do seek and receive care from a wide variety of health care providers outside the network of NTP services. In urban areas most of the patients seek medical care from a diverse range of facilities including tertiary care, NGO networks, private qualified doctors, and unqualified care providers (quakes) who are mostly not following National Guidelines. Thus the current TB case notifications involving mainly public and few private health facilities may under-estimate the true burden of TB in Pakistan. The recently completed inventory study in Pakistan provided evidence that there is 68% under reporting from private providers when the results are extrapolated at the country level in the study all non NTP health providers were involved in the data collection in 12 selected districts and in the sampled population of selected districts the under-reporting was 28% out of total TB patients detected and not notified to NTP the proportion notified were 32% only[3].

2.2. Tuberculosis Epidemiological Situation in Pakistan

TB is a life-threatening disease with 8.7 million incident cases and 1.4 million deaths annually worldwide. Pakistan ranks 5th among the 22 high TB burden countries . The World Health Organization (WHO) estimated incidence and prevalence rate of all forms of TB were revised in 2012 from 181 and 329 /100,000 population to 231 and 350/100,000 population, respectively[4].
In 2011, 270,394 TB patients (all types) were notified to the NTP. In the same year, according to WHO estimates, the total number of new cases of TB (including HIV/AIDS) would be 410,000 with an incidence of 231 per 100,000 population (95% Confidence Interval (CI): 190-276), and the number of prevalent TB cases was estimated at 620,000, corresponding with a prevalence of 350 per 100,000 (95% CI 158-618). In 2011, the total number of TB patients with known HIV status reported to NTP was 8,322 (3%), and 33 (0.4%) of these were HIV co-infected. Among notified pulmonary TB cases, there were 7,100 (3.4%) MDR-TB cases in 2012. However, since only a marginal proportion of TB patients are tested for HIV co-infection or MDR-TB, these figures probably underestimate the real burden of disease.

National TB Control Program Case Notifications and Disease Burden

The NTP of Pakistan consists of a well-defined Central Unit, five TB control Units at provincial level and one TB coordinator for each of the 139 districts who account to provincial managers for reporting and district level facilitation. TB services are fully integrated into the primary health care system. Effective implementation of the WHO recommended directly observed treatment, short course (DOTS) strategy started after revival of the NTP in the year 2000. DOTS coverage was expanded at accelerated pace to cover the public sector by May 2005. Case notification has shown a remarkable improvement since 2001 with an increase in cases with all types of TB from

20,707 in 2001 to 267,969 in 2010 (Figure 2), which was mainly due to DOTS expansion between 2005 and 2006 and thereafter because of increasing private sector involvement.

2.3. Pakistan: Demographics and Geography

Pakistan comprises a total land mass of 796,096 square kilometers. The Gross Domestic Product (GDP) in Pakistan expanded 3.59% in the fiscal year 2012-13 from the previous year. The GDP annual growth rate in Pakistan, reported by the Pakistan Bureau of Statistics, averaged 4.9% from 1952 until 2013, reaching an all time high of 10.2% in June of 1954 and a record low of -1.8% in June of 1952[9]. Sixty percent of Pakistan's population is living below the poverty line and the Gini index is 30[6].

The estimated population size of Pakistan in 2011 was over 178 million[5], making it the world's sixth most-populous country[6]. This population estimate was the result of projecting census data of 1998 using an average estimated annual population growth rate of 2-3% (National Institute of Population Study (NIPS), unpublished data). The male to female ratio is 1.06 males per female. The Pakistan population pyramid (Figure 3) with the distribution of the population over sex and age groups, shows the typical shape of an expanding population which is beginning to stabilize.

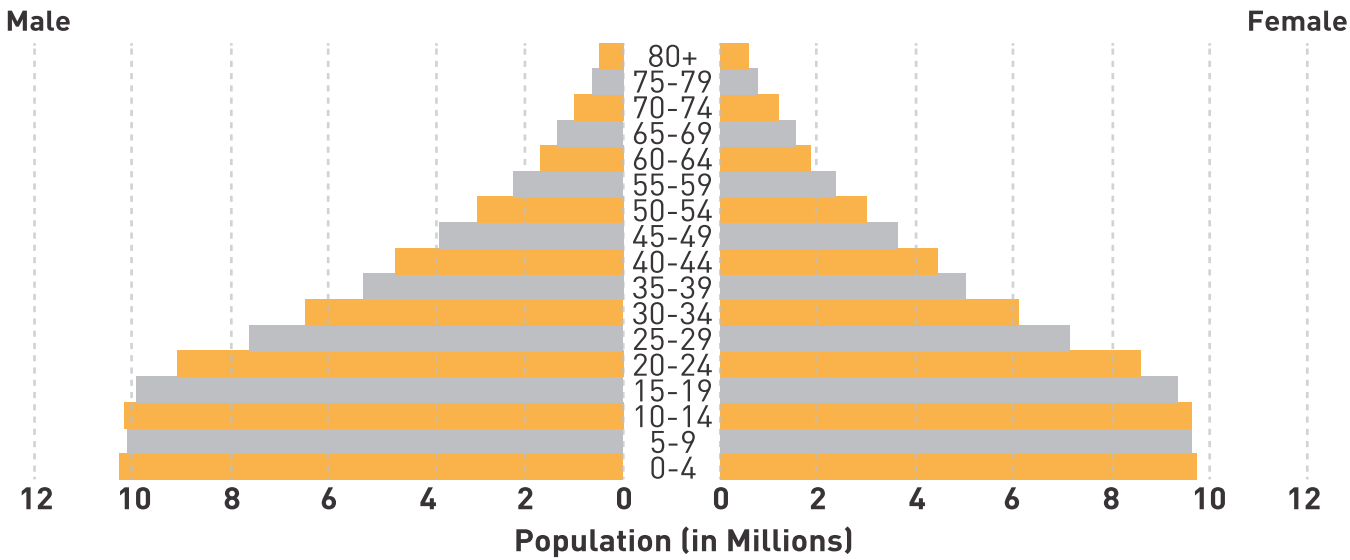


Figure 3. Population of Pakistan by Sex and 5-year Age Groups, 2010.
Source: US Census Bureau, International data base

Figure 4 .The administrative units of Pakistan (second tier of administrative division)

The country is divided into five provinces known as Sindh, Punjab, Balochistan, Khyber Pakhtoon Khwa (before 2011 known as North West Frontier Province) and Gilgit-Baltistan (before 2012 known as the Federally Administered Northern Areas). Apart from these provinces, the country also has three special areas: the federally administered tribal areas, Azad-Jammu-Kashmir, and the federal capital territory. At the third tier of administrative division, the country is divided in 139 districts, and it has 387 *tehsils* (fourth tier) (Figure 4). *Tehsils* are further divided into union councils (UC).

2.4. Nationwide and Sub National TB Prevalence Surveys

Pakistan has carried out three TB disease and TB infection prevalence surveys that took place specifically in 1960-62, 1974-78 and 1987-1989. The screening methodology of the last survey included symptom screening, chest X-ray and sputum smear microscopy of TB suspects. The sampled clusters were stratified by province and by urban and rural areas, but the exact sampling technique was not documented and remains unclear. A total of 41 clusters were sampled with approximately 989 individuals per cluster, targeting a total sample size of 40,549 participants, resulting in a smear positive TB prevalence of 170/100,000 population[7].

Since the last survey, major interventions for TB control have been implemented, including expanding DOTS interventions throughout the country, increasing case detection by engaging private providers, improving treatment adherence through treatment supporters, provision of social support to multidrug resistant TB (MDR-TB) patients, and implementing childhood TB interventions.

3 RELEVANCE OF THE SURVEY

The TB burden in Pakistan is currently based on estimates from the WHO, that use both notification data, previous surveys, trends in surrounding countries and expert opinion. In order to get a more precise understanding of the TB burden, there was a need to conduct a new prevalence survey; all the more important because more than two decades have passed since the last TB prevalence survey was conducted in Pakistan. The information about registration status of detected cases, health seeking behaviour and socioeconomic status of TB patients will enable the Ministry of Health and National program to develop the appropriate future strategy for TB Control. In 2007, the WHO Global Task Force on TB Impact Measurement developed a set of criteria to identify countries that can be considered eligible to carry out nationwide surveys of the prevalence of TB disease and Pakistan was among those. The preparations for the fourth national TB prevalence survey started in 2009 while the field work took place from December 2010 to December 2011. The pilot was conducted in August 2010.

4 SURVEY OBJECTIVES

4.1. Overall Objective

To estimate in a nationwide representative survey, the prevalence of bacteriological confirmed pulmonary tuberculosis among the adult population (∑15years) in Pakistan during 2010.

4.2. Specific Objectives

To determine the prevalence of sputum smear positive pulmonary TB.
To determine the prevalence of bacteriologically confirmed pulmonary TB.
To inform the Pakistan NTP about health care seeking behavior of TB suspects and TB cases.

This report will cover the first two objectives. The last objective will be covered in separate publications.

5 METHODS

5.1. Survey Procedures

The survey was a community survey with a cross sectional design using multistage cluster sampling. The survey was designed following the prevailing prevalence survey guidelines ("red book") outlining the international agreed procedures . Full details about the sampling strategy are described in section 5.3.

At the field cluster site, households within the selected area were visited and all eligible persons living in these households were enumerated in the census register until the target number of 1,400 adults was reached. All those enumerated and who consented to participate in the survey (see section 5.6.2) were screened for TB symptoms and signs by means of a short questionnaire and a chest X-ray, to assess if they could be identified as TB suspects.

The screening interview was done at the reception desk, mainly by Lady Health Workers (LHWs), who were part of flexible teams and in each cluster designated LHWs performed this task. The interview was structured and was done using the short symptom screening questionnaire consisting of five different questions covering current TB treatment, current cough and the duration of this cough, smoking behavior and consent for X-ray examination. After the screening interview, all survey participants were subjected to chest X-ray examination, except pregnant women, those not able to have an X-ray because of physical disabilities, and those not consenting to X-ray.

Those eligible for sputum examination were:
i) on TB treatment at the time of the survey
ii) those with cough of more than two weeks
iii) cough of any duration and no X-ray result
iv) those with chest X-ray abnormalities.

They were subjected to a second in-depth interview with questions on for example, TB symptoms and on health seeking behavior.

After the in-depth interview, TB suspects were asked to submit two sputum samples (spot and morning from the next day). The spot specimen was examined in the field for the presence of acid fast bacilli (AFB) using direct smear microscopy with Ziehl Neelsen staining. The morning specimen was transported in cold chain within 72 hours to the National TB reference laboratory (NTRL) in Islamabad for AFB direct smear microscopy and culture examination using the modified Kudoh method . If a TB suspect could only provide the spot specimen, this specimen was sent to the NTRL instead of the morning sample. All cultures isolated were examined for morphology and tested for sensitivity to paranitrobenzoic acid (PNB). In the case of doubtful results further confirmation was done by strip test (MPB64) or nuclear acid amplification test (NAAT). If cultures were overgrown or not done while at least one smear result was positive, NAAT was conducted directly on the smear.

Full details of all the procedures described above are given in more detail in Chapter 6 (organization of data collection).

5.2. Study Setting

The study sample included a total of 95 clusters with an intended sample size of 1,400 adults (aged ∑15 years) per cluster. The primary sampling unit consisted of *tehsils* (sub-districts). During the preparation of the fieldwork we had to consider the risk that a worsening security situation could result in non-accessibility of selected clusters. It was therefore decided to add five "extra" *tehsils* to the original sampling frame (see Annex 2).

Although the aim was for a nationwide survey, there was a need to exclude the following areas from the nationwide sampling frame because of serious security threats: the Federally Administrative Tribal Areas, district Dera Bugti in Balochistan and 17 *tehsils* of Khyber Pakhtoon Khwa, which together are inhabited by 6.4% of the country's population (Federal Bureau of Statistics (FBS), 2010 unpublished). An international company,

called 'Risk and Strategic Management Consulting', was contracted to carry out the security assessment in some of the high risk areas based on criteria decided by the Provincial TB Program (PTP) and the NTP for high, moderate and low risk areas. This company submitted a report to the NTP focusing on strategic threat assessment, fundamental mitigation measures and detailed district/*tehsil*/union council level information about the selected survey field locations. Seventeen districts, 18 *tehsils* and 18 UCs (fifth tier of administrative division) were visited and assessed. As per recommendations of the report, a security forum was constituted at the NTP and an NTP booklet on survey safety and security was developed for the survey field staff. As an outcome of security situation assessment a guideline document was developed with complete information regarding emergency measures to be taken and numbers to be contacted if there was any security threat so the document was used as a guidance throughout the survey.

5.3. Sample Size and Sampling Strategy

A target sample size of 133,000 enumerated adults (∑15 years) was calculated based on 95% confidence interval (CI) boundaries of 170 to 256 per 100,000 population for an observed prevalence of smear positive TB in the adult population of 213 per 100,000 population, a design effect due to cluster sampling of 2.5 (based on results of the recent TB prevalence surveys of Vietnam and Philippines), 25% precision, and an expected participation rate of 85%, we used the following formula:

$N= \{z^2_{1- /2}(1-P)\}/d^2$, in which

$z_{1- /2}=1.96$,

P= the expected prevalence of pulmonary TB in the adult population which was WHO's prevalence estimate for smear-positive TB in 2006 (132 per 100,000) divided by 0.62 since 62% of Pakistan's population was 15 years or older in 2008.

d=precision (25%) of P = P*0.25.

Full details of the sample size calculation can be found in Annex II.

Ninety-five clusters and 5 extra clusters were selected by probability proportional to the population size sampling. The five extra clusters were selected as replacement clusters that could be included if one of the 95 clusters proved to be too insecure to visit in the course of the field work. Therefore, each *tehsil* with its projected population size for 2010 (based on the 1998 census (FBS, unpublished data, 1998)) was listed. The *tehsils* were listed in alphabetical order. A sampling interval of 128044962/95=1347842/100= 1,337,075.64 (included population size/number of clusters) was calculated. Starting at a random number x, each *tehsil* having the starting number + 1,337,075th inhabitant was then selected in the sampling frame. The random number was selected from the random number table and was smaller than the sampling interval. After selection of the primary sampling units by the FBS, in each cluster, one UC was randomly selected by an international consultant using a random number table and a list of all UCs obtained from the National Expanded Program for Immunization (NEPI).

During the fieldwork sketched maps (micro plans) of all selected UCs were obtained from the NEPI Program to be able to select an area including 400 households (assuming that the mean household size was 3.5 adults). From the micro plan, one cluster of households was randomly selected using a random number table. For selected rural *tehsils*, clusters of households were formed of villages (one or more) having approximately 350-400 households using the micro plan. In urban areas all enumeration blocks (200-260 houses) demarcated within the jurisdiction of the selected UC were identified on the micro plan, and two adjacent blocks were grouped to form one cluster of households. Such a cluster of households was drawn randomly using a random number table. In the selected cluster of households, all households were listed from the east corner moving in a clock-wise direction, until the desired number of adults (1,400) was obtained. In the scenario that the sample size of 1,400 adults was not obtained, enrolment was continued in the east adjacent cluster of households or the adjacent west cluster of households if the selected cluster of households was the most eastern cluster of households within the boundaries of the UC.

As the clusters were selected with a probability proportional to population size, the majority of clusters (57) were in Punjab, which is the most populous province, and 23 clusters were located in Sindh, nine in Khyber Pakhtoon Khwa, three in Azad-Jamu and Kashmir, two in Balochistan, and one in the Federally Administered Northern Areas (now known as Gilgit-Baltistan) (Table 1).

Table 1. Number of Selected and Included Clusters by Province in the Pakistan Tuberculosis Prevalence Survey.*

	Punjab	Sindh	KPK	Balochistan	FANA	AJK	Total
Selected *tehsils*	56	23	9	4	1	2	95
Selected extra *tehsils*	2	1		1		1	5
Tehsils in which survey was conducted	57	23	9	2	1	3	95

* Abbreviations used in this Table: KPK: Khyber Pakhtoon Khwa; FANA: Federally administered Northern areas (now known as Gilgit-Baltistan); AJK: Azad-Jammu and Kashmir.

5.4. Survey Population

Smear positive TB is rare in persons under 15 years of age and it is difficult to collect sputum samples from children. Therefore most prevalence surveys focus on individuals of 15 years and older[9]. The survey population included all residents aged 15 years or more living in households within the selected clusters (nationals or not) who had slept in the household the night before the census and who gave consent to participate in the survey. Individuals suffering from mental illness and/or unable to provide informed consent were excluded, along with individuals who were incarcerated, institutionalized, in military camps, refugee camps and homeless. Pregnant woman and those physically unable to get a chest X-ray were excluded from chest X-rays, but were requested to submit sputum samples if they had a cough.

Survey Field Work in One of the Clusters

5.5. Survey Definitions

The following definitions were applied in the survey:

Eligible as a Participant:
The population eligible for the survey included individuals of 15 years or older who had slept in the household the night before the census of the household was taken.

Participant:
Participants were eligible persons enumerated in the census who had been screened on symptoms (i.e. at least one question of the screening questionnaire answered) and/or had received X-ray examination (i.e. X-ray examination form available).

Suspect Eligible for Sputum Examination:
TB suspects were defined as survey participants having a cough for more than two weeks and/or abnormal shadows on their chest X-ray image or being on TB treatment at the time of screening. If a chest X-ray was either not done or not interpretable, a TB suspect was identified based on a cough of any duration.

A definite survey TB case (bacteriologically confirmed survey TB case) was defined as having at least:
Culture positive with five or more *M o a terium tu er ulosis* (MTB) colonies; **OR**
Culture positive with less than five MTB colonies either in combination with one or more positive smears or an abnormal chest X-ray result consistent with TB; **OR**
Any smear positive plus a positive nuclear acid amplification test result produced by the Genotype®MTBDR*plus* assay or the GeneXpert MTB/RIF test and no isolation of non-tuberculous mycobacteria (NTM).

An AFB-smear positive survey TB case (smear-positive TB survey case) was defined as having at least:
Two positive smears (in two different samples) but no MTB-positive culture or NAAT result (this was also defined as a *pro a le* TB case);**OR**
One positive smear AND an abnormal chest X-ray result consistent with TB, but no MTB-positive culture, nor a positive NAAT result, nor NTM grown on culture (this was also defined as a *pro a le* TB case); **OR**
One positive smear plus an MTB-positive culture or a positive NAAT result (this was also defined as a *e nite* TB case).

A probable TB case was defined as having at least:
One positive smear in combination with a chest X-ray abnormal finding in lung at central reading BUT not MTB culture- (or NAAT) positive NOR NTM grown on culture; **OR**
Two positive smears from two different specimens BUT not MTB culture- (or NAAT) positive nor NTM grown on culture.

Multidrug resistant tuberculosis (MDR-TB):
A definite TB case with drug resistance to at least isoniazid and rifampicin.

5.6. Ethical Considerations

5.6.1. Risks and Benefits of Participation

The risks and benefits of participation were clearly explained in the information sheet with individuals having the right to choose not to participate. It was explained that all the tests such as smear microscopy and chest X-ray were done free of charge and that free treatment would be provided for any symptoms the individual might have. Although there were no direct risks from participation in the survey, the survey might have included women who did not know that they were pregnant, or did not disclose their pregnancy to the survey field staff, and who were then exposed to X-Ray. To avoid radiation hazards, protective measures were taken for all the procedures involving risk. Psychological harm might have occurred to persons being diagnosed with malignancies on X-Ray.

Moreover, those diagnosed with TB in the survey might face stigma since their status might be known to other participants because of the mass nature of the survey.

5.6.2. Informed Consent

During the household visits of the census and before deciding on participation, each potential survey participant was informed of the aims, methods and sources of funding of the survey, any possible conflicts of interest, institutional affiliations of the researchers, the anticipated benefits and potential risks of the study, and discomfort it may entail. The subject was informed of the right to abstain from participation in the survey or to withdraw consent to participate at any time without reprisal. After ensuring that the subject had understood the information, the investigator then obtained the subject's written informed consent with a signature or thumb print. For some households in which the head of the household considered obtaining informed consent from all individual household members insulting or unnecessary, while he/she was giving informed consent for the complete household, informed consent was only obtained from the household head. Receipt of informed consent was checked in the appropriate column of the census register. For participants who were not at home when the census of that household was taken, written consent was obtained at the screening interview location.

5.6.3. Ethical Approval

The survey started after the receipt of written ethical clearance from the ethics committee of the Pakistan Medical and Research council.

5.6.4. Data Confidentiality

All forms and registers contained a personal identification number (PIN), the name of the participant and the name of the father or husband of the participant, along with sex and age of the participant. PINs consisted of cluster-, household-, and participant (subject) number so that they were unique for each participant and could be used to identify the participants. Names and father names were only used within the Data Management Unit (DMU) to verify both the correctness of the PINs and merges with the PIN.

Storage of forms at field level

Each evening, at the cluster sites, the survey field teams checked all filled forms for completeness and consistency, after which they were sorted and filed (by type of annex) and put in locked iron boxes. After finalizing the field work in a cluster, the forms were sent to the DMU at central level by courier in these locked boxes. The central DMU for the survey was established with arrangements for protecting the confidentiality by limiting access to the data. The investigators also preserved confidentiality by aggregating individual data into tables or diagrams.

Storage of forms and registers at central level

As explained above, the names of persons were registered in combination with test results on forms. These names and the combination of names with test results is highly confidential data. Therefore, in the DMU, all forms and registers were kept in a room that was locked by a coded lock, and for which only the data manager and his assistant knew the code.

Processing of Confidential Data at Central Level

Names and father/husband names of registered persons were not to be entered in the final data file. However since problems were observed with administration of PINs in the field, it was decided to enter this information in data files. Within the DMU, this data was needed for validation and merging of forms and registers as explained in section 5.7. Data files containing names and father/husband names were kept within the DMU and were not shared with others. If these needed to be shared over internet, the file was zipped with a password, and while the data file was sent over e-mail, the password needed for unzipping the file was sent separately. Identifying information was discarded from the digital files when consolidating data for purposes of statistical analysis.

Archiving and Destroying Confidential Data after Data Analysis and Reporting

These identifiable variables were and will not be used outside the DMU. After completion of data management activities, all essential documents pertaining to the prevalence survey should be stored safely at least 3 years after the final report has been published[11]. There should be a clear procedure for if, when, and how the paper documents will be archived or destroyed, always ensuring that confidentiality is not violated. Equally, there should be a clear plan for if, when, and how the electronic data files will be archived or destroyed, always ensuring that confidentiality is not violated. Ideally, the final validated survey electronic data file should be archived so that it can be used or re-analyzed in future.

5.7. Quality Assurance and Survey Monitoring

5.7.1. Quality Assurance

A protocol with a data management plan and standard operating procedures (SOPs) for all procedures applied in the survey was prepared before the start of the survey. Quality assurance procedures are explained below.

Quality Assurance of Subject Interviews

Interviewers were trained to administer questionnaires in a standardized manner before the start of the survey and the number of interviewers was kept to a minimum to reduce the interpersonal variation. Within each cluster, the field team leader was supposed to check each questionnaire, register and form for completeness and consistency and to sign it if this was considered adequate.

In principle on daily basis in each cluster, one census, one symptom screening interview and one TB suspect interview was repeated blindly by the field team leader and the outcome was cross- checked with the TB suspect register. All data were entered using appropriate forms with correct personal identification numbers (PINs). The original and re-interview results were then compared. Results of re-interview were unfortunately not routinely included in the field monitoring reports.

Quality Assurance of X-ray Reading

After being read by one medical officer, all X-rays were reread by a second medical officer. All X-ray images reported with any abnormal shadows and 20% of X-ray images reported as normal (by taking every 5th film starting from a random number between 0 and 4) were saved on a CD and transported to the central X-ray reading facility by courier every alternate day, where they were blind re-read by a radiologist.

Any X-ray which resulted in a discordant conclusion between the field and the central radiologist was reread by a senior radiologist for a final decision. Results were summarized in a monthly X-ray report by the radiology coordinator and presented to the Data Monitoring Committee (DMC) on regular basis.

On completion of a cluster's field work, all X-ray images were transported to central level and stored in a central server.

Quality Assurance of Smear Microscopy

In the mobile field laboratory, all positive smears were reread by a second lab technician and at the end of the cluster's field work, all spot smears slides were transported to the NTRL. All positive and a randomly selected sample (20%) of negative smear were re-examined blindly for quality assurance. For any discrepancy between the first reader and the first controller, final reading was done by a second controller which was considered as final.

Besides those, the following smears were also re-examined:

- All negative smears (spot and morning) with a positive culture result
- Both spot and morning smears in case different results were reported on two specimens of same subject e.g. if the result was positive on one smear and negative on another, or if a quantitative difference in positive smear results of two smears was found
- All negative smears for a cluster in which a high false negative (HFN) error was found by the NTRL on rereading of a 20% sample of slides reported as AFB-negative.

At the end of TB prevalence survey when all clusters had been completed, a random sample of morning smears was reread following the lot quality assurance system.

In response to the high proportion of false negative errors observed in spot smears rechecked at the NTRL, panel testing was conducted to test the proficiency of field laboratory technicians. For this purpose, a panel composed

of 10 stained smear slides and 10 unstained smears (each including 4 negative, 2 scanty and 4 positive slides) was sent to each lab technician. Panels were sent once only, after completion of field work in the first 40 clusters in June 2011. Laboratory technicians showing poor performance were given on-the-job training on sputum smear examination by provincial laboratory supervisors. When there was discrepancy between the field lab and the NTRL, NTRL results were used as final.

Quality Assurance of Culture

Culture quality indicators, including specimen transport time from the date of collection to the date that the specimen was received at the NTRL (the indicator was the proportion of specimens being received within 72 hours), culture recovery in smear positive cases (the target for this indicator was a recovery rate of B90%) and the proportion of smear negative TB cases (no target was set) and the culture contamination rate (<5%), were regularly monitored. Results of cultures, smear rereading and culture quality indicators were summarized in a monthly laboratory report.

Quality Assurance of Drug Susceptibility Testing (DST)

Drug susceptibility testing was performed in the NTRL on pure MTB isolates with more than 5 colonies. For quality assurance, all strains showing resistance to any of the drugs and 10% of susceptible strains were sent to the TB laboratory of Agha Khan University Hospital (AKUH) in Karachi, which has been nominated by the WHO as candidate supranational reference laboratory (SNRL).

Quality Assurance of NAAT

As a quality control of the Genotype®MTBDRplus test, negative and positive controls were run with each batch. For the Xpert MTB/RIF assay no quality control measures were taken, as the equipment is fully automated with built-in internal quality control.

5.7.2. Monitoring of the Field Work in each Cluster

A field manual was developed with a description of the tasks and responsibilities of each individual at all stages. Monitoring of the field activities was carried out to ensure that activities were being implemented according to the approved protocols and regulations, and that the reported data was accurate, complete and verifiable against the source documents.

Both on-site monitoring and desk monitoring was done on regular basis by national staff. International consultant epidemiologists monitored the first pilot and visited another cluster during survey field work in December 2011, whereas monitoring from central level was done once by an epidemiologist in April 2011. The international data management consultant visited Islamabad four times during data collection to provide technical assistance to the central DMU.

On-site Monitoring: A survey monitoring module including a standardized monitoring checklist was developed. The checklist was tested by the international consultant epidemiologist during their first monitoring visit in December 2010 and was then reviewed and finalized and subsequently used by national staff. Each time before the start of field activities simultaneously in six clusters, monitoring plans were developed. The monitoring plan was developed in such a way that each cluster was monitored independently by one central level survey team member (either the Monitoring and Evaluation (M&E) officer of KNCV (local branch), or one of the survey coordinators (overall survey coordinator, X-ray survey coordinator or laboratory coordinator) and a provincial monitor (a district TB program officer). All clusters received a one-day visit from such a monitoring team. If possible, the training that was organized for local flexible team members (mainly LHWs) was also monitored by the monitoring team.

The monitoring visit usually took place between the 3rd and the 14th day of the field work in a cluster. Monitoring was conducted as much as possible in the presence of the field team leader for the purposes of capacity building. During the monitoring visit, the standard monitoring checklist was filled. Monitoring visits usually consisted of meetings with the field team leader and local authorities, monitoring of all field work activities following the survey flow (see section 6.1.3), interviewing field team members (especially the LHWs) about their satisfaction with and confidence in the work they were assigned to do, and a debriefing for the complete field team, in which observations of the monitor were shared and an action plan for observed problems was discussed. Monitors tried to observe all in-depth interviews conducted on the monitoring day, as usually there were around 10 suspects every day.

Every evening the survey coordinator received reports from all teams by text message with the number of participants and suspects enrolled that day.

Independent monitoring of field activities was also conducted by USAID in 35 clusters, and by the Federal Bureau of Statistics (FBS) in some clusters.

Desk Monitoring: Each day on completion of that day's field work, team leader were directed to send information via text message to the survey coordinator on key parameters (number of household census completed, number of subject screened, number of X-rays done, number of suspects identified, number of spot and morning specimens collected, number of positive cases reported).

A cluster summary report was prepared by the team leader on completion of field work and was sent to the survey coordinator along with other data. This report was used to monitor the progress of the field work on daily basis and to monitor the field team's performance and included the number of households and number of persons enumerated in the census, the number of participants screened, the number of suspects identified and the number of spot and morning samples collected and the number of smear-positive cases reported.
The hierarchy of the flow of responsibilities and the generation of reports was followed to ensure that information from the field was received regularly and timely and immediate action could be taken, if needed (see Figure 5). Table 2 summarizes the tools for monitoring.

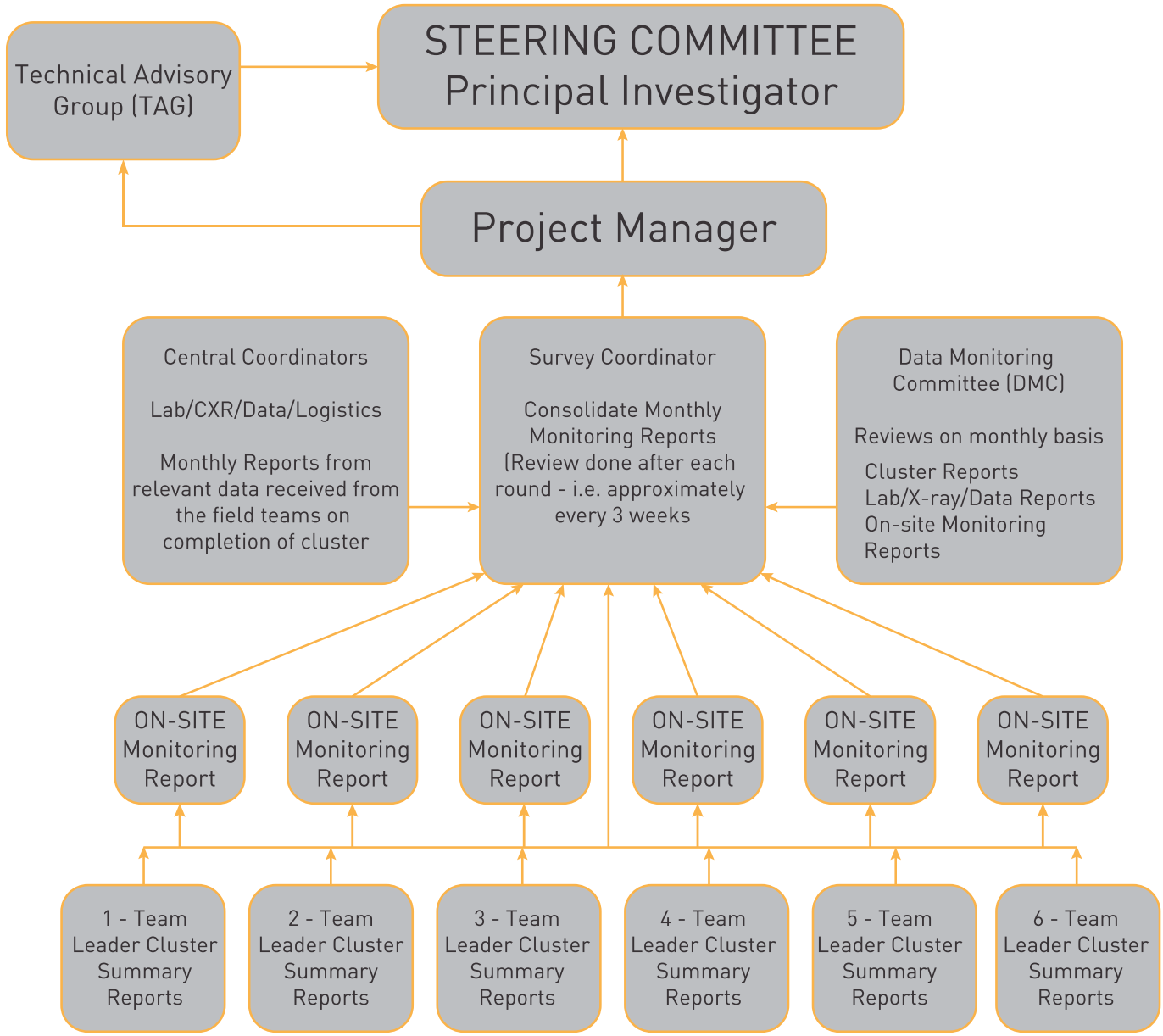


Figure 5. Prevalence Survey Monitoring.

Table 2. Tools for monitoring used in the TB prevalence survey.

Tools for monitoring	Prepared by	Rationale
Cluster summary reports	Team leader	Desk monitoring for analysis of performance indicators and to get insight into performance and performance trends of survey team.
Checklist and Monitoring reports	Survey Monitor	Provide detailed assessment of the technical procedures applied in the survey.
Reports on quality control and data validation procedures (re-examination of smears and X-rays and consistency checks on data entered into the database)	Central lab, radiology and data coordinator	Assess the performance of field laboratory , X-ray reading staff and data collection teams in the field

A Data Monitoring Committee (DMC) was constituted. The members of the DMC included the principal investigator, the survey manager, the survey coordinator, research coordinator NTP, Central Coordinators (Lab, X-ray, Data and Logistics), a project management officer and KNCV's M&E officer in Islamabad.

The DMC met roughly every three weeks after completion of field work in six simultaneously running clusters. The performance indicators of these clusters (as described above) were presented by the survey coordinator and issues were discussed. Examples of issues discussed were any specimen transport delays, low numbers suspects identified, or problems with X-ray machines. Recommendations were formulated, which were then followed up by the respective survey coordinators and communicated to the field team leaders by the survey coordinator.

Expert Review Committee

After completion of the field work, on 6th and 7th March 2012 an expert review committee reviewed potential TB cases with a single positive culture result with less than 5 colonies or a smear positive result that was not confirmed by a positive culture or NAAT result. The expert committee members comprised of (inter)national laboratory experts (Dr San Jae Kim, Dr Sabira Tahseen), a national radiology expert (Dr Aisha Ursani) and two national chest physicians (Dr Amanullah Ansari and Dr Abdul Ghafoor). Cases were discussed one by one and all available information including laboratory and radiology findings along with information on cough symptoms and treatment history of each potential TB case was reviewed to reach a decision on the potential TB case's final status: either definite survey TB cases, probable survey TB cases, or no TB cases.

5.8. Data Management

Before the start of the survey, the Data Management Plan (DMP) was defined, describing the procedures and processes to create accurate and complete data that are verifiable with source documents (primary data). The DMP was set up as advised in the TB Prevalence Surveys: A Handbook. It described all data management processes and procedures as well as the organizational structure of the DMU such as staffing and responsibilities, the data sources, data linking, data monitoring and data flow, data transfer, data sorting and filing, data entry, data cleaning and validation, database development and data processing tools.

The data collection and data processing were piloted at the time of the first pilot in the field (August 2010). During the survey, data collection took place at both field and central level. At both levels, paper forms and registers were used for data collection (see also Annex II for a complete list of annexes for data processing). The data flow is depicted in Figure 6.

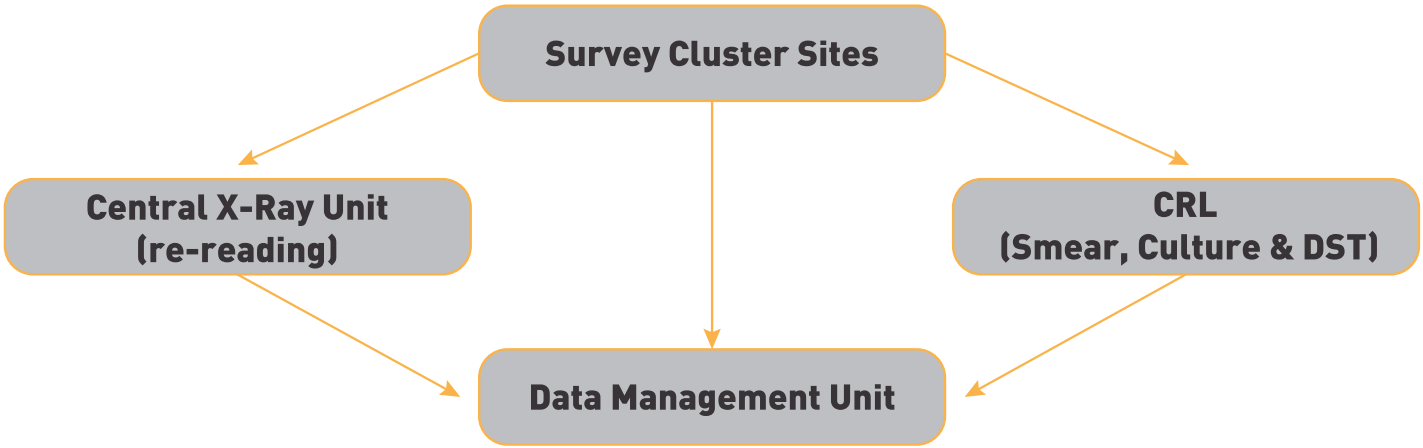


Figure 6. Data Flow in the TB Prevalence Survey.

5.8.1. Data Collection, Monitoring of Data Collection in the Field and Transport of Data to Central Level

The data collection in the field was done by the field team members. Pre-printed carbonated paper sheets were used for each form and register. After filling, the copies were separated into two packages. The first package contained the originals and was forwarded to the DMU as soon as the field activities in one cluster had been finished. The second package (copies) stayed with the field team until the next cluster had been finished. After this next cluster had been finished, the original package and the photocopies of the previous cluster were forwarded to the DMU together. After the last cluster, both the original and photocopies packages were forwarded to the DMU at once. All forms and registers were counted before the (original) forms and registers were transferred to the DMU by truck. A transport list was used to monitor the number of forms and registers in transit. All copies of forms and registers were stored and used as a backup if any form or register was lost.

No trained data checker was appointed to check the data before the field team left the cluster site. Instead, at field level, the data collection processes were monitored by the field team leaders. Data monitoring was done in the field, immediately after the field work for each cluster concerned to verify the paper-based primary data (forms and registers) for completeness and consistency. However, during data validation it was observed that this monitoring of completeness and consistency at the field level had not prevented forms and registers containing incomplete and inconsistent records from going to the DMU. After data monitoring, the forms were transported from the field to the DMU at central level. At field level, a standardised report form was used to report the number of forms and registers that were transported from the respective cluster.

5.8.2. Data Collection, Monitoring of Data Collection at Central Level and Transport of Data

At central level, data collection took place at the Central X-ray Unit (CXRU) and the NTRL. At the CXRU, no data checkers were appointed, and all X-ray reading results were collected on paper forms and transferred to the DMU for data processing and storage. At the NTRL, the data collection took place in laboratory registers used for routine testing and data were entered in Excel and data files were transferred to the DMU for data processing. The survey central laboratory registers stayed at the NTRL.

The data manager kept a data management register and a progress register. During the survey some backlog was observed in data processing which led to an increase of the number of data entry clerks to solve the backlog.

5.8.3. Data Sorting, Counting, Numbering and Filing

All data forms and registers received at the DMU were sorted, numbered and counted before filing. The total number of received forms and registers were crosschecked with the total number on the report forms from the field. In the case of discrepancies the data manager contacted the prevalence survey coordinator to solve any inconsistency between counts at DMU and field level with the field team leaders.

5.8.4. Data Processing

All forms and registers, once filed and eligible for data entry (Annex II), were processed by the data entry clerks. For data entry, EpiData version 3.1 (http://www.epidata.dk) predefined data entry screens were used. More than 1 million data entry points from 10 forms and registers were processed.

All data forms and registers eligible for data entry were entered. A random sample of 10% of all forms and registers was double entered in a separate database. After data entry of these 100% and 10% data files, the files were compared and checked for discrepancies. Discrepancies occurred in less than 1% of the records for all key variables, so that complete double data entry was considered unnecessary. The discrepancies were corrected if necessary and the results were documented by cluster and type of form or register by the data manager.

5.8.5. Data Merging

All individual forms and registers eligible for data analysis from the field level, CXRU and NTRL and all clusters were merged record by record into one data file. To be able to merge the forms and registers of one cluster, the PINs were checked for duplicates and corrected. Forms and registers were first merged on the PINs per cluster. Subsequently, all clusters were appended in the complete data file. The merge process was done with IBM SPSS Statistics version 21 (IBM corporation, NY) after all forms and registers were exported from EpiInfo to SPSS.

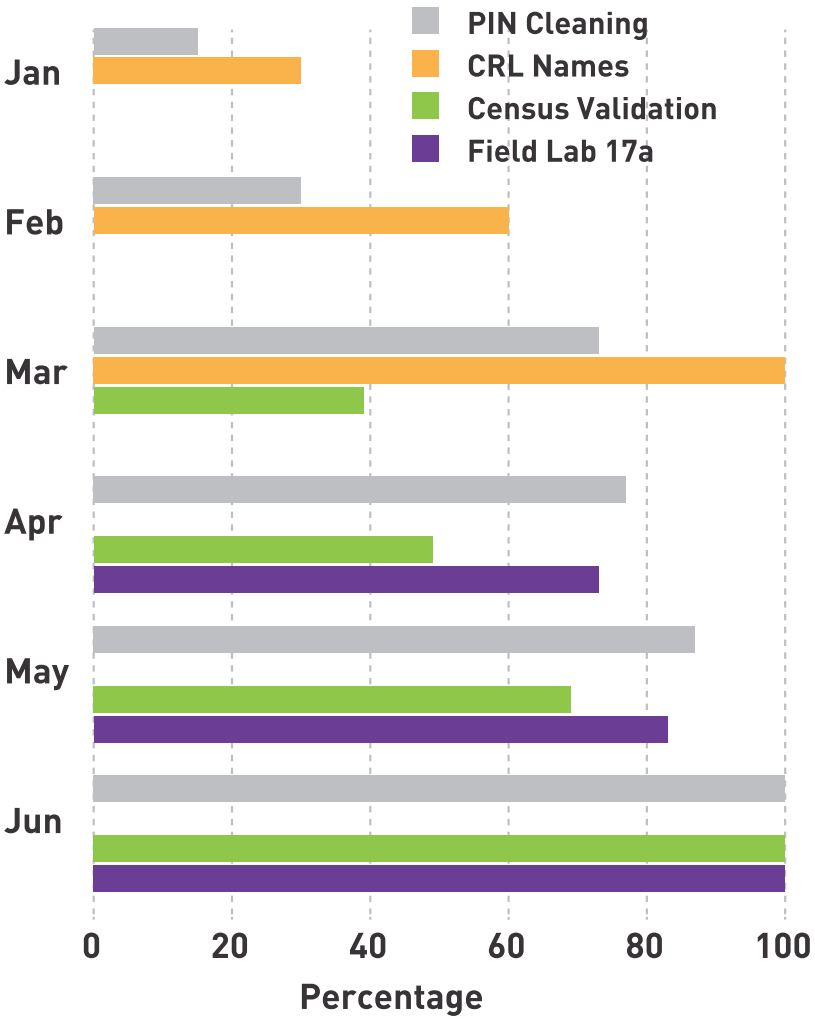


Figure 7. Progress in data cleaning showing the different steps in data cleaning between January and June 2013.
Steps are explained in the text.

5.8.6. Data Cleaning and Validation

PIN Cleaning

After the merge per cluster was completed, the merged result was checked for all records by comparing the names and father names of the participants on all forms and registers. It was observed that the use of the PINs to merge the forms and registers record by record did not always result in correct merges, which led to a correction process of all PINs by using a combination of name, father name and age on each form, starting in January 2013 and ended in June 2013 (see Figure 7, PIN cleaning).

Names with NTRL Laboratory Results (CRL names)

The NTRL data file with the NTRL laboratory results did not include the names of the participants. To enable correction of invalid PIN merges by participant name in the merged data file, it was decided to include the names in the file with the NTRL data. After including the names, all records of the NTRL file were merged again, names were checked to verify PIN merges and, in case of invalid merges, PINs were corrected in the merged data file. This step is referred to as "CRL names" in Figure 7.

Census Validation

The DMU checked if all persons enumerated in the census had been entered by comparing the data file with the paper census register. This validation step was done to assure that all members of a household on the paper based census register were included in the data file of the merged data.

Re-check Field Laboratory Data (field lab 17A)

During the validation process, it was observed that in the field laboratory register data from one of the variables had been dropped during the data validation process in 2012. This variable was re-entered in the digital field laboratory data file and re-merged with the final data file using PINs.

Validating the Data for Completeness and Consistency

Data were validated in Stata version 11 SE (Stata Corporation, Texas, USA). Data validation concerned checking of incomplete and inconsistent information (within and between forms and registers) against the information on the paper forms. An example of an inconsistency within a form is inconsistency on cough questions, e.g. the answer on Question 2 of the symptom screening questionnaire, "Do you have cough?" was "No" while there was also duration of cough. An example of inconsistencies that occurred between paper forms is differing information on age on the different forms and annexes. Queries were made in Stata and checked with the paper forms by the DMU staff. Then, data entry errors were listed in Excel files together with the corrected information. These Excel files were then integrated in the final data file, in which the corrected information replaced the data entry errors. For inconsistencies that existed on the paper form and were thus the result of errors made during field work, rules were made. For the example above, the rule was to assume that the answer on the question "Do you have cough?" should have been "Yes" instead of "No" if there was cough duration, since this was considered the most specific information a person could give.

5.9. Data Analysis

Data were analyzed in Stata version 11 SE (Stata Corporation, Texas, USA). Descriptive analyses included tabulation of the proportion of participants by sex, age group and cluster, the proportion of suspect by screening method and by sex, age group, and cluster and a description of smear and culture results.

To take the cluster design into account, logistic regression models were applied using the Stata svy commands including weights. This results in adjusted point prevalence and standard errors for the cluster design of the survey using population weights. The cluster population weight was calculated by dividing the total adult population in the sampling frame by 95 times the adult population in selected *tehsil*.

Point prevalence estimates and 95% confidence intervals, as well as design effects, were calculated using the Stata "svy" commands. These commands incorporate the effect of clustering when computing the variance, standard error and confidence intervals.

In the primary analyses, we ignored any sputum results of persons whose sputum was analyzed but who did not qualify as a TB suspect according to the suspect definitions based on symptom screening and/or X-ray screening result (see section 5.5) since these should not have submitted a specimen.

Since TB was found among persons who had submitted sputum but who were not eligible for sputum smear examination according to information obtained in the symptom screening interview and the chest X-ray, or who had no screening forms available (no symptom screening and no X-ray screening), in a secondary sensitivity analysis, we included all sputum examination available, irrespective of whether eligibility for sputum examination and compared the results to those of the primary analysis.

We calculated the design effect for the different TB case definitions, which is the ratio of the true variance of a statistic to the variance derived under simple random sampling assumptions (thus, Design Effect = ($\hat{V}_{survey}/\hat{V}_{simple\ random\ sampling}$) = [1 + ρ(n-1)] with $\hat{V}_{survey}$ as the true variance of a statistic given the survey design, $\hat{V}$simple random sampling denoting the variance estimate obtained for the statistic under simple random sampling assumptions, ρ being the intra-cluster correlation coefficient, and n the average cluster size)[13].

The patient detection rate (PDR) was calculated by dividing the number of notified cases to the regular programme per 100,000 persons per year by the prevalence rate of the survey per 100,000 persons. The case detection rate (CDR) was then obtained using the formula CDR = PDR / (PDR + 0.5).[14]

5.10. Bias Control Strategies and Survey Precision

Bias control strategies were undertaken during the selection of clusters: a proper sampling design was developed making five extra *tehsils* available to be selected in case of any replacement needed because of security reasons or operational difficulties. Blinding of the laboratory staff was done so that they were not aware whether the person submitting sputum was a TB suspect or not. During the survey implementation certain actions were taken to improve participation after the lesson learned from low participation in the first pilot (54%) and the strategies were adopted to increase participation in second pilot (81%). For example, cluster field work was sometimes done in the evening so that those working men who could not come in morning could come in evening to avoid gender bias. Secondly, census teams were trained to improve their communication, so they were able to clearly explain the purpose of the survey, properly invite the household members to survey sites, and to explain the benefits of the survey. Benefits were provision for free of routine medicines like cough syrup, antibiotics and antipyretics to symptomatic participants. Thirdly, the local community leaders were engaged to help increase participation and to increase the trust of the local community in the survey team which facilitated the implementation of field work. Fourthly, free transportation was provided to persons living relatively far away from the screening site. Fifthly, children were provided with a small present at the screening site, which encouraged their parents to visit the screening site.

6 ORGANIZATION OF DATA COLLECTION

6.1. Organization of Data Collection at Field Level

The first pilot was conducted in August 2010 in a cluster that was part of the total number of selected clusters. Because of the low participation rate obtained in this first pilot, we decided to schedule another pilot in November 2010, including strategies to increase participation rate. These strategies included better communication and involvement of influential persons and religious and community leaders. The second pilot was conducted in November 2010 in another cluster which was part of total number of selected clusters and showed that the interventions improved the participation rate (81%).

6.1.1. Survey Field Teams

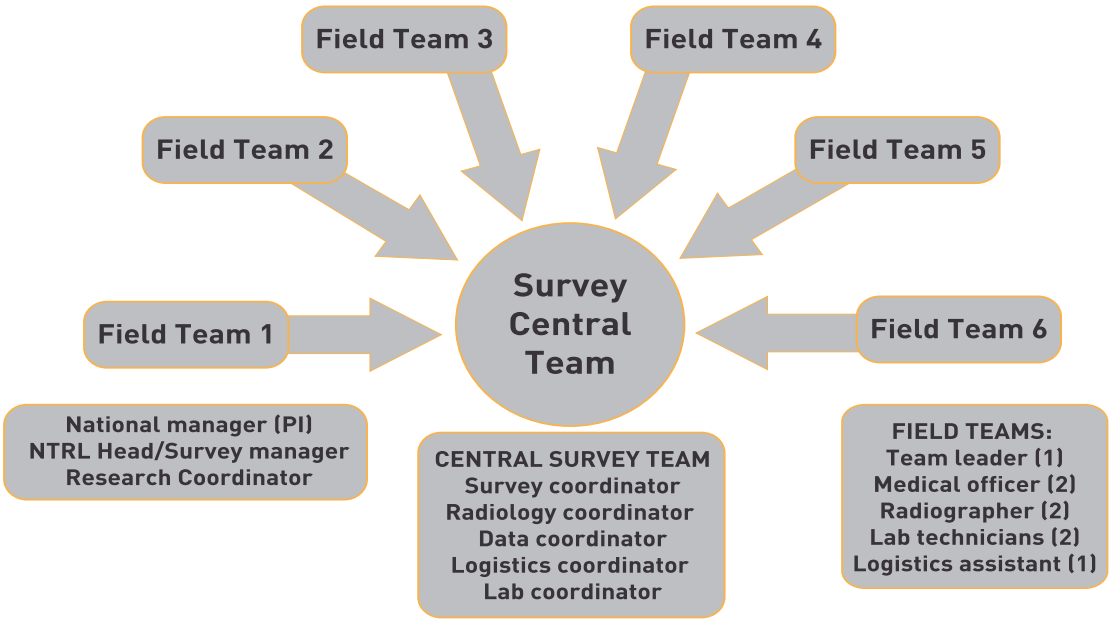


Figure 8. Schematic Diagram of the Survey Organization.

Each field team consisted of a team leader, two medical officers, two radiographers, two laboratory technicians and a logistics assistant (Figure 8). The team leader was responsible for training, overall management and quality control of the field team's work, while medical officers assessed X-ray images and conducted in-depth interviews with TB suspects. Radiographers registered participants for X-ray and took X-ray images, whereas the logistics assistant was responsible for all logistical procedures involved in the field work. This "fixed" team was completed by local team members (LHWs and vaccinators). This "flexible" team was routinely working in the cluster area and only collected data in the cluster that they usually worked in. LHWs were involved in the mobilization of the population for participation, census taking and symptom screening. Vaccinators administered tuberculin skin tests (TSTs) to children until the tuberculin survey was stopped halfway the TB prevalence survey, and later were involved in the mobilization of the population.

6.1.2. Preparation of Field Work

Training

Before the first pilot, the "fixed" field teams and central teams were received 7 days training on all survey processes and procedures at field and central level by the central survey coordination team, existing of the survey coordinator, the radiology coordinator, the laboratory coordinator, the logistics coordinator, the head of the DMU from NTP, and the KNCV M&E officer. Plenary sessions were followed by in-depth training sessions for specific job descriptions (e.g. field and central laboratory staff received training on sputum smear preparation and examination, and field procedures).

National TB program officials responsible for TB control activities in 4 or 5 districts, were also trained in a 3-day training about survey management and operation.

Subsequently, all survey teams received 2 days of training at the first pilot site to learn how the survey field work would be done in practice.

All survey field teams consisted of fixed team members who travelled from cluster to cluster, and of 8-12 local team members, who were added to the team at each cluster site. These non-fixed team members were LHWs and local vaccinators who worked in the survey area. LHWs were supposed to mobilize the cluster's population to participate in the survey, and to conduct the census and the symptom screening interview. Before the start of the field work in each cluster, LHWs received one day of training by the field team leader on all aspects of the survey they were to be involved in. Vaccinators administered tuberculin skin tests to children (TST; not discussed in this report as this work was stopped after a few clusters had been included). In some clusters, members of local vaccination teams were assisting the LHWs with mobilization of the cluster's population.

Preparation of the Field Work in a Cluster

Two to four weeks before the start of the field work in a cluster, the survey coordinator informed local and national TB officials about the name of the cluster (district, *tehsil*, UC) and its geographical location. Letters were also sent to district health officers to inform them about the survey objectives, organization, dates and support needed. Then the team leader visited the cluster to discuss and prepare the survey (pre-survey visit) including logistical arrangements with the district TB coordinator and the executive district officer for health (EDOH) in the 1-week interval after finishing one cluster and starting the next cluster. A district FBS official joined the pre-survey visit to mark the exact borders of the selected area to guide the census takers. In consultation with district officials, an appropriate health facility was identified in the vicinity of clusters for referral of individuals that needed further medical assistance during the survey. The pre-survey visit also aimed to identify the survey population within the selected cluster and to do sub sampling using a sketched map of the area and micro plans obtained through the polio vaccination program.

6.1.3. Survey Field Work

Census

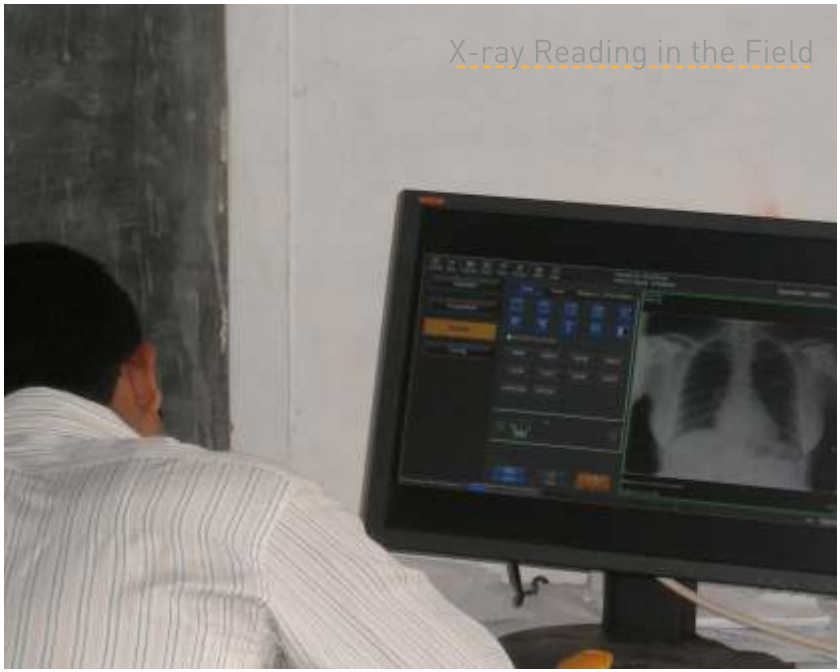
The fieldwork took 14 days per cluster and started with the household census. All households in the selected area were visited by a fixed team member and a LHW until the household with the 1400th eligible inhabitant was registered. Only eligible persons were registered (persons of 15 years and older who had slept in the household the night before the survey). During the household visits, relevant information about all the eligible individuals was entered in the census register including name, father or husband name, position in the household, age, sex, ethnic group to which the person considered him/herself, education level and occupation. Eligible subjects who consented to participate were issued a survey identification card with information on time and place of screening, on which the name, father/husband name and personal identification number (PIN) of the subject were written, and were invited to the field cluster site for screening. If the eligible person was not available during the census this was written in a specific column in the census register. The survey identification card was left at the home of such a person and when the person visited the site for screening the consent was taken at the screening site. Also, the census register contained columns for checking if enumerated should be excluded (e.g. because of mental illness), and for checking if enumerated persons had been screened on symptoms and by X-ray.
Filled census register forms were sent to the symptom screening area and were kept there during the field work, since all participants had to be cross-checked with the census register and each form contained all persons of a household.

Participant Flow at the Survey Site

At the field cluster site, all eligible survey participants were screened for TB symptoms and signs by means of a short questionnaire and a chest X-ray to assess if they could be identified as TB suspects. The symptom screening was done in the survey's reception area by LHWs using a structured questionnaire. PINs were copied to questionnaires from the person's survey identification card or from the census register if the person had lost the survey identification card. In the census register, PIN, name and father/husband name were checked. The LHW conducting the interview registered the person as having participated in symptom screening and having been referred for X-ray (or not having been referred for X-ray if the person did not consent to or was not eligible for X-ray screening). The symptom screening interview contained 5 fixed questions: whether the person was currently on TB treatment, whether the person had cough, and, if yes, for how long, whether the person was smoking, and whether the person consented to X-ray screening. Although intended and repeatedly tried, the interview was not done in private and it was usually done under time pressure, since other participants were waiting. This often led to problems with symptom interviews, such as missed questions and inconsistencies between questions.

After completion of the symptom screening interview, the filled questionnaire was given to another LHW who handed the questionnaire to the radiographer and referred the participant to the X-ray screening room. This LHW was responsible for checking if the person consented to X-ray screening. If the person did not consent or was pregnant, the person was sent directly to a medical officer to check the outcome of symptom screening and to decide whether the person should be listed as a TB suspect.

The radiographer copied the PIN and the participant's name from the survey identification card (or the symptom screening questionnaire if the survey identification card was not available) onto the X-ray screening form and into the digital X-ray file. Then, the person was invited into the X-ray room where his/her name was checked before making an X-ray image.


X-ray Reading in the Field

A medical officer read the X-ray and filled the result on the X-ray screening form. After this, the medical officer decided if the person should be regarded as a TB suspect based on the information from symptom and X-ray screening.

TB suspects were defined as survey participants having cough for more than two weeks and/or abnormality on chest X-ray image or being on TB treatment at the time of screening. If chest X-ray was either not done or not interpretable, a TB suspect was identified based on cough of any duration.

TB suspects were sent to a separate, private room for the in-depth interview, which was done by a second medical officer using a structured questionnaire. PINs were copied from the survey identification card of the participant, or from the symptom screening questionnaire or the X-ray screening form. Before or after the in-depth interview, the medical officer registered the TB suspect in the TB suspect register based on information in the symptom screening questionnaire and the X-ray screening form. The in-depth interview included questions on cough duration and other respiratory symptoms, on TB treatment, and on health seeking behavior. After the in-depth interview, the TB suspect was referred for sputum collection with a referral slip with PIN and name. At the survey field laboratory, TB suspects were registered in the laboratory register and PINs were copied from the referral slip.

Field Laboratory Work

All TB suspects registering at the field laboratory site received information on how to produce a sputum specimen and a container with a screw cap marked with the participant's PIN and name. Two sputum specimens, one spot and one morning sample, were collected from TB suspects. The spot sample was examined in the field laboratory using AFB direct smear microscopy with Ziehl Neelsen staining. Its appearance and volume were checked and noted. For the morning sample, also appearance and volume were assessed and recorded, after which it was transported to the NTRL for sputum smear examination and culture. Of those TB suspects who

failed to submit a morning sample by 10.00am the next day, the spot specimen was sent in cold chain to the NTRL for smear and culture.

Sputum Collection and Storage

Sputum specimens were collected in the open air, for women the specimens were collected at a predefined place secluded by a screen in the open air. Sputum containers with screw caps were labeled with the PIN and name of the suspect. After collection of the spot specimen, a second sputum container was given to TB suspects for collection of a morning specimen at their own premises.

All collected specimens were kept in refrigerators or cool boxes till transportation. After smear preparation, the spot specimen containers were carefully closed and stored till the next day.

X-ray Screening at Cluster Level

Sputum Microscopy

Direct smear from spot specimen was prepared and stained using the Ziehl-Neelsen (ZN) staining technique and examined under a bright field light microscope. Smears were read by a trained laboratory technician, and a second reading was done of all positive slides by another laboratory technician to confirm the result of the first technician. All results of the spot specimen were entered in the field laboratory register.

Sputum Transport

For transportation, sputum specimens were packed in a transparent leak proof sealed vinyl bag together with two layers of cleansing tissue. This package was then placed in another vinyl bag and which was then placed in a cooler or cool box with ice packs. A dispatch list of specimen was placed in a separate vinyl bag. This list contained all PINs of TB suspects for whom a specimen was sent, along with their names and father names, age, sex, type of specimens sent (spot or morning), and collection date and physical appearance of these specimens. Specimens were transported by courier on daily basis from the cluster sites, to arrive in Islamabad within 72 hours after collection.

Follow up of TB Suspects

Participants eligible for sputum examination (TB suspects) who failed to submit two samples and/or to attend the suspect interview, were traced and invited for further screening the day after having been identified and registered as a TB suspect. For this, the TB suspect register was checked every evening for completeness of data against the suspects entered in the register. TB suspects who failed to submit a morning sample were traced and encouraged to submit a morning sample on the next morning. The field survey team was informed in case of sputum contamination or specimen loss during transportation. If this occurred, the field survey team was requested to collect and send an extra sputum specimen from the respective TB suspect.

Storage of Received Samples at the NTRL

Storage and Transport of Forms, Registers, X-ray Images and Slides

Each evening, the field team and team leader checked the forms and registration from each participant on consistency and completeness. After this, forms were filed by annex and put in locked iron boxes, and sent to central level by courier at end of field work in these boxes. After completion of 31 clusters (March 2011), the procedure of filing forms was changed in some teams, and the survey identification card and all forms for one participant (symptom screening questionnaire, X-ray result form and in-depth questionnaire (for TB suspects only)) were stapled since this facilitated the field team's task in checking if all information for a participant was complete and consistent. At the DMU, the forms were then un-stapled and counted and filed by annex.

All abnormal X-ray images and a random sample of 20% of the X-ray images scored as normal were stored on DVDs were sent to the central DMU On every alternate day for quality assurance purposes. All images were sent after finalizing the field work in a cluster and stored in a central server.

All examined spot smear slides were stored in serial order and on completion of the cluster's field work, were sent by courier to the DMU in Islamabad along with a two copies of the field laboratory register, while the original laboratory register stayed with the survey field team. The DMU forwarded the slides with a copy of the field laboratory register to the NTRL.

6.2. Organization of Data Collection at Central Level

6.2.1. X-ray Reading

Lists with PINs and names were prepared for reading at central level. All abnormal and 20% of the normal X-ray images were read at central level by expert radiologists/pulmonologists in a blinded fashion. Reading of X-ray images with abnormal shadows was needed to ensure that the X-ray image contained abnormalities and to classify the type of abnormalities. Air space infiltrates, consolidation, cavitation, pleural effusion, mediastinal or hilar lymph node involvement, milliary mottling and apical involvement were concerned as abnormalities suggestive for TB. The result of X-ray reading was marked on the list, after which one form per X-ray image was filled. After completion of the forms, the results were compared to the result of field X-ray screening. If the reader in the field had judged the image to be normal, while the central reader had registered it as an abnormal image, or vice versa, a third reading was organized at the central level. This third reading result overruled the results of previous readings.

6.2.2. Sputum Examination (morning specimen)

On arrival at the NTRL the sputum containers were checked for the tightness of the screw caps and any contamination outside the container, which was cleaned with 70% alcohol. All specimens were processed in a bio-safety cabinet under standard safety conditions. In cases where a specimen was found to be leaking and/or labeling was not readable, the specimen was not processed and the field team was requested to a repeat (extra) sample.

All morning specimens were processed for smear and culture in the NTRL. A direct smear was prepared and stained with ZN stains. After smear preparation, specimens were processed for culture by a simple technique without centrifugation (Figure 9). Four percent sodium hydroxide (NaOH) was used for decontamination and two slopes of acid Ogawa medium were inoculated. All cultures slopes were incubated at 37°C and examined twice in the first week (for detection of contamination or rapid growth), and then on weekly basis until they showed growth. All negative cultures were incubated for nine weeks.

Identification of Culture Colonies

The *Mycobacterium* species in culture positive samples was identified based on colony morphology (growth rate, color, appearance and serpentine cording under microscope). Those samples with a morphology, color and growth rate concordant with MTB were set up for culture on a paranitrobenzoic acid (PNB) containing medium to identify susceptibility to PNB. Strip identification by MPB64 (Becton Dickinson, Franklin Lakes, NJ, USA) and/or GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) testing was performed in case the initial identification result using morphology and growth was not correspondent with the PNB result.

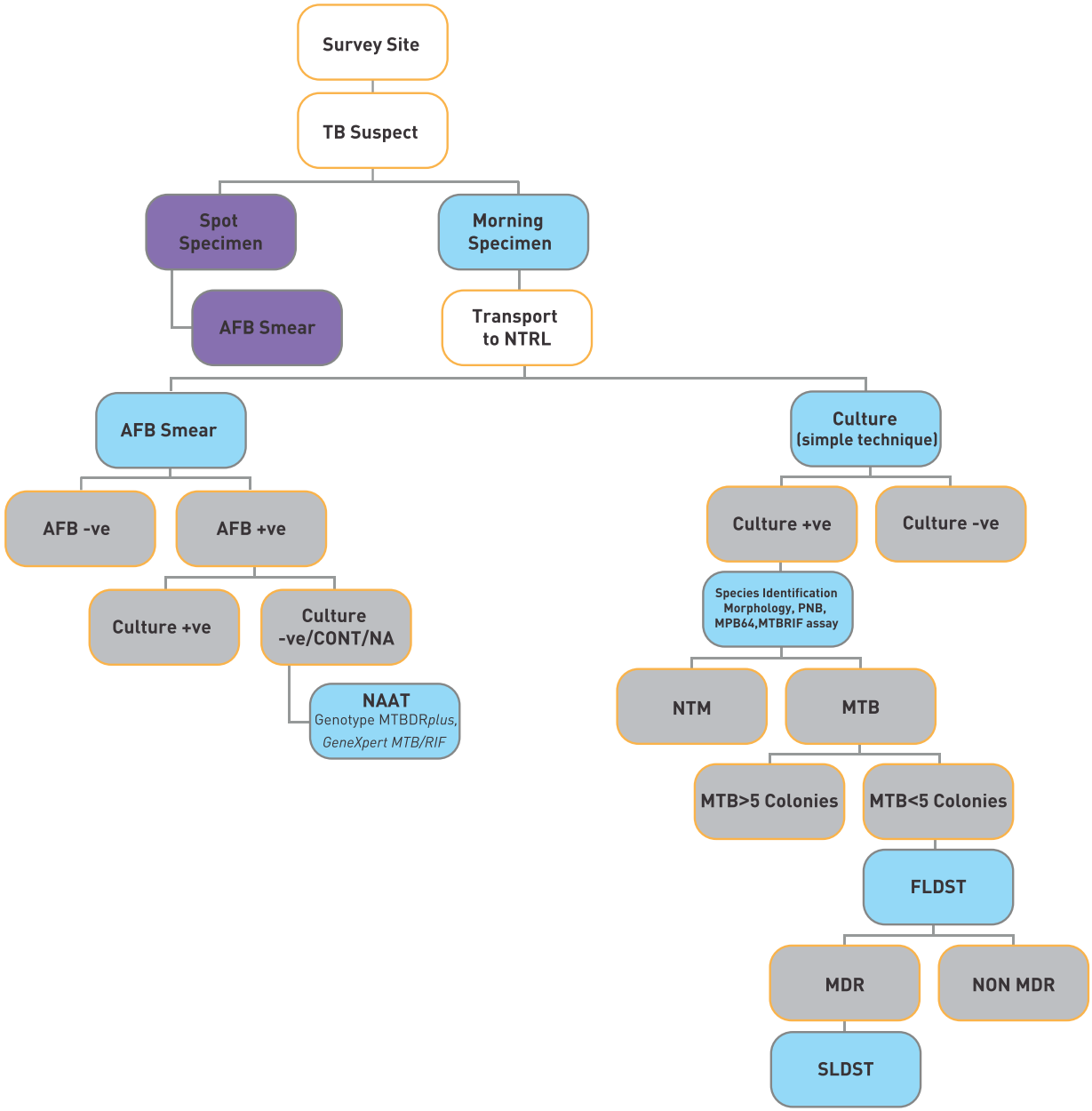


Figure 9. Flow Chart of Field and Central Laboratory Activities.
All tests done in the National Tuberculosis Reference Laboratory are given in blue boxes, while field work is depicted by purple boxes, gray boxes indicate results

Abbreviations used in this chart: +ve positive; –ve negative; AFB: acid fast bacilli; FLDST: First line drug susceptibility testing; MDR: Multi-drug resistant tuberculosis; MTB: Mycobacterium tuberculosis; NAAT: nuclear acid amplification test; NTRL: National Tuberculosis Reference Laboratory; NTM: Non-tuberculous Mycobacteria; SLDST: Second line drug susceptibility testing.

Identification of MTB on smears using nucleic acid amplification tests (NAAT) .


Incubator at the NTRL

For TB suspects with a negative culture, or for whom no culture was grown, as there was no sputum, not enough sputum to prepare a culture, or the culture was contaminated, but for whom one or more positive smear results were obtained, the smear slide was subjected to either Genotype®MTBDRplus (Hain Lifescience GmbH, Nehren, Germany) or GeneXpert MTB/RIF assay testing. All such slides for which the culture result was known before 25 November 2011 were sent in one batch to the SRL in Korea for Genotype®MTBDRplus testing. After the end of laboratory work for the prevalence survey (March 2013), similar slides for which culture results were obtained after 25 November 2011 were subjected to GeneXpert MTB/RIF testing. This was also done for second positive slides from the TB suspects of whom slides had tested negative on the Genotype®MTBDRplus assay plus, if available. Isolates from cultures that had grown less than five colonies and isolates from where other identification test results were not available were also subjected to GeneXpert MTB/RIF testing.

Drug Susceptibility Testing (DST)

DST was performed on culture isolates showing pure M. tuberculosis (MTB) isolates and growth of 5 or more colonies. All such isolates were first tested for first line drug susceptibility (isoniazid, rifampicin, ethambutol and streptomycin). Isolates showing resistance to both isoniazid and rifampicin (multidrug resistant (MDR) isolates) were subjected to second line drug susceptibility testing (ofloxacin, amikacin, kanamycin, and capreomycin) using the modified proportion method on Löwenstein Jensen media.

6.3. Case Management

Positive smears from the spot specimen were reported by the field laboratory technician to the medical officers and the team leader. Positive smear results from morning samples were reported by NTRL staff via text message on cell phone to the field laboratory technicians and the team leader. All individuals diagnosed as a TB case during the course of the survey were referred by the team leader or medical officer to a pre-identified basic management unit (BMU) in the patient's residential area to register for TB treatment (Figure 10).

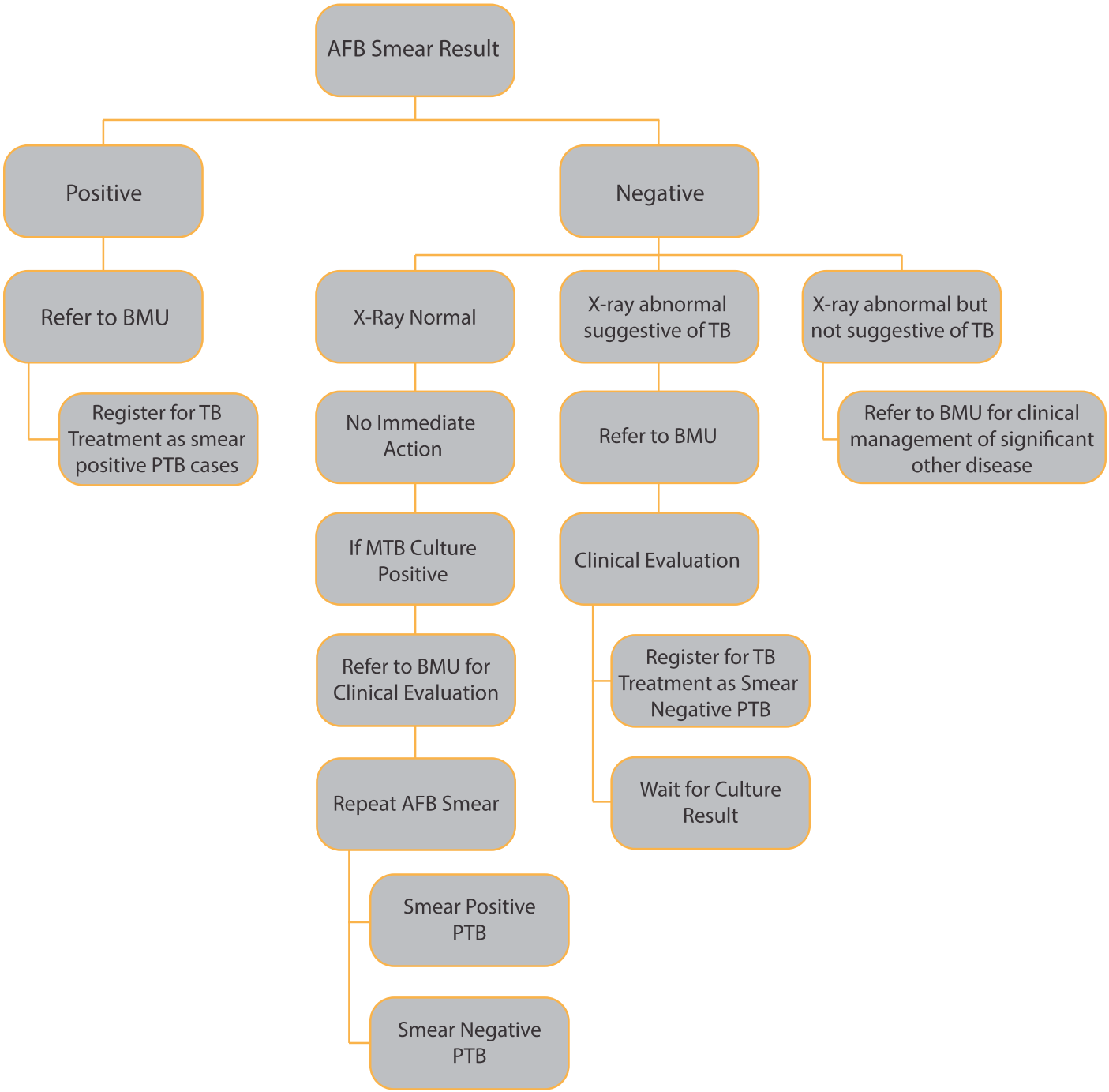


Figure 10: Schematic Diagram Representing Case Management in the TB Prevalence Survey.
Abbreviations used in this diagram: AFB: Acid fast bacilli; BMU: Basic management unit; PTB: Pulmonary TB.

Smear Positive TB Cases

Cases identified with positive smear on spot sample were informed on arrival of patient for submission of morning sample and referred for registration. If the spot smear was negative but the morning smear was reported positive by NTRL staff, the patient was located by a LHW using the data from the census register and referred to the nearest BMU to register for TB treatment.

6.4. Survey Timeline

The inception of the TB disease prevalence survey took place in March 2008 as a result of participation of the NTP team in workshop organized by Impact Measurement Unit at the Head Quarters of the WHO. Commitment of funds by USAID (through TB CAP and subsequently TB CARE I) for conducting this survey was obtained in 2009. Survey preparation took more than a year which included the setting up of a KNCV office in Islamabad, development of the prevalence survey protocol, standard operating procedures (SOPs) including a DMP with data collection tools, procurement of X-ray equipment and other laboratory items, recruitment of staff and training of staff.

Survey workflow was tried out with simulation exercises before start. All survey procedures and data collection tools were pretested in a first pilot in one cluster being part of the sampling frame of 95 clusters (cluster 2) that was conducted in August 2010. Since the participation rate to that first pilot was much lower than expected, a second pilot was done in another cluster being part of the sampling frame (cluster 15) in November 2010. The full scale survey was launched in December 2010. The field work was completed in all clusters in January 2012. Reporting of the culture and DST results was completed in March 2012. The timeline is described in Table 3.

Table 3. Survey Timeline.

Activity	Time
Survey inception	March 2008
Setting up of KNCV office	June 2009
Survey preparation (Protocol , data collection tools, standard operating procedure documents, procurement, recruitment and training of staff)	2009 -2010
First pilot	August 2010
Second Pilot	November 2010
Field work in 93 survey clusters	December 2010 - Jan 2012
Completion of lab work	March 2012
Data cleaning and validation	March 2012 - August 2013
Data analysis	June 2013 - August 2013
Final report	July 2013 - August 2013

6.5 Funding and Procurement

Funding

The TB disease prevalence survey was conducted with financial support from USAID. These funds were received through TBCAP (APA 4 and APA 5) TB CARE I (APA-1). In 2010 financial support for local staff was obtained from the Ministry of Health. Between 2010 and 2012, a total amount of 3.2 million US dollar (USD) was spent on the survey, corresponding to 24 USD per eligible person, 30 USD per participant, 305 USD per TB suspect and over 9,200 USD per TB case.

Procurement

Procurement of 7 mobile X-ray units was conducted through an international tender process with the technical support of KNCV.

Laboratory items including transport boxes, laboratory reagents, supplies and other items were procured through a national tender process.

A contract was established with a courier service for specimen transport from the field to the NRL and for transport of empty transport boxes back to cluster sites. The same courier service was used for the transportation of data collection forms and registers to the new cluster site as well as of the completed forms and registers from the field back to the DMU after completion of field work in a cluster.

Radiographer in a Field Cluster Site

Vehicles for use in field services were obtained through contracting a car rental service after an open tender process.

7 RESULTS

7.1. Census, Enrollment and Participants

In total, 131,377 persons were registered in the census, which was 98.8% of the calculated sample size of 133,000. Only persons aged 15 years and above who slept in the household were registered. However, due to misunderstandings, 43 children younger than 15 years of age were registered and had to be excluded. Also, 3 persons with mental illness were excluded as per protocol. This brought the total eligible study population to 131,331 persons. Of the eligible population, 47.1% was male and 52.8% was female. The median age was 30 years (interquartile range, 21-45 years).

105,915 persons (80.6%) had either a symptom screening questionnaire available, or a field X- ray done, or both (Figure 11). These were classified as participants. The remainder missed both screening methods. There were 261 persons for whom a combination of forms was available, but not the screening forms. These were regarded as non-participants in the primary analyses though they were included in the sensitivity analyses. The participation rate by cluster and team over time is given in Figure 12. The first pilot cluster had by far the lowest participation rate (54%). Higher participation rates of mostly between 70% and 80% were achieved in later clusters.

Women were more likely to participate than men (the participation rate was 88% among women and 72% among men, $p>0.0001$). There was no marked difference in participation rates among different age groups, province, type of area or field team. Figure 13 compares the eligible population with the participating population with respect to age and sex. It can be clearly seen that men were underrepresented among the participants within all age groups.

7.2. Screening Results

As described in section 6.1.3, all participants were screened on signs and symptoms of TB by answering questions in a short screening interview and chest X-ray. In total, 105,913 eligible persons (80.7%) participated in symptom screening, X-ray screening, or both. Most persons participated in both screening procedures. An overview of screening coverage is given in the columns 'symptom screening done' and 'X-ray screening done' of Table 4.

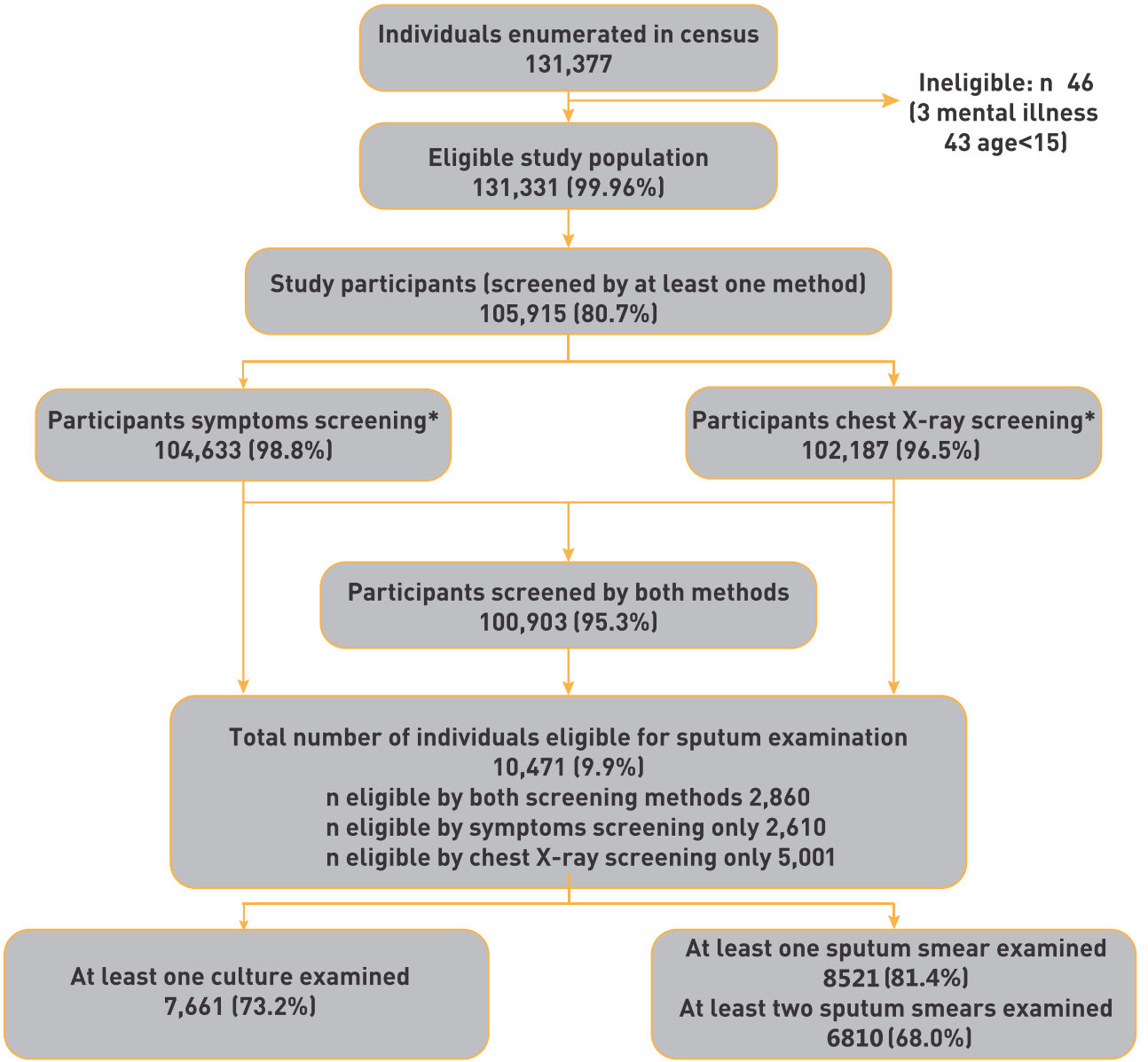


Figure 11. Survey Flowchart.

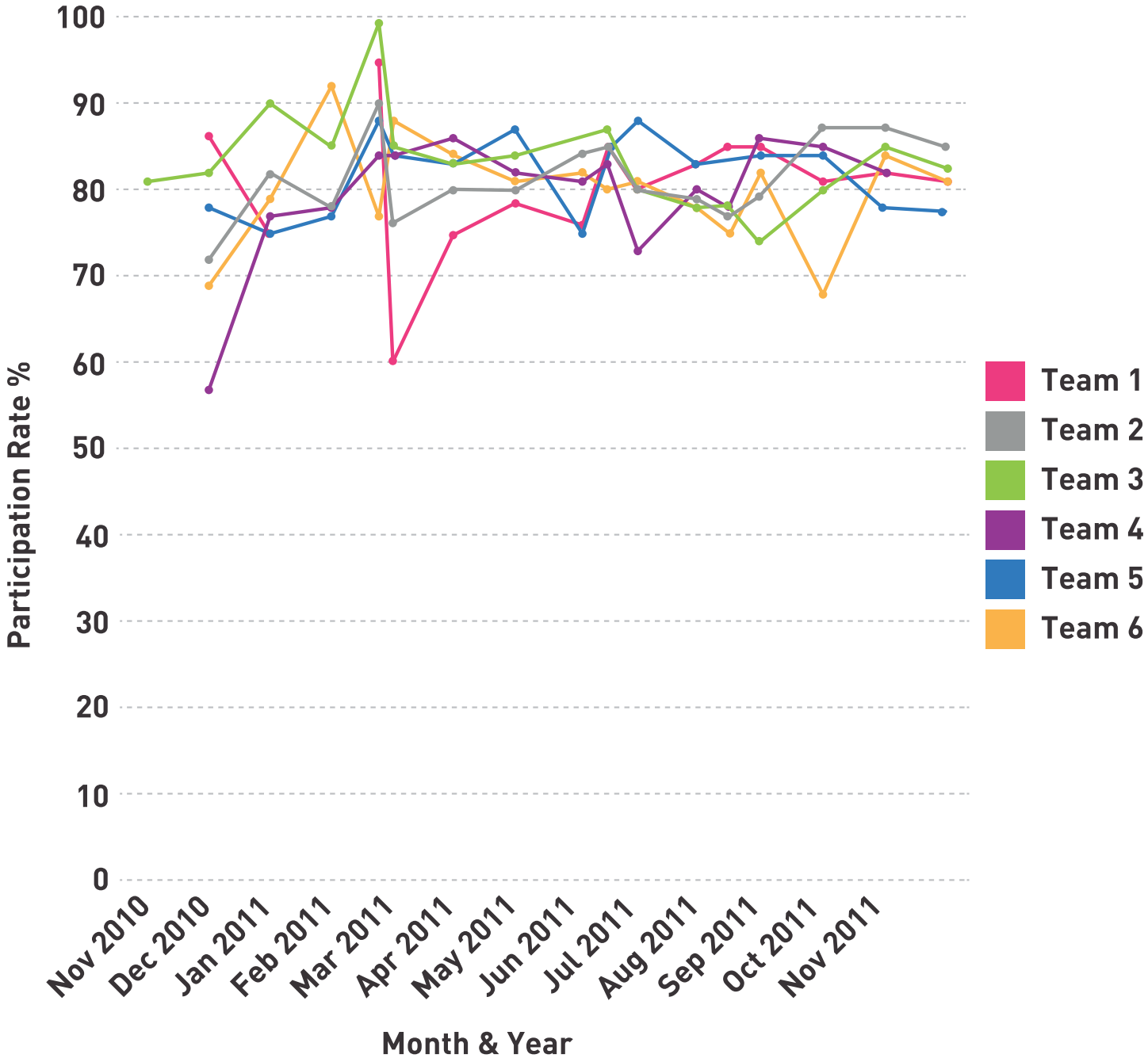


Figure 12. Participation Rate Per Cluster and by Team Over Time.

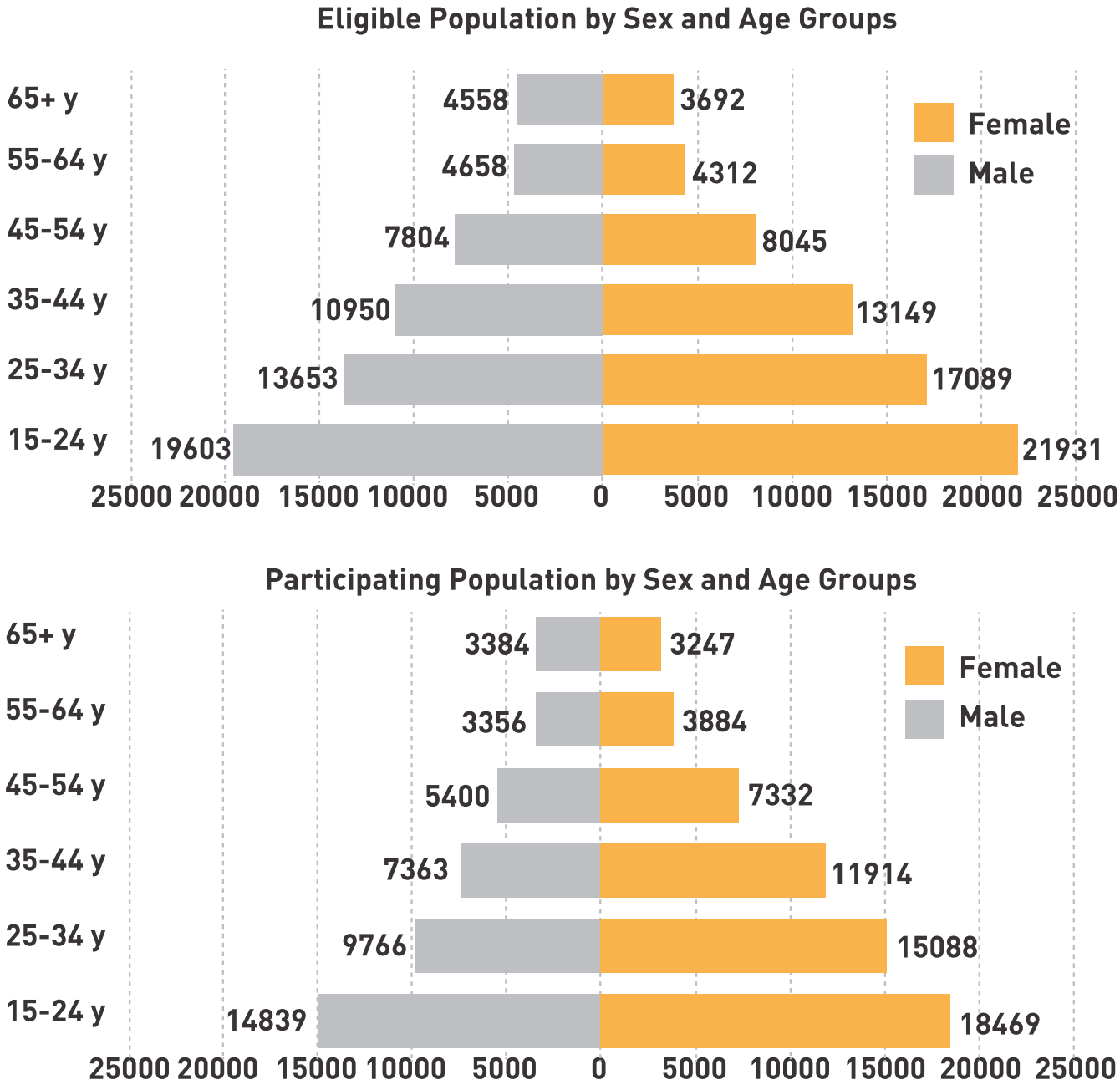


Figure 13. Eligible Versus Participating Population by Sex and Age Groups.
Y axis depicts the age classes, X-axis depicts the number of persons.

Table 4. Characteristics of Participants and Non-participants to the TB Prevalence Survey.

Characteristic		Non Participants		Participants		Among Participants:				Total Eligible
						Symptom Screening Done		X-ray Screening Done		
		n	%	n	%	n	%	n	%	n
Sex	Male	17,124	27.7	44,786	72.3	44,222	71.4	43,343	70.0	61,910
	Female	8,290	12.0	61,067	88.0	60,350	87.0	58,787	84.8	69,357
	Unknown	4	6.3	60	93.8	60	93.8	55	85.9	64
Age	15-24y	8,227	19.8	33,336	80.2	33,013	79.4	32,169	77.4	41,563
	25-34y	5,888	19.1	24,863	80.9	24,608	80.0	23,857	77.6	30,751
	35-44y	4,822	20.0	19,287	80.0	19,059	79.1	18,621	77.2	24,109
	45-54y	3,118	19.7	12,735	80.3	12,543	79.1	12,343	77.9	15,853
	55-64y	1,731	19.3	7,242	80.7	7,106	79.2	7,061	78.7	8,973
	65+ y	1,620	19.6	6,634	80.4	6,502	78.8	6,418	77.8	8,254
	Unknown	12	0.7	1,816	99.3	1,801	98.5	1,716	93.9	1,828
Province	Punjab	15,208	19.2	64,134	80.8	63,425	79.9	61,693	77.8	79,342
	Sindh	6,178	19.8	25,067	80.2	24,619	78.8	24,243	77.6	31,245
	Balochistan	438	16.9	2,160	83.1	2,132	82.1	2,080	80.1	2,598
	Azad-J. & Kashmir	979	22.7	3,343	77.3	3,319	76.8	3,281	75.9	4,322
	K. Pakhtoon Khwa	2,402	19.2	10,076	80.8	10,013	80.2	9,769	78.3	12,478
	Gilgit-Baltistan	213	15.8	1,133	84.2	1,125	83.6	1,121	83.3	1,346
Type of Cluster	Rural	13,738	19.1	58,104	80.9	57,446	80.0	55,870	77.8	71,842
	Urban	11,680	19.6	47,809	80.4	47,187	79.3	46,317	77.9	59,489
Field Team	1	5,023	21.7	18,132	78.3	17,944	77.5	17,080	73.8	23,155
	2	4,184	18.8	18,085	81.2	17,884	80.3	17,418	78.2	22,269
	3	3,943	17.0	19,230	83.0	19,008	82.0	18,698	80.7	23,173
	4	4,254	20.6	16,430	79.4	16,240	78.6	15,794	76.4	20,684
	5	3,578	18.2	16,093	81.8	15,724	80.0	15,668	79.7	19,671
	6	4,472	19.9	17,953	80.1	17,832	79.5	17,527	78.2	22,425

Characteristic		Non Participants		Participants		Among participants:				Total Eligible
						Symptom Screening Done		X-ray Screening Done		
		n	%	n	%	n	%	n	%	n
Survey Round	Pilot 1	929	46.1	1,086	53.9	1,063	53.0	1,024	51.0	2,015
	Pilot 2	275	19.2	1,157	80.8	1,156	80.8	1,134	79.3	1,432
	Round 1	2,255	26.3	6,317	73.7	6,228	72.7	6,172	72.1	8,572
	Round 2	1,789	20.6	6,879	79.4	6,775	78.2	6,497	75.0	8,668
	Round 3	1,178	17.6	5,498	82.4	5,352	80.2	5,279	79.1	6,676
	Round 4	890	11.2	7,045	88.8	6,946	87.5	6,769	85.3	7,935
	Round 5	1,714	20.7	6,580	79.3	6,521	78.6	6,358	76.7	8,294
	Round 6	1,512	18.2	6,789	81.8	6,767	81.5	6,543	78.9	8,301
	Round 7	1,460	18.0	6,668	82.0	6,603	81.2	6,428	79.1	8,128
	Round 8	1,598	19.3	6,679	80.7	6,621	80.0	6,415	77.5	8,277
	Round 9	1,393	16.9	6,864	83.1	6,835	82.8	6,351	76.9	8,257
	Round 10	1,680	20.1	6,668	79.9	6,628	79.4	6,545	78.4	8,348
	Round 11	1,622	19.6	6,658	80.4	6,529	78.9	6,461	78.0	8,280
	Round 12	1,661	20.1	6,586	79.9	6,516	79.0	6,457	78.3	8,247
	Round 13	1,490	18.1	6,721	81.9	6,593	80.4	6,549	79.8	8,211
	Round 14	1,711	20.5	6,644	79.5	6,548	78.4	6,401	76.6	8,355
	Round 15	1,350	16.9	6,618	83.1	6,516	81.8	6,428	80.7	7,968
	Round 16	947	17.5	4,466	82.5	4,436	82.0	4,376	80.9	5,413
Total		**25,418**	**19.4**	**105,913**	**80.6**	**104,632**	**79.7**	**102,185**	**77.8**	**131,331**

Screening Interview Results

The screening interview consisted of 5 questions, three of which were used to find TB suspects. The first question was "Are you on TB treatment now?". The second and third question inquired about cough and the duration of cough. Information was available for 104,633 of 105,915 participants (98.8%). Depending on cluster, between 0% and 10% of the participants said that they were currently on TB treatment. This large variation was probably due to misunderstanding of the question, since the questionnaire had not been translated in local languages, and translation was done on the spot by LHWs. The cluster with the highest rate of persons being "currently on TB treatment" was located in Sindh, where mainly Sindhi is spoken. Another indication that the question had been misunderstood by LHWs conducting the symptom screening interview was that the majority of persons being "currently on TB treatment" was not listed as a suspect in the TB suspect register. We combined information on current TB treatment from the symptom screening questionnaire, the in-depth interview, and the TB suspect

register to decide who was probably currently on TB treatment, and who was probably not. This way, we listed 105 (0.1% of all participants) persons as being most likely on TB treatment at the time of the survey, realizing that this underestimates the true number of persons currently on TB treatment by an unknown proportion.

Table 5. Symptom Screening Results by Sex, Age Groups and Field Teams.

Characteristic		Annex 9 available	Cough for > 2 weeks		Cough of any duration, no X-ray		Currently on TB treatment		Any of these*	
		N	n	%	N	%	n	%	n	%
Sex	Male	44,222	2,343	5.30	242	0.55	48	0.11	2,498	5.65
	Female	60,351	2,720	4.50	391	0.65	57	0.09	2,972	4.92
	Unknown	60	0	0.00	0	0.00	0	0.00	0	0.00
Age	15-24 y	33,013	809	2.45	151	0.46	29	0.09	918	2.78
	25-34 y	24,608	790	3.20	159	0.65	19	0.08	887	3.60
	35-44 y	19,059	896	4.70	113	0.59	15	0.08	975	5.12
	45-54 y	12,543	887	7.07	88	0.70	13	0.10	942	7.51
	55-64 y	7,106	736	10.33	41	0.58	11	0.15	760	10.67
	65+ y	6,502	888	13.66	58	0.89	18	0.28	920	14.15
	Unknown	1,801	57	3.16	23	1.28	0	0.00	68	3.78
Team	1	17,944	1,225	6.82	218	1.21	22	0.12	1,347	7.50
	2	17,884	543	3.03	82	0.46	7	0.04	598	3.34
	3	19,008	986	5.19	66	0.35	17	0.09	1,039	5.47
	4	16,240	856	5.26	108	0.67	21	0.13	923	5.68
	5	15,724	444	2.82	63	0.40	8	0.05	473	3.01
	6	17,832	1,009	5.66	96	0.54	30	0.17	1,090	6.11
Total		**104,633**	**5063**	**4.84**	**633**	**0.60**	**105**	**0.10**	**5,470**	**5.23**

*Numbers in the left columns do not sum up to the numbers presented in this column because persons could have a combination of signs and symptoms.

5,062 (4.8% of all persons screened on symptoms) complained of cough for >2 weeks and 354 persons (0.3%) had cough for a shorter or unknown duration, but were listed as TB suspects because no X-ray result was available. Details by sex, age groups and field teams are given in Table 5. Men more often complained of cough than women ($p<0.001$), and the prevalence of cough increased with age ($p<0.001$).

X-ray Screening Results

X-ray images were taken from 102,187 participants (96.5%). The majority of X-ray images showed no abnormal shadows, but 7,846 persons (7.7%) had any abnormality on X-ray image. The proportion of men with an abnormal X-ray image was slightly higher than the proportion of women (p=0.001). X-ray abnormalities were increasingly diagnosed with increasing age: 2.8% of those between 15 and 24 years of age had abnormal shadows in their lungs, compared with 25.4% of those aged 65 years and above (p<0.0001; Table 6). The proportion of abnormalities found among the screened population varied per team, with Team 5 finding abnormalities in 6% of the screened population and Teams 4 and 6 concluding that 8.3% of the screened population had any abnormality on chest X-ray (p<0.001, Table 6).

Table 6. Field X-ray Results by Sex, Age Group and Field Team.

Characteristic		Field X-ray result						Total
		Normal		Abnormal		Unknown		
		n	%	n	%	n	%	N
Sex	Male	39,523	91.2	3,498	8.1	323	0.7	43,344
	Female	53,990	91.8	4,347	7.4	451	0.8	58,788
	Unknown	53	96.4	1	1.8	1	1.8	55
Age group	15-24 y	31,034	96.5	903	2.8	233	0.7	32,170
	25-34 y	22,602	94.7	1,041	4.4	214	0.9	23,857
	35-44 y	17,015	91.4	1,476	7.9	130	0.7	18,621
	45-54 y	10,826	87.7	1,433	11.6	84	0.7	12,343
	55-64 y	5,714	80.9	1,298	18.4	49	0.7	7,061
	65+ y	4,730	73.7	1,633	25.4	55	0.9	6,418
	Unknown	1,645	95.8	62	3.6	10	0.6	1,717
Field team	1	15,533	90.9	1,246	7.3	301	1.8	17,080
	2	15,947	91.6	1,363	7.8	108	0.6	17,418
	3	17,089	91.4	1,534	8.2	76	0.4	18,699
	4	14,337	90.8	1,314	8.3	144	0.9	15,795
	5	14,683	93.7	936	6.0	49	0.3	15,668
	6	15,977	91.2	1,453	8.3	97	0.6	17,527
Total		**93,566**	**91.6**	**7,846**	**7.7**	**775**	**0.8**	**102,187**

TB Suspects

Using the screening information listed above, 10,471 persons could be listed as TB suspects. The overlap between the different signs and symptoms of TB among those eligible for sputum examination is shown in Figure 14.

The TB suspect register (different from screening information) listed 9,845 persons, of whom 1,445 did not qualify for sputum examination based on the information available in the symptom screening questionnaire or on the X-ray form (see section 5.4). On the other hand, the suspect register missed 1,814 persons qualifying for sputum examination based on the screening information. All 1,445 participants being listed in the TB suspect register were included in the field laboratory register, and 1,338 (92.6%) of them provided at least one sputum specimen for examination. The results of these persons are included in the sensitivity analysis (section 7.10).

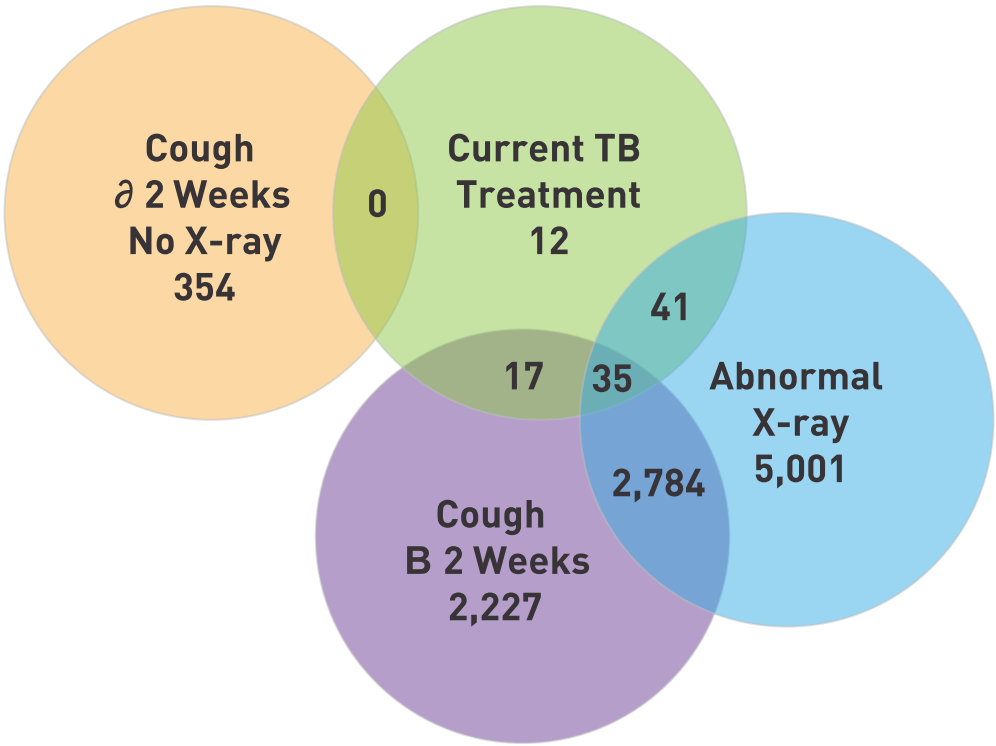


Figure 14. Venn Diagram Showing the Overlap of Occurrence of Different Types of Symptoms.

7.3. Laboratory Examinations

As explained in section 5.9, our primary analysis included only the laboratory results of participants being eligible for sputum examination because of symptoms or signs of TB according to survey definitions for TB suspects (see section 5.4). Smear grading results of participants eligible for sputum examination are depicted in Table 7; 180 spot smears and 199 morning smears were positive (respectively accounting for 2.1% and 2.9% of all smears received). Of the positive smears, the majority was graded 1+ or higher (84.4% of the spot and 88.9% of the morning smears respectively).

Table 7. Final Grading Results of the Spot Smear* and of the Morning Smear Among Participants being Eligible for Sputum Examination

Smear Grading	Spot Specimen		Morning Specimen	
	n	%	n	%
Negative	8,198	78.29	6,615	63.17
Scanty	28	0.27	30	0.29
1+	68	0.65	80	0.76
2+	42	0.40	43	0.41
3+	42	0.40	46	0.44
Not available*	2,093	19.99	3,657	34.93
Total	**10,471**	**100.00**	**10,471**	**100.00**

* This includes 99 persons not able to produce a sample and 142 persons who refused to produce a sample

In total, there were 317 positive cultures, of which 312 were of persons being eligible for sputum examination (Table 8). Results of all positive cultures are presented in section 7.10 (sensitivity analysis). Among persons eligible for sputum examination, 259 confirmed MTB-positive cultures were found while 19 positive cultures had a provisional MTB identification result only, and 34 positive cultures grew NTM.

Table 8. Culture Results of Participants Qualifying for Sputum Smear Examination.

Culture Result	None	MTB	NTM	MTB on provisional identification only*	Total
1-4 colonies		27	12	4	43
5-9 colonies		22	4	5	31
1+		97	16	7	120
2+		44	1	1	46
3+		66	1	2	69
Mixed growth		3	0	0	3
Total with positive culture result		259	34	19	312
Culture negative after 8 weeks	6,909				
No culture results**	3250				

* These are cultures in which the identity was assessed on culture growth rate and morphology only, but was not confirmed by strip test (MPB64) or nuclear acid amplification test; these include n=2,447 persons not listed in the NTRL register, n=363 for whom culture could not be done since not enough sputum was available for culture, and n=440 for whom the culture was contaminated.

7.4. Expert Committee Consultation Results

On 7 and 8 March 2012, the expert review committee (ERC) reviewed 118 cases including 46 culture positive cases with 5 or more colonies, 27 culture positive cases with less than 5 colonies, 33 subjects with a negative culture, 6 subjects with a contaminated culture result and 6 subjects for whom culture was not done (Table 9). All those with a negative or contaminated culture or no culture done had one AFB positive smear. For 16 of these, although the final smear results were negative, one of the smears had been reported as scanty positive (1-2 AFB) by the initial reader. All these suspects were declared having no TB by the ERC.

Table 9. Summary of Cases Discussed in Expert Review Committee.

Reason for Discussion in Expert Review Committee	Decision reached by Expert Review Committee				Total
	Definite TB case	Probable TB case	Not a TB case	NTM	
Culture MTB Positive, ∑ 5 Colonies					
2 smears negative	35			1	36
1 smear negative, 1 not done	5				5
2 smears positive	1				1
1 smear positive, 1 not done	4				4
Culture MTB Positive, < 5 Colonies*					
2 smears negative	14*		8	1	23
1 smear negative, 1 not done	4*				4
Culture Negative					
1 smear positive, 1 smear negative		11*	4		15
1 smear positive, 1 not done			3		3
2 smear negative, but 1 smear reported positive on initial reading			13		13
1 smear negative, 1 not done, but smear reported positive on initial reading			2		2
Culture Contaminated					
1 smear positive, 1 negative	2**	3*			5
1 smear positive, 1 not done		1*			1
Culture Not Done					
1 smear positive, 1 not done	1**	4*			5
2 smears negative, but 1 smear reported positive on initial reading		0	1		1
Total	**66**	**19**	**31**	**2**	**118**

* All those had an abnormal X-ray result indicative of TB; ** all those had a positive NAAT result.

7.5. TB Cases Identified in the Survey Including DST

In total, using the definitions listed in section 5.4, 345 TB cases were identified in the survey, of which 236 (68.4%) had smear-positive TB and 317 (91.9%) had definite TB (Table 10). The majority of the definite cases presented with 5 or more colonies. When taking only participants being identified as TB suspects on information available in the screening forms into account, 341 TB cases remained, of whom 233 (68.4%) were smear-positive (two cases occurred among persons that had no screening information available) and 315 (92.4%) had definite TB. These latter figures were used for calculating final prevalence rates.

Table 10. Definite TB cases Identified in the TB Prevalence Survey by Case Definition.

Definite TB Cases Case description	Participant Eligible for Sputum Examination?		Total	%
	No	Yes		
MTB culture positive, 5+ colonies	0	244	244	77.0
MTB culture positive, <5 colonies in combination with either one or more positive smears, or an abnormal X-ray	1	25	26	8.2
NAAT positive and 1 or more positive smears	1	46	47	14.8
Total	**2**	**315**	**317**	**100**

Table 11 shows the 236 smear-positive TB cases by case definition. Out of these 236 cases, 233 occurred among participants qualifying for sputum examination. The majority of these also had a positive culture with MTB and/or a positive NAAT result.

Table 11. Smear-positive Definite and Probable TB Cases Identified in the TB Prevalence Survey by Case Type and Per Suspect Definition.

Smear-positive TB Cases Case Description	Participant Eligible for Sputum Examination?		Total	%
	No	Yes		
2 positive smears, no culture/NAAT confirmation (probable TB)	0	7	7	3.0
1 smear positive and abnormal X-ray, no culture/NAAT confirmation (probable TB)	2	19	21	8.9
Smear-positive and culture/NAAT positive (definite TB)	1	207	208	88.1
Total	**3**	**233**	**236**	**100**

One of the definite TB cases was not considered a participant since no screening information was available for this case and it was therefore not included in the main analysis. Strikingly, 125 (40%) of the definite TB cases, of which 82 had smear-positive TB (out of 233 definite smear-positive TB cases, 35%), had no abnormalities on the chest X-ray image identified that were indicative of TB. Eighty-one of the 125 cases with no abnormalities indicative of TB complained of cough with a median duration of 30 days (range: 3-672 days). Fifty-nine of those with X-ray images that showed no indications of TB according to the Central X-ray Reading Unit had been scored as abnormal in the field.

We also applied the case definitions provided in the Tuberculosis prevalence surveys handbook , including as definite (bacteriologically-confirmed) survey TB cases those cases which had one culture or NAAT positive TB specimen combined with at least one of the following conditions:

- AFB-smear positive on at least one slide (smear-positive, culture-positive TB definite case);
- MTB culture or NAAT positive in another specimen (note that this definition could not be applied to the Pakistan survey as only one specimen was cultured, and NAAT was either done on the culture isolate if there were less than 5 colonies, or on the smear-positive slide, and in both cases, this concerned the same instead of another specimen);
- Chest X-ray abnormal finding in lung at central audited reading.

The WHO definition for smear-positive TB cases includes those cases with at least one AFB-smear positive specimen AND at least one of the following conditions:

- MTB culture positive (definite survey TB case);
- AFB-smear positive in another specimen BUT not MTB culture positive AND no isolation of NTM (probable TB case);
- Chest X-ray abnormal at central reading BUT not MTB culture or NAAT positive AND no isolation of NTM (probable TB case).

This resulted in the identification of 272 definite TB cases and 236 smear positive survey TB cases, of which 208 (88.1% of 236 and 76.5% of 272 cases) were definite survey TB cases and 28 were probable TB cases (Table 12).

Table 12. Overview of Cases Identified that are Consistent with the TB Prevalence Survey Handbook Survey TB case Definitions[15].

WHO Definition	Suspect on Screening Annexes?		Total
	No	Yes	
Definite survey TB case			
MTB culture or NAAT positive and smear positive	0	207	208
MTB culture or NAAT positive and abnormal chest X-ray	1	63	64
Total	**1**	**270**	**272**
Smear positive survey TB case			
Smear positive and MTB culture or NAAT positive	0	207	208
2 different smears positive, no MTB culture nor NAAT positive	0	7	7
Smear positive and abnormal chest X-ray, no MTB culture nor NAAT positive	2	19	21
Total	**2**	**233***	**236**

*Used in final prevalence calculation

Drug Susceptibility Test (DST) Results

DST was applied on cultures with pure growth of 5 or more MTB colonies. In total, there were 318 cultures showing growth[1]. Thirty-five of these were growing NTM only[2], while 283 cultures were identified as growing MTB. Among these, there were 34 cultures growing less than 5 colonies, and 3 cultures with mixed growth. DST was applied on 194 (78.8%) of 246 eligible culture isolates. DST was not applied in 38 culture isolates due to failure to isolate pure growth or loss of primary culture slopes. DST results were available for 183 (94/3%) of the 194 MTB cases.

Phenotypic DST results for isoniazid, rifampicin, streptomycin and ethambutol were available for 193 strains, while NAAT results were available for 278 strains (71 Genotype®MTBDRplus assay results, and 229 GeneXpert MTB/RIF results were available), summing up to a total of 357 isolates for which drug susceptibility was tested. Two-hundred-fifty-four of these isolates were MTB isolates. The results of the latter are summarized in Table 13. MDR-TB occurred in 5 MTB of 182 strains (2.8%) tested both for susceptibility to rifampicin and isoniazid. All of the latter strains were from participants who were eligible for sputum examination.

1 Note that one of these is not included in this report since they were from persons who were not listed in the census register and who were therefore considered ineligible; the total number of positive cultures analyzed is thus 317, as presented in section 7.3.

2 This includes one culture from a person not listed in the census register and therefore considered ineligible.

Table 13. DST Results for 254 MTB Isolates.

Drug	Resistant		Susceptible	Total
	n	%	n	N
Rifampicin	6	2.4%	247	253
Isoniazid	17	9.3%	166	183
Streptomycin	38	20.8%	145	183
Ethambutol	7	3.8%	176	183
Multi-drug resistant TB	5	2.8%	177	182

7.6. Outcome of Quality Assurance

Outcome of Spot Smear Rechecking

All positive spot smear slides prepared and read at field level and around 20% of the negative smears were re-read at the NTRL. The results of this rereading are shown in Table 16. Since screening is aimed at obtaining a high sensitivity while the specificity of screening is of lesser importance, high false negative results (i.e. smears were scored as negative in the cluster field laboratory, while these were found positive (1+, 2+ or 3+) by the central team in the NTRL) were considered as the most serious errors. Out of 2,399 slides that were scored as negative in the field and reread at the NTRL, 24 (1.0%) were found to be positive: 10 of these were scored as scanty (1-9 AFB per 100 fields) and 14 (0.6%) were scored as 1+ to 3+ (Table 14). If a high false-positive error was found, the laboratory technicians and the field team leader were informed and all negative smears of that cluster were re-read by NTRL laboratory staff.

Banner at Field Survey Site

Table 14. Results of Rereading of Spot Smears Initially Read in the Survey Field Laboratories by the National Tuberculosis Reference Laboratory (NTRL).

		Spot NTRL Re-checking Result						
		Not done	**Negative**	**Scanty**	**1+**	**2+**	**3+**	**Total**
Spot smear field Microscopy result	Negative	6,699	2,375	10	9	4	1	9,098
	Scanty	0	21	17	7	1	0	46
	1+	0	1	2	47	3	2	55
	2+	0	0	1	1	32	4	38
	3+	0	0	0	4	3	35	42
	Smear not done	9	0	0	0	0	0	9
Total		**6,708**	**2,397**	**30**	**68**	**43**	**42**	**9,288**
SUMMARY								
# Smear Re-checked				Number of Errors Reported				
Total	Positive	Scanty	Negative	HFN	HFP	LFN	LFP	QE
2580	**153**	**30**	**2,937**	**14**	**1**	**10**	**21**	**8**

* Abbreviations used in this Table: HFN: high false-negative, HFP: high false-positive, LFN: low false-negative, LFP: low false positive, QE: quantification error

Culture Quality Indicators

Table 15 summarizes quality indicators of culture that was performed at the NTRL. Slightly more than 5% of the cultures were contaminated, which was in range of the expected value. Cultures from spot specimens were less often contaminated than cultures from morning specimens, while the time lag between collection of a spot specimen and receipt of the specimen at the NTRL was usually longer than for morning specimens. This can probably be explained by the fact that spot specimens were directly stored appropriately in cold chain, while morning specimens were collected at the homes of the TB suspects and were probably exposed to higher temperatures for a longer time before they were brought to the survey field site for appropriate storage. Almost 5% of the samples received at the NTRL were not cultured, since the amount received was too small or sputum containers showed leakage and there was a high probability of cross-contamination.

Table 15. Summary of Culture Results at the National Tuberculosis Reference Laboratory (NTRL).*

Type of specimens for culture	Number of specimens received for culture	Number of specimens NOT applied for culture**		Culture Result Reported							
				Positive (MTB+NTM)		Negative		Contamination		Total Reported	
	n	n	%	n	%	n	%	n	%	N	%
Morning	8,157	356	4.4	281	3.6	7,060	90.5	460	5.9	7,801	95.6
Spot	1,098	77	7.0	36	3.5	947	92.8	38	3.7	1,021	93.0
Extra	28	0	0.0	0	0.0	28	100	0	0.0	28	100
Morning and spot	5	0	0.0	1	20	4	80	0	0	5	100
Total	**9,288**	**433**	**4.7**	**318**	**3.4**	**8,039**	**86.6**	**498**	**5.4**	**8,855**	**95.3**

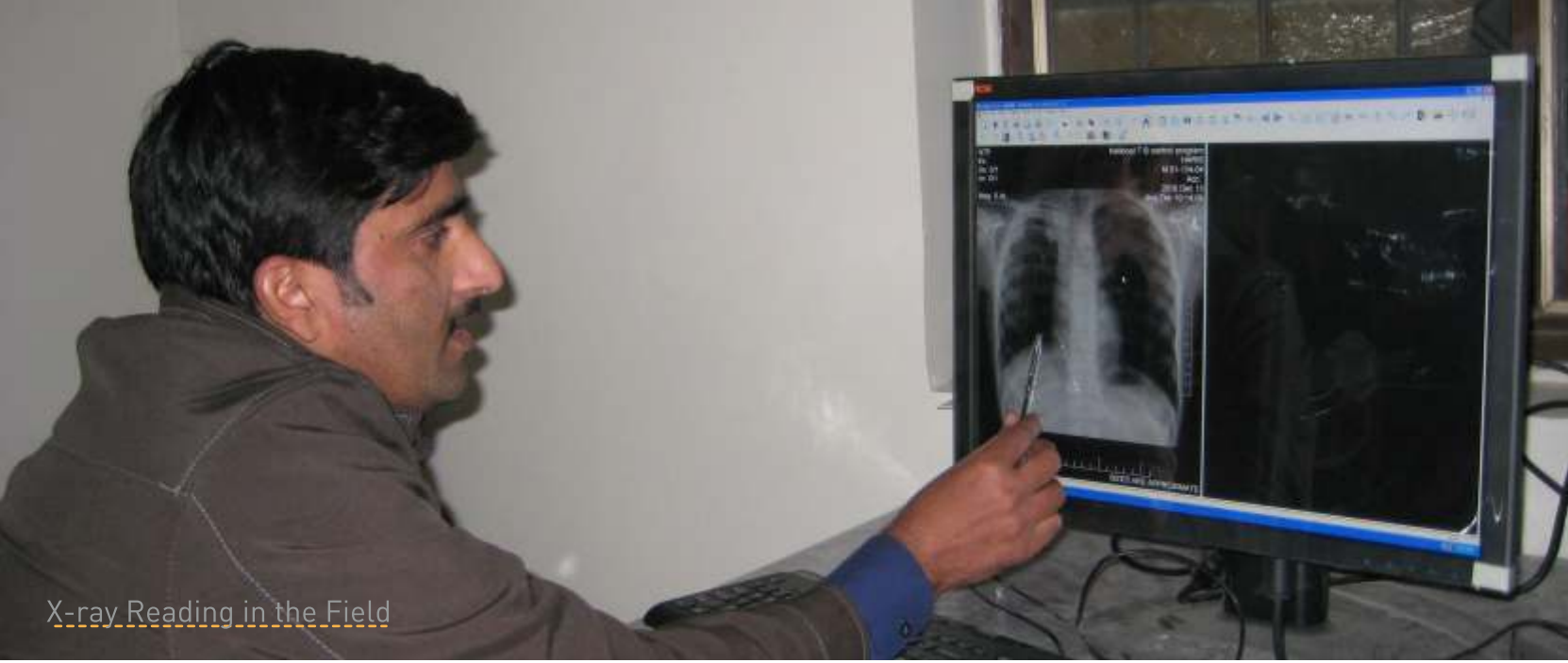
* Abbreviations used in this table: MTB: Mycobacterium tuberculosis; NTM: non-tuberculous mycobacteria, **Due to empty containers received or specimen leakage during transport.

Outcome of Quality Assurance of Drug Susceptibility Testing

For EQA, 65 out of 183 strains tested for drug susceptibility, including all strains with resistance to any drug and 10% of susceptible strains, were sent to the TB laboratory of Agha Khan University Hospital. Table 16 shows a summary of the EQA results. Though the sensitivity of isoniazid and rifampicin susceptibility testing was relatively low, the accuracy was sufficiently high.

Table 16. Summary of Drug Susceptibility Quality Assurance Results.

	Streptomycin	Isoniazid	Rifampicin	Ethambutol
Total number of correct results	50	55	61	61
Truly resistant	20	10	5	6
Falsely resistant	11	4	0	1
True susceptible	30	45	56	55
False susceptible	2	4	2	1
Sensitivity	91%	71%	71%	86%
Specificity	73%	92%	100%	98%
Accuracy	79%	87%	97%	97%



X-ray Reading in the Field

Outcome of Quality Assurance of X-ray Reading Result at Central Level Compared to X-ray Result at Field Level.

It was aimed to reread all X-ray images judged to have abnormal shadows by the field teams and 20% of X-ray images scored as normal for quality assurance. Table 19 shows that 7,466 out of 7807 (95.6%) of abnormal images and 17,631 of 93,445 (18.9%) normal images were reread at central level. Of the 7,466 abnormal images, 4,615 (61.8%) were scored as normal by the Central X-ray Reading Unit, while of 17,631 normal images, 693 (3.9%) were scored as abnormal (among which were 466 (2.6%) images with abnormalities suggestive of TB). Thus, as intended, the sensitivity of reading at the field level was high (96.1%) whereas the specificity was lower.

Table 17. Comparison of X-ray Reading Results at Field Level with Reading Results at Central Level.

X-ray result of central reading	X-ray Result after Field Reading			Total
	Abnormal	Normal	Unknown*	
Abnormalities suggestive of TB	2,277	462	14	2,753
Other abnormalities	576	227	4	807
No abnormalities	4,623	16,932	140	21,695
Number not read	352	75,805	775	76,932
Total	**7,828**	**93,426**	**933**	**102,187**

* This includes X-rays that were not reread and X-rays for which the reading result remained unknown.

7.7. Prevalence Rates of TB

Figure 15 gives a summary of the cluster-specific TB prevalence rates. Most clusters had a prevalence of definite TB of between 0 and 500 per 100,000 population. There was one cluster (56) with 14 definite TB cases, leading to a prevalence of definite TB of above 2000 per 100,000.

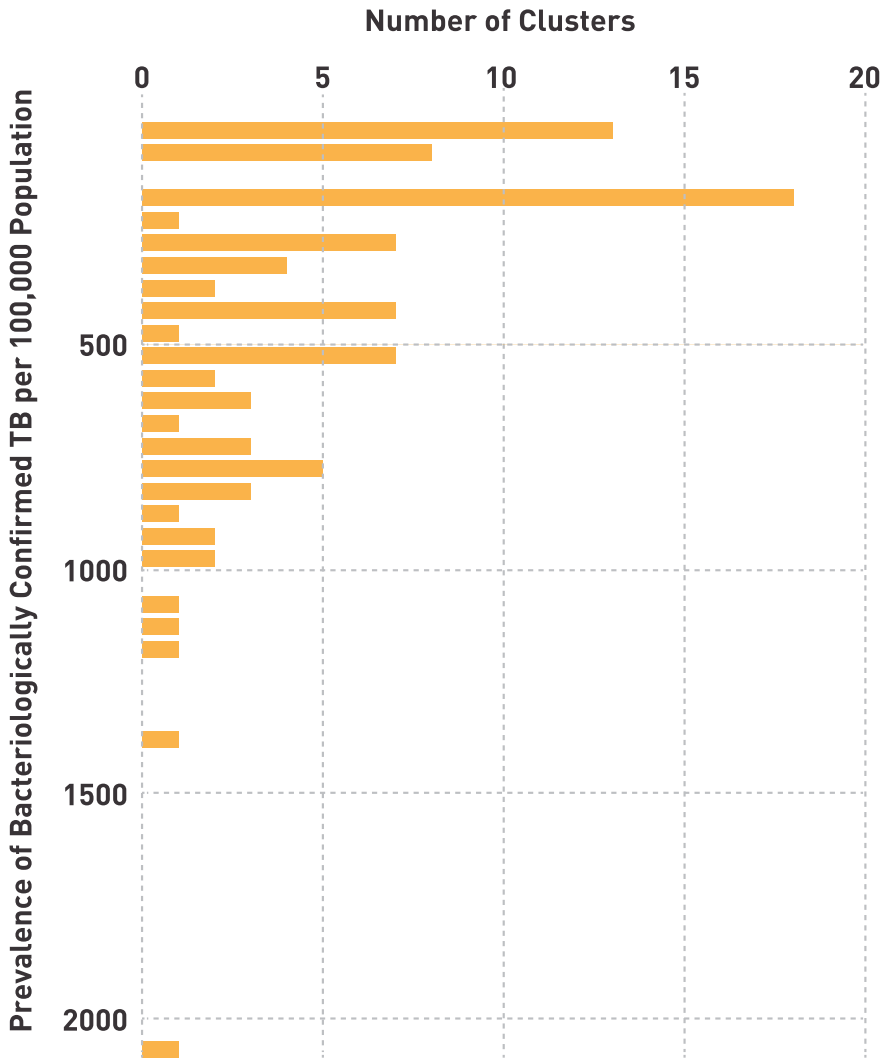


Figure 15. Distribution of Cluster-specific Prevalence of Definite TB over Clusters.
Table 18 gives the prevalence rate of definite TB, smear-positive TB and all bacteriologically confirmed TB per suspect group. The prevalence estimate was 297/100,000, with a 95% confidence interval from 248 to 345/100,000 population.

Table 18. Prevalence Estimates of Definite TB, Smear-positive TB and all Bacteriologically Confirmed TB.

Type of TB	Prevalence estimate	95% confidence interval	
Definite TB	296.6	248.1	345.2
Smear-positive TB	219.1	175.2	262.9
Definite and probable TB	321.1	268.9	373.3

TB was more prevalent among men, increased with increasing age, and was more prevalent in the rural areas than in the urban areas of the sampled areas of Pakistan (Table 19).

Table 19. Prevalence of Definite TB (Bacteriologically Confirmed TB) by Sex and Age Groups.

Characteristic		OR	95% CI		Point Prevalence Estimate	95% CI	
Sex	Male	1	REF		365.1	293.7	436.6
	Female	0.68	0.53	0.85	246.9	196.2	297.6
Age group	15-24 y	1	REF		174.8	124.7	225
	25-34 y	0.88	0.55	1.41	154.6	99.1	210.2
	35-44 y	1.65	1.09	2.49	287.4	194.6	380.1
	45-54 y	2.26	1.52	3.35	393.8	272.7	514.8
	55-64 y	2.75	1.78	4.25	479.6	303.6	655.5
	65+ y	6.76	4.69	9.74	1170.2	870.8	1469.5
Type of Area	Rural	1	REF		351.8	280.9	422.7
	Urban	0.65	0.47	0.9	229.9	171.6	288.2
Province	Punjab	1	REF		291.8	234.4	349.2
	Sindh	1.07	0.69	1.67	313.2	190.6	435.8
	Balochistan	0.64	0.51	0.79	185.8	170.7	200.9
	AJK	1.04	0.62	1.74	304.3	159.6	449
	KPK	1.1	0.63	1.91	320.2	154.6	485.9
	Gilgit-Baltistan	0.6	0.5	0.74	176.5	--	--

Design Effect

The design effect of the cluster design varied between 2.14 for the prevalence of definite TB and 2.28 for all TB (i.e. including definite and probable TB) when ignoring laboratory results of those not eligible for sputum smear examination. When including all those with sputum examination, as was done in the sensitivity analysis described below, the design effect varied between 2.22 for the prevalence of definite TB and 2.44 for the prevalence of all TB. In general, for a well designed study, the design effect usually ranges from 1 to 3 . Design effects calculated for this study point out that there was intra-cluster correlation with respect to the prevalence of TB, but that these effects were within limits.

7.8. Patient Diagnostic Rate and Case Detection Rate

As shown above, the point prevalence estimates for TB in this survey were was 297 per 100,000 population for bacteriologically confirmed TB and 219 per 100,000 population for smear-positive TB, respectively. In 2011, 105,733 TB cases were notified with smear-positive TB (of which 3,895 cases occurred in children aged below 15 years)[4] in a total population of around 177 million[3], of which 63.3% were aged 15 years or above[17]. The sample we included covered 93.5% of Pakistan's population (FBS 2010, unpublished data). Assuming that the prevalence of TB is similar in the excluded areas[4], this leads to an estimated PDR of [(105733-3895)/(0.633*1770)]/219=90.9/219=41.5%, and a CDR of 0.444/(0.444+0.5)=45.4%. However, it should be noted that this may be an underestimate of the true PDR and CDR, since the TB prevalence survey did not cover the whole Pakistani population as 6.5% of the population was excluded. Moreover, the estimate is dependent on the estimated population size and on the proportion of adults in that population, and both estimates vary over sources, as is explained in footnote 3 on the previous page. Using the CIA World Fact Book estimate of 184.4 million, the PDR would be estimated at 39.8% and the CDR at 44.3%.

7.9. Cases Detected in the Routine TB Program

Of 317 definite TB cases, 24 (7.6%) were on TB treatment at the time of the survey, while this remained unknown for 1 case due to inconsistencies between answers on different questions about current TB treatment. The proportion of patients being currently on TB treatment among smear positive TB cases was slightly higher: 11.0% (26/236) of the cases was on TB treatment at the time of the survey. Among probable TB cases, 4 of 28 (15.4%) patients were on TB treatment at the time of the survey.

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3 Note that the WHO factsheet[4] mentions this number, while the CIA World Fact Book estimates the total population at 184,404,791 in July 2010.

4 It is likely that the prevalence in the excluded areas was somewhat lower than the overall TB prevalence, since excluded *tehsils* were located in Balochistan, Gilgit-Baltistan and Khyber Pakhtoon Khwa, of which Balochistan and Gilgit-Baltistan had a relatively low TB prevalence in this study. This points to a slight underestimation of the PDR and the CDR.

7.10. Sensitivity Analysis

In the analyses above, we only included the sputum examination results of those who qualified for sputum smear examination. However, the TB suspect register listed 9,890 persons, 8,414 (85.1%) of whom were eligible for sputum examination based on information obtained during the screening. Thus the register missed over 2,000 persons qualifying for sputum examination based on information collected during screening whereas it listed 1,476 persons not participating in screening or not qualifying for sputum examination. We expected the screening forms to be more reliable than the suspect register, since the latter was copied from these forms and was sometimes filled directly after the in-depth interview. The suspect register was used to check the completeness of sputum collection. Therefore, of all those listed in the TB suspect register, 96.3% also occurred in the field laboratory register, whereas of all those qualifying for sputum examination, only 82.4% occurred in the field laboratory register.

In the sensitivity analysis presented here, we compare the results presented above with the results when including all those having submitted at least one sputum specimen. Among those with a positive smear result, only 2 spot smear results were scanty positive and 1 of the morning smear results was scanty in one and 1+ in another smear (Table 20).

Table 20. Comparison of Sputum Smear Results of those Qualifying for Sputum Examination and those Submitting at Least One Sputum Specimen for Examination.

Smear Grading	Participants eligible for sputum examination		Persons submitting at least one sputum specimen		Difference between these two groups	
	Spot specimen	Morning specimen	Spot specimen	Morning specimen	Spot specimen	Morning specimen
NEG	8,198	6,615	9,592	7,601	1,394	986
Scanty	28	30	30	31	2	1
1+	68	80	68	81	0	1
2+	42	43	42	43	0	0
3+	42	46	42	46	0	0
Not available*	2,093	3,657	355	2,327	NA	NA
Total	**10,471**	**10,471**	**10,129**	**10,129**	-342	-342

* This includes 99 persons not able to produce a sample and 142 persons who refused to produce a sample; respectively 126 and 169 (for all persons submitting a sputum sample)

Persons not qualifying for sputum smear examination but having a positive culture result all had low graded cultures (1-4 colonies in case of MTB, and 1-9 colonies if NTM was found). It is possible that these persons were in early stages of TB. In-depth analysis of their potential signs and symptoms of TB is warranted.

Table 21. Comparison of Culture Results of those Qualifying for Sputum Examination and those Submitting at Least One Sputum Specimen for Examination.

Culture Result	Taking only TB suspects on screening				Taking all those with sputum into account				Difference between these two groups; MTB and "MTB" combined
	MTB	NTM	"MTB"*	Total	MTB	NTM	"MTB"*	Total	
1-4 colonies	27	12	4	39	29	13	5	45	3
5-9 colonies	22	4	5	31	22	5	5	31	0
1+	97	16	7	120	97	16	7	120	0
2+	44	1	1	46	44	1	1	46	0
3+	66	1	2	69	66	1	2	69	0
Mixed growth	3	0	0	3	3	0	0	3	0
Total	**259**	**34**	**19**	**312**	**261**	**36**	**20**	**317**	**3**

* These are cultures in which the identity was assessed on culture growth rate and morphology only, but was not confirmed by strip test (MPB64) or nuclear acid amplification test.

In Tables 10 and 11, the number of TB cases found is compared between those qualifying and those not qualifying for sputum examination. Among those not qualifying for sputum examination, two definite (Table 10) and two probable TB cases were found (Table 11).

In Table 22, the TB prevalence estimates for definite TB, smear-positive TB and all pulmonary TB are provided. In the first rows, prevalence estimates are provided if all laboratory results of those not qualifying for smear examination are ignored (i.e., it is assumed that there are no TB cases among those not qualifying for sputum examination. In the middle rows of the table, all sputum results are included. The point prevalence estimate was lower when taking all sputum results into account than the prevalence estimate presented above, since all eligible persons were included and not only the participants. This was done because 134 'non-participants' had participated in sputum examination of whom one had two positive smears and was qualified as a definite TB case. When repeating the analyses including participants only, the prevalence estimates were very similar to those produced in the primary analyses.

Table 22. Prevalence Estimates of Definite TB, Smear-positive TB and all Bacteriologically Confirmed TB for those Qualifying for Sputum Examination and those Submitting at Least One Sputum Specimen for Examination.

Persons included in the analysis*	Type of TB	Prevalence estimate	95% confidence interval	
Suspect as according to info on screening forms	Definite	296.6	248.1	345.2
	All smear-positive	219.1	175.2	262.9
	Definite and probable TB	321.1	268.9	373.3
Any *person* submitting a sputum specimen	Definite	239.7	199.8	279.6
	All smear-positive	178.4	142.3	214.5
	Definite and probable TB	261.1	217.9	304.2
Any *parti ipant* submitting a sputum specimen	Definite	297.6	248.9	346.2
	All smear-positive	221.2	177.2	265.2
	Definite and probable TB	324.1	271.7	376.6

* See text above this table for explanation.

A workshop on final analysis of the tuberculosis prevalence survey was carried out in Geneva from 26th - 28th November, 2013. During this workshop, three different methods that were recently recommended as best practice methods[1] were applied to the data of the Pakistan prevalence survey. The first method included only participants with smear and culture results and applied robust standard errors. Results of the first method were similar to those presented in table 22, but not identical due to program calculation differences. The second method included all individuals eligible for participation in the survey, irrespective of participation, applying multiple missing value imputation. The third method, which is regarded as the most robust method currently, applied multiple missing value imputation for those participants for whom smear and/or culture results were missing, followed by inverse probability weighting to represent all individuals eligible for participation. The results of these three methods are provided in Table 23.

[1]Floyd S, Sismanidis C, Yamada N, Daniel R, Lagahid J, Mecatti F, Vianzon R, Bloss E, Tiemersma E, Onozaki I, Glaziou P, Floyd K. Analysis of tuberculosis prevalence surveys: new guidance on best-practice methods. Emerging Themes in Epidemiology 2013, 10:10

Table 23: Prevalence of pulmonary tuberculosis among persons aged 15 years and older in Pakistan, excluding non-accessible areas.

Type of TB	Method 1	Method 2	Method 3
All bacteriologically positive TB	**328**	**361**	**396**
95% CI	*275-381*	*308-414*	*332-458*
Smear positive TB	**224**	**252**	**270**
95% CI	*179-269*	*205-298*	*217-322*

Subsequently, the figures produced by method 3 were extrapolated using the notification data of the country for all ages and all forms of TB. This resulted in a final prevalence estimate for all forms of TB of 348 per 100,000 population, and an incidence of all forms of TB of 276 per 100,000 population per year.

Table 24: Estimated prevalence and incidence of all forms of TB for all ages for Pakistan.

Estimate	**Point estimate**	**95% confidence interval**
TB prevalence (all forms, all ages), per 100,000 population	348	287-409
TB incidence (all forms, all ages), per 100,000 population per year	276	158-424

8 DISCUSSION

8.1. Summary of the Survey Results

To our knowledge, this survey was the second largest TB prevalence survey conducted ever. Only a survey in China was larger, including 176 clusters, a total population of 263,281 persons and 252,940 participants, and 347 pulmonary TB cases.

The 4th Pakistan TB prevalence survey enumerated 131,331 eligible persons in 95 clusters, of whom 105,915 (81%) participated. A total of around 10,000 sputum smears were examined, over 7,500 cultures were performed, and a total of 345 TB cases were identified (of which 341 TB cases had been eligible for sputum examination), leading to a prevalence estimate for the population of 15 years and older of 296.6 per 100,000 population (95% confidence interval, 248.1-345.2) for definite TB and an estimated patient diagnostic rate of 41.5% and a case detection rate of 45.4%. This estimate is valid for the country with exception of the unsafe areas that were excluded.

The prevalence of sputum smear positive TB in the survey was 219.1 (95% CI, 175.2-262.9), which is higher than the prevalence found in the previous TB prevalence survey held in Pakistan in 1986/1987. However, it is difficult to compare results of these two surveys, as the exact sampling design and the methodology used for screening in the 1986/1987 survey remain unknown.

The prevalence found in this survey of definite and probable TB combined was 321.1 (95% CI, 268.9-373.3) and is within the ranges estimated by WHO, which estimates a prevalence of all types of TB of 350 per 100,000 , with a wide confidence interval of 158 to 618 per 100,000 population. The information produced by this survey may be used to decrease the size of these confidence intervals.

8.2. Eligibility, Enrollment and Participation

It was calculated that a sample size of 133,000 adults was needed, assuming that 85% of these would participate in the survey (Annex II). Based on this sample size and the number of clusters, a sampling interval was calculated and a sample of clusters was drawn. However, as since 1998, no census had been done in Pakistan, the population list was the result of population projection, assuming a constant growth rate of the population of 2-3% per year, and assuming that this was similar for all *tehsils*. These assumptions are questionable, but since the next survey, intended to take place in 2011 was not (yet) conducted, these assumptions cannot be checked. In the first pilot cluster, the participation rate remained well below the expected participation rate of 85%. Although major interventions were introduced and continuous efforts were done to mobilize the clusters' populations, the participation rate (80,7%) remained below 85% in 74 of the 95 clusters. On the other hand, the prevalence of smear-positive TB was slightly higher, and the design effect was smaller than expected (see Annex II).

8.3. Screening Outcome

Symptom screening was done by LHWs who interviewed participants using a structured questionnaire. This was done in the reception area of the survey. In practice, the LHW (who was usually well known to the area's population) was often surrounded by participants awaiting their symptom screening while conducting the interviews and this may have lead to the inconsistencies and missing data observed on the questionnaires. Such problems also had been encountered during monitoring visits, and were repeatedly addressed with the field team leader. However, it remained practically impossible to organize a one-to-one person interview in the reception area.

Symptom screening resulted in identification of 10,471 (9.9%) participants eligible for sputum examination: 5.2% of these were identified by symptom screening and 7.7% had abnormal shadows on chest X-ray. This is in line with what was expected and similar to rates observed in other prevalence surveys[15].

8.4. Laboratory Examinations and Chest X-ray

At the time that the protocol was written (2009 and 2010), the Tuberculosis prevalence surveys handbook[15] was not available. Introduction of that Handbook triggered the introduction of NAAT tests in the survey. First, slides for all cases with at least one positive smear result, but a negative, contaminated or no culture result, were sent to the SNRL in Korea for Genotype®MTBDRplus testing directly on the slide. Meanwhile, the GeneXpert MTB/RIF became available to the Pakistan NTP, which proved to be more sensitive. Therefore, GeneXpert testing was done for cases with at least one positive smear result, but a negative, contaminated or no culture result, as well as for cases with a positive MTB culture with less than 5 colonies. Also, during the survey, MPB64 was introduced as an additional test for the species identification of positive cultures. However, introduction of these new techniques was not formalized in adapted protocols.

Also, during the survey the CXRU decided to change the central X-ray reading forms since some inconsistencies were identified. While the form in its original format had been used in different (early) clusters, only the 2nd pilot cluster had been entered in the old format data entry sheet, while more clusters were using old format forms. Though this led to some confusion when cleaning and validating the data, it was possible to resolve all inconsistencies and everything which wasn't clear.

The proportion of high false-negative errors was relatively high, whereas the culture contamination rates were acceptable. If a high false-negative error was detected in a cluster, all negative smears were re-read and the field team leader was contacted.

The 'sensitivity' of X-ray screening at field level was high at a cost of a lower specificity, which is preferable in surveys where it is intended to maximize suspect finding. Surprisingly, the proportion of bacteriologically confirmed, and even of sputum smear positive TB cases with a normal X-ray was much higher than is usually found in surveys. This may partially be the result of different case definitions: the TB prevalence surveys handbook (Lime Book) requests confirmation of an MTB positive culture result with an AFB-positive smear, an

abnormal X-ray, or another specimen being MTB culture-positive, whereas in the Pakistan survey, confirmation of a positive culture was not required for being defined as a definite TB case if there were more than 5 colonies. This finding needs further analysis.

8.5. Prevalence Rates of TB

TB was more prevalent among men, increased with increasing age, and was more prevalent in the rural areas than in the urban areas of the sampled areas of Pakistan.

While the male to female ratio is 1 among notified TB cases, this was 1.5:1 in the TB prevalence survey, suggesting that TB is more often under diagnosed in men than in women. Alternatively, male TB cases might be missed more often by the NTP than female cases. This finding needs in-depth analysis. A higher male to female ratio than expected based on notification data was also found in the Vietnam TB prevalence survey[19]. The most probable explanation for this finding in Vietnam was that men are less likely to seek health care in the public sector than women, and, although women had longer health care seeking delays than men[20], an analysis of TB laboratory data in the north of Vietnam suggested that women were more likely than men to have or request a sputum smear examination[21]. As expected, the TB prevalence increased with age. This phenomenon fits with an aging epidemic and does also occur in notification data of Pakistan. It was also described in other TB prevalence surveys (e.g. Hoa et al, 2010[18]). While TB was less prevalent in urban than in rural areas, it was more prevalent in the densely populated provinces Punjab and Sindh than in Western provinces of Balochistan and Khyber Pakhtoon Khwa. In-depth analysis of these data is needed to explain these patterns.

8.6. Strengths and Limitations of the Survey and Analysis

Strengths

This TB prevalence survey was one of the biggest of its type, and it was conducted under difficult conditions, including severe flooding of huge areas of Pakistan in July and August 2010 when the preparations of the prevalence survey were in full swing, long distances and inclusion of areas that were difficult to reach, extreme weather conditions (both in winter in the Northern areas, and in summer in all areas), security threats, populations that were speaking different languages and some could hardly speak Urdu (the lingua franca of Pakistan), low education level especially of the flexible field team members and initial high staff turnover. In the light of these challenges, fulfilling the data collection for this TB prevalence survey has been a great achievement of the Pakistan NTP.

Name, father or husband name, age and sex had been collected on almost all data collection forms and registers and this made it possible to check and correct wrong PIN merges.

Challenges Addressed

With respect to the flood disaster, the NTP of Pakistan developed an assessment format to review the situation. It

was decided to proceed with the survey since the majority of 95 clusters were not affected by the flood. However 12 clusters, mostly in Sindh, were either mildly (1), moderately (9) or severely (2) affected, defining affection as damage to infrastructure, displacement of population and return to normal situation in more than 6 months time (severe), within 3 months (moderate) and within 4 weeks time period (mild). The affected clusters were visited last so that by the time field work started the situation had returned to normal.

Another challenge for implementation of the prevalence survey was the constant security concern in some of the areas in Pakistan. A complete security assessment was done and a security and safety handbook was prepared in consultation with international consultants in May 2010 with clear guidelines about the safety measures that should be observed during field work to avoid any untoward situation. The security assessment was done in some of the high risk areas based upon the criteria agreed upon by the Provincial TB control Programs and NTP. During the implementation of the survey, three *tehsils* from Balochistan (Lehri, Quetta and Awaran) were replaced by three clusters from the five clusters that had been selected as backup: Sharda in Azad-Jammu and Kashmir, Khan Pur in Punjab, and Hub in Balochistan due to security concerns.

Further challenges included managing transport of field teams and equipment. The maintenance of chest X-ray equipment was challenging in remote areas, as for minor trouble shooting the X-ray maintenance team had to visit the cluster. Fortunately, problems with X-ray equipment remained limited and the progress of the field work was not affected. The most remote clusters in Sindh were reached in hot weather in July which, together with the relatively long transportation time, contributed towards contamination of sputum specimens in these specific clusters. Maintaining cold chain before and during specimen transportation in extremely hot weather (May-July with temperatures touching 45 C/113 F and above) was challenging.

Initially, there was a relatively high staff turnover which was dealt with by increasing the staff's motivation by improving salaries, installing incentives and offering (re)training.

At central level, during the data collection period, the NTP had to move several times to another location. This lead to unrest among the team, time loss because of restructuring, and challenges in storing and retrieving all forms.

Challenges with Data Cleaning and Validation

When analyzing the data in 2012, major problems were discovered with PIN allocation. The most common error in PIN coding was assigning the correct cluster and household number, and allocating a new (higher) PIN. A new, higher PIN was probably given to persons who lost their survey identification card. Therefore, before presenting the results of the survey, PINs on all forms and registers were checked against names, father names and age and corrected if needed. On all data collection forms, except for X-ray images and forms, at least the participant's name, age and sex were available, and in the census register, on the symptom screening questionnaire and the in-depth questionnaire, also father name was available. Together, this information uniquely identified participants within clusters. The weakest link in this PIN check actions formed the X-ray forms. X-ray images were stored with PIN and participant name; no age and sex were available of the participant

on the form or with the digital image. Since participant name alone was not always unique within a cluster, it is possible that X-ray forms were not always merged to the correct person. There were 231 records with information about central X-ray reading while field X-ray reading information was not available in the data file. On the other hand, these records may also belong to persons for whom an X-ray was made but no form was filled. We randomly checked the presence of a digital image in the server for 20% of these X-rays and discovered that 76% of these were available as a digital image while the form was missing.

Cleaning the PINs resulted in a marked increase in successful and complete merges, and thus a decrease in the enumerated population from 133,440 to 131,377 persons and an increase in the participation rate from 79.6% to 80.7%.

Table 23 briefly summarizes the most important challenges and the actions taken to address these.
TB prevalence estimates: primary versus sensitivity analysis

As explained in section 7.10, there was much inconsistency between the information occurring on the screening forms compared to the information listed in the TB suspect register. Since information given on data collection forms of participants was likely to be the most precise, we used the information available on the screening forms to define persons eligible for sputum examinations using the definitions listed in section 5.4. (i.e. participants (having at least information on the symptom screening or the X-ray screening form) being currently on TB treatment, having cough for more than 2 weeks, having abnormal shadows on the X-ray image, or a combination of those; or having cough of any duration while no X-ray was done). When using this definition for the calculation of TB prevalence estimates, all laboratory results of those not complying with this suspect definition were ignored. A sensitivity analysis included all sputum examination results. This resulted in prevalence estimates that were fairly similar to those produced in the primary analysis.

We conclude that a prevalence of definite TB of 297 per 100,000 population is the best estimate for the prevalence of bacteriologically confirmed TB in this survey population.

8.7. Future Statistical Analysis

This report presents the initial key findings of the Pakistan prevalence survey. Further in-depth analysis will be needed to digest all the valuable information the survey generated. Additional statistical analyses, like estimation of the point prevalence and surrounding confidence intervals using missing value imputation and random effects modelling, and in-depth analysis of patterns observed, will be conducted by a PhD student and will be presented in separate peer-reviewed publications.

Table 23 Challenges of the Pakistan TB Prevalence Survey and Actions Taken to Mitigate Their Influence.

Challenge	Possible cause(s)	Actions taken to address the challenge (if any)
Flooding in summer of 2010		An assessment was made for all clusters. Worst affected clusters were visited last.
Security concerns		A security plan was developed and the least secure areas were not included in the sampling frame.
Low motivation of field teams	High workload, security situation, weather conditions, low understanding of aims of survey	Increased salary, offering re-training
Problems with PINs	Chaotic situation at reception area; participants not bringing their survey identification cards while LHW knew them	During data validation, check all merges by name, father name and age, and correct wrong PINs with the PIN appearing on the census register.
Low participation rate	Mistrust of official bodies	Evening timing of cluster field work; routine medicines such as cough syrup, antibiotics, and antipyretics provided for free to symptomatic cluster site participants; involving local leaders were involved to increase trust of local community in survey team; free transportation for persons living relatively far away from the screening site; giving small present to children living in the selected area
Inconsistencies within and between forms and questionnaires, and missing data	Absence of independent data checker in each field team	Field team leader was requested to check and sign each form, but work load was too high to permit thorough checking.
Misunderstanding of specific questions (Q1 of the symptom screening questionnaire - are you on TB treatment now?)	Questionnaire only existed in Urdu and was not translated into major local languages, while the majority of the population is not fluent in Urdu. LHW probably made own translations of the question.	At data validation, compare the information collected in the screening interview, the suspect register and the in-depth questionnaire to decide who was probably on TB treatment at the time of the survey.
Introduction of new forms, procedures and techniques	Introduction of new forms, procedures and techniques was not formalized in protocol and SOPs	

Challenge	Possible cause(s)	Actions taken to address the challenge (if any)
Listing non-suspects in the suspect register and missing suspects	Suspect register was often filled after the in-depth interview, so that information collected during this interview may have influenced the medical officer.	In the data analysis, two models were run: the first model ignored laboratory results of non-participants and non-TB suspects, the second did not ignore any laboratory data.
Problems with mis-merges and date variables from laboratory	Laboratory Data were entered in Excel	PIN by PIN checks of all suspects with laboratory data.

9 PROGRAM IMPLICATIONS

The bacteriologically confirmed TB prevalence estimate for the population ∑15 years of 297 per 100,000 population (95% confidence interval, 248-345) shows that TB is still prevalent in the country and remains a public health problem. The government of Pakistan should continue to keep TB high on the agenda of the Ministry of Health and develop a comprehensive plan to combat TB with more commitment and resources.

The estimated patient diagnostic rate of 41.5% for adults >15 years and an overall case detection rate of 45.4% suggest that a high proportion of cases present in the community are being missed. There is a need to further strengthen and expand diagnostic and treatment services including augmented and context specific case finding strategies.

The high number of undiagnosed cases detected in the community is alarming. It indicates that people may not be aware of the symptoms of TB and the presence of diagnostic and treatment services. The National Program should develop strategies to enhance the awareness of TB symptoms and the presence of TB services in the community by a comprehensive advocacy, communication and social mobilization (ACSM) strategy and to engage communities effectively.

A higher prevalence of TB cases in males as compared to females (365 versus 246 per 100,000 population) further implies that TB care needs to be expanded to the private sector. The public health care facilities currently offer services only in the morning hours, which is a challenge for the working population.

Similarly, the high prevalence in older age groups and in rural areas demonstrate the need for improved case finding by actively engaging trained community health workers in suspect identification and referral.

68% of all TB cases diagnosed in the survey were smear positive, and out of these more than 85% were reported as 1+ or above and most of them were not on treatment. This important finding indicates that the target of 70% case detection can be achieved by improving access to TB care and that further expansion of microscopy network is needed.

Around 45% of smear positive cases were reported high positive, suggesting significant delays in diagnosis. This may indicate that patients do not seek health care even when their disease has reached an advanced stage or, when they do come forward, are not recognized as having TB symptoms by the health care providers. This finding calls for improving awareness about disease symptoms, availability of free TB care and contact tracing in the community. Further analysis of questions on health seeking behavior and awareness among the health care providers, will help the program to design interventions to improve health seeking behavior.

In 32 of the 95 clusters, the prevalence estimate was found to be more than 500/100,000 population, which included five clusters having a prevalence of more than 1000/100,000 population. This requires the NTP to design and implement context specific interventions for enhanced case finding in these clusters on a priority basis.

The specimen transport system was successfully used for first time in the survey for the transport of specimens in cold chain from field cluster sites to the NTRL. Experience gained in the survey will be implemented in the regular program for the transportation of specimens from lower level laboratories to higher level laboratories for advance testing.

The NTP Pakistan recruited a PhD student who will develop the report into scientific publications and do further detailed analysis of the survey that will give guidance on policies. KNCV has committed to give technical support for this process.

Lady Health Workers at a Survey Reception

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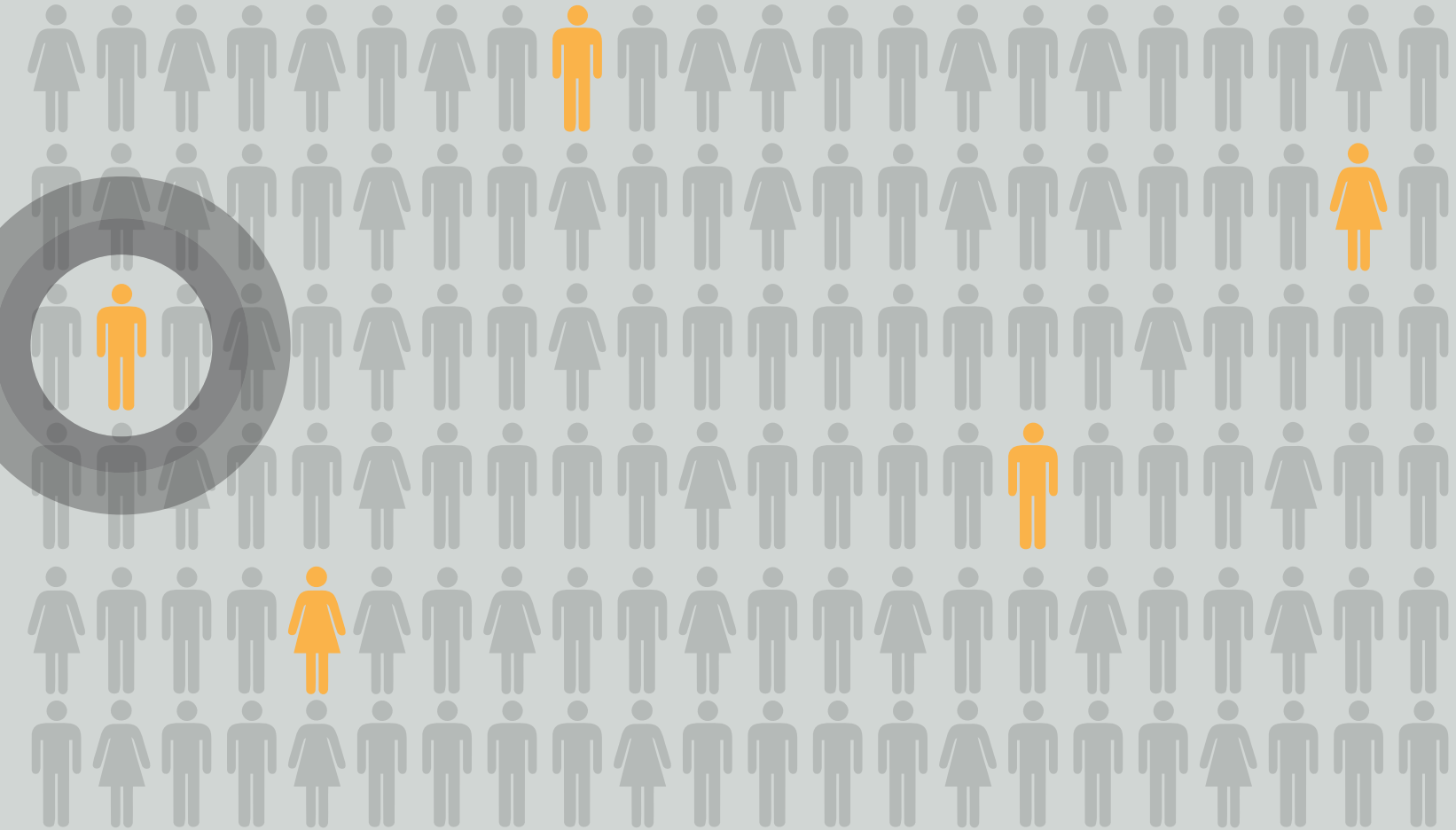

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