Recruitment, loss to follow-up and morbidity:Lessons for infant TB vaccine trials from an epidemiological cohort study in western Kenya

Steve Otieno Wandiga

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Steve Otieno Wandiga

aus (Geburtsort)
born in (place of birth)

Kenya

am (Tag an dem die Dissertation abgeschlossen wurde)
submitted on (day of finalization of the thesis)

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Supervisors LMU:

Habilitated Supervisor Prof. Dr. Ulrich Mansmann

Direct Supervisor Prof. Dr. Christian Heuman

3rd LMU Supervisor Dr. Guenter Froeschl

Supervisor External:

Local Supervisor Dr. Kayla Laserson

Reviewing Experts:

1st Reviewer Prof. Dr. Ulrich Mansmann

2nd Reviewer Prof. Dr. Christian Heumann

Dean: Prof. Dr. med. dent. Reinhard Hickel

Date of Oral Defence: 25th November 2015

Recruitment, loss to follow-up and morbidity: Lessons for infant TB vaccine trials from an epidemiological cohort study in western Kenya.

Affidavit

Wandiga, Steve

Surname, first name

KEMRI CGHR, Off Kisumu-Busia, P. O. Box 1578

Street

40100, Kisumu

Zip code, town

Kenya

Country

I hereby declare, that the submitted thesis entitled

Recruitment, loss to follow-up and morbidity:

Thesis Title

Lessons for infant TB vaccine trials from an epidemiological

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Recruitment, loss to follow-up and morbidity: Lessons for infant TB vaccine trials from an epidemiological cohort study in western Kenya.

by

Steve Otieno Wandiga

PhD in Medical Research – International Health

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CIH^{LMU} Center for International Health

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(infants, recruitment, enrollment, follow up and morbidity)

Abstract

Objective:
To evaluate the recruitment, follow up and morbidity patterns of infants enrolled in the tuberculosis (TB) epidemiological cohort study in Siaya County, western Kenya in preparation for future TB pediatric trials.

Methods:
An observational prospective TB cohort study that enrolled 2900 infants. Infants were recruited through birth notifications and screened for eligibility. Mothers were consented prior to infants' enrollment into the study. Upon enrollment, mothers were asked for antenatal profiles, demographic and baseline information. Follow-up visits were 1-2 years at six weeks and thereafter four-monthly. Mothers whose enrolled infants fell sick were reimbursed transport when they came to study clinic. Univariate analyses on infant and maternal characteristics. Bivariate and logistic regression models were performed to identify factors associated with place of delivery, loss to follow up (LTFU) and unscheduled visits. Kaplan Meier curves and Cox Proportional Hazards were used to explore LTFU factors.

Results:
Village reporters contributed 98% of the birth notifications, 98% of infants were enrolled from home, 63% of infants were home delivered, 82% of mothers had primary education and 14% of mothers were HIV positive. BCG coverage was improved from 25% to 87% post enrollment. Cohort retention was 91.5%.

No or lower maternal education, farming mothers, salaried mothers, mothers engaged in fishing, mothers living in mud houses, mothers living in semi-permanent housing and receipt of antenatal care was associated with home delivery.

Home delivered infants, infant birth weight, mothers engaged in farming, mothers employed as salaried workers, mother living in mud type of housing and mothers with ≥ 3 children were associated with loss to follow up (LTFU).

Infant delivered at home, infants residing within the HDSS area, no maternal or primary education, HIV positive mothers, and maternal age were associated with unscheduled visits.

Inpatient symptoms were fevers, fast breathing and vomiting whereas outpatient symptoms were breathing difficulty, fevers and fast breathing. Malaria and upper respiratory tract infections were the leading impressions. Common prescriptions dispensed were antimalarials, analgesics and antihistamines. Unscheduled visits identified TB suspects and TB cases that would have been missed.

Conclusion:
Infant cohort retention was impressive. Study withdrawals and choice of place of delivery require further qualitative assessment. Consideration of factors associated with LTFU and unscheduled visits to inform the planning and implementation of future pediatric studies.

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Abbreviations

Aeras – Aeras Global TB Vaccine Foundation

AFB – acid-fast bacilli

ANC – Ante Natal care

ARV – antiretrovirals

AZT – zidovudine

BCG - Bacille Calmette-Guérin

BSL 3 – Bio-Safety Level 3

CDC – Centers for Disease Control and Prevention

CFR – Code of Federal Regulation

CGHR – Center for Global Health Research

CRDR – Center for Respiratory Disease Research

CRFs – Case Report Forms

CTLC – County Tuberculosis and Leprosy Coordinator

CVW – Case Verification Ward

CXR – Chest X-ray

DNA – Deoxyribo Nucleic Acid

EU – European Union

GCP – Good Clinical Practice

GIS – Geographic Information System

HDSS – Health and Demographic Surveillance System

HIV – Human Immuno-deficiency Virus

ICH – International Conference on Harmonization

IEC – Independent Ethics Committee

IQR – Inter Quartile Range

IRB – Institutional Review Board

KAIS – Kenya AIDS Indicator Survey

KDHS – Kenya Demographic Health Survey

KEMRI – Kenya Medical Research Institute

LFTU – Loss To Follow Up

LTBI – latent TB infection

M TB – mycobacteria tuberculosis

MCH – Maternal Child Health

MDG – Millennium Development Goal

MoH – Ministry of Health

NLTP – National Leprosy and Tuberculosis Program

NTM – Non Tuberculous Mycobacteria

NTP – National Tuberculosis Programs

PCR – Polymerase Chain Reaction

PDA – Personal Digital Assistant

PEPFAR – US President's Emergency Plan For AIDS Relief in Africa

PMTCT – Prevention of Mother to Child Transmission

PNC – post natal care

PSC – Patient Support Center

RFLP – Restriction Fragement Length Polymorphism

SCRH – Siaya County Referral Hospital

SIN – Study Identification Number

SRL – Supranational Reference Laboratory

SSA – sub Saharan Africa

SSC – Scientific Steering Committee

TB – Tuberculosis

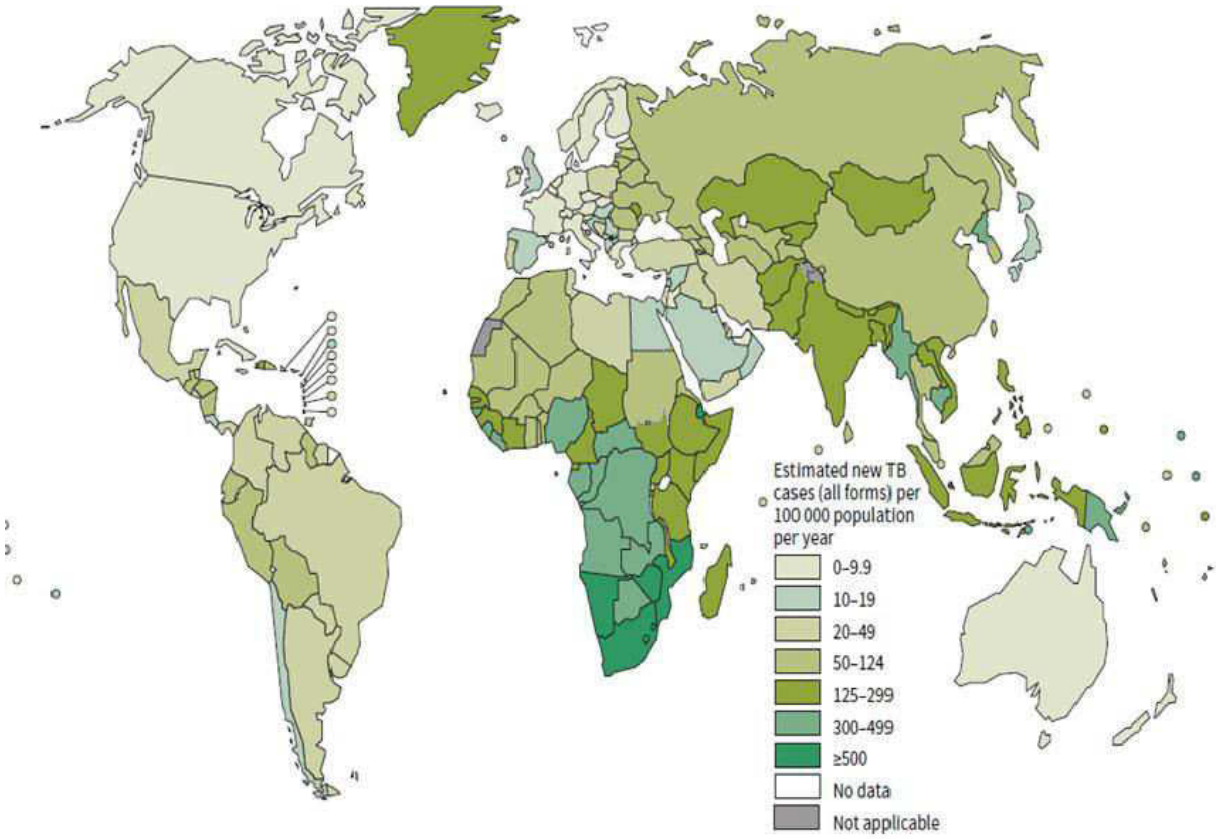
USA – United States of America

WHO – World Health Organization

1. Introduction

Tuberculosis (TB) remains a major global health problem, responsible for ill health among millions of people each year. TB ranks as the second leading cause of death from an infectious disease worldwide after human immunodeficiency virus (HIV). There are 22 TB high burden countries that altogether contribute about 80% of the global TB burden. The greatest burden of TB is in sub-Saharan Africa and South East Asia regions (WHO, 2014).

Figure 1. Estimated TB incidence rates, 2013



Source: World Health Organization TB Report, 2014

Kenya is ranked 15th among the 22 TB high burden countries that collectively contribute to about 80% of the world TB cases and also ranks 5th in Sub Saharan Africa (WHO, 2011). Various factors have been identified as risk factors for TB disease (Lonnroth et al., 2009) but in Kenya majorly attributed to the HIV pandemic (Chintu et al., 2002, Gilks et al., 1997). Historically, paediatric tuberculosis has been neglected largely due to the challenges

associated with uncertainty in diagnosis and low priority accorded by national TB control programs (Marais and Pai, 2007). TB disease in a child is considered a sentinel event because it usually represents disease resulting from recent transmission from an adult with infectious pulmonary TB (Myers et al., 2006, Swaminathan and Rekha, 2010). One of the main hurdles hampering efforts to control TB globally is the ability to identify and diagnose cases especially poor among children (Trunz et al., 2006). The epidemiology of TB in young children (<5 years old), a vulnerable population where diagnosis and treatment are most challenging, is not well understood, especially in countries with limited public health resources (Jain et al., 2013). In addition, pediatric TB is complicated by the unique challenges that it poses to TB control (Cardy et al., 2012). Children generally make up only a small proportion of TB cases, but in some high-incidence countries up to 15% of all TB cases occur in children (Nicholson et al., 2011). The fact that children rapidly progress to active disease and represent a potential pool for disease in their adult life, further underlines the importance of analysing the epidemiology of childhood TB (Sandgren et al., 2011). The risk of developing disease has been estimated to be 24% in children 1–5 years of age, and unfortunately as high as 43-50% in infants less than a year of age (Hesseling et al., 2009). The youngest children carry the biggest burden for three main reasons: firstly, they are more prone to develop severe extrapulmonary TB disease such as meningitis and miliary TB; secondly, they tend to develop severe pulmonary disease with bronchial obstruction; and thirdly, they are more likely to develop disease after being infected (Weber et al., 2000). Bacille Calmette Guerin (BCG) vaccine given at birth has benefits that clearly outweigh its risks in most settings and populations (Abu-Raddad et al., 2009), there is an urgent need for a more effective and safer TB vaccine for children (Rowland and McShane, 2011). However, studies on the epidemiology, clinical profile, and treatment outcomes of childhood TB from developing countries are lacking (Edmond et al., 2006, Anderson et al., 2014, Newton et al.,

2008). The significant TB-related morbidity and mortality suffered by children in high-burden settings (Trunz et al., 2006), underlines the need to obtain a more comprehensive picture of childhood TB at the community level in high-burden settings. Childhood TB nevertheless remains neglected, as the perception in resource limited settings rule out children as not a public health risk (Marais et al., 2006, Hesseling et al., 2009, Swaminathan and Rekha, 2010).

1.1. Recruitment of infants into studies

Observational studies in children with TB are often hospital-based, which may result in considerable bias, preventing accurate extrapolation to the community level (Marais and Pai, 2007). Multicentric pediatric trials are urgently required to help develop improved diagnostic strategies and formulate shorter, more effective, safe, and evidence-based regimens for treatment and prevention of drug susceptible and drug-resistant TB (Swaminathan and Rekha, 2010). Although reporting of the recruitment and retention of participants in randomised trials has improved with the advent of the CONSORT Statement (Altman et al., 2001, Moher et al., 1994, Schulz et al., 2010), detailed protocols and the lessons learnt are rarely described (Toerien et al., 2009). A review of 114 trials reported that only 38 (31%) achieved their original recruitment target (McDonald et al., 2006). When consent rates are low and additionally loss to follow up and withdrawals may result in high attrition, the potential for selection bias and retention bias is increased. Both biases limit the generalisability of results, and reduce the successful translation of any positive outcome effects into practice (Fewtrell et al., 2008). In a study, where interviewed parents about their decision for or against enrolling their child in a vaccine study, the data analysis suggests that parents' ability to evaluate a vaccine study depended on how attuned they are with science and medicine, either professionally or as consumers of health services. Familiarity does not predispose parents to enrolling their child in research; rather, it is a predictor of parents' confidence in their

decision making (Chantler et al., 2007). Neonatal research is made more complex by the issue of proxy consent. Obtaining valid informed consent for entry of an infant into a research project needs to deal with this complexity. New evidence on the role and responsibilities of parents in giving consent has implications for all clinical staff that are considering embarking on and/or recruiting infants in research projects (McKechnie and Gill, 2006). A cross sectional survey to quantify refusal rates and factors of refusing pertaining to studies and recruiting pediatricians showed that refusal to participate in clinical research was low and influenced by factors associated to the objectives, conduct of the studies and factors related to the characteristics and perceptions of the recruiting pediatricians (Kaguelidou et al., 2013).

For many pediatric studies, the relative scarcity of potential study participants means that it often takes considerable effort that requires lots of creativity to enroll and retain sufficient numbers of children who meet the criteria for study participation. It may also mean that studies must extend for quite long periods just to secure enough participants. One recently published article on the prevention of fungal infections in children and adults with chronic granulomatous disease reported that it took 10 years to enroll 39 participants, most of whom were children at the time that the study started (Gallin et al., 2003). A review of randomized controlled trials published in the Archives of Diseases in Childhood from 1982 to 1996 reported that about half of the studies recruited less than 40 children, with medians of 80 children for multicenter trials and 36 children for single-center studies (Campbell et al., 1998, Pattishall, 1990, Freiman et al., 1978, Moher et al., 1994). The authors comment that these small studies “have inadequate power to detect small or moderate treatment effects and result in a significant chance of reporting false-negative results”(Campbell et al., 1998). In recent years, various initiatives have aimed at furthering paediatric research, as the absence of adequate studies in the paediatric population has hampered progress in the care of children

(Burns, 2003, Koren et al., 2003). A key determinant of the success of a clinical trial is the recruitment and retention of a study population of an adequate sample size. Low rates of recruitment and retention have a number of negative implications, such as longer durations of the clinical trial, which may lower staff and participant morale; a costlier clinical trial, since extra resources may need to be dedicated to the recruitment effort; and less statistical power for both the study and the validity of the results would be in doubt. In some cases, inadequate accrual of subjects may result in the termination of the trial (Ross et al., 1999, Hunninghake et al., 1987, Lovato et al., 1997). Poor clinical trial recruitment and retention is therefore likely to impede the successful evaluation of new and existing interventions, and prevent greater efficiency in clinical development (Spilker and Cramer, 1992). There is scarce data about recruitment and retention in TB cohort studies especially among infants. Researchers, healthcare providers, and parents acknowledge the importance of research, but effective recruitment into paediatric clinical trials remains a difficult task (Caldwell et al., 2002, Singhal et al., 2004, McKechnie and Gill, 2006). Recruiting infants into clinical studies can be challenging and there is need for researchers to acrrue target participants in an effective and timely fashion in order to represent valid results. Several studies have examined their recruitment experience in various ways (Kaur et al., 2012, Vollmer et al., 1992, Heinrichs et al., 2005, Bailey et al., 2004, Strunk R, 1999). There are reports from trialists reporting their recruitment experience, methods and strategies (Finne et al., 2009). Little has been documented about recruitment and strategies employed to complete studies in time (Galbreath et al., 2008). In a proof of concept study to assess the novel T-cell activation marker–tuberculosis assay for diagnosis of active tuberculosis in children, children aged older than 6 months and younger than 16 months were referred from peripheral health facilities and local hospitals (Portevin et al., 2014). In yet another study conducted in Africa to diagnose childhood tuberculosis and host RNA expressions were recruited from children admitted and

undergoing TB evaluation in the hospital (Anderson et al., 2014). In neighbouring Uganda, a site under Makerere University Demographic Surveillance System, recruited and enrolled infants ≤ 8 weeks either at health facilities or at home through village scouts (Nabongo et al., 2014).

1.2.Place of Delivery

Mothers in developing countries still prefer home delivery unattended by skilled health workers to health facility delivery (Montagu et al., 2011). In several contexts, individual factors including maternal age, parity, education and marital status, household factors including family size, household wealth, and community factors including socioeconomic status, community health infrastructure, region, rural/urban residence, available health facilities, and distance to health facilities determine place of delivery (Stephenson et al., 2006). A study looked at antenatal care and delivery care among women in western Kenya and demonstrated that older women, high parity, lower socioeconomic status, low education levels and more than an hour walking distance were associated with delivery outside health facilities (van Eijk et al., 2006). Health or home delivery was significant in determining the subsequent steps by study such as administration of BCG vaccination, whether to enroll at the home or health facility. Understand the differences between home and health facility enrolled infants in terms of follow-up and unscheduled visits attendance using baseline variables from infants and mothers. One challenging task for the tuberculosis cohort study was to demonstrate the ability to demonstrate the capacity to recruit and enroll 2900 participants in a rural setting.

1.3.Loss to Follow Up in Pediatric Studies

Loss to follow up (LTFU) can be problematic and lead to bias (Kristman et al., 2004). A multicenter cohort study in Europe of children aged 2-9 years old found that being overweight, low education, single parenthood and low-well being were determinants of attrition (Hense et al., 2013). In the Avon Longitudinal Study of Parents and Children, an association was established between socio-economic position and study outcomes (Howe et al., 2013). In a large HIV-1 perinatal transmission cohort study in Malawi established infants with low birth weight and singletons were less likely to return for follow-up. Parents from farming background, educated and students were also less likely to be loss to follow-up (Ioannidis et al., 1999). In an isoniazid prophylaxis study with 2 cohorts, HIV infected and HIV exposed and not infected, a larger household and presence of an elder was associated with decreased LTFU. Among HIV negative, younger maternal age increased risk for loss to follow up whereas maternal history of TB reduced the risk of LTFU (Beneri et al., 2013). In a study of Fetal Malnutrition in Nigeria, attrition at 4 weeks follow-up was higher among mothers who had no antenatal care, teenage mothers, primiparous mothers, low social class, mothers of female children, among clinically well babies and mothers of babies without fetal nutrition (Olusegun et al., 2007). In a study to describe attrition comprehensively in an asthma intervention study, they found out that caregiver age predicted dropout (Zebracki et al., 2003). In a 5 year follow up of mother and child pairs in prevention of mother to child transmission of HIV (PMTCT) study, HIV negative mothers were LTFU (Kurewa et al., 2012). In the "Making Our Mealtimes Special" (MOMS) study targeting low income populations knew that successful recruitment and retention of low-income and/or minority participants present a unique set of challenges (Nicholson et al., 2011). In the HIV Prevention Trials Network (HPTN) 064 study, factors associated with missed study visits were unstable housing and later

enrollment whereas black race, recruitment from an outdoor venue and financial responsibility for children increased likelihood of attendance of study visits (Haley et al., 2014). Older maternal age was associated with better study participation in a large clinical trial to reduce prevention of mother to child transmission of HIV (PMTCT)(Sellers et al., 2014). In the Avon Longitudinal Study of Parents and Children, loss to follow-up was associated with socio-economic position (SEP) and outcomes such as birth weight and length, breastfeeding, preterm birth, maternal obesity, smoking during pregnancy, educational attainment (Howe et al., 2013).

1.4.Morbidity patterns among infants

Influenza is major cause of pediatric hospitalisation but recruiting sufficient controls was problematic and in the future (Dixon et al., 2010). Recruiting for research studies is always a challenge, particularly in paediatric studies. In this study, experiences recruiting children to five studies through primary care demonstrated evidence that participants who had participated in previous studies were more likely to participate again (Cardy et al., 2012). In sub-Saharan Africa (SSA), fever remains a major public health problem. Fever is a symptom of diseases especially predominant among children under age five (Nnedu et al., 2010). Such diseases include malaria, diarrhoea, pneumonia, measles, polio and tuberculosis, to mention a few. These diseases are main contributors to deaths among children under age five in the sub-Saharan African region (Black et al., 2003). The World Health Organization's (WHO) 1999 report on infectious diseases posits that these diseases cause most of the deaths from infectious diseases. Respiratory diseases are a leading cause of hospital admissions in both HIV-1-positive and HIV-1-negative children worldwide (Chintu et al., 2002). Previous research has shown that various factors influence child health and survival, including place of

residence, breastfeeding, place of delivery, access to postnatal care, and maternal age and education (Doctor, 2001, Swenson et al., 1993, Teka et al., 1996, Edmond et al., 2006, Black et al., 2003, Manda, 1999, Nath et al., 1994). Observational studies have reported substancially increased diarrhea in artifically fed infants, with highest risk being noted in the first 2 to 3 months of life (Jason et al., 1984, Feachem and Koblinsky, 1984). In a study that looked at diarrhea among children with known HIV status, it was found that diarrhea was frequent among HIV positive children (van Eijk et al., 2006). Low birth weight is a risk factor for infant mortality. WHO 1998 report states that "fetal anaemia is also an important risk factor for anaemia in the first six months of life" and may contribute to mortality risk (Wilcox, 2001). "As low birth weight and fetal anaemia have been related to malaria in pregnancy" (McCormick, 1985, le Cessie et al., 2002, Verhoeff et al., 1999, Brabin, 1992). It is important to establish their association with infant morbidity in malaria endemic areas. In a health seeking behavior, mothers classified childhood illnesses into four categories: not serious – coughs, colds, diarrhea; serious but not life threatening – malaria; sudden and serious – pneumonia; chronic and therefore not requiring immediate action – malnutrition, tuberculosis, chronic coughs (Amuyunzu-Nyamongo and Nyamongo, 2006).

The different determinants of infant morbidity and mortality include age, sex, plurality, mode of delivery, gestational age, infant birth weight, parity of mother, vaccination, maternal education, age, birth spacing and socioeconomic conditions. Breast feeding is an important determinant which lowers the rate of infection related morbidities (Kramer et al., 2001). WHO Expanded Programme on Immunization has reduced infant mortality by controlling vaccine preventable diseases (Kabir et al., 2003). There are only a few reports of morbidity associated with underweight at childhood and adolescence in industrialized societies (Booth, 1991, Kraemer et al., 1978).

The articles reviewed mainly assessed children who made return visits to the hospital emergency unit (Alessandrini et al., 2004, Goldman et al., 2006, Imsuwan, 2011, English et al., 2003). Previous studies reviewed majorly addressed, unscheduled return visits at emergency departments in hospitals with the view to minimize these visits (Singer et al., 2014, Cohen et al., 2014) whereas others targeted adult populations (van der Linden et al., 2014, O'Connor et al., 2014, Akenroye et al., 2014). Infant where not breastfed were associated with increased risk of serious infections between 0-2.9 months of age in a HIV-exposed, uninfected infants in the first 6 months of life (Bork et al., 2014). A study comparing the frequencyof hospitalizations between breastfed, partially breast-fed and bottle-fed infants showed that breastfed infants had fewer admissions (Shiva et al., 2007). Upper respiratory tract infections (URTIs) was identified as one of the leading extreme events especially among prematurely born infants (Al-Kindy et al., 2009). Leading infections in a prospective follow-up of children attending municipal day centers in Helsinki found that URTI was one of the most diagnosed infection (Ponka et al., 1991). Younger age and day care outside home were identified as contributors to infectious morbidity. There was no difference in symptom days between children with or without older siblings (Hedin et al., 2010). Incidences of morbidities were more among partially immunized babies and among mothers with lower socio-economical and educational status (Joseph et al., 2013). Morbidity was more among boys and in the second half of infancy. Respiratory tract infections, diarrhea and skin infections were the commonest morbidities (Joseph et al., 2010). Infants with lower tract infections were hospitalized more frequently (Mussi-Pinhata et al., 2007). Diarrhea and malaria were most common between 6-12 months of age and especially during rainy season whereas acute respitatory illness reportedly frequent between 1-3 months of age and in the cold season (Vaahtera et al., 2000). In a study in Nairobi slum settlements, morbidity was influenced by child age, ethnicity and type of toilet whereas health seeking behavior was linked to child's

age, type and severity of illness, survival of father and mother, mother's education, mother's work status and wealth class (Ndugwa and Zulu, 2008). Respiratory disease is the major cause of mortality and morbidity worldwide, with infants and young children especially susceptible (Zar and Ferkol, 2014). Tuberculosis is a large source of morbidity and mortality among children. However, limited studies characterize childhood tuberculosis disease, and contact investigation is rarely implemented in high-burden settings (Jaganath et al., 2013). There is very little reported literature on post-birth newborn morbidity and therefore a secondary analysis of data from a randomized clinical trial assessed infants' health in the first eight weeks and established that the most common infant morbidity was upper respiratory illness (Hannan, 2014).

1.5. Categorization of cited studies due to key criterions

Literature review was conducted and there are studies that were carried out and identified to be linked to the thesis criterions as follows:

Table 1. Categorization of cited studies according to thesis criterions

Outcome	Country	Sample size	Study Type	Year of Completion	Author(s)
Recruitment of infants into studies	Uganda	2500	Observational cohort	2010	Nabongo et. al., 2014
	South Africa, Malawi and Kenya	2955	Prospective tuberculosis study	2011	Anderson et. al., 2014
	Tanzania	290	Proof-of-concept study	2012	Portevin et. al., 2014
	United States of America	39	Prospective study	2001	Gallin et. al., 2003
Place of Delivery	Demographic Health Survey (DHS) data		Secondary analysis	2003 – 2008	Montagu et. al., 2011

	from 48 countries				
	Demographic Health Survey (DHS) data from 6 countries		Secondary Analysis	1999 – 2000	Stephenson et. al., 2006
	Kenya	730	Survey	2002	van Eijk et. al., 2006
Loss to Follow Up in Pediatric Studies	8 countries in Europe	31543	Population based intervention study	2010	Hense et. al., 2013
	United Kingdom	12000	Longitudinal study	Ongoing	Howe et. al., 2013
	Malawi	2156	Prospective cohort	1994	Ioannidis et. al., 1999
	South Africa	1351	Secondary analysis	2006	Beneri et. al., 2013
	Nigeria	473	Prospective study	2002	Olusegun et. al., 2007
	United States of America	327	Randomized control trial	2001	Zebracki et. al., 2003
	Zimbabwe	1050	Prospective cohort study	2010	Kurewa et. al., 2002
Morbidity patterns among infants	Australia	76	Case control study	2008	Dixon et. al., 2010
	Sierra Leone	175	Prospective study	2005	Nnedu et. al., 2010
	Zambia	264	Descriptive study		Chintu et. al., 2002

2. Rationale and Objectives

2.1. Rationale

About 1.5 million new tuberculosis (TB) cases occur per year in Sub-Saharan Africa, where the prevalence of the disease continues to rise by 3-4% annually and where HIV has been the single most important factor determining the increased incidence of TB in the past 10 years. The age-specific incidence of TB in developing countries is characteristically high before 5 years, peaks in the second year, is low between 5 and 12 years of age and then gradually rises through adolescence to peak in young adults. BCG given at birth is thought to confer only incomplete and variable protection against pulmonary TB. There are also significant safety concerns when BCG is used in HIV-infected and other immune-suppressed infants. This has prompted the search for new, safer and more efficacious TB vaccines. Several of these vaccines are being tested in Phase I and IIA clinical trials, two are planned to start Phase IIB proof of concept clinical trials in 2008 and one or more will likely be ready for efficacy testing in a Phase III trial within the next 3-5 years. In order to conduct Phase III efficacy trials of new TB vaccines, trial sites are needed which have the capacity to enroll and vaccinate large numbers of infants, retain them during follow up, and be capable of detecting all incident cases of TB and safety endpoints. For example, the ability to vaccinate infants within a specified period after birth in an area where the majority of deliveries occur at home would be required in a Phase III TB vaccine trial.

In addition, until a validated surrogate marker of protection following vaccination, such as an immunologic assay, is available, clinically diagnosed TB is likely to be the endpoint for comparison of efficacy of a new vaccine with a control. Planning trials will require knowledge of the baseline incidence of TB in the population under study. This will be crucial

for determining the sample sizes which will be needed to detect a statistically significant reduction in TB incidence in trial populations receiving new TB vaccine candidates. The proposed epidemiological cohort study will provide important information needed for the accurate planning of future TB vaccine efficacy trials e.g. rates of TB infection and disease in the target population. In addition it will help to develop capacity at the site to recruit, vaccinate and follow- up a large cohort of infants in such a way as to ensure that all endpoints are detected in a timely fashion, properly verified and properly documented. The formative qualitative research in the proposed study will provide insights for tailoring efforts to optimize recruitment, retention and acceptability; in a future TB vaccine trial and strategies for enhancing care seeking during acute illness episodes. A Phase IV BCG trial conducted in South African infants showed a cumulative incidence of definite, probable or possible TB of 6.31% over 2 years. Given this high incidence, infants are a promising target group for future large TB vaccine efficacy trials, because they are at high risk for TB and would stand to benefit the most from the new technology. Pediatric TB is often under-diagnosed and poorly managed due to challenges in case detection and limited pediatric drug formulations. The -under-utilization of children in clinical research has led to this dearth of tools for pediatric TB prevention, diagnosis, and treatment.

In TB research, efforts are geared towards urgently finding the replacement for Bacille Calmette Guerin (BCG) vaccine more than 100 years old. Finding research sites in developing countries that are ready for the conduct of phase 3 clinical trials is a daunting task. Additionally, pediatric research studies are a novel field in developing countries and unexplored. Recruitment of newly born infants into research in Africa can be a challenge as few deliveries take place in the hospitals. Loss to follow up reduces the sample size thereby

compromising the statistical power of the study. Monitoring sick visits in pediatric populations is useful in mitigating severe outcomes and minimizing deaths. European and Developing countries Clinical Trials Partnerships (EDCTP) partnered with Kenya Medical Research Institute (KEMRI) and Centers for Disease Control and Prevention (CDC) to develop capacity of KEMRI/CDC site in western Kenya for late phase TB clinical trials by funding a TB epidemiological study among infants to document annual TB incidence.

2.2.Primary objective:

To evaluate the recruitment, follow-up and morbidity among infants enrolled in a tuberculosis epidemiological cohort study in western Kenya for future TB vaccine trials.

2.3.Specific objectives:

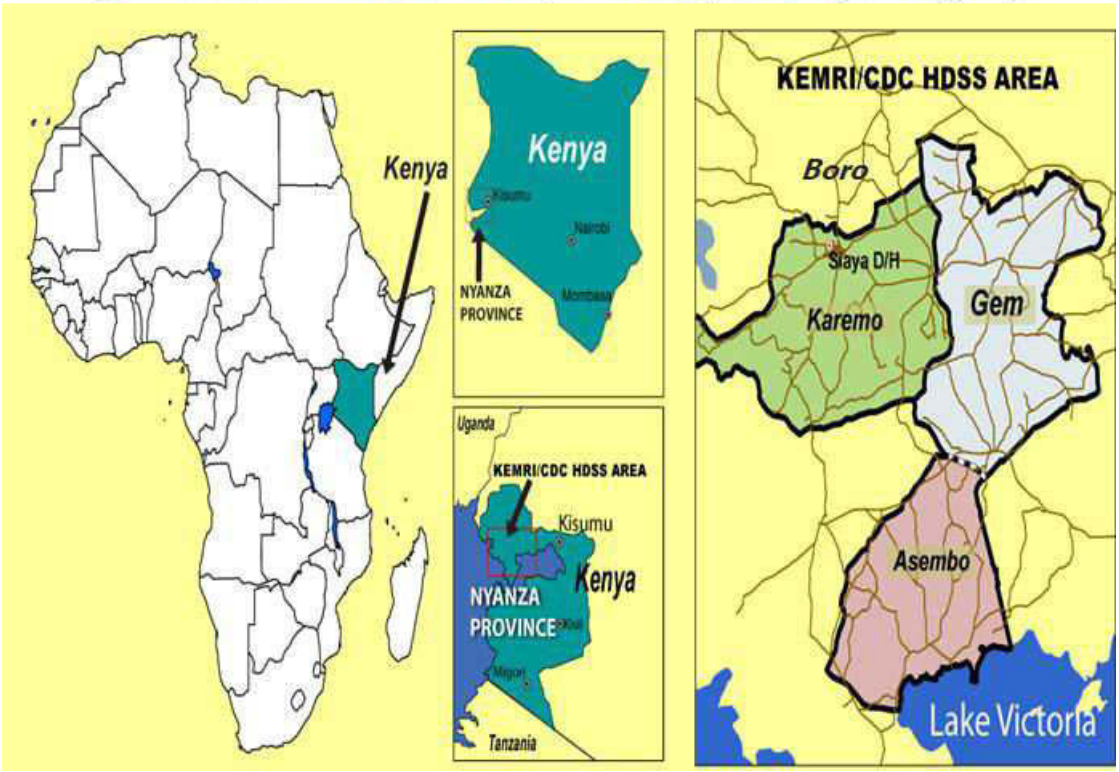
1. To assess the recruitment outcomes, accrual trends and baseline characteristics of infants enrolled into TB cohort study
2. To determine the factors associated with place of delivery among mothers of infants in this TB cohort study
3. To evaluate the predictors of loss to follow up in an infant TB cohort study
4. To highlight factors influenced by attendance of unscheduled visits in the infant cohort study
5. To describe the morbidity burden among infants enrolled utilizing unscheduled visits in an infant TB cohort study

3. Methods

3.1.Study area

KEMRI and CDC collaboration is based in East Africa, Kenya in the third largest lake side city called Kisumu at 1,131 meters (3,711 feet). Kisumu is situated in the western part of Kenya in the former Nyanza Province. KEMRI/CDC runs a health and demographic surveillance system (HDSS) about 70 kilometers from Kisumu in Siaya County at 1,224 meters (4,019 feet). The HDSS covers Gem, Asembo and Karemo areas that are typically rural populations (*Figure 2*). Western Kenya experiences long rains from March to June and short rains from October to December.

Figure 2. Infant Tuberculosis Cohort Study Site at KEMRI/CDC in Siaya County, Kenya



Source: KEMRI/CDC Health and Demographic Surveillance System, 2010

3.2.Study site

The study was conducted in Karemo division and contiguous areas e.g Boro, Gem etc, Siaya District where the Siaya County Referral Hospital (SCRH) is located. All diagnostic procedures were conducted in a case verification ward (CVW) at SCRH. An outpatient annex was built at SCRH with laboratory capacity to do basic hematological, chemical, serological, and microbiological (excluding all TB) tests for this study. Kenya Medical Research Institute (KEMRI) and Centres for Disease Prevention and Control (CDC) already operates a health and demographic surveillance system (HDSS) in Gem and Karemo (in Siaya District) and Asembo (in Bondo District), which provides general demographic and health information as well as disease- or intervention-specific information. KEMRI and CDC operates a health and surveillance within out-patient and in-patient public health facilities serving residents of Karemo which records symptoms, diagnosis, and treatment information for all children <10 years of age. KEMRI and CDC initiated a TB prevalence survey in Asembo and Gem in August 2006 with a fluorescent microscopy (FM) TB smear laboratory based at KEMRI Center for Global Health Research (CGHR) research station in Kisian. The TB laboratory was upgraded to a biosafety level 3 (BSL-3) laboratory and create TB culture capacity.

3.3.HDSS Platform

KEMRI and CDC ran a Health and Demographic Surveillance System (HDSS) is situated in Kisumu, Kenya within East Africa, which is a longitudinal, population-based health and vital event registration system that monitors demographic (e.g., births, deaths, pregnancies, and migrations) and health (e.g., clinic attendance and hospital admission) events in a geographically defined population with timely production of data. This continuous surveillance makes it possible to easily and clearly define risks of demographic and health

events for individuals over time. The HDSS can provide cause-specific mortality and morbidity profile that is demographically or geographically stratified, permitting rational resource allocation to priority diseases in defined target groups. Accurate sampling frames can be generated from HDSS data at multiple levels (individual, house, village) and by several strata (age, sex, geographic location) to permit unbiased, population-based sampling. The longitudinal morbidity, mortality, and fertility data generated from the HDSS can help generate hypotheses on the causes of disease and death in the population and evaluate the impact of public health interventions. Finally, significant efficiencies may be achieved when multiple research program evaluation activities operate from the same infrastructure and population base.

3.4.Study Population

The study population were infants born to women who are residents of Karemo division and contiguous areas e.g Boro, Gem, etc. in Siaya District, Nyanza Province, in western Kenya. The total population in Karemo division is approximately 73,000 and the annual birth cohort 3600, of which 80% take place at home. More than 95% of the population is of Luo ethnicity, with subsistence farming as the main source of income. Families live in compounds; each compound has been mapped using Geographic Information System (GIS) coordinates and each individual has been given a unique identification number as part of the HDSS.

3.5.Study Design

A prospective, observational cohort study that targeted infants aged 0-6 weeks old and born to women who resided within the study area was conducted. Infants were recruited through birth notifications followed by prescreening and screening for eligibility. Mothers gave assent

prior to enrollment of their children into the study. Upon enrollment, antenatal profile, demographic and baseline information was collected from mothers and infants. Follow up was scheduled at six weeks upon enrollment and thereafter four-monthly between 1-2 years depending on time of enrollment. Mothers were allowed to make unscheduled visits to the clinic at any time they found it necessary for the wellbeing of the infant.

Loss to follow was defined as follows: three or more documented attempts to contact the participant without success; participant confirmed to have moved from study area and not traceable; no contact established by the time the study was completed such that there was insufficient information to determine the participant's status irrespective of enrollment period.

Unscheduled visits were defined as those visits outside the study visits (scheduled visits) where mothers brought in their infants to be treated for various illnesses. During these visits, the infants were examined for signs and symptoms related to TB or not and investigations were requested by study clinicians as deemed necessary.

3.6. Materials and methods

Study site was situated at Siaya County Referral Hospital. The BCG vaccination administered to infants was sourced from Ministry of Health. Electronic case report forms were completed on personal digital assistants (PDAs) or hand held computers and laptops on a customized data entry interface. Source documentation was also maintained for study participants to allow for source data verification with entries in the electronic case forms. The data was saved onto an SQL database and exported into STATA 12.0 for statistical analyses.

3.7.Study Procedures

3.7.1. Participant selection criteria

3.7.1.1. Inclusion Criteria:

Enrolled infants must have met all of the following criteria: Infant was born within the Karemo Division and contiguous areas e.g Boro, Gem etc ; Infant was vaccinated with BCG within six weeks of birth ; Infant weighs 1700g or more at the time of vaccination ; Infants born to mothers diagnosed with TB will not be excluded from the study but have delayed BCG vaccination after INH prophylaxis.

3.7.1.2. Exclusion Criteria:

Enrolled infants must have had none of the following: In the opinion of the attending clinician, participation in the study may adversely affect the health of the infant (includes infants with advanced HIV disease); Parent/guardian declines to give informed consent; Parent/guardian plans to move from the study area within the next year.

3.7.2. Recruitment strategy and process

HDSS households are visited every four months in order that general demographic and health information, including reports of pregnancies, may be collected. This system of reporting pregnancies was adopted and utilized for study recruitment. Furthermore, village reporters (VRs) were utilized as an extra surveillance system outside of the regular HDSS visits to identify pregnant women in their villages. Once pregnant women had been identified through one of the two approaches above, study staff contacted the pregnant women in the community, and asked for informed consent in writing for the tuberculosis cohort study. This contact was used as a health education opportunity, an opportunity to describe the planned

epidemiological study, and the women were encouraged to have an attended delivery and to obtain BCG and other EPI vaccines for their children.

During pre-delivery enrollment, as part of study procedures, the study staff attempted to determine the expected date of delivery (EDD). This was done through the use of the date of the pregnant woman's last menstrual period (LMP) and/or the estimation of the fundal height by a study nurse, where the date of the LMP cannot be verified. A database was developed in to track of EDDs. The database was stored in a server with password-protected access to ensure confidentiality of participants' record. This system created an automatic alert for study staff 4 weeks before the EDD to contact VRs who reside in villages with potential upcoming deliveries. The VR's were expected to be extra vigilant closer to the delivery date in order to notify the study staff within 72 hours of a pregnant woman giving birth. VRs were provided with a cellphone contact number with which to notify study staff once a delivery occurs; this was over and above the birth notification form they will fill as part of the HDSS. The time and date of notification was recorded by study staff and compared to when the birth notification was received by the HDSS. This was a quality control system to monitor the efficiency of the process of birth notification to the study and utilized for feedback to the VRs. Information on the study was provided during antenatal clinics.

The main study was presented again to mothers after delivery either at home or in health care facilities (80% of deliveries in the study area occur at home, the majority attended by traditional birth attendants (TBAs). Two thousand nine hundred (2,900) healthy infants were enrolled. Since the majority of VRs were TBAs, notification of births to study staff was expected to be quite efficient. A home visit was conducted by study staff within 96 hours of delivery of the infant to administer standard Ministry of Health (MoH) BCG vaccine.

All infants born from consenting mothers who have been in Karemo and contiguous areas e.g Boro, Gem, etc. for at least one month were eligible for the study unless the parents stated that they intended to move from the area within the next year. Although the objective was to vaccinate all infants within 96 hours after birth, infants not vaccinated or vaccinated late were also enrolled and followed to evaluate the success of the aim of the study in reporting home deliveries and provision of BCG vaccination within 96 hours of birth, and since they contributed to TB incidence in the study area. All participants, regardless of when vaccinated, were entitled to the same services provided by this study.

After birth notification, a team of nurses were dispatched on motor bikes to the same geographic area targeting several deliveries at a time whenever possible. Detailed instructions of the village and compound location were obtained from the DSS GIS data and the VRs and relayed to the vaccinating team. To improve the speed of identifying households, village reporters from villages with targeted enrollments were involved in identifying the actual households at enrollment. Contacts were made with health facilities providing maternity services in Karemo and contiguous areas e.g Boro, Gem etc. Once deliveries had occurred in a health facility, study teams were dispatched to enroll the infants. It was anticipated that for deliveries occurring at health facilities; those infants will be provided with BCG vaccination at the time of delivery (i.e. within 96 hours). The recruitment process was fine tuned by development of a recruitment study operating procedure (SOP) and improved overtime with the changes in recruitment strategies being tracked through different versions of the recruitment SOP.

3.7.3. Informed Consent

A scripted informed consent was used to provide study information to the mother/parent or guardian. Signed informed consent to enroll the infant was obtained from the infant's mother during pregnancy and reconfirmed after delivery. For women not enrolled during pregnancy, the consent process took place after delivery in the event of a live birth. During the informed consent process, the study staff confirmed with the parent/guardian that they understood the study before signing the consent form. In particular, it was determined that the infant's parent/guardian understood that as part of study procedures, study staff would access and review the mother's and the infant's health records, including information on HIV. The risks and benefits section of the consent form was reviewed to ensure that the infant's parent/guardian understood. If it was unclear that the parent/guardian never understood the consent form, the infant was not enrolled into the study but the parent was revisited after 2 weeks by a study team member to review the consent form and ascertain his/her understanding of the study. Study staff kept track of the number of parents/guardians who withdrew consent after they were reminded of the above information and the study staff ensured they understood. If the parent/guardian indicated that s/he needed more time to think about his/her infant's participation, the study staff made arrangements to visit him/her again. Infants of parents who required more time to think about study participation were referred for BCG vaccination at the nearest maternal child health (MCH) clinic.

3.7.4. Enrollment and Baseline Evaluations

No evaluations were performed before the written informed consent of the infant's parent/guardian was obtained. All baseline evaluations either occured at home for infants born at home or within maternity child health units for infants born at health facilities.

All participants were assigned a study identification number (SIN). At the time of BCG vaccination, information was collected by interview and health record inspection about: The infant's birth (weight, length, head circumference, jaundice, birth information, etc.); BCG vaccination; Adults in the household who had TB or symptoms of TB; Parents' demographic characteristics (gender, age, occupation, education); Maternal HIV/PMTCT status.

This information was verified and additional information such as household size was obtained by interviewing mother or guardian. Information on HIV/PMTCT status of the mother/child was obtained from the antenatal card and from health facility maternity records. The parent/guardian was given information about TB and referred to the case verification ward (CVW) at Siaya District Hospital if the infant has symptoms, signs, or history of contact with an adult TB case.

At the end of the baseline evaluation, all parents/guardians were given comprehensive health education for early diagnosis of TB symptoms in infants and advised to contact study staff or report to the Siaya District Hospital (SDH) CVW, if any of these symptoms occurred. The study handed over education material to the parents that described the relationship between TB and HIV, and explained that infants with HIV infection or malnutrition may have had a false TB negative infection response to the tuberculin skin test (TST). In addition, education and educational material was given to the parents describing the "early warning signs" of childhood illness (IMCI) and they were advised to seek health care by coming to study clinic should they detect any of the danger signs in their infant.

3.7.5. Participant Follow Up

All participants were followed for a minimum of one year after enrollment and BCG vaccination, up until 2 years. During this time, four ways were used for all TB case surveillance (home visits, register review, self report and private sector) :

3.7.5.1. Home visits

Study staff arranged to meet mothers or guardians at the health facilities they routinely attended, every fourth month. If the mother never made it to the health facility, a study nurse carried out a home visit. Study staff made at least three attempts to visit the home of the infant. If the family had moved, neighbors were approached for directions to their new address. At each visit: The participant's parent/guardian was asked: Whether any member of the household had symptoms of TB; Whether the participant had been hospitalized since the last visit ; Whether the participant had any household contact with an infectious case of TB in the last 2 years ; Whether the participant had an HIV test and whether they would like to be tested if they declined the last offer for testing. If previously tested, what was the result? The participant underwent a physical examination which included (Height, weight, head circumference, mid-upper arm circumference). Targeted examination for signs of TB e.g. enlarged, matted lymph nodes. All relevant clinical, demographic and contact details of the mother and infant were entered onto the case report form. At each visit, the participant's parent/guardian were asked to contact the study office or the VR in the event of their infant/child was diagnosed with TB, being admitted to hospital, sufferred a serious or life threatening illness or if their infant died.

3.7.5.2. Register review

Documents were reviewed at the following institutions: TB registers at local TB clinics and at all health centers and hospitals in the study area (SCRH, Ng'iya Health Centre, Ting'wang'i Health Centre, Kadeng Ratuoro Health Centre, Nyangoma Kogelo Dispensary, Bar Olengo Community Centre) and at some hospitals outside the study area but within 50 km of Siaya District Hospital (Bondo District Hospital, Kisumu District Hospital and the Provincial General Hospital). Admission and discharge records at all hospitals, chest x-ray records, laboratory records (for those with positive smears who did not start treatment), the DSS IPD (In-patient department)/OPD (Out-patient department) registers and patient support center (PSC) records. The purpose of the review was to: To identify study participants diagnosed with TB at these facilities ; To identify study participants started on TB treatment at these facilities ; To retrieve and capture details of hospitalizations (including dates, length of stay and discharge diagnoses) ; To find individuals suffering from TB who are contacts of study participants by mapping their HDSS identification numbers (HDSS IDs) to households and seeing whether any of our enrolled infants resides in those households ; All participants identified with suspected or diagnosed TB or a history of contact were notified to the study field office and invited to the CVW to undergo further evaluation for TB. Every attempt was made to admit them to the CVW as early as possible after notification.

As part of the HDSS, all in-patient and out-patient facilities in Karemo had ongoing pediatric in-patient (IPD) and out-patient (OPD) surveillance. This captured information including symptoms and signs of TB that was critical in catching missed TB cases that presented to those health facilities. Using HDSS IDs IPD/OPD data was searched to see whether any our participants had been to those facilities. Any participant discharged with a diagnosis of

HIV/AIDS, moderate or severe malnutrition or a lower respiratory tract infection e.g. pneumonia, bronchiolitis, etc. in the study area was followed up and examined weekly, to assess response to treatment: if the response was poor the infant was offered admission to the CV ward to exclude underlying tuberculosis.

Study staff took note of those referred out of the study area and if referred to neighboring divisions or the Provincial General Hospital; every attempt was made to track those cases. Case notes and results of laboratory tests were collected and summarized. Referral to the nearest TB clinic for investigations for TB (sputum smear plus chest x-ray examination if indicated) was offered to any symptomatic household member of the family during follow-up visits every four months or to symptomatic mother/guardian during admissions at the case verification ward.

3.7.5.3. Self Report

In between regularly scheduled follow-up and unscheduled visits, contact was maintained with the participants' parents/guardians to ensure prompt follow-up of study participants in the event that they experience an illness, symptoms of TB, or have TB diagnosed in a household member (i.e. TB suspects for CVW admission). At the time of the participant's enrollment, each parent/guardian was given a "phone card" with the telephone number of the study office printed on the back for use in such a circumstance. In addition, the village reporters or other study staff contacted the participant's parent/guardian to follow-up on the participant, or during a well-baby visit at a health center.

3.7.5.4. Private Sector

Area medical practitioners and hospital personnel were sensitized about the study, and contacted regularly by staff to review their records to check whether any participating infant presented with symptoms or signs suggestive of TB disease.

3.7.6. Mortality surveillance, determining cause of death, severity and causal relationship

At enrollment, mothers of infants were informed of the need that should their infant die, to determine the cause of death accurately and were asked to contact study staff as soon as possible if a death occurred. Relevant staff at all hospitals, clinics and mortuaries and other individuals who might find out about an infant's death shortly after it occurred were asked to contact a study team member about the death. The study team determined whether the deceased infant was a study participant. Any participant enrolled into the study discovered dead would have the following done, if possible: All relevant case notes accessed from hospitals, clinics or private practitioners copied and evaluated by a study clinical officer for cause of death. A copy of the participant's death certificate and autopsy report, if performed, obtained, and evaluated by a study clinical officer for cause of death. (Pathological autopsies are seldom performed in this population). As part of the HDSS, a "verbal autopsy" questionnaire was administered to the primary caregiver (or a family member where the primary caregiver was not accessible) at least four weeks after death. This was evaluated by two HDSS clinical officers for cause of death using a standardized algorithm. In the case of disagreement, a third HDSS clinical officer/medical officer was used. In addition, study clinical officers from this study performed additional reviews of verbal autopsy forms for all

study infants in order to further confirm these reviews. Referral to the nearest TB clinic for investigations for TB (sputum smear plus chest X-ray examination if indicated) was offered to any household member of the deceased participant who was symptomatic.

3.7.7. Schedule of Participant Evaluations

All participants were assigned a study identification number (SIN) were followed up according to the protocol unless consent for follow-up was withdrawn. The sponsors and local/international regulatory bodies were notified of any deviations from protocol visits or evaluations and these evaluations, if applicable, were rescheduled or performed at the nearest possible time to the original schedule.

3.7.8. HIV counseling and testing

HIV counseling and testing was routinely offered to all mothers in the study area during pregnancy, during labour or post delivery. This was facilitated through the US President's Emergency Plan For AIDS Relief in Africa (PEPFAR). Provider initiated HIV testing and counseling (PITC) and HIV care and treatment facilities were available in Karemo in Ng'iya Health Centre, Ting'wang'i Health Centre, Nyangoma Kogelo Dispensary, Bama Nursing Home (BNH) and Bar Olengo Community Centre, which doubled as TB treatment facilities. At SCRH, there was a voluntary counseling and testing (VCT) center which served as an alternative testing site for those reluctant to have PITC at recommended sites. Furthermore, the PEPFAR program initiated home-based, door to door HIV testing in the study area in 2008.

For this study, consent was obtained prior to conducting HIV testing. If a mother was enrolled during pregnancy, the study nurse enquired whether she had been tested in the two months preceding the day of the interview. If the result was negative and the test done more than 2 months prior to the interview date, the study nurse encouraged her to go to her nearest PITC or VCT center for re-testing. If the result was positive, she was given information on PMTCT, advised on the health benefits of hospital delivery and referred to the nearest PSC for care.

If enrollment was done during or shortly after delivery and the mother had not been tested for HIV in the previous 2 months, she was referred for HIV testing and if the result is positive, she was given nevirapine in labour, where possible, and the infant was given a single dose of nevirapine within 72 hours. If the mother has been tested for HIV in the previous 2 months and the result was positive, she was supplied with nevirapine to take at the onset of labour, and to give to her infant within 72 hours of birth.

All infants exposed to maternal HIV were referred to a patient support centre in order to receive AZT for 4 weeks, cotrimoxazole throughout the breastfeeding period; and the mothers were adequately advised on safe infant feeding practices. All mothers of infants enrolled in the study were asked to bring the infants to SCRH for HIV PCR at 6 weeks of age. If an infant was enrolled after discharge from the hospital or at home, included in the consent form, permission was sought to extract information on PMTCT status from the health facility maternity records. Participant privacy and confidentiality was assured at all times, and special care taken to ensure the privacy of the HIV counseling process and the confidentiality of the data. All parents of infants were told about the HIV care and treatment services available at SDH and in their area and encouraged to seek them. Linkages were formed with PSCs to check whether parents and infants referred for care reached the referral points.

3.7.9. TB treatment and follow-up of TB diagnoses

For infants, if a diagnosis of active TB was made, the case was reported to the Ministry of Health's Division of Leprosy, Tuberculosis and Lung Disease (DLTLD) via the County Tuberculosis and Leprosy Coordinator (CTLC) based at the SCRH. Treatment was started at the hospital and parents/guardians were given a two-week supply of anti-TB medication, following the treatment guidelines of the DLTLD, instructed to contact their nearest treatment center, and given a referral letter. Study staff notified the respective treatment centre and followed up the patient up to ensure that s/he subsequently reported to the referral center. If a study participant was diagnosed with TB in another health facility, the infant was offered admission to the SCRH CVW for further investigations and to obtain sputum for culture.

Study staff abstracted from the TB register and recorded in study documents, as applicable, whether treatment was started, what treatment was prescribed, the date it was started, the date it was stopped and the treatment outcome. Outcomes will be recorded using the standard WHO DOTS program definitions of cured, completed, failure, default, transferred out and died. Study staff assisted the clinics to trace any treatment defaulters and conduct home visits to assess outcome.

3.7.10. Laboratory Procedures

Induced sputa and gastric lavage specimens were digested using Nalc/NaOH and concentrated for better yield by centrifugation. Smears were prepared and read using the FM microscopy technique to identify AFB. Digested sputa were inoculated into solid culture, Lowenstein Jensen (LJ) and liquid culture (MGIT 960) media. Detailed instructions for laboratory procedures were developed separately in standard operating procedures. Isolates of positive cultures were kept and subjected to drug susceptibility testing and fingerprinting if new

funding becomes available. Through collaboration with San Raffaele Scientific Institute, Supranational Reference Laboratory (SRL), Milan, Italy; quality assurance and quality control were ensured through proficiency panel testing. At the initial stages of the project; inactivated TB bacteria were shipped to the SRL for spoligotyping and DNA finger printing. The SRL's support included feed back on cross contamination based on the list of samples handled at given periods during the study. All positive cultures will be speciated by PCR to exclude disease caused by non-tuberculous mycobacteria (NTM) and BCG disease.

3.7.10.1. Specimen collection

Detailed instructions for specimen management, including collection, processing, storage, shipment and infection control, were developed separately in a Specimen Management section of the standard operating procedures.

3.7.10.2. Specimen Storage

After processing, if the relevant consent had been received, aliquots of sputum specimens were stored at **Kisian KEMRI CGHR TB Laboratory** in a -70^{0}C freezer for up to 20 years after completion of the study. During storage, the samples were delinked from participant identifiers (anonymized). These specimens may be used in the future to carry out other tests for TB or conditions related to TB, including HIV, if new funding becomes available. Separate IRB approval will be sought for any such future evaluations.

3.7.10.3. Routine Laboratory Evaluations

Study participants suspected of having TB were referred for diagnostic procedures which included some or all of the following: HIV-1 rapid test and HIV-1 PCR test ; two serial early morning induced sputum specimens and two serial early morning gastric lavage specimens for detection of acid fast bacilli (AFB) by fluorescent microscopy and for culture for *M. TB;* their household members who are symptomatic were referred to neighboring TB diagnostic facilities for further workup and HIV counseling and testing.

3.7.11. Safety considerations

3.7.11.1. Adverse Events

Since there was no experimental pharmaceutical product being administered in this study, there was no formal safety surveillance. However, adverse reactions may occur during clinical procedures such as sputum induction e.g. bronchospasm or TB skin testing e.g. ulceration. The onset and resolution times and dates of each event and the action taken in response to the reaction was documented. All serious adverse events were closely monitored and reported to the IRB/ERC. Expected adverse events included: Infant deaths due to non-TB causes (HIV, malaria, ARI, tetanus, diarrheal disease, etc.) (250 deaths expected - 8.9%) ; Infant deaths due to TB ; BCG disease following BCG vaccination (fewer than 1 case expected).

Study-related Adverse Events included: Bronchospasm during sputum induction and local reactions to tuberculin testing.

3.7.12. Completion of Study and Loss to Follow-up

Enrolled infants were considered to have completed the study if followed up between 1 year upto a maximum of 2 years. It was specified on the case report form whether or not the participant completed the study follow-up procedures. Interim participant status was noted per protocol. A participant were considered lost to follow-up if: three or more documented attempts to contact the participant were made without success, and/or the participant was confirmed to have moved from the study area and was not traceable and/or no contact was established by the time the study was completed such that there was insufficient information to determine the participant's status depending on enrollment period. At this time, a documentation of lost to follow-up was entered on this participant's Study Completion CRF. All attempts were made to trace participants who moved out the study area and they were only be labeled as lost to follow up if they were untraceable. Investigators documented attempts to re-establish contact with missing participants throughout the study period. If contact with a missing participant was re-established, follow-up was resumed according to the protocol.

3.7.13. Final Study Visit

At the final visit, all parents/guardians were given information about TB and informed of the need to seek treatment if they or their infant developed symptoms or signs of TB. All parents/guardians were asked if the participant had any episode of TB during the study.

3.7.14. Statistical Considerations

The sample size for this study was calculated using current birth estimates in the target population. The total population in the study area was estimated by the time as 181,272 with 80% of births taking place at home. The infant mortality rate was estimated to be 86/1000 (Ref, MOH data, Siaya District 2007). Assuming a 5 percent exclusion rate and 15% refusal rate, the enrollment target for this study is 2,900 infants (~3600 X 80%). The estimated annual notification rate of total TB (all types) is 440/100,000 (0.4%) among the general population. The annual incidence of culture-positive TB among infants less than 1 year of age is expected to be 0.5% based on experience at another African site. Given 2900 infants, a 95% confidence interval for the above would be 0.5% (0.29%, 0.85%) based on a binomial approximation.

Most successful strategies for recruiting infants into the tuberculosis cohort study assessing the methodologies employed and BCG profiles (vaccination at time of enrollment, effort time taken to vaccinate infants, vaccinations percentages for cohort) will be tabulated. Of interest also is to draw a flow chart that describes the recruitment numbers and percentages from notifications, deferrals, refusals, screenings, consents, ineligible and enrolled. Loss to follow-up (at 6 weeks and four monthly visits), withdrawals, deaths and retention will also be included in the flow chart. Reasons for screen failures and ineligible will also be documented. Recruitment and enrollment numbers by month and year are also graphically represented to monitor the trends with time.

3.7.15. Baseline Demographics and Other Characteristics

Case report forms (CRFs) designed for collection of information that was saved into the study database. Potential infants and their parents/guardians were screened prior to enrollment to establish eligibility. The following information was gathered from the parents/guardians: weight (≥ 1700 grams) changed to kilograms in subsequent analysis; source of knowing about the study (village reporter, antenatal clinic, other – specify). From enrollment CRFs: date of enrollment was recorded and used to calculate ages of infants, fathers and mothers from provided dates of births if known. Housing type (mud, semi-permanent, permanent, other). Mother's occupation (subsistence farming, commercial farming, salaried worker, small business e.g. sell maize, business owner e.g. duka or shop, skilled laborer e.g., carpenter, unskilled laborer e.g. construction worker; fishing; not working; housewife. Father's occupation (subsistence farming, commercial farming, salaried worker, small business e.g. sell maize, business owner e.g. duka or shop, skilled laborer e.g., carpenter, unskilled laborer e.g. construction worker; fishing; not working. Level of education obtained by parents – mother and father (none, primary, secondary, tertiary or unknown). How many living brothers and sisters does this child have? (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, >10). Birth details; place of birth (hospital, traditional birth attendants, home, other). Numbers of babies born (1, 2, 3). Sex of Baby (Male, Female). Infant's estimated gestational age at birth (in weeks). Baby's weight in grams. Baby's length at birth in centimeters. Was it a difficult delivery (yes or no). If yes state which one, prolonged labor, fits before giving birth, mother had febrile illness at the time of giving birth or the waters broke ≥ 24 hours before delivery. Maternal TB and HIV status: Did the mother have oral thrush during the last one year (Yes or No)? Has the mother been tested for HIV in the last 2 months (Yes or No)? What was the result? (Positive or Negative). If negative (and tested greater than 2 months before) or not tested, refer for repeat

test or test respectively. If mother referred for test; result of HIV antibody test: Positive, negative or not done. Did mother receive ARVs during pregnancy? (Yes or No). Did mother receive ARVs during labor? (Yes or No). Did child receive ARVs within 3 days of birth? (Yes or No). Has the mother been diagnosed with TB during the last one year? (Yes or No). If yes, is she currently on treatment? (Yes or No). How long has she been on treatment? (≤2 weeks or ≥2 weeks). Is Isoniazid prophylaxis needed for the baby? (Yes or No). BCG vaccination status: Date of BCG vaccination, Time of BCG vaccination, Not vaccinated, reasons for not receiving vaccination (parent declined, baby too sick, other – specify). Inform mother, the baby will be tested for HIV using PCR at 6 weeks of age. Baby given appointment for 6 week HIV PCR test (Yes or No). Name of clinic or facility where sample for HIV PCR collected. Appointment for delivering HIV PCR results scheduled (Yes or No). HIV PCR results (Positive, Negative or Not done).

Follow-up CRFs collected the following information: Was this study visit completed? Yes or No. If No, reason why not (Consent annulment/ withdrawal, Loss to follow up, Moved out of study area, Died). Date of follow-up visit. Does the child currently have any of the following signs or symptoms? Yes or No. If yes, check the following (Cough ≥ 2 weeks, Fever ≥2 weeks, Night sweats ≥2 weeks, Wheeze≥2 weeks, Failure To Thrive, Loss of appetite, Unusual fatigue or weight loss (maternal assessment). If any of the above symptoms is ticked, schedule a case verification ward admission and notify the follow-up and clinical coordinators. Since the last visit, has s/he been taken to any clinic other than for vaccines and well baby visit? Yes or No. If yes, what was the diagnosis / reason for visit: Pneumonia, Bronchiolitis, Malnutrition, HIV/AIDS or Other – specify. If infant referred to the case verification ward for TB Diagnostic workup. What is the infant‟s HIV status (Positive, Negative or unknown). If negative or unknown and 18 months, request for HIV PCR. Result

of infant PCR (Positive, Negative or Not done). If negative or unknown and >18 months of age, request for HIV antibody test. Result of HIV antibody test (Positive, Negative, or Not done). Household TB Contact Details: Is there anyone living in the same household as the child have any TB symptoms? No or Yes. If yes, How many? 1 person, more than 1 person or unsure.

Is the person suffering from TB in the household currently on TB treatment? Yes or No. If yes, does s/he take it everyday? Yes or No. Where does s/he collect the treatment? SDH, Ng‟iya, Akara, Kadenge, Ting‟wang‟I, Kogelo Nyang'oma. Which date did s/he start taking TB treatment? If there was any history of TB contact, refer to the case verification ward.

Overall status of the child: Child is well and there is no history of TB contact ("W"); Child is well but there is a history of TB contact ("C"); Child has symptoms +/ signs of TB but no history of TB contact ("S"); Child has symptoms +/ signs of TB and a history of TB contact ("CS"); Child on TB treatment ("TB Rx"); Child on INH prophylaxis or Child has died ("D").

TB Referral: After last visit: Has your child been in the CV ward? Yes or No. Was s/he diagnosed with TB? Yes or No. With this visit: Has the child been booked into Siaya District Hospital CV ward for TB diagnosis? Yes or No. TB Diagnostic Evaluation/CV Ward.

Information from referral letter/mother: How did we find out this child might have TB? Routine home visit day; Hospital records; Self report; Private, mission, NGO sector report. Has the child been diagnosed with TB? Yes, No or Unsure. Has the child been started on TB treatment? Yes, No or Unsure. Has the child been started on Isoniazid prophylaxis? (Yes, No or Unsure).

Case verification Ward Admission: Which date was infant admission? How many times has this child been admitted to the CV ward before? (0, 1, 2 or 3). History from the Road to Health Card. RTH Card seen (Yes or No). Comment on the child's growth. (Good, Danger, Very dangerous or No RTHC). Length baby was breastfed in (months). Is baby still breastfeeding? (Yes or No). Has your child been coughing more than usual during the last month? (No or Yes); How many days if more than 7 days how many wks or Unsure. Has your child been wheezing more than usual during the last month? (No or Yes); How many days if more than 7 days how many wks or Unsure. Has your child been having breathing difficulties? (No or Yes); How many days if more than 7 days how many wks or Unsure. If yes does you child have fast breathing? (No or Yes); How many days if more than 7 days how many wksor Unsure.

Does your child have in-drawing of the chest while breathing? (No or Yes); How many days if more than 7 days how many wks or Unsure. If yes, has your child received treatment? (No or Yes); How many days if more than 7 days how many wks or Unsure. If yes, is the child‟s breathing getting better with treatment? (No, Yes or Unsure).

Has your child been hot to touch more than usual during the last month? (No or Yes); How many days if more than 7 days how many wks or Unsure. Has your child been sweating at night more than usual during the last month? (No or Yes); How many days if more than 7 days how many wks or Unsure.

Has your child been growing well? (Gaining weight, not gaining not losing, losing weight or don't know). Has your child lost weight? (Yes or No). Has your child been eating more poorly than usual during the last month? (No or Yes); If yes, how many days if more than 7 days

how many wks or Unsure. Has your child been tired (decreased playfulness) more than usual during the last one month? (No or Yes); If yes, how many days if more than 7 days how many wks or Unsure. Have you taken your child to any health facility due to any of the above symptoms? (No or Yes). If yes, which health facility? State the facility.

TB contact details: Is there anyone living in the same household as your child whom you think may have TB or may have had TB in the last year? (No or Yes). If yes, 1 person, more than 1 person or unsure. What is the relationship of the main person with TB to your child? [i.e. the person mentioned above who has the most contact with the child]. (Mother, Father, Sibling, Granny, Grandpa, Child, Other family, other person (not family) or not applicable (NA). Is the person suffering from TB in the household currently on TB treatment? (Yes or No). If yes, does s/he take it regularly? (Yes or No). Where does s/he collect the treatment? State the health facility.

HIV status: What your infant‟s HIV status? Positive, negative or unknown. If negative or unknown and 18 months, request for HIV PCR. Result of infant PCR: Positive, Negative or not done. If negative or unknown and >18 months of age, request for HIV antibody test. Result of HIV antibody test: Positive, Negative or not done.

General Information and Examination; Weight in kilograms. Height in centimeters. Mid upper arm circumference [MUAC] in centimeters. Head circumference in centimeters. Oedema, (Present or Absent). Status of participant: Well and there is no history of TB contact (“W”). Well but there is a history of TB contact (“C”). Has symptoms +/ signs of TB but no history of TB contact (“S”). Has symptoms +/ signs of TB and a history of TB contact (“CS”). Was diagnosed with TB infection? Was diagnosed with TB disease? If the participant was a

confirmed TB case, where was it first diagnosed? (Study team through the CVW; Public health care facility; Private health care facility; Verbal autopsy). How was the TB case detected? Through: (Study screening and specimen collection, DSS surveillance (Verbal Autopsy), IPD/OPD surveillance, Self/VR referral. Participant status: On TB treatment, Defaulted, Lost to follow-up (date of last contact below), Withdrew Consent (fill in date of withdrawal below), Death (fill in death details below) ("D"). Date of last contact and date of withdrawal.

Baseline demographics of the participants (infant) and parents' demographic characteristics were selected from the information collected using the CRFs (e.g, infant sex (male or female), infant age (calculated by subtracting enrollment date (year, month and day) from date of bith (year, month and day), age of parents (calculated by subtracting enrollment date (year, month and day) from date of bith (year, month and day), mothers' occupation (subsistence farming, commercial farming, salaried worker, small business, business owner, skilled laborer, unskilled laborer, fishing, not working, housewife), place of residence (generated by looking at places of origin to categorize as HDSS (Gem, Asembo or Karemo) or non-HDSS area (Boro), attendance of unscheduled visits ((infants who attended unscheduled visits for sick infants versus those who did not turn up for unscheduled visits), mother's education (none, primary, secondary, tertiary or unknown), type of housing (mud, semi-peermanent or permanent), place of birth (home or health facility), place of enrollment (at home, traditional birth attendant,health facility or other place where infant was born), mother's HIV status (positive, negative or not done), mother's attendance of antenatal care (yes or no), numbers of infants delivered (singletons, twins or triplets), number of mother's other children (other siblings i.e. 1-10 and >10) and infant's weight at birth (grammes was converted) was summarized for all participants in two frequency tables for infant and maternal characteristics.

Fathers were excluded in the selection of variables because of scant information collected from their spouses. Housing type was collapsed into three categories i.e., mud, semi-permanent and permanent. Mother occupation was collapsed into five categories i.e., farming (subsistence and commercial farming), salaried worker, business (small business and business owner) and laborer (unskilled and skilled laborers). Maternal education was collapsed into four categories i.e., none, primary, secondary and tertiary. Infant place of birth was collapsed into home and health facility. Number of infants born was categorized into singletons and twins. Age was calculated in days for infants. Birth weight was converted to kilogrammes. Number of other siblings of infant by mother was categorized into ≤ 3 and > 3 children to describe family size. Mother's HIV status was classified into two variables i.e., positive and negative. Mother's age was calculated in years. Detailed Variables description, codes, values and names are in Table 20 in the appendices section page 130. Average duration of study follow-up (time from enrollment to study completion, death, or withdrawal of consent) was calculated for all participants. Number (percentage) of participants who left the study area was summarized. Reason for outmigration, as available, was presented by key demographic parameters, and for all participants. In addition, missing data will be included in the frequency tables.

3.7.16. Statistical Analyses

Baseline demographics of the infant and parents' demographic characteristics were collected and summarized by frequency and corresponding percentages for categorical variables and descriptive statistics (median, IQR) where applicable. Bivariate analysis was performed using Pearson's chi-square test (p-values) whereas logistic regression was done using backward elimination to study the effect of co-variates on outcome of interest to get a parsimonious

model. Considering follow-up as an event of interest, time to being lost to follow-up was calculated and explored using Kaplan-Meier curves and the difference between the curves determined using log-rank test for binary variables and Generalized Wilcoxon test for > 2 variables.

The outcome in logistic regression analysis is coded as 0 or 1, where 1 indicates that the outcome of interest is present, and 0 indicates that the outcome of interest is absent. We defined p as the probability that the outcome is 1; the multiple logistic regression model can be formulated as follows:

$$p(y = 1|X) = \hat{p} = \frac{\exp\,(b_0 + b_1X_1 + b_2X_2 + \cdots + b_pX_p}{1 + \exp\,(b_0 + b_1X_1 + b_2X_2 + \cdots + b_pX_p}$$

Where $\hat{p}$ is the expected probability that the outcome is present; X_1 through X_p are distinct independent variables; and b_0 through b_p are the regression coefficients to be estimated from the model.

A proportional hazard model (Cox (1972)) defined as:

$$h(t, \boldsymbol{X}) = h_0(t)\exp\,(\sum_{i=1}^{p} \beta_i X_i)$$

Where $h_0(t)$ is the unspecified baseline hazard function at time *t*, **X**= $(X_1, X_2 \ldots, X_p)$ are the explanatory/predictor variables and β_i are the regression coefficients for the *i*th individual.

3.7.17. Assessment of Objectives

3.7.17.1. Analysis of Objectives

Characterize recruitment and enrollment activities to document notifications, prescreening and screening outcomes prior to enrollment by way of a flow chart illustrating numbers and percentages as appropriate. A graphical representation of recruitments and enrollments by month and year to assess the trends over the study period. Tabulate recruitment strategies used in the study and profile BCG vaccinations at enrollment, effort time taken to vaccinate and evaluate vaccination status post-enrollment. Run frequencies for infant and maternal

characteristics to tabulate distribution. Assess distribution of infant and maternal characteristics (infant age at birth in days, infant birth weight in kilogrammes and maternal enrollment age in years).

Factors associated with place of delivery (home versus health facility) was analysed using maternal variables (education, occupation, housing type, received antenatal care, HIV status, number of children mother has of her own, place of residence, mother's age). This analysis was carried out using logistic regression for crude and adjusted odds ratios.

Factors associated with loss to follow up was analyzed using infant variables (place of enrollment, place of birth, infant sex, number of infant delivered, attendance of unscheduled visits, birth weight, infant age at enrollement and maternal variables (education, occupation, housing type, attendance of antenatal care, HIV status, number of children mother has of her own, place of residence, mother's age). Plot Kaplan Meier estimates of significant variables.

A descriptive analysis of signs, symptoms, clinical impressions and investigations documented during the unscheduled visits for infants enrolled in the tuberculosis cohort study. Frequencies, interquartile ranges (IQRs) and means were ran as applicable. A graphical representation of unscheduled visits by day of the week. An evaluation of prescriptions dispensed to infants during unscheduled visits. Tabulate suspects, TB cases and deaths identified from unscheduled visits.

Factors associated with unscheduled visit attendance (yes versus no) was analyzed using infant characteristics (place of enrollment, place of birth, infant sex, number of infant delivered, unscheduled visits, birth weight, infant age) and maternal characteristics (maternal

education, maternal occupation, housing type, mother's receipt of antenatal care, HIV test status, number of children mother has of her own, place of residency and maternal age). These analyses were carried out using logistic regression for crude and adjusted odds ratios.

3.7.17.2. Participant Populations

All infants enrolled in the study were used in the analysis of the primary objective and in all exploratory or supportive analyses. A separate sub-analysis for mothers of infants was carried out to assess the relationship of maternal variables with study objectives.

3.7.17.3. Computer Methods

Statistical analysis was performed using STATA 12.0 under a Windows operating system.

3.7.18. Data Collection and Monitoring

A detailed description of how data was collected and monitored is provided in summary is as follows : Source documents included infant medical records, TB registers, HIV registers/clinic records, HDSS verbal autopsy data, out-patient surveillance data, inpatient surveillance data and study documents. Data was captured onto personal digital assistants (PDAs), laptops or paper case report forms (CRFs). Data collected on PDAs were uploaded to laptop computers and later transferred into the study database. Paper CRFs were checked and scanned, and the data verified and then exported to the main database. The study database was stored on a server on a secure network with nightly backup at the KEMRI CGHR offices in Kisian. Programmatic edit checks facilitated data cleaning and generate data queries for discrepant values. Data was evaluated and stored in such a way as to maintain confidentiality

of infants and their families. All study records are kept secured for a minimum of 5 years following the completion of the study. The investigator permitted study monitors to access the network server location where the entered data and all study-related documents are stored.

3.7.19. Human Subjects

3.7.19.1. Ethics and Regulatory Considerations

The study was conducted according to the Declaration of Helsinki, ICH-GCP, local regulatory requirements, Protection of Human subjects (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312). The protocol and informed consent form were reviewed and approved by the Institutional Review Boards (IRBs) or Independent Ethics Committees (IECs) of the participating clinical site (Center for Respiratory Diseases Research (CRDR) Scientific Committee (SC) ; Kenya Medical Research Institute (KEMRI) Scientific Steering Committee (SSC) and Kenya Medical Research Institute (KEMRI) ERC ; Centers for Disease Control and Prevention (CDC) IRB and IRB used by the Aeras Global TB Vaccine Foundation (Aeras). The investigator regularly informed the IRB/IEC on the progress of the study, at minimum once a year. Aeras had an independent IRB review and approval of the protocol and informed consent form and kept the IRB informed of the progress of the study. Written informed consent was obtained from each participant prior to any protocol-specified procedures being conducted. To maintain confidentiality, study ID numbers were used to identify the participant's laboratory specimens and study documents. All study records were maintained in a secured location. Clinical information was not released without written permission from the participant except as necessary for monitoring or auditing of the study by Aeras or its designee or applicable regulatory authorities.

3.7.19.2. Institutional Review Board or Independent Ethics Committee

All the documents the IRB/IEC needed to fulfill its responsibilities, such as the protocol, protocol amendments, information concerning participant recruitment, payment or compensation procedures, etc., were submitted to the IRB/IEC by the investigator. The IRB's/IEC's written, unconditional approval of the study protocol and the informed consent form were in the possession of the investigator/clinical site staff prior to the conduct of any protocol-specified procedures. Modifications to the protocol were not implemented without prior written IRB/IEC approval (the Kenya Medical Research Institute (KEMRI) scientific steering committee (SSC) and the IRB's/IEC's of KEMRI, CDC and Aeras) except when necessary to eliminate immediate hazards to the participants or when the modification involves only logistical or administrative aspects of the study. Such logistical or administrative modifications were submitted to the IRB/IEC in writing by the investigator, and a copy of the correspondence to verify the submission was maintained. Documentation of IRB/IEC approval was sent to the sponsor immediately upon receipt. The investigator informed the IRB/IEC of any modifications to the informed consent form or any other documents previously submitted for review/approval and of any new information that adversely affected the safety of the participants or the conduct of the study, and provided an annual update with or without a request for re-approval, and advised the IRB/IEC when the study has been completed. Any documents or forms provided to the participant (e.g., information cards, form letters from the investigator), and all forms of study advertising (flyers, brochures, print advertisements, radio or television scripts, etc.) were approved by the IRB/IEC prior to the documents or forms being provided to any participant.

3.7.19.3. Informed Consent

Informed consent was obtained in accordance with the current edition of the Declaration of Helsinki and ICH-GCP prior to any protocol-specified procedures being conducted. Informed consent was documented in writing on a consent form approved by the IRB/IEC. All relevant information was provided in both oral and written form in a way that is understandable to the participant's parent/ guardian. Ample time and opportunity was given for the participant's parent/ guardian to inquire about details of the study. The written consent document embodied the elements of informed consent as described in the Declaration of Helsinki and complied with local regulations**.** The investigator or the investigator's qualified designee explained the nature of the study and informed the participant's parent/ guardian that participation was voluntary and that they could withdraw the participant at any time. The participant's parent/ guardian were informed about the study's purpose including why the participant was selected to participate, the goals of the study, the expected benefits and risks of participating, including unforeseeable risks. The individual was provided with a description of the procedures and the estimated duration of time required to participate in the study, as well as alternative interventions or courses of treatment, if applicable. The participant's parent/ guardian received an explanation as to whether any compensation and any medical treatments are available if injury occurs and, if so, what they are, where further information may be obtained, and whom to contact in the event of a study-related injury. Individuals were told whom to contact for answers to any questions related to the study. All parents and guardians of participants were informed that their child's participation was voluntary and that they were free to withdraw them from the study for any reason at any time without penalty or loss of benefits to which they are otherwise entitled. The extent of the confidentiality of participant records was defined and the participant's parent/ guardian informed that applicable data

protection legislation applied. The parents and guardians of participants were informed that monitor(s), auditor(s), IRB/IEC members, and the applicable regulatory authorities may be granted direct access to the participant's original study medical records for verification of protocol-specified procedures and/or data, without violating the confidentiality of the participant to the extent permitted by the applicable laws and regulations. The participant's parent/ guardian were informed that his/her signature on the informed consent form indicated that s/he has decided to participate in the study, having read and discussed the information presented. All parents or guardians were be told that they may withdraw from the study at any time. Any such withdrawal was fully documented by the study site. The original, signed informed consent form for each participant was maintained by the investigator as part of the participant's study records. A copy of the signed informed consent form was provided to each participant's parent/ guardian.

3.7.20. Relationship with Regional Health Services in the study area

3.7.20.1. Study awareness

The regional and local TB control program staffs were made aware of the study and their assistance sought in raising general awareness of and support for the study. This was done through the regional and district TB and HIV coordinators.

3.7.20.2. Treatment of infants with latent TB infection

The policy of the Kenyan Division Leprosy, Tuberculosis and Lung Disease (DLTLD) is to offer treatment for latent infection to individuals under the age of 5 years. Those found to be infected but with no evidence of active disease were offered isoniazid therapy.

3.7.20.3. Treatment of children with TB

Those who have microbiological or other evidence of TB were prescribed the standard TB treatment regimen recommended by the DLTLD and the WHO. TB treatment and care is available free of charge in all public sector facilities in this community.

3.7.21. Management of TB suspects

Those infants with symptoms and/or signs of TB or a history of contact with a TB case were referred to the case verification ward (CVW) at SCRH for further investigations. The results of the investigations were communicated in writing to the parent/guardian and to the nearest TB clinic. The parent/guardian was given a referral letter to take the infant to the nearest public sector TB treatment clinic. The results of smears and cultures were reported to the DLTLD. Symptomatic members of the family were referred to the nearest TB treatment clinic for sputum smears, chest x-rays and treatment if found to have TB.

3.7.22. Compensation and incentives

The study reimbursed the costs of transportation for the parent/guardian of infants attending the study clinic, admission to the CVW at the SCRH according to distance travelled from home to central clinic. It ranged from Kenya Shillings (KES) 300-500 (€ 2.5-4.2). The study

provided a small incentive to mothers of children admitted to the CVW in the form of mother/baby products, the value did not exceed KES 500 (€ 4.2) upon discharge. Infants were enrolled at home, clinics or hospitals by study nurses. Recruitment teams were issued with blocks of study identification numbers (SIN). If an infant is eligible for the study, individual written parental consent was obtained. Infants were screened by study staff to assess eligibility for entry into the study. A master log was maintained of all screened infants. On enrollment day (study day 0), the investigator confirmed that the participant met all eligibility criteria. Only infants whose parent/guardian had signed an informed consent and who met eligibility criteria were enrolled into the study. A participant was considered to be entered into the study upon assignment of a SIN.

3.7.23. Confidentiality

Every effort was made to keep the infants' study records strictly confidential, in particular results of HIV test results. To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records were identified by a coded number and participant's initials only. All study records were kept in a locked filing cabinet and code sheets linking a participant's name to a study identification number was stored separately in another locked filing cabinet. Clinical information was not be released without the written permission of the participant's parent/ guardian, except as necessary for monitoring by the relevant regulatory agency or the sponsor of the clinical trial. In regard to the intended use of fingerprinting technology, fingerprint readers pose no greater threat to confidentiality than issuing a study participation card with the study ID on it to a research participant and much less threat to confidentiality than a card with name or picture on it; such a card had already been approved for use in the HDSS. The principal investigator also complied with all applicable privacy

regulations (e.g. Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

3.7.24. Expected application of the results

An assessment of recruitment, loss to follow-up and morbidity among infants enrolled in an infant tuberculosis cohort study will inform the planning and conduct of future vaccine trials. Elicit a better understanding of the epidemiology of TB in infants from populations with a high burden of HIV. Establish better understanding of recruitment and follow-up of infants into TB research. Describe morbidity patterns of infants in this cohort during the 1-2 years follow up to inform planning of pediatric studies and trials.

4. Results

4.1. Recruitment outcomes, accrual trends and baseline characteristics of infants enrolled into TB cohort study

4.1.1. Notifications, recruitment and retention of infant cohort

Community entry process began in February 2009 where stakeholders from the Ministry of Health and staff, Provincial Administration and 159 village reporters, 20 CAB members, 20 administrative officials were engaged. Community meetings and ongoing community mobilization activities drove the recruitment of potential participants and subsequent enrollment of infants into the cohort study. Strong collaborations were established with maternal child health clinics and hospitals providing antenatal care, preganancy delivery and post natal care services.

Figure 3. Recruitment, enrollment, follow-up and retention

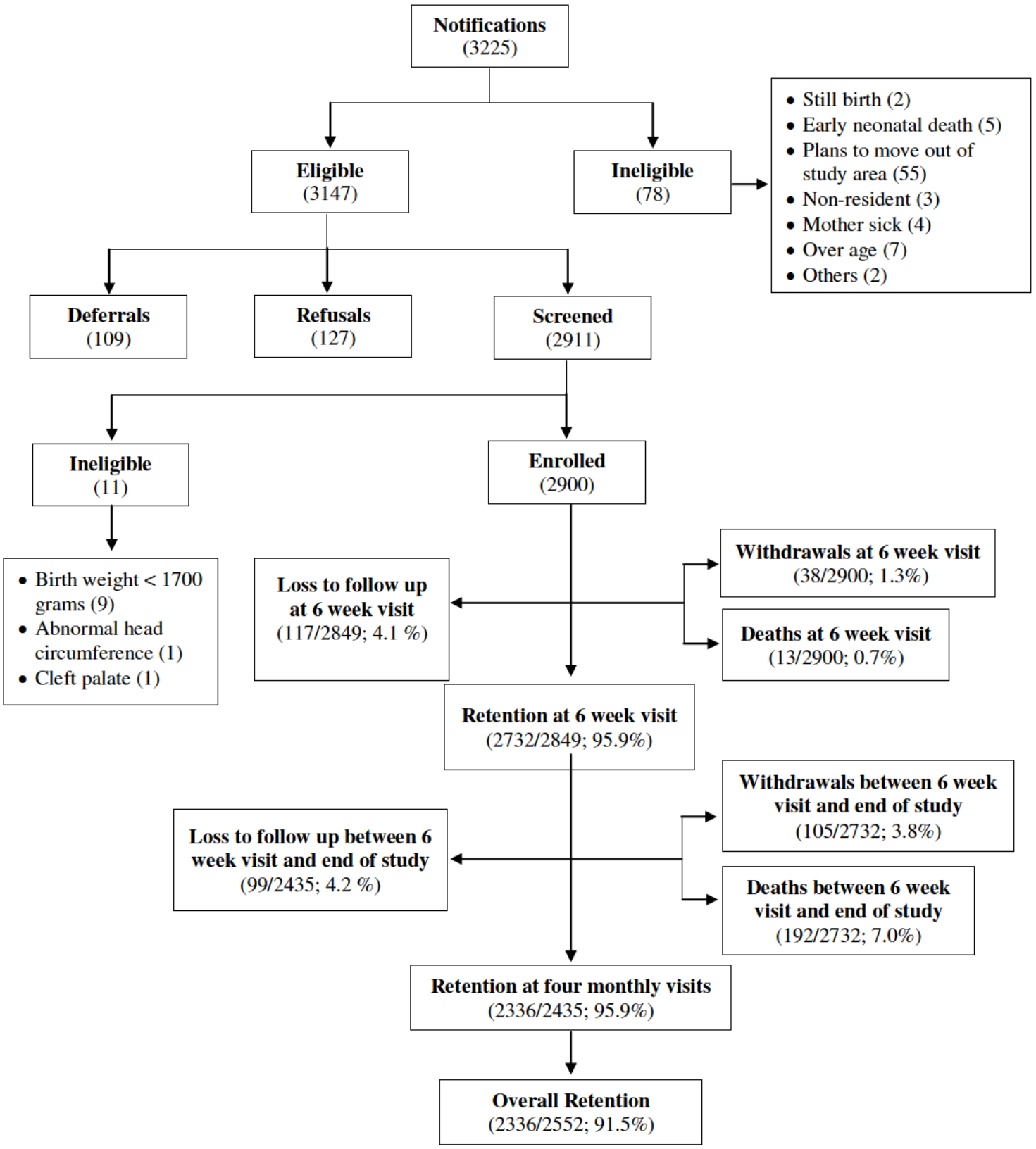
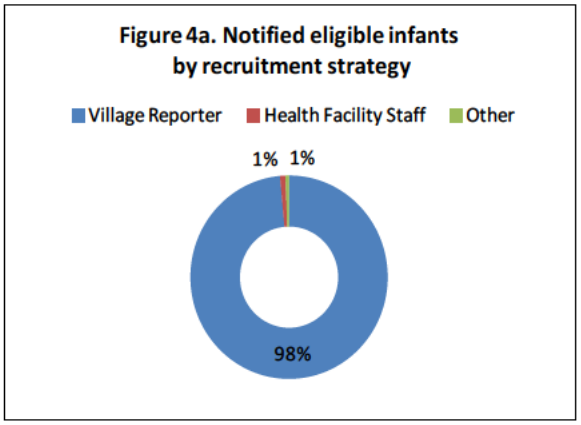
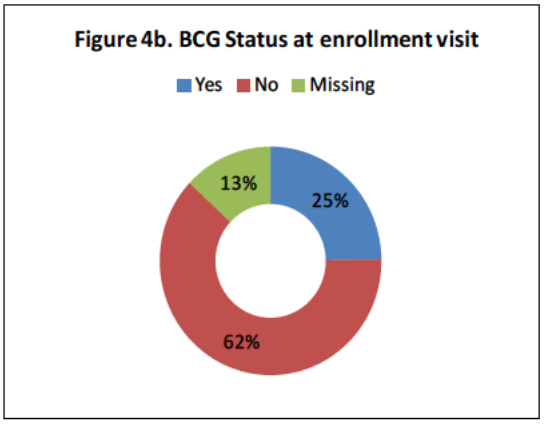
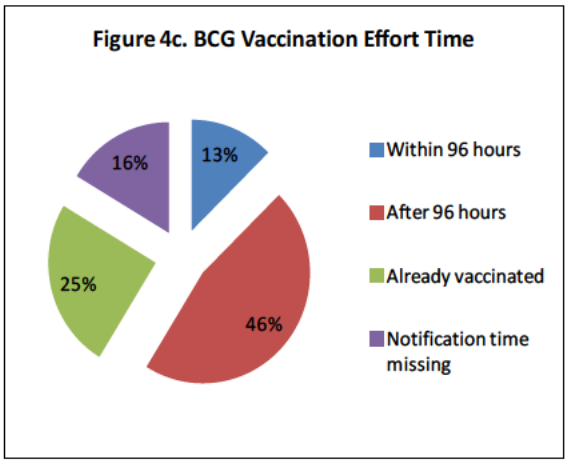


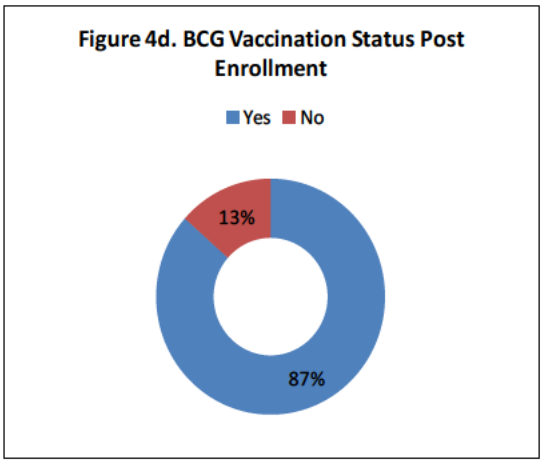
Figure 3 shows that the study approached 3225 infants as would-be potential study participants. During pre-screening exercise, 78 were identified as ineligible and of these ineligible, 55/78 (71%) had plans to move out of study area followed by over age infants 7/78 (9%), early neonatal death 5/78 (6%), mother sick 4/77 (5.2%), non-resident 3/77 (3.9%), still birth 2/77 (2.6%) and other reasons that were not listed by respondents. Infants found to be eligible were 3147. When subjected to study screening, 109 deferred for various reasons, mostly noted to consult their spouses or family at home and 127 refused participation in the study whereas 2911 were screened and eligible for consenting for study participation. Eleven were found ineligible with reasons identified as low birth weight 9/11 (82%), abnormal head circumference 1/11 (9%) and cleft plate 1/11 (9%). The study enrolled 2900 infants into the tuberculosis cohort. Study follow up was 1-2 years depending of time of enrollment. Upon enrollment into the study, infant withdrawals were 38/2900 (1.3%) and infant deaths were 13/2900 (0.7%) before or as at 6 week visit. Loss to follow up before or as at at 6 week visit was 117/2849 (4.1%). Retention as at 6 week visit was 2732/2849 (95.9%). We also assessed infant study participation during the four monthly follow up visits that took place after the 6 week visit to end of study. We found that withdrawals were 105/2732 (3.8%) and deaths were 192/2732 (7.0%) whereas loss to follow up was 99/2435 (4.2%). Retention during four monthly follow up visits was 2336/2435 (95.9%). Overall study retention having incorporated infants seen during unscheduled visits was 2336/2552 (91.5%).

4.1.2. Recruitment strategy and BCG vaccinations profiles









The highest notifications that were eligible were received from village reporters and health facility referrals (Figure 4a). Over 60% of infants were not BCG vaccinated on enrollment (Figure 4b). The study managed to vaccinate only 13% of infants within 96 hours (Figure 4c). Post enrollment, a total of 2510 (86.6%) were BCG vaccinated (Figure 4d).

Table 2. Recruitment mode and BCG vaccination profiles of enrolled infants

Infant cohort recruitment strategy	**N (3147)**	**%**
Village Reporter	3104	98.6
Health Facility Staff	28	0.9
Other	15	0.5
Infant BCG immunized at enrollment visit	**N (2900)**	**%**
Yes	719	24.8
No	1803	62.2
Missing	378	13.0
BCG vaccination effort time	**N (2900)**	**%**
Within 96 hours	365	12.6
After 96 hours	1340	46.2
Already vaccinated	719	24.8
Notification time missing	476	16.4
BCG Vaccination status post-enrollment	**N (2900)**	**%**
Yes	2510	86.6
No	390	13.4

The study employed two main recruitment strategies that utilized notifications through village reporters 3104/3147 (98.6%) and health facility staff 28/3147 (0.9%). We also found out that there were mothers who were informed about the study through other means such as snowball effect where a mother of an already infant 18/3147 (0.6%) informed a potential mother about the infant study. The study staff verified the road to health card to ascertain whether the infants had received BCG vaccination or not. The verification revealed that infants who had received BCG vaccination were 719/2900 (24.8%) and those who had not received BCG vaccination were 1803/2900 (62.2%). There were infants 378/2900 (13%) whose verification cards were not available. The study had a target to vaccinate enrolled infant within 96 hours. Efforts to achieve this target were ; within 96 hours 365/2900 (12.6%) and after 96 hours 1340/2900 (46.2%) whereas we had 719/2900 (24.8%) had already received BCG vaccinations by the time the nursing teams arrived at infant home or health facility and 476/2900 (16.4%) whose notification times were not recorded. BCG vaccination status was

reviewed after study efforts to assist vaccination infants not yet vaccinated and found that 2510/2900 (86.6%) were BCG vaccinated and 390/2900 (13.4%) had no records to verify vaccination status post enrollment. Thirty-five percent of birth notifications were enrolled within 96 hours of receiving the information of birth, we hypothesize that this was attributable to many of the infants being delivered at home and partially, lack of involvement of men during recruitment (*Table 2*).

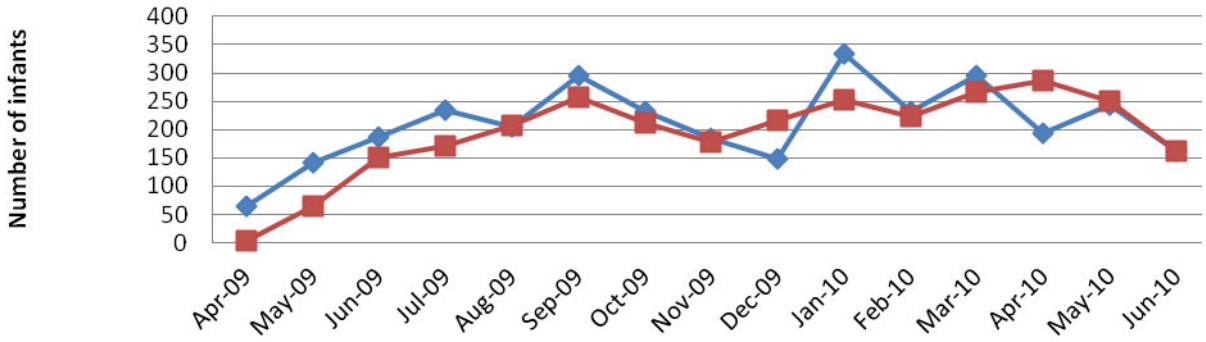
4.1.3. Recruitment and accrual trends

Birth notifications were received in the study from April 2009 and recorded upto June 2010. The study protocol was amended in December 2009 to extend study area following decreased accrual in the months of October to December 2009. January 2010 recorded the highest notification followed by September 2009 and March 2010. The study reported high enrollments in the months of April 2010, May 2010 and September 2009. A study team comprising of nurses and community health workers met village reporters at designated areas to visit the mother who had delivered. We targeted to enroll 2900 infants within 1 year though the study extended slightly with a month having enrolled the first infant on 25th May 2009 and enrolled the last infant on 28th June 2010 owing to the initial challenges the study faced in accruing infants into the study.

Initially, the recruitment strategy at this time was that the Recruitment Supervisor received all the notifications in a centralized approach then channeled this communication to the field and nursing teams to facilitate a visit where study information was shared, BCG vaccinations administered if not yet done and locator information taken. The centralized approach was not able to handle the numerous notifications that were received via mobile phone. It slowed down the enrollment numbers as the individual manning the phone verified details of the

notifications before releasing a team of nurses and filed workers. One of the things the study sought during this drop in recruitment and enrollment activities was an amendment to extend the study area to cover other areas contiguous to the study area. The other adjustment was to devolve the recruitment and enrollment activities to the nursing and field teams to reduce the turn around in responding to directly to notifications from community based workers referred to as 'Village Reporters'. These changes were responsible for the high numbers recorded in January 2010. Considerable efforts were put in place to educate the community on the objectives of the study and reasons behind targeting healthy infants; the highest notifications were recorded in January 2010 following a protocol amendment that extended the study area to include contiguous regions. The amendment was initiated after noting a decline in notifications between October - December 2009. Initially, the study sought to recruit pregnant mothers by informing them about the study with a follow up visit thereafter after delivery but this was logistically challenging due to personnel time and costs incurred in the two visits prior to delivery and post-delivery. The study team amended the recruitment standard operating procedure (SOP) to drop approaching pregnant mothers and only focus on notifications of mothers who had successfully delivered. Some of the challenges faced during recruitment period included communication bottlenecks between study team and community health workers as ownership of personal mobile phones was very low and therefore depended upon relatives or neighbours to relay birth notifications to the study; some terrains in the study area were hard to penetrate or access during rainy seasons experienced around March to May (long rains) and October to December (short rains) where we recorded decreased notifications and enrollments; motorbike riders in the study were slapped with a speed limit control (30 kilometers/hour) due to an increase in road accidents involving study motorbikes in the study area; birth notifications were at times fewer than expected such as May, June and November of 2009.

Figure 5. Infant recruitment and enrolment by month and year



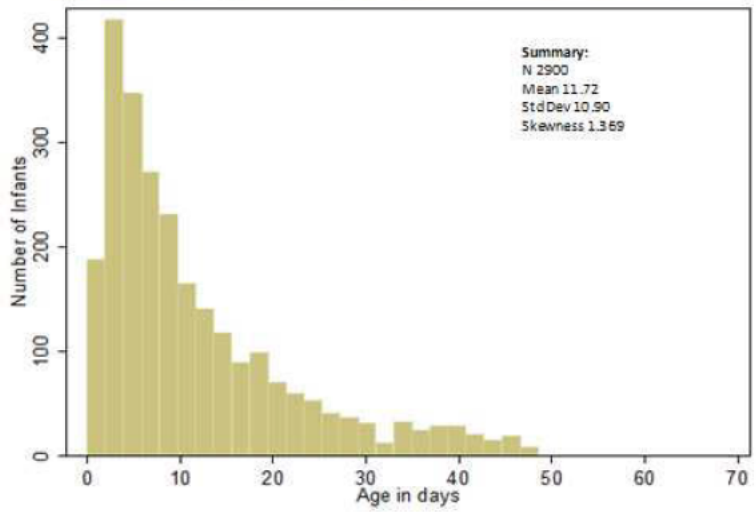
	Apr-09	May-09	Jun-09	Jul-09	Aug-09	Sep-09	Oct-09	Nov-09	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10	Jun-10
Notifications	64	141	187	234	204	296	231	184	149	334	231	296	193	244	162
Enrollments	4	64	150	171	207	257	211	178	216	252	224	267	286	251	162

Figure 5, is a graphical presentation of recruitment and enrollment by month and year. There was a sustained increase in the recruitment and enrollment activities from April 2009 to September 2009 and thereafter, in October 2009 the study started experiencing a continual decrease in recruitment and enrollment efforts until December 2009. The highest recruitment months were January 2010 (334), September 2009 (296), March 2010 (296), July 2009 (234) and February 2010 (231). The highest enrollment months were April 2010 (286), March 2010 (267), September 2009 (257), January 2010 (252) and May 2010 (251) respectively.

4.1.4. Distribution of infants by age in days

Majority of infants enrolled into the cohort were aged 0-9 days as captured in histogram (*Figure 5*). Range of infant age at enrollment was 0-66 days old. There was an outlier (infant aged 66 days) in the cohort. Infant cohort had a population distribution skewed (1.369) to the left. Distribution is skewed to the left with a mean age of 11.72 days with 51 infants were classfied as outliers between 43-60 days as study targeted to enroll 0-6 week olds.

Figure 6. Histogram on infant age distribution in the cohort



4.1.5. Infant characteristics in the tuberculosis cohort study

In *Figures 7 a-e,* infants characteristics were as follows;

Figure 7a. Place of Enrollment

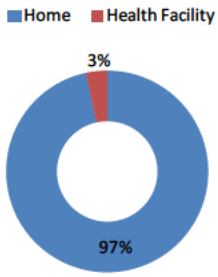


Figure 7b. Place of Birth

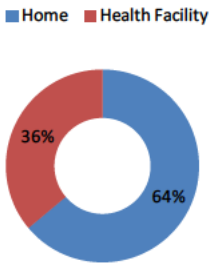


Figure 7c. Infant sex

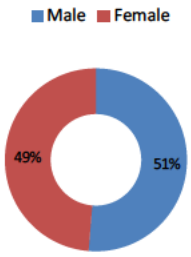


Figure 7d. Number of infants delivered

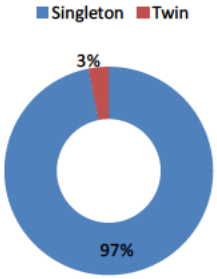
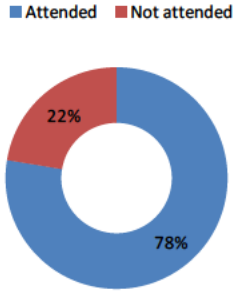


Figure 7e. Unscheduled visits



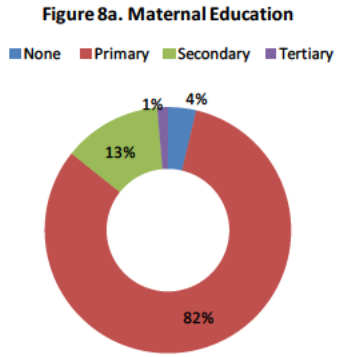
Characteristics of infants enrolled into the study were as follows: majority of enrollments were carried out in the homes; more deliveries took place at home than health facility; male infants were more than females; most of the infants were delivered as singletons and over three-quarters of infants attended unscheduled visits (Figure 6a, 6b, 6c, 6d, 6c). Home enrollments 2801/2900 (96.7%) compared to health facilities 97/2900 (3.3%) when place of enrollment was assessed. Place of birth was evaluated and we found that 1837/2900 (63.9) and 1038/2900 (36.1%) were born at home and health facilities respectively. Infant sex at birth were recorded as 1488/2900 (51.3%) and 1412/2900 (48.7%) being males and females respectively. Number of children delivered as singletons were 2810/2900 (96.9%) and those delivered as twins were 90/2900 (3.1%). Infants brought for unscheduled visits were 2251/2900 (77.6%) while 649/2900 (22.4%) did not participate in unscheduled visits (*Table 3*).

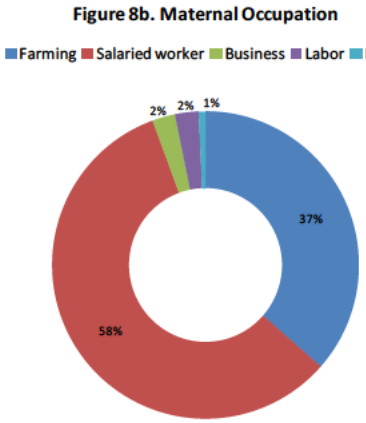
Table 3. Infant characteristics in the tuberculosis cohort study (N=2900)

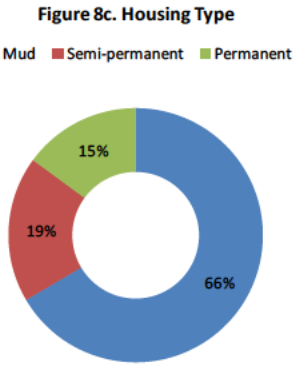
Variable	Frequency	Percent
Place of Enrollment		
Home	2801	96.7
Health Facility	97	3.3
Missing	2	
Place of Birth		
Home	1837	63.9
Health Facility	1038	36.1
Missing	25	
Infant Sex		
Male	1488	51.3
Female	1412	48.7
Infants Delivered		
Singleton	2810	96.9
Twin	90	3.1
Unscheduled visit attendance		
Yes	2251	77.6
No	649	22.4

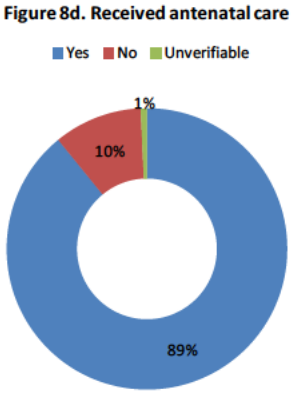
4.1.6. Maternal characteristics in the tuberculosis cohort study

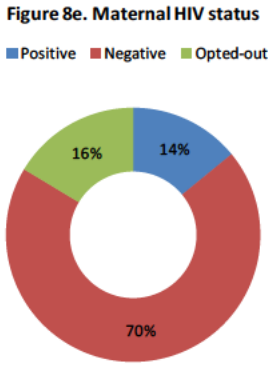
In Figures 8 a-g, maternal characteristics are shown

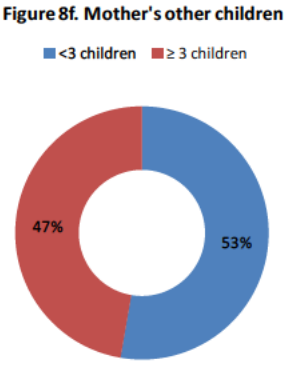


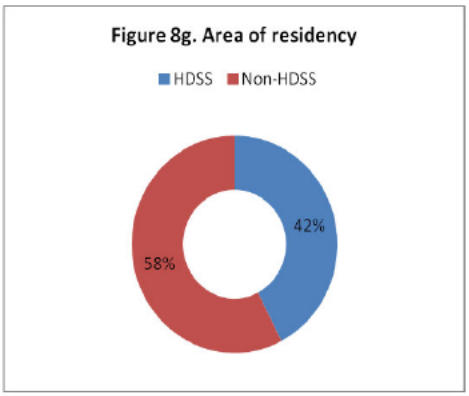












Maternal characteristics were such that primary level of education had majority (82%); mothers were occupied with salaried work (58%) followed by farming (37%); about two-thirds of the mothers lived in mud-type of housing; almost 90% of mothers in this cohort

received antenatal care; HIV infected mothers were 14%; over 50% of mothers had family sizes < 3 children; about 58% of mothers in the cohort had no HDSS identification or lived outside the HDSS area (Figure 8a, 8b, 8c, 8d, 8e, 8f, 8g). Education status showed that mothers without education were 105/2828 (3.7%), those with primary education were 2319/2828 (82%), those with secondary education were 365/2828 (12.9%) and those with tertiary education were 39/2828 (1.4%). Maternal occupation revealed that farmers were 1033/2828 (36.5%), salaried workers were 1637/2828 (57.9%), businesswomen were 68/2828 (2.4%), laborers were 70/2828 (2.5%) and fishmongers were 20/2828 (0.7%). Housing types where mothers resided were as follows; those residing in mud houses were 1877/2900 (66.4%), those residing in semi-permanent housing were 524/2828 (18.5%) and those living in permanent houses were 421/2828 (14.9%). Uptake of antenatal care (ANC) clinic visits was assessed and we found that mothers who received ANC clinics were 2521/2828 (89.1%) compared to 287/2828 (10.2%) who never attended ANC clinics. HIV testing was provided and the outcome was as follows; 393/2791 (14.1%) were HIV positive, 1941/2791 (69.5%) were HIV negative whereas 457/2791 (16.4%) opted out and non testers. The study evaluated family size and established that in this cohort, mothers with < 3 children were 1491/2828 (52.7%) versus mothers with ≥ 3 children who totaled 1337/2828 (47.3%) (*Table 4*).

Table 4. Maternal characteristics in the tuberculosis cohort study (N=2828)

Variable	Frequency	Percent
Maternal Education		
None	105	3.7
Primary	2319	82.0
Secondary	365	12.9
Tertiary	39	1.4
Maternal Occupation		
Farming	1033	36.5
Salaried worker	1637	57.9
Business	68	2.4
Labor	70	2.5
Fishing	20	0.7
Housing Type		
Mud	1877	66.4
Semi-permanent	524	18.5
Permanent	421	14.9
Other	6	0.2
Received Antenatal Care		
Yes	2521	89.1
No	287	10.2
Unverifiable	20	0.7
HIV status		
Positive	393	14.1
Opted-out	457	16.4
Negative	1941	69.5
Mother other children		
<3 children	1491	52.7
≥ 3 children	1337	47.3
Residency		
HDSS	1201	42.5
Non-HDSS	1627	57.5

Interquartile range (IQR) for infant age in days (4, 17); infant weight in grams (2900, 3700) and maternal age in years (20.3, 29.9) (*Table 5*).

Table 5. Infant and maternal characteristics summarized in percentiles

Variable	N	p25	p50	p75
Infant Age (days)	2900	4	8	17
Infant Weight (grams)	2900	2900	3300	3700
Mother Age (years)	2828	20.3	25.3	29.9

Table 4, summarises infant age in days as follows; 25th percentile was 4 days, 50th percentile was 8 days and 75th percentile was 17 days. Infant weight in kilograms (kgs) analyzed as follows; 25th percentile was 2.9 kgs, 50th percentile was 3.3kgs and 75th percentile was

3.7kgs. Maternal age in years (yrs) was as follows; 25th percentile was 20.3 yrs, 50th percentile was 25.3 yrs and 75th percentile was 29.9 yrs.

4.2. Factors associated with place of delivery among mothers in tuberculosis cohort

Maternal characteristics analyzed by place of delivery using bivariate and logistic regression to look at maternal characteristics associated with choice of delivery place. Bivariately, maternal occupation : farming, odds ratio (OR)=6.14, 95% confidence interval (CI) [3.56, 10.57] ; salaried worker, OR=4.5, 95% CI [2.63, 7.7] ; fishing, OR=3.29, 95% CI [2.1, 18.66] versus labor. Housing type : mud, OR=4.39, 95% CI [3.51, 5.48] ; semi-permanent, OR=2.65, 95% CI [2.03, 3.46] versus permanent. Mothers receiving antenatal care, OR=0.19, 95% CI [0.13, 0.28] versus mothers who did not receive antenatal care. HIV positive status, OR=0.64, 95% CI [0.51, 0.79] versus HIV negative status. HDSS residency, OR=1.42, 95% CI [1.22, 1.67] versus non-HDSS residency and mother age, OR=1.04, 95% CI [1.03, 1.06] were associated with place of delivery. Lack of education, primary, secondary levels of education versus tertiary ; mothers involved in business as an occupation versus labor and family size (≥3 children) versus < 3 children were not statisically associated with place delivery (*Table 5*).

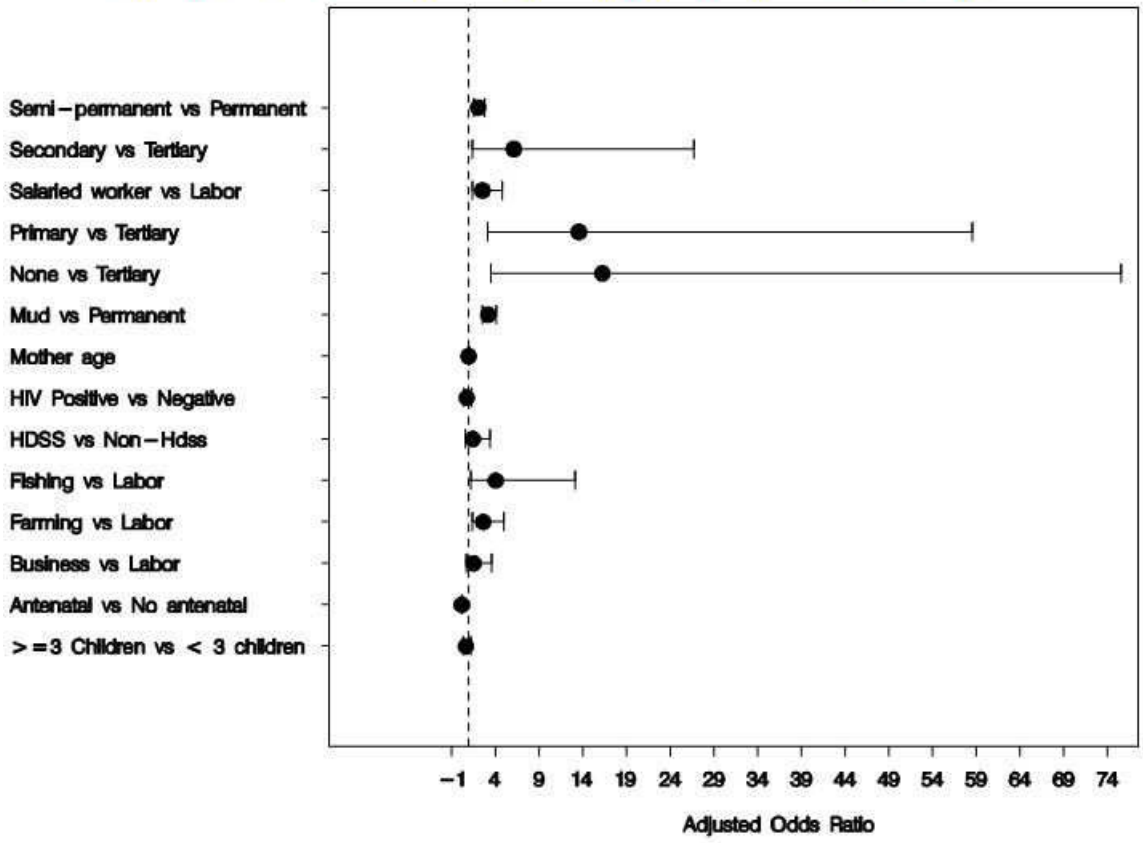
Table 6. Maternal characteristics by place of delivery (health facility versus home)

Variable	Health Facility Delivery n (%)	Home Delivery n (%)	COR* OR	COR* 95% (CI)	COR* P value	AOR** OR	AOR** 95% (CI)	AOR** P value
Mother Education (N=2803)								
None	24 (23.1)	80 (76.9)	61.7	(13.84, 274.77)	0.469	16.28	(3.51, 75.59)	<.0001
Primary	746 (32.4)	1553 (67.6)	38.5	(9.26, 160.22)	0.396	13.6	(3.16, 58.54)	<.0001
Secondary	207 (57.3)	154 (42.7)	13.8	(3.27, 57.98)	0.858	6.16	(1.42, 26.72)	0.015
Tertiary	37 (94.9)	2 (5.1)		Ref			Ref	
Mother Occupation (N=2803)								
Farming	311 (30.4)	711 (69.6)	6.14	(3.56, 10.57)	<.0001	2.66	(1.41, 5.02)	0.002
Salaried worker	606 (37.3)	1017 (62.7)	4.5	(2.63, 7.7)	<.0001	2.57	(1.37, 4.82)	0.003
Business	40 (58.8)	28 (41.2)	1.89	(0.92, 3.84)	0.084	1.57	(0.69, 3.56)	0.278
Fishing	6 (30.0)	14 (70.0)	3.29	(2.1, 18.66)	0.001	4.08	(1.26, 13.17)	0.019
Labor	51 (72.9)	19 (27.1)		Ref			Ref	
Housing Type (N=2797)								
Mud	535 (28.8)	1322 (71.2)	4.39	(3.51, 5.48)	<.0001	3.23	(2.52, 4.13)	<.0001
Semi-permanent	209 (40.1)	312 (59.1)	2.65	(2.03, 3.46)	<.0001	2.08	(1.56, 2.78)	<.0001
Permanent	268 (64.0)	151 (36.0)		Ref			Ref	
Mother Received Antenatal Care (N=2783)								
Yes	973 (39.0)	1523 (61.0)	0.19	(0.13, 0.28)	<.0001	0.21	(0.14, 0.31)	<.0001
No	31 (10.8)	256 (89.2)		Ref			Ref	
HIV test status (N=2783)								
Positive	177 (45.3)	214 (54.7)	0.64	(0.51, 0.79)	<.0001	0.78	(0.48, 1.29)	0.332
Negative	827 (34.6)	1565 (65.4)		Ref			Ref	
Number of children mother has of her own (N=2803)								
>= 3 children	478 (36.2)	844 (63.8)	1	(0.86, 1.17)	0.985	0.68	(0.39, 1.20)	0.185
< 3 children	536 (36.2)	945 (63.8)		Ref			Ref	
Residency (N=2803)								
HDSS	377 (31.6)	818 (68.4)	1.42	(1.22, 1.67)	<.0001	1.49	(0.65, 3.42)	0.344
Non HDSS	637 (39.6)	971 (60.4)		Ref			Ref	
Mother Age			1.04	(1.03,1.06)	<.0001	0.99	(0.95, 1.04)	0.728

** Crude Odds Ratio*
*** Adjusted Odds Ratio*

Multivariate logistic regression: No maternal education, OR=16.28, 95% CI [3.51, 75.59], maternal primary education, OR=13.6, 95% CI [3.16, 58.54], maternal secondary education, OR=6.16, 95% CI [1.42, 26.72] versus maternal tertiary education ; mothers engaged in farming, OR=2.66, 95% CI [1.41, 5.02], mothers employed as salaried workers, OR=2.57, 95% CI [1.37, 4.82], mothers engaged in fishing, OR=4.08, 95% CI [1.26, 13.17] versus mothers working as laborers ; mothers living in mud type of housing, OR=3.23, 95% CI [2.52, 4.13], mothers living in semi-permanent housing, OR=2.08, 95% CI [1.56, 2.78] versus mothers living in permanent type housing and mother receipt of antenatal care, OR=0.21, 95% CI [0.14, 0.31] versus mothers non-receipt of antenatal care (Figure 9).

Figure 9. Maternal characteristics by place of delivery (health facility versus home)



4.3.Predictors of loss to follow up

4.3.1. Infant characteristics by loss to follow up versus retained

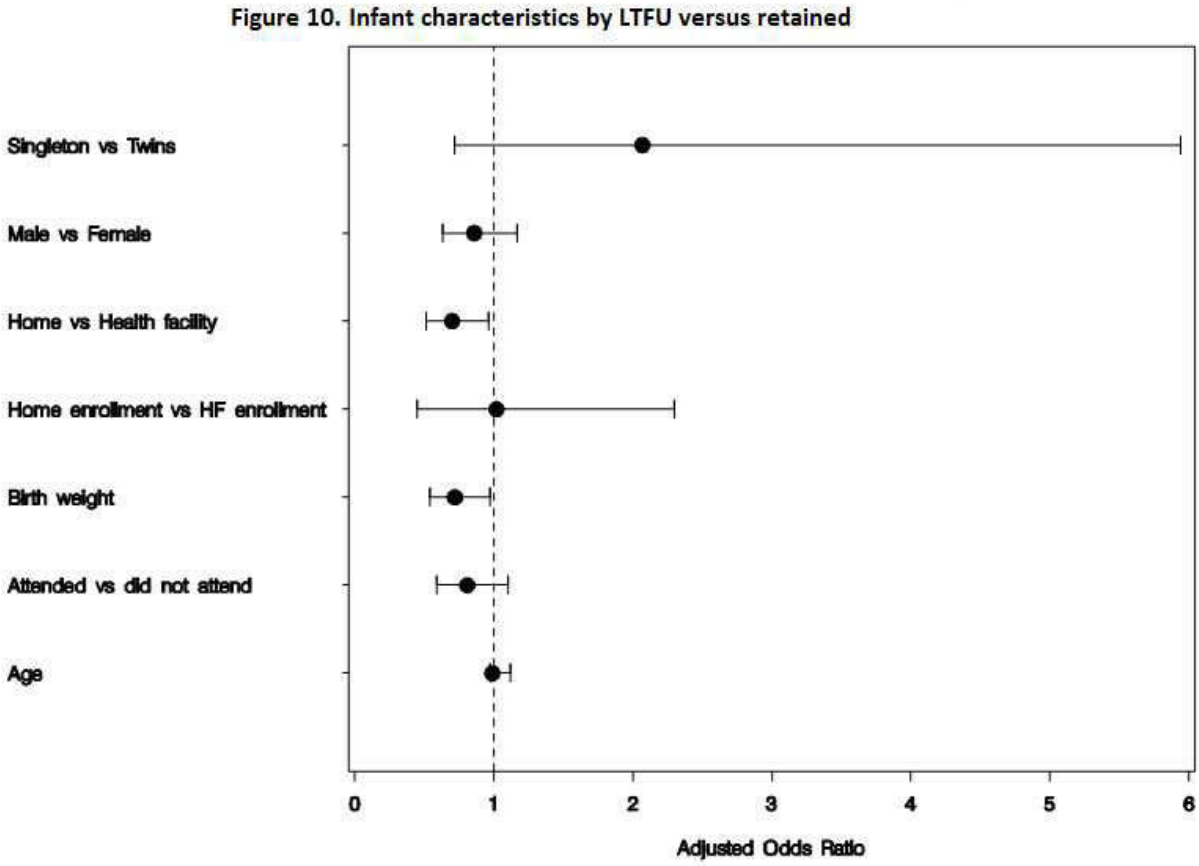
Infant characteristics of loss to follow up by bivariate and multivariate analyses. Bivariate analyses established that: home delivery, OR=0.76, 95% CI [0.57, 1.00] versus health facility delivery ; a unit increase in infants birth weight and age respectively implied that both were statistically significant. However, if place of enrollment was at home, male infants, being born as singletons, and attendance of unscheduled visits were not associated with LTFU were not associated with LTFU in bivariate and mutlivariate analysis (*Table 6 and Figure 10*).

Table 7. Infant characteristics by LTFU versus retained

				OR*			AOR**	
Variable	**LFTU n (%)**	**Retained n (%)**	**COR**	**95% (CI)**	**P value**	**AOR**	**95% (CI)**	**P value**
Place of Enrollment (N=2551)								
Home	207 (8.4)	2255 (91.6)	0.82	(0.40, 1.65)	0.571	1.02	(0.45, 2.30)	0.969
Health Facility	9 (10.1)	80 (89.9)		Ref			Ref	
Place of Birth (N=2530)								
Home	125 (7.7)	1492 (92.3)	0.76	(0.57, 1.00)	0.054	0.70	(0.51, 0.96)	0.026
Health Facility	91 (10.0)	822 (90.0)		Ref			Ref	
Infant Sex (N=2552)								
Male	111 (8.5)	1201 (91.5)	1.00	(0.76, 1.32)	0.995	0.86	(0.63, 1.17)	0.343
Female	105 (8.5)	1135 (91.5)		Ref			Ref	
Number of infants delivered (N=2552)								
Singleton	208 (8.4)	2262 (91.6)	0.85	(0.40, 1.79)	0.669	2.07	(0.72, 5.94)	0.174
Twins	8 (9.8)	74 (90.2)		Ref			Ref	
Unscheduled Visits								
Attended	108 (8.7)	1129 (91.3)	0.82	(0.60, 1.12)	0.210	0.81	(0.59, 1.10)	0.180
Did not attend	75 (10.4)	643 (89.6)		Ref			Ref	
Birth Weight	-	-	0.66	(0.53, 0.83)	<.0001	0.72	(0.54, 0.97)	0.032
Age	-	-	0.97	(0.96, 0.99)	0.001	0.99	(0.97, 1.12)	0.118

** Crude Odds Ratio (COR)*
***Adjusted Odds Ratio (AOR)*

Multivariate logistic regression: home delivered infants, OR=0.70, 95% CI [0.51, 0.96] versus health facility delivered infants ; infant birth weight, OR=0.72, 95% CI [0.54, 0.97] ; mothers engaged in farming, OR=0.35, 95% CI [0.17, 0.74] (*Figure 10*).



4.3.2. Maternal characteristics by loss to follow up versus retained

Maternal characteristics of loss to follow up by bivariate and multivariate analyses. These analyses revealed that mothers engaged in farming activity OR=0.27, 95% CI [0.14, 0.52] and mothers who are salaried OR=0.31, 95% CI [0.16, 0.59] versus laborers ; mothers living in mud type of housing OR=0.46, 95% CI [0.33, 0.66] versus mothers living in permanent housing and mothers living within the HDSS OR=2.00, 95% CI [1.51, 2.65] versus mothers not residing within the HDSS were significant bivariately whereas maternal education, receipt of antenatal care, HIV status, number of children mother has of her own and mother

age were not statistically significant. Maternal education ; mothers engaged in businesses and fishing ; mothers residing in semi-permanent type of housing ; mothers receipt of antenatal care ; HIV status ; place of residency and maternal age were not associated with loss to follow up (*Table 7*).

4.3.3. Separation or combining of infant and maternal characteristics by LTFU versus retained

An assessment of the strategy to decide whether to separate or combine infant and maternal characteristics was carried out by analyzing both models and the following was established: The combined model lost a significant amount of sample size of about 450. The combined model had four maternal characteristics that were statistically significant variables i.e., housing type (mud), housing type (semi-permanent), HDSS residency and mother's age. None of the infant characteristics were statistically signficant. In the literature review, the tendency was to separate the two models as distinct to understand individual characteristic contribution to the outcome of interest. Our exploratory analysis supports the strategy used in literature of studies evaluated.

In the separate model, infant characteristics: place of birth and birth weight were statistiscally significant whereas maternal characteristics: occupation (farming and salaried worker), housing type (mud) and number of children mother has (≥ 3 children) were statistically significant. The difference between the two models i.e. combined is that combined has no infant characteristics that are significant and shows that housing type (semi-permanent) is statistically significant whereas for separate model, infant characteristics (place of birth and birth weight) were significant.

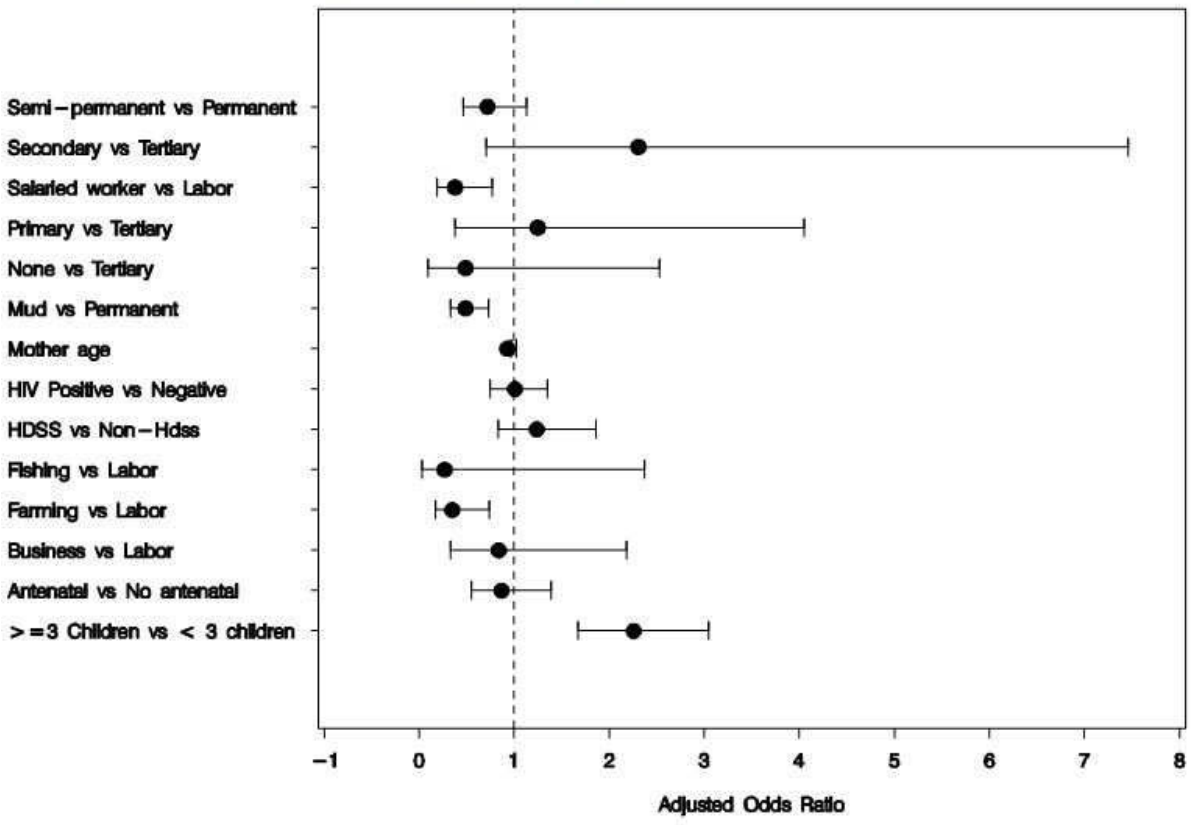
Table 8. Maternal characteristics by LFTU versus retained

Variable			COR*			AOR**		
	LFTU n (%)	**Retained n (%)**	**OR**	**95% (CI)**	**P value**	**OR**	**95% (CI)**	**P value**
Maternal Education (N=2480)								
None	3 (3.1)	95 (96.9)	0.22	(0.05, 1.05)	0.057	0.49	(0.95, 2.53)	0.395
Primary	157 (7.7)	1875 (92.3)	0.59	(0.20, 1.69)	0.323	1.25	(0.38, 4.05)	0.712
Secondary	51 (16.0)	267 (84.0)	1.34	(0.45, 3.98)	0.601	2.31	(0.71, 7.46)	0.162
Tertiary	4 (12.5)	28 (87.5)		Ref			Ref	
Mother Occupation (N=2480)								
Farming	68 (7.3)	862 (92.7)	0.27	(0.14, 0.52)	<.0001	0.35	(0.17, 0.74)	0.006
Salaried worker	120 (8.4)	1306 (91.6)	0.31	(0.16, 0.59)	<.0001	0.38	(0.19, 0.77)	0.008
Business	13 (24.5)	40 (75.5)	1.10	(0.46, 2.65)	0.832	0.84	(0.33, 2.18)	0.724
Fishing	1 (7.1)	14 (92.9)	0.26	(0.03, 2.18)	0.215	0.27	(0.03, 2.37)	0.237
Labor	13 (22.8)	44 (77.2)		Ref			Ref	
Housing Type (N=2475)								
Mud	119 (7.0)	1573 (93.0)	0.46	(0.33, 0.66)	<.0001	0.49	(0.33, 0.73)	<.0001
Semi-permanent	47 (10.8)	386 (89.2)	0.75	(0.49, 1.15)	0.183	0.72	(0.46, 1.13)	0.153
Permanent	49 (14.0)	301 (86.0)		Ref			Ref	
Mother Received Antenatal Care (N=2460)								
Yes	192 (8.7)	2016 (91.3)	0.90	(0.58, 1.41)	0.660	0.87	(0.55, 1.39)	0.558
No	24 (9.5)	228 (90.5)		Ref			Ref	
HIV test status (N=2808)								
Positive	34 (9.7)	315 (90.3)	1.14	(0.78, 1.68)	0.493	1.01	(0.75, 1.35)	0.962
Negative	182 (8.6)	1929 (91.4)		Ref			Ref	
Number of children mother has of her own (N=2828)								
> =3 children	105 (8.9)	1075 (91.1)	1.06	(0.80, 1.40)	0.699	2.26	(1.67, 3.05)	<.0001
< 3 children	110 (8.5)	1190 (91.5)		Ref			Ref	
Residency (N=2828)								
****HDSS*	122 (12.0)	898 (88.0)	2.00	(1.51, 2.65)	<.0001	1.24	(0.83, 1.86)	0.292
Non HDSS	93 (6.4)	1367 (93.6)		Ref			Ref	
Mother Age	-	-	1.00	(0.98,1.02)	0.932	0.93	(0.97, 1.02)	0.824

** Crude Odds Ratio (COR)*
*** Adjusted Odds Ratio (AOR)*
****Health and Demographic Surveillance System (HDSS)*

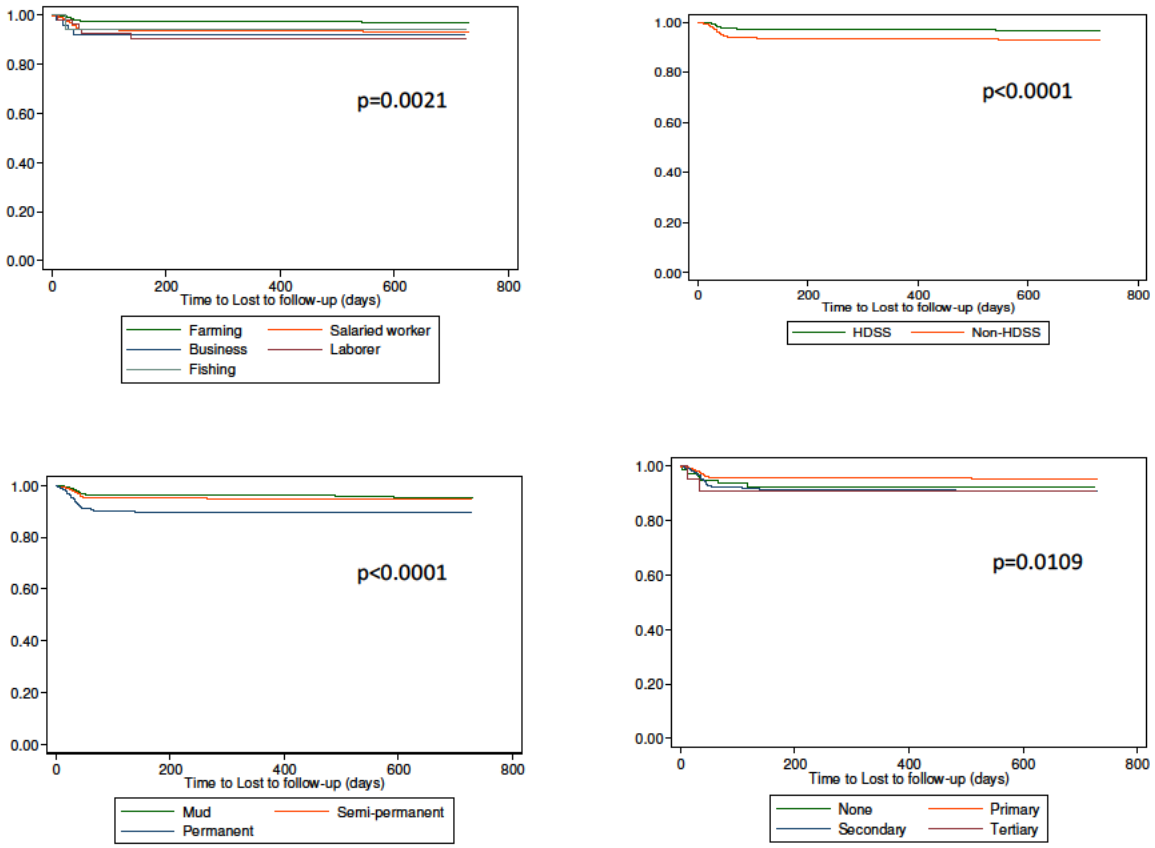
Mutlivariate analyses showed that mothers engaged in farming OR=0.35, 95% CI [0.17, 0.74], mothers working as salaried workers OR=0.38, 95% [0.19, 0.77] versus mothers working as laborers. Mothers living in mud type of housing OR=0.49, 95% CI [0.33, 0.73] versus mothers living in permanent type of housing and mothers with ≥ 3 children OR=2.26, 95% CI [1.67, 3.05] versus < 3 children were statistically significant. Maternal characteristics by LTFU versus retained showed that mothers employed as salaried workers, OR=0.38, 95% CI [0.19, 0.77] versus mothers working as laborers ; mother living in mud type of housing, OR=0.49, 95% CI [0.33, 0.73] versus mothers living in permanent type housing and mothers with ≥ 3 children, OR=2.26, 95% CI [1.67, 3.05] versus mothers with < 3 children (Table 7 and *Figure 11).*

Figure 11. Maternal characteristics by LTFU versus retained



Kaplan Meier estimates show that maternal occupation (*p=0.0021*), place of residence (*p<0.0001*), housing type (*p<0.0001*) and maternal education of follow-up (*p=0.0109*) (*Figure 12*).

Figure 12. Kaplan Meier curves for exploration of factors associated with LTFU



4.4. Risk of loss to follow up by infant and maternal characteristics by Cox Proportional Hazards

Additionally, Cox Proportional hazards analysis was carried out to assess the risk of LTFU by infant and maternal characteristics (*Tables 8 and 9 respectively*).

Table 9. Infant characteristics by LTFU using Cox Proportional Hazards

Variable	LFTU n (%)	*HR	95% (CI)	P value
Place of Enrollment (N=2551)				
Home	207 (8.4)	0.71	(0.31, 1.62)	0.198
Health Facility	9 (10.1)		Ref	
Place of Birth (N=2530)				
Home	125 (7.7)	0.74	(0.51, 1.08)	0.118
Health Facility	91 (10.0)		Ref	
Infant Sex (N=2552)				
Male	111 (8.5)	0.78	(0.55, 1.13)	0.198
Female	105 (8.5)		Ref	
Number of infants delivered (N=2552)				
Singleton	208 (8.4)	1.60	(0.38, 6.73)	0.520
Twins	8 (9.8)		Ref	
Birth Weight	-	1.26	(0.57, 2.77)	0.565
Age	-	1.01	(0.99, 1.03)	0.059

** Hazards Ratio (HR)*

None of the infant characteristics were found to be statistically significant by Cox Proportional Hazards (*Table 8 and Figure 13*).

Figure 13. Infant characteristics by LTFU using Cox Proportional Hazards

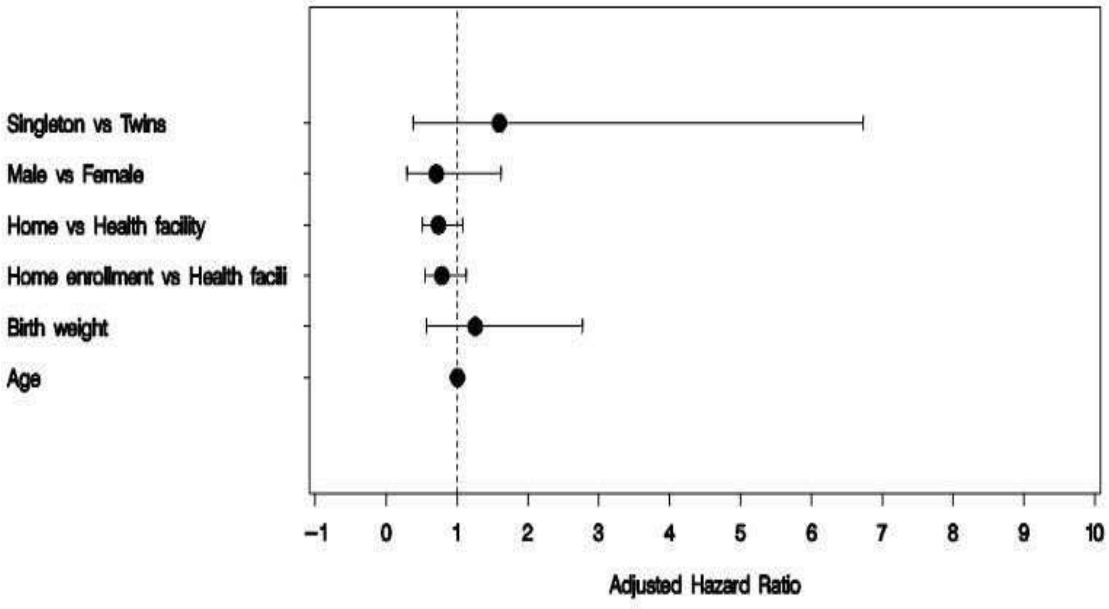


Table 10. Maternal characteristics by LFTU using Cox Proportional Hazards

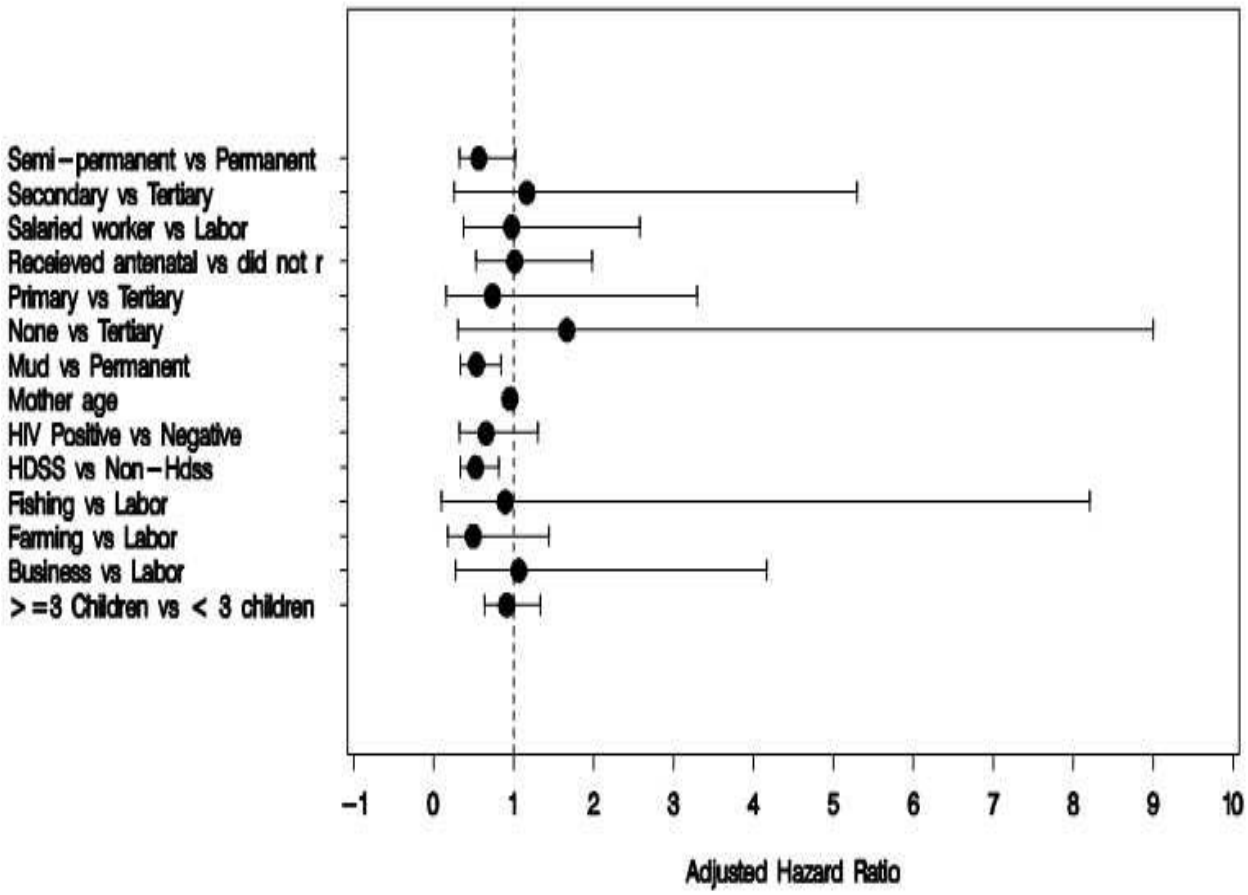
Variable		HR*		
	LFTU n (%)	HR	95% (CI)	P value
Maternal Education (N=2480)				
None	3 (3.1)	1.67	(0.31, 8.97)	0.550
Primary	157 (7.7)	0.74	(0.17, 3.31)	0.697
Secondary	51 (16.0)	1.15	(0.25, 5.18)	0.857
Tertiary	4 (12.5)		Ref	
Mother Occupation (N=2480)				
Farming	68 (7.3)	0.50	(0.18, 1.42)	0.195
Salaried worker	120 (8.4)	0.98	(0.38, 2.57)	0.981
Business	13 (24.5)	1.07	(0.28, 4.16)	0.923
Fishing	1 (7.1)	0.90	(0.10, 8.03)	0.926
Labor	13 (22.8)		Ref	
Housing Type (N=2475)				
Mud	119 (7.0)	0.54	(0.34, 0.84)	0.007
Semi-permanent	47 (10.8)	0.57	(0.33, 1.01)	0.054
Permanent	49 (14.0)		Ref	
Mother Received Antenatal Care (N=2460)				
Yes	192 (8.7)	1.11	(0.51, 2.45)	0.789
No	24 (9.5)		Ref	
HIV test status (N=2808)				
Positive	34 (9.7)	0.88	(0.49, 1.63)	0.701
Negative	182 (8.6)		Ref	
Number of children mother has of her own (N=2828)				
> =3 children	105 (8.9)	0.91	(0.62, 1.32)	0.606
< 3 children	110 (8.5)		Ref	
Residency (N=2828)				
****HDSS*	122 (12.0)	0.53	(0.34, 0.82)	0.004
Non HDSS	93 (6.4)		Ref	
Mother Age	-	0.95	(0.93, 0.99)	0.015

** Hazards Ratio (HR)*

Infant and maternal variables were analysed using Cox Proportional Hazards to evaluate factors associated with LTFU only. Maternal characteristics that were significant were: Mothers living in mud type of housing, Hazards Ratio (HR)=0.54, 95% CI [0.34, 0.84] versus mothers living in permanent housing; Residing within the HDSS, HR=0.53, 95% CI [0.34, 0.82]. A unit increase in maternal age, HR=0.95, 95% CI [0.93, 0.99] (*Table 9).*

Maternal characteristics that were significant were: Mothers living in semi-permanent houses, mothers living in mud houses, mothers residing within the HDSS and a unit increase of maternal age had a decreased risk for LTFU (*Figure 14*).

Figure 14. Maternal characteristics by LTFU using Cox Proportional Hazards



5. Factors influencing attendance of unscheduled visits in the infant tuberculosis cohort study

Table 11. Infant characteristics by unscheduled visits (yes vs. no)

			COR*			AOR**		
Variable	**Unscheduled visits**		**OR**	**95% (CI)**	**P value**	**OR**	**95% (CI)**	**P value**
	Yes n (%)	**No n (%)**						
Place of Enrollment (N=2898)								
Home	628 (22.4)	2173 (77.6)	0.90	(0.54, 1.48)	0.676	0.84	(0.51, 1.40)	0.504
Health Facility	20 (20.6)	77 (79.4)		Ref			Ref	
Place of Birth (N=2875)								
Home	387 (21.1)	1450 (78.9)	1.21	(1.01, 1.45)	0.035	1.22	(1.01, 1.46)	0.036
Health Facility	254 (24.5)	784 (75.5)		Ref			Ref	
Infant Sex (N=2900)								
Male	306 (20.6	1182 (79.4)	1.04	(0.87, 1.24)	0.686	1.00	(0.84, 1.20)	0.970
Female	299 (21.2)	1113 (78.8)		Ref			Ref	
Number of infants delivered (N=2900)								
Singleton	627 (22.3)	2183 (77.7)	1.20	(0.73, 1.94)	0.468	1.21	(0.73, 2.00)	0.453
Twins	23 (25.6)	67 (74.4)		Ref			Ref	
Residency (N=2900)								
HDSS	565 (23.3)	1859 (76.7)	1.40	(1.08, 1.80)	0.009	0.08	(0.04, 0.13)	<.0001
Non-HDSS	85 (17.9)	391 (82.1)		Ref			Ref	
Birth Weight (N=2900)	-	-	0.92	(0.80, 1.05)	0.210	0.64	(0.82, 1.15)	0.731
Age (N=2900)	-	-	0.99	(0.98, 0.99)	0.027	0.99	(0.98, 1.00)	0.105

**Crude Odds Ratio (COR)*
*** Adjusted Odds Ratio (AOR)*

Infant and maternal variables were analysed to evaluate factors associated with unscheduled visit attendance. Infants born at home OR=1.21, 95% CI [1.01, 1.45] versus infants born at health facility; Infants residing in HDSS OR=1.40, 95% CI [1.08, 1.80] versus infants residing outside the HDSS area and a unit increase in age OR=0.99, 95% CI [0.98, 0.99] were bivariately associated with unscheduled visits. Multivariate analyses revealed that infants delivered at home OR=1.22, 95% CI [1.01, 1.46] versus infants delivered in health facility; infants residing in HDSS OR=0.08, 95% CI [0.04, 0.13] versus infants residing outside the HDSS were associated with unscheduled visits (*Table 10 and Figure 15*).

Figure 15. Infant characteristics by unscheduled visits (yes versus no)

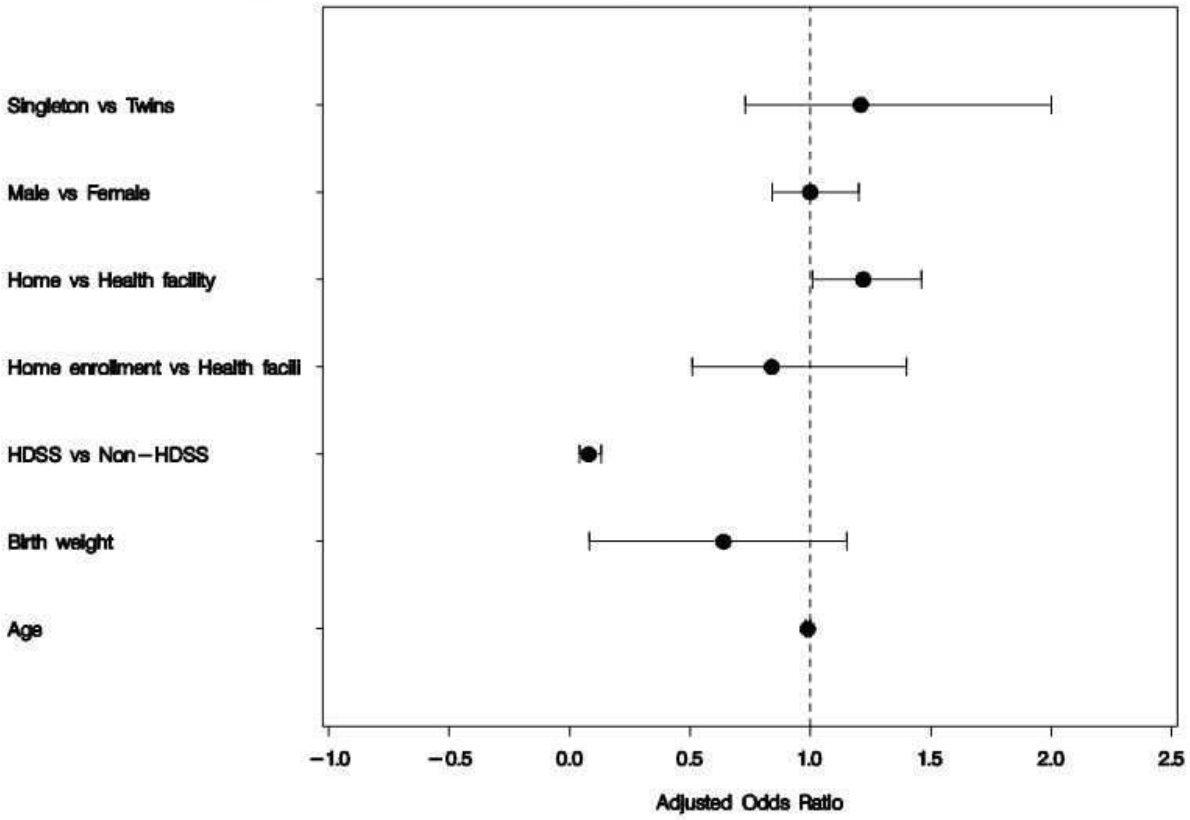
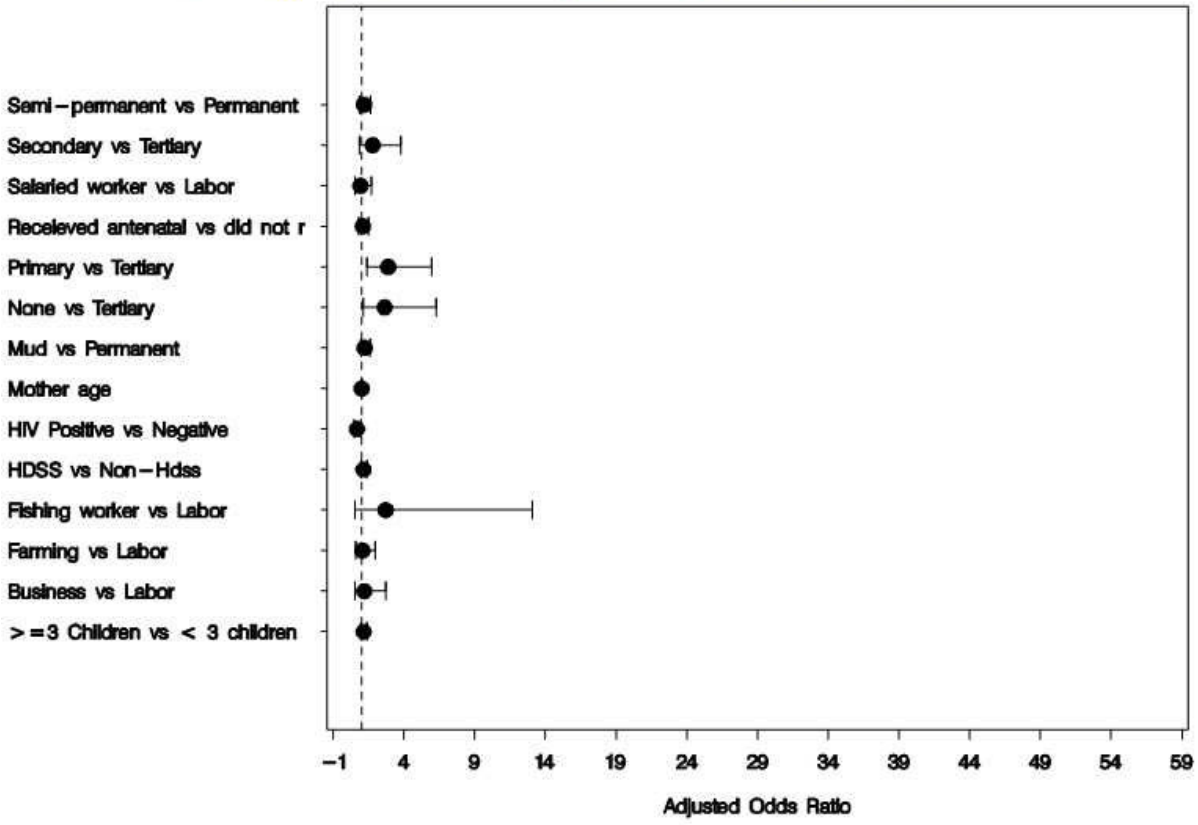


Table 12.Maternal characteristics by unscheduled visits (yes vs. no)

Variable	Unscheduled visits		COR*			AOR**		
	No n (%)	Yes n (%)	OR	95% (CI)	P value	OR	95% (CI)	P value
Maternal Education (N=2830)								
None	22 (21.2)	82 (78.8)	3.02	(1.36, 6.67)	0.006	2.65	(1.11, 6.28)	0.027
Primary	480 (20.7)	1842 (79.3)	3.10	(1.63, 5.93)	0.001	2.89	(1.40, 5.94)	0.004
Secondary	112 (30.6)	254 (69.4)	1.84	(0.93, 3.61)	0.079	1.81	(0.87, 3.75)	0.111
Tertiary	17 (44.7)	21 (55.3)		Ref			Ref	
Mother Occupation (N=2830)								
Farming	201 (19.4)	836 (80.6)	1.89	(1.08, 3.18)	0.024	1.08	(0.59, 1.97)	0.799
Salaried worker	391 (23.9)	1243 (76.1)	1.42	(0.84, 2.41)	0.192	0.94	(0.52, 1.68)	0.828
Business	16 (22.9)	54 (77.1)	1.51	(0.71, 3.22)	0.289	1.21	(0.55, 2.70)	0.636
Fishing	2 (9.5)	19 (90.5)	4.24	(0.91, 19.90)	0.067	2.72	(0.56, 13.08)	0.212
Labor	21 (30.9)	47 (69.1)		Ref			Ref	
Housing Type (N=2824)								
Mud	393 (20.9)	1491 (79.1)	1.50	(1.18, 1.91)	0.001	1.23	(0.95, 1.59)	0.122
Semi-permanent	116 (22.3)	404 (77.7)	1.38	(1.02, 1.85)	0.034	1.19	(0.88, 1.63)	0.262
Permanent	119 (28.3)	301 (71.7)		Ref			Ref	
Mother Received Antenatal Care (N=2810)								
Yes	564 (22.3)	1962 (77.7)	1.03	(0.77, 1.38)	0.830	1.10	(0.81, 1.48)	0.547
No	65 (22.9)	219 (77.1)		Ref			Ref	
HIV test status (N=2810)								
Positive	75 (19.2)	316 (80.8)		(0.43, 0.79)	<.0001	0.69	(0.50, 0.95)	0.023
Negative	554 (22.9)	1865 (77.1)		Ref			Ref	
Number of children mother has of her own (N=2830)								
> =3 children	311 (23.3)	1025 (76.7)		(0.92, 1.32)	0.305	1.16	(0.95, 1.41)	0.142
< 3 children	320 (21.4)	1174 (78.6)		Ref			Ref	
Residency (N=2830)								
HDSS	241 (20.0)	964 (80.0)	1.26	(1.05, 1.51)	0.012	1.15	(0.95, 1.39)	0.153
Non HDSS	390 (24.0)	1235 (76.0)		Ref			Ref	
Mother Age (N=2830)	-	-	1.02	(1.01, 1.03)	0.003	1.02	(1.00, 1.03)	0.026

** Crude Odds Ratio (COR)*
***Adjusted Odds Ratio (AOR)*

Figure 16. Maternal characteristics by unscheduled visits (yes versus no)



Maternal characteristics associated with unscheduled visits were no maternal education OR=3.02, 95% CI [1.36, 6.67] and primary level of education OR=3.1, 95% CI [1.63, 5.93] versus tertiary level of education ; mothers engaged in farming OR=1.89, 95% CI [1.08, 3.18] versus laborers ; mothers living in mud type of housing OR=1.50, 95% CI [1.18, 1.91] and mothers living in semi-permanent OR=1.38, 95% CI [1.02, 1.85] versus mothers living in permanent houses ; positive HIV status OR=, 95% CI [0.43, 0.79] versus negative HIV status ; mothers residing in the HDSS OR=1.26, 95% CI [1.05, 1.51] versus mothers residing in non-HDSS area ; a unit increase in age OR=1.02, 95% CI [1.01, 1.03] were statistically significant by bivariate statistics. Secondary level of education, mothers working as salaried workers or in business or in fishing, mothers receipt of antenatal care, number of children mother has of her own, residency and unit increase in age were not associated bivariately with unscheduled visits. Multivariately, no maternal education OR=2.65, 95% CI [1.11, 6.28] and primary level of education OR=2.89, 95% CI [1.40, 5.94] versus tertiary level of

education ; positive HIV status OR=0.69, 95% CI [0.50, 0.95] versus negative HIV status ; a unit increase in age OR=1.02, 95% CI [1.00, 1.03] were associated with unscheduled visits. Secondary level of education, mothers engaged in farming or working as salaried workers or in business or in fishing, type of house mother is living in, mothers receipt of antenatal care, number of children mother has of her own and place of residency were not associated multivariately with unscheduled visits ((*Table 11 and Figure 16*).

5.1. Separation of infant and maternal characteristics by unscheduled visits (yes) and unscheduled visits (no)

An assessment of the strategy to decide whether to separate or combine infant and maternal characteristics by unscheduled visits (yes) and unscheduled visits (no) was carried out by analyzing both models and the following was established: The combined model lost a small amount of sample size of about 58. The combined model had one infant characteristic i.e. HDSS residency as statistically significant whereas four maternal characteristics that were statistically significant i.e., maternal education (none) and maternal education (primary), HDSS residency and mother's age. In the literature review, the tendency was more focused towards infant characteristics to evaluate morbidity and very little of parent characteristics.

In the separate model, infant characteristics: place of birth and HDSS residency were statistiscally significant whereas maternal characteristics: maternal education (none) and maternal education (primary), maternal HIV status (positive) and mother age were statistically significant. The difference between the two models i.e. combined is that combined has infant characteristic i.e. HDSS residency as statistically significant and not so in the separate model. In the separate model, maternal characteristic i.e. maternal HIV status

(positive) is statistically significant. HDSS residency is captured in both models though place of birth and HIV status is unique to the seprate model.

5.2. Morbidity burden among infants utilizing unscheduled visits in the tuberculosis cohort study

5.2.1. Inpatient versus outpatient symptoms and signs for infants attending unscheduled visits

A total of 25,262 unscheduled visits were made to the Siaya County Referral Hospital to address signs and symptoms observed by the mothers. Out of the 2900 infants enrolled into this cohort study, 2265 (78.1%) participated in these unscheduled visits. Provision of medical services for the infants attending these unscheduled visits was provided for by 18 members of staff from the clinical team. Vomiting accounted for 4361 visits in this cohort, with 1371 (62.3%) and 409 (44.7%) being seen as out-patients and in-patients respectively. Of these visits with vomit, 189 vomitted everything and out of these 63 (2.9%) and 113 (12.4%) who were out-patients and in-patients respectively. Infant visits associated with lethargy were 293 and of these visits, 108 (4.9%) and 166 (18.2%) were out-patients and in-patients respectively. One-hundred and forty visits were associated with convulsions and 37 (1.3%) and 86 (9.4%) were seen as out-patients and in-patients respectively (*Table 12*).

Table 13. Signs and symptoms from infants attending unscheduled visits

Inpatient symptoms and signs	Inpatients (914; n, %)	Inpatient visits	Outpatient symptoms and signs	Outpatients (2200; n, %)	Outpatient visits
Fever	846 (92.6)	6637	Breathing difficulty	2083 (94.7)	17354
Fast breathing	457 (50.2)	717	Fever	1779 (80.9)	945
Vomiting	409 (44.7)	470	Fast breathing	1490 (67.7)	3704
Breathing difficulty	370 (40.5)	851	Diarrhea	1452 (66)	4607
Nasal flaring	326 (35.7)	378	Vomiting	1371 (62.3)	3891
Chest indrawing	311 (34)	208	Palmor pallor	389 (17.7)	522
Diarrhea	310 (33.9)	344	Oral thrush	334 (15.2)	438
Palmor pallor	240 (26.3)	266	Nasal flaring	284 (12.9)	315
Lethargic	166 (18.2)	175	Diarrhea (bloody)	244 (11.1)	358
Vomiting everything	113 (12.4)	121	Chest indrawing	181 (8.2)	365
Convulsions	86 (9.4)	99	Pus from ear	150 (6.8)	208
Sunken eyes	78 (8.5)	84	Measles last 3 months	126 (5.7)	141
Oral thrush	63 (6.9)	72	Lethargic	108 (4.9)	118
Severe wasting	62 (6.8)	74	Vomiting everything	63 (2.9)	63
Bulging fontanelle	62 (6.8)	67	Burns	63 (2.9)	64
Abdomen skin	55 (6)	61	Severe wasting	60 (2.7)	68
Stridor	26 (2.8)	28	Sunken eyes	57 (2.6)	61
Measles last 3 months	25 (2.7)	27	Abdomen skin	50 (2.3)	53
Oedema (both feet)	18 (2)	23	Convulsions	37 (1.3)	41
Stiff neck	15 (1.6)	16	Swelling behind ear	23 (1)	25
Diarrhea (bloody)	13 (1.4)	15	Bulging fontanelle	15 (0.7)	16
Burns	10 (1.1)	11	Stiff neck	16 (0.7)	16
Pus from ear	5 (0.5)	5	Stridor	10 (0.5)	12
Swelling behind ear	2 (0.2)	2	Oedema (both feet)	4 (0.2)	5
Mouth ulcers	0 (0)	0	Pus from eyes	3 (0.1)	3
Pus from eyes	0 (0)	0	Cornea clouding	2 (0.1)	2
Cornea clouding	0 (0)	0	Mouth ulcers	0 (0)	0
Respiratory rate	(48, 64)	-	-	(38, 48)	-
Temperature (IQR)	(36.3, 37.6)	-	-	(37, 39.1)	-

Severe wasting was recorded in 142 visits and of these visits, 60 (2.7%) and 62 (6.8%) infants accounted for out-patient and in-patient visits respectively. Twenty-eight visits had

oedema that was defined as swelling of both feet and 4 (0.2%) and 18 (2%) infants showed up with this sign during out-patient and in-patient visits respectively. Burns in this study were reported in 75 visits with 63 (2.9%) and 10 (1.1%) infants being seen at out-patient and in-patient visits respectively. Breathing difficulty was reported in 18205 visits and observed among 2083 (94.7%) out-patients and 370 (40.5%) in in-patients participating in unscheduled visits. In table 8, where normal ranges are given as 30-60 breaths/minute for less than 1 year old child and 24-40 breaths/minutes for 1-2 years old child, the study found that 1659 infant-visits were above the normal respiratory rate and only 1 infant-visit was below the normal respiratory rate. Inter quartile range was 40-52 breaths/minutes with mean of 46.83 breaths/minute. Fast breathing was seen in 4421 visits but recorded among 1490 (67.6%) out-patients and 457 (50.2%) in-patients. Chest indrawing was observed in 573 visits and documented among 181 (8.2%) out-patients and 311 (34%) in-patients. Infants presenting with stridor were 10 (0.5%) and 26 (2.8%) among out-patients and in-patients respectively and 40 visits were associated with stridor. Nasal flaring was observed in 693 visits from 284 (12.9%) and 326 (35.7%) out-patients and in-patients respectively. Diarrhea was reported in 4951 visits in 1452 (66%) and 310 (33.9%) out-patients and inpatients respectively. In the cohort shows the duration of diarrhea and number of infants, where a majority reported having had diarrhea for the past 3 days, followed by 2 days. Thirty seven (37) infant visits had diarrhea greater than 1 week. The inter quartile range (IQR) for duration of diarrhea was (2-3 days). The range between the minimum and maximum duration of diarrhea was (0-90 days). Bloody diarrhea was observed in 373 visits among 244 (11.1%) and 13 (1.4%) out-patients and in-patients respectively. Sunken eyes was seen in 145 visits among 57 (2.6%) and 78 (8.5%) out-patients and in-patients respectively. Abdomen skin was observed in 114 visits among 50 (2.3%) and 55 (6.0%) of out-patients and in-patients respectively. Fever was recorded in 7578 visits among 1779 (80.9%) and 846 (92.6%) out-patients and in-patients

respectively. The fevers were further categorized into hypothemia (36.8), normal temperature (36.8 ºC - 37.4ºC), low grade fever (37.5 ºC - 38 ºC) and high grade fever (>38 ºC) as shown in *Table 12* shows that 3705/7578 (49%) had high grade fever followed by 1751/7578 (23%) had low grade fever. Oral thrush was observed in 510 visits among 334 (15.2%) and 63 (6.9%) of out-patients and in-patients respectively. Palmar pallor was seen in 788 visits among 389 (17.7%) and 240 (26.3%) out-patients and in-patients respectively. Swelling behind the ear was seen in 27 visits among 23 (1%) and 2 (0.2%) out-patients and in-patients respectively. Pus from ear was seen in 213 visits among 150 (6.8%) and 5 (0.5%) of out-patients and in-patients respectively. Bulging fontanelle was observed in 83 visits among 15 (0.7%) and 62 (6.8%) of out-patients and in-patients respectively. Stiff neck was recorded in 32 visits among 16 (0.7%) and 16 (1.6%) out-patients and in-patients respectively. Measles last 3 months was observed in 168 visits among 126 (5.7%) and 25 (2.7) out-patients and in-patients respectively. No mouth ulcers were observed in this infant cohort. Pus from eyes was seen in 3 visits in 3 (0.1%) out-patients. Cornea clouding was observed in 2 visits in 2 (0.1%) out-patients.

5.2.2. Inpatient versus outpatient clinical impressions from infants attending unscheduled visits

Gastroenteritis was an impression recorded in 4768 visits among 1416 (63.4%) and 269 (29.4%) out-patients and in-patients respectively. Upper respiratory tract infections (URTI) were recorded in 10643 visits among 1873 (85.1%) and 69 (7.5%) out-patients and in-patients respectively. Malaria was suspected in 10481 visits among 1862 (84.6%) and 678 (74.2%) of out-patients and in-patients respectively. In summary, 23985 out-patient visits were seen from 2200 infants who attended the unscheduled visits and the top four reasons for out-patient visits was as follows: breathing difficulty (2083, 94.7%), fever (1779, 80.9%), diarrhea (1452, 66.0%) and vomiting (1371, 62.3%). One thousand and seventy seven visits resulted in hospitalization. Of these visits, 914 infants were seen during these in-patient visits,

the top four reasons for visits were associated with: fever (846, 92.6%), vomiting (409, 44.7%), breathing difficulty (370, 40.5%) and diarrhea (310, 33.9%). Leading impressions observed by clinical team for for out-patient visits was upper respiratory tract infection (URTI) and for in-patient visits was malaria (*Table 13*). A Kilifi based study found that causes of all severe morbidity before 98 days included pneumonia, gastroenteritis and malaria (English et al., 2003)

Table 14. Clinical impressions of infants attending unscheduled visits

Inpatient clinical impressions	**Inpatients (914; n,%)**	**Outpatient clinical impressions**	**Outpatients (2200; n,%)**
Impression Malaria	678 (74.2)	Impression URTI**	1873 (85.1)
Impression GE*	269 (44.7)	Impression Malaria	1862 (84.6)
Impression URTI**	69 (7.5)	Impression GE*	1416 (63.4)

*GE – Gastroenteritis ** URTI – Upper Respiratory Tract Infection

5.2.3. Inpatient versus outpatient clinical investigations requested during unscheduled visits

Haemoglobin test was ordered in 2091 visits among 889 (40.4%) and 464 (50.8%) out-patients and in-patients respectively. Full haemogramme was investigated in 1133 visits among 136 (6.2%) and 750 (82.1%) out-patients and in-patients respectively. Liver function test (LFT) was requested in 10 visits among 4 (0.2%) and 4 (0.4%) out-patients and in-patients respectively. Urea EC was done in 14 visits among 11 (0.5%) and 2 (0.2%) out-patients and in-patients respectively. Blood smears for malaria parasites (BSMPS) was performed in 3283 visits among 1227 (55.8%) and 716 (78.3%) out-patients and in-patients respectively. Random blood sugar/fasting blood sugar (RBSFBS) test was requested in 399 visits for 109 (5%) and 249 (27.2%) out-patients and in-patients respectively. Cross matching

for blood (GXM) was performed in 105 visits among 10 (0.5%) and 88 (9.6%) out-patients and in-patients respectively (*Table 14*).

Table 15. Laboratory investigations requested by clinicians during unscheduled visits

Inpatient investigations	**Inpatients (914; n,%)**	**Outpatient investigations**	**Outpatients (2200; n,%)**
BSMPS$^{\times}$	716 (78.3)	BSMPS$^{\times}$	1227 (55.8)
Hemoglobin	464 (50.8)	Hemoglobin	889 (40.6)
Full haemogramme	750 (82.1)	Full haemogramme	136 (6.2)
RBSFBS$^{\gamma}$	249 (27.2)	RBSFBS$^{\gamma}$	109 (5)
GXM $^{\Psi}$	88 (9.6)	Urea EC $^{\alpha}$	11 (0.5)
Urea EC $^{\alpha}$	2 (0.2)	GXM $^{\Psi}$	10 (0.5)
LFT$^{\beta}$	4 (0.4)	LFT$^{\beta}$	4 (0.2)

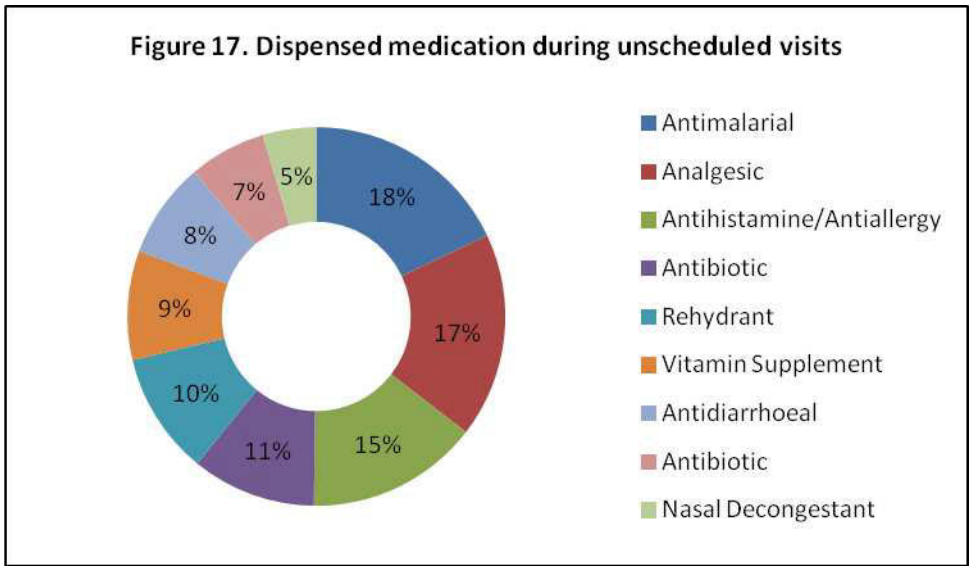
BSMPS$^{\times}$ – Blood smear for malaria parasites
RBSFBS$^{\gamma}$ – Random blood sugar/fasting blood sugar
Urea EC$^{\alpha}$ – Urea ecotoxicity
GXM$^{\Psi}$ – Glucuronoxylomannan
LFT$^{\beta}$ – Liver function test

5.2.4. Prescriptions dispensed to infants utilizing unscheduled visits

A list of prescribed drugs that were dispensed during the unscheduled visits for infants who required treatment in the following ascending order; anti-malarials, analgesics, antihistamines/antiallergies, antibiotics (amoxyxillin syrup), rehydrants, vitamin supplements, antidiarrhoeal, antibiotics (cotrimoxazole syrup) and nasal decongestants (*Table 15 and Figure 17*).

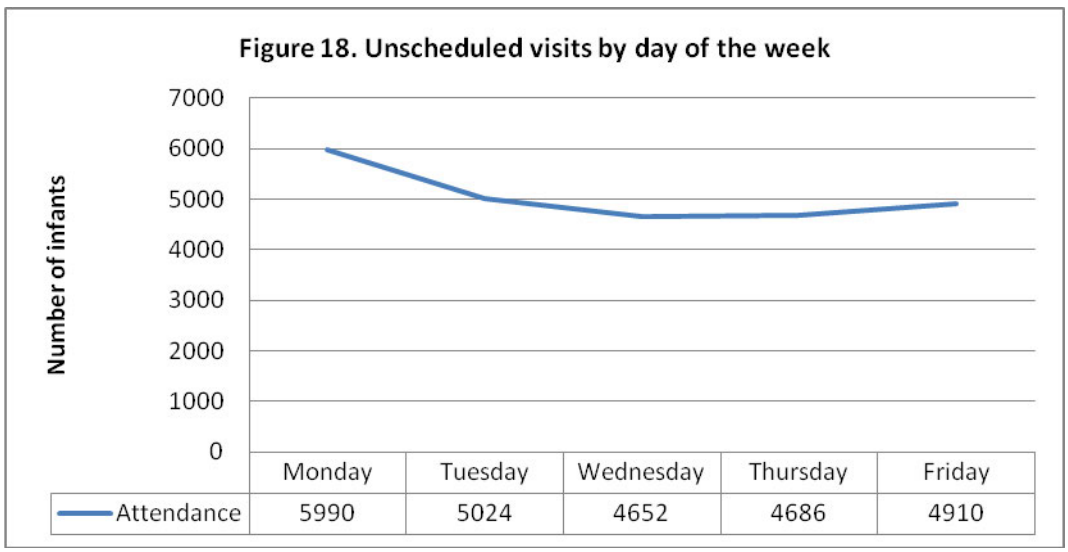
Table 16. Prescriptions dispensed to infants attending unscheduled visits

Prescriptions	**Quantity**	**Class**	**Formulation**
Artemether/Lumefantrine Tablets	8970	Antimalarial	Tablets
Paracetamol Suspension	8721	Analgesic	Suspension
Chlorpheniramine suspension. 125mg/5mls.	7364	Antihistamine/Antiallergy	Suspension
Amoxycillin syrup	5310	Antibiotic	Suspension
Oral Rehydration Salts	5217	Rehydrant	Suspension
Multivitamin Syrup	4647	Vitamin Supplement	Syrup
Zinc	4094	Antidiarrhoeal	Tablets
Cotrimoxazole Syrup	3297	Antibiotic	Suspension
Normal Saline Nasal Drops	2282	Nasal Decongestant	ENT Preparation



5.2.5. Association of unscheduled visit days and market day

We also assessed unscheduled visits by day of the week to see if there was an association between these visits and the market day. Monday (5990 visits) was most frequented day of the week followed by Tuesday (5024 visits), Friday (4910 visits), Thursday (4686 visits) and Wednesday (4652 visits) in that order. The market day for Siaya County is Wednesdays and therefore they study found no relationship between visits and market day (*Figure 18*).

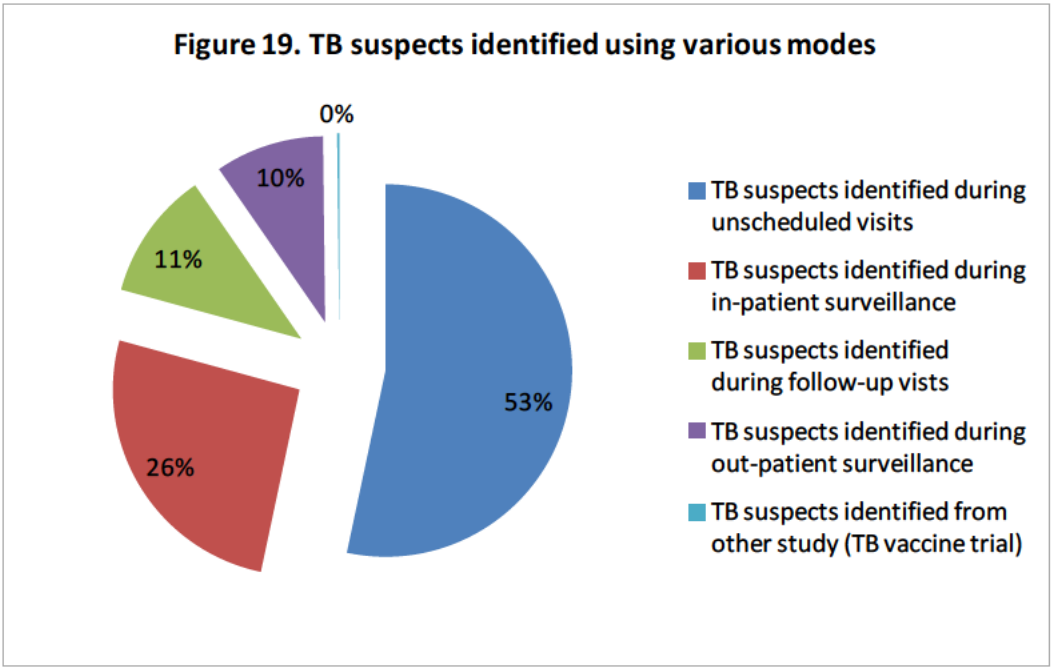


5.2.6. TB suspects, TB cases and deaths with unscheduled visits history

TB suspects, TB cases and deaths from unscheduled visits shows that TB suspects were identified through 5 approaches that included: 609/1142 (53.3%) were identified during unscheduled visits, 296/1142 (25.9%) identified through in-patient surveillance, 127/1142 (11.1%) were identified at follw-up visits, 108/1142 (9.5%) were identified through out-patient surveillance and 2/1142 (0.2%) were identified through a TB vaccine trial study. Of the total 48 cases of TB that were confirmed through culture and clinical evaluation, 18 cases were referred from the unscheduled visits. Out of the 205 deaths, 132/205 (64.4%) were infants who attended unscheduled visits. Proportion of male infants who died were 78/132 (59.1%) (*Table 17 and Figure 19*).

Table 17. TB suspects with unscheduled visits history

Description of activity	Proportion (n/N)	Percent
TB suspects identified during unscheduled visits	609/1142	53.3
TB suspects identified during in-patient surveillance	296/1142	25.9
TB suspects identified during follow-up vists	127/1142	11.1
TB suspects identified during out-patient surveillance	108/1142	9.5
TB suspects identified from other study (TB vaccine trial)	2/1142	0.2



Unscheduled visits contributed 38% of TB cases, infants who died with a history of visits for ancillary care were 64% and male infants with unscheduled visits history were 59% (Table 17). Adverse events information that may be related to infants who attended unscheduled visits and later on died can provide further details of these deaths.

Table 18. TB cases and deaths with history of unscheduled visits

Description of activity	Proportion (n/N)	Percent
TB cases identified from unscheduled visits	18/48	37.5
Infants unscheduled visits history who died	132/205	64.4
Males who died with history of unscheduled visits	78/132	59.1

6. Discussion

Initially, the study approached pregnant mothers before they delivered to discuss with them about the study and importance of prevention of mother to child (PMTCT) but this was shelved as it was not cost effective as it had implications personnel time and also slackened recruitment rates. The study decided to target deliveries using village reporters who would notify the study through a phone call and the study staff would then call back to take the

details of the compound where the delivery had taken place to initiate a home visit by a study team. Leveraging on existing structures within the HDSS platform assisted the study in reaching the community and enrolling this cohort. Village reporters (VRs) notified the study on most of the births in the study area. The VRs are trusted members the community residing in the same community as the mothers and infants. In a similar cohort study in neighbouring Uganda, village scouts were used to recruit and enroll infants into the study (Nabongo et al., 2014). Almost all of the infants were enrolled from their homes. Many infant studies enrolled from health facilities. Home deliveries were higher than health facility delivery even though majority of mothers received antenatal care during last pregnancies. This discrepancy brings the need to qualitatively assess factors influencing place of delivery. Three out of 10 deliveries occurred in the health facility but only about three out hundred infants were enrolled at the health facility. The study set up various field teams each comprising of nurses and community health workers as a strategy to succeed in home enrollments and motorbike transportation made penetration into community easier. Over three-quarters turned up for unscheduled visits thereby increasing contact between study staff and infants in need of clinical evaluation and treatment. The ancillary visits provided rich information for evaluating the free medical care visits where transport was reimbursed. Many mothers worked as salaried employees and lived in mud houses in this rural setting. Mothers with HIV positive results in this cohort mirrored the regional data. Mothers in this cohort tended to have smaller families. Mothers in the cohort were younger in age. Infant accrual into the study took 13 months and January 2010 is singled out as the most successful month owing to extension of study through a protocol amendment. The infant cohort study improved BCG coverage by administering BCG vaccine to the community to target infants delivered in the homes. Reasons for study withdrawals require further qualitative investigations in the future

to understand why participants and mothers leave. The study demonstrated capacity to recruit and retain the infant cohort with an LFTU rate less than 10%.

Low levels of education; mothers engaged in farming, salaried work and fishing; mothers living in mud type and semi-permanent houses were more likely to be associated with home delivery compared to health facility in this cohort. Conversely, mothers receiving antenatal care were less likely to deliver at home than health facility. This finding is ironical as majority of mothers received antenatal care from health facilities in the community. Reasons for preferring home delivery to health facility even after receiving antenatal care need to be evaluated. Education levels and antenatal care have been identified elsewhere as factors influencing place of delivery. Socio-economic status (SES) data was not part of information collected in the study though type of residence may be a proxy that indicates wealth status and there are studies that have found that SES affects choice of place of delivery. A study in Kericho, Kenya found that education and using of antenatal care services determined the place of delivery (Kitui et al., 2013) Overall, there is an indication that more awareness is necessary to increase uptake of skilled birth attendant at health facilities where only thirty percent of mothers in this cohort had their infants delivered. Maternal occupation such as those engaged in fishing, farming, salaried with tendency to deliver at home would require further investigation as these mothers may have been financially better placed than mothers working as laborers. Qualitative research on preference for home delivery is required as majority of the mothers in the tuberculosis cohort attended antenatal care services at facilities in the study area.

Infants delivered at home had less LTFU due to the increased contact and rapport created by village reporters and study teams that visited the families in their homes upon birth

notifications where study information was shared, BCG vaccination administered and locator information taken for future reference. Increased infant weight at birth had reduced LTFU as these mothers may have been intrinsically motivated to participate with the view of monitor the wellbeing of children. Mothers who were engaged in farming and salaried work were less likely to be LTFU because they accessed more stable sources of income, had flexibility in time schedules than mothers working as laborers. Mothers living in mud housing compared to those living in permanent are hypothetically economically disadvantaged and therefore the provision of free medicare and the reimbursement of transport costs for these sick visits may have enhanced their participation which is the converse of a Nigerian nutrition study that found that low income mothers had increased LTFU (Olusegun et al., 2007). Mothers with more children had increased LTFU compared to mothers with fewer children probably due to conflict in balancing domestic and study expectations. Mothers residing within the HDSS area had less LTFU compared non-HDSS residents participating in the cohort study. HDSS has a mapping infrastructure for the homes within its community that enhance easy tracing and additionally, HDSS residents are better exposed to research carried out within the KEMRI and CDC collaboration compared to non-HDSS participants in the cohort. Older mothers had reduced LTFU compared to younger mothers probably because younger mothers tend to be more mobile and change locations thereby miss scheduled visits (Beneri et al., 2013). Older mothers may have had increased level of awareness as they have better experience in raising up children that motivated them to participate for the welfare of their children. Prioritizing follow up efforts is critical as part of study activities to avoid attrition of study participants during the conduct and up to closure of the study. Retention strategies such as clinic staff providing good medical care to infants, transport reimbursements for scheduled visits, indicating the next visit date on study card, issuing reminders, physical tracing and open days were utilized.

Home delivered infants attended more unscheduled than health facility delivered infants. Mothers who delivered at the health facility had a better health seeking than mothers who delivered at home. However, we posit that the latter mothers may have taken advantage of a medical care system that was free and transport reimbursed for sick visits. Similarly mothers with lower education levels have may not necessarily sought medical care for any other reason than the instrinsic benefits (Ndugwa and Zulu, 2008). On the other hand, mothers residing in the HDSS had less unscheduled visit attendances compared to those residing outside the HDSS may be due to previous experience with research activities from KEMRI and CDC collaboration and therefore better able to differentiate between research and medical services. HIV positive mothers had fewer unscheduled visits compared to HIV negative probably due to better health seeking behavior and therefore better positioned to discriminate the need for an unscheduled visits or not.

The leading symptoms for inpatient visits were fevers (Nnedu et al., 2010), fast breathing and vomiting whereas for outpatient unscheduled visits were breathing difficulty (Chintu et al., 2002), fevers and fast breathing. Inpatient visits had higher temperatures and respiratory rates compared to outpatient visits. Leading clinical impressions were malaria and upper respiratory tract infection for inpatient and outpatient visits respectively. For all unscheduled visits, the commonly requested laboratory investigations were blood smears for malaria, hemoglobin and full haemogramme. Antimalarials, analgesics and antihistamines were the most prescribed medication during these sick visits. We evaluated the relationship between unscheduled visits and market day in the community which was Wednesday and found no correlation as most visits were made on Mondays, Tuesdays and Fridays. TB suspects were identified during the unscheduled visits and referred for further investigation at the case

verification (CV) ward in Siaya County Referral Hospital. TB cases were identified that would have otherwise been missed without provision and facilitating of free medical care visits.

Table 19. Thesis objectives by relevant variables, target variables and references

Objective	Relevant variables	Target variable	Reference
Recruitment outcomes - notifications	Village reporters, health facility staff or other strategies deployed to refer potential participants to the study	Village reporters similar to scouts used in Uganda neonatal study for referring infants	Nabongo et. al., 2014
Factors associated with place of delivery	Maternal education (none, primary and secondary) Mother occupation (farming, salaried worker and fishing) Housing type (mud, semi-permanent) Receipt of antenatal care (yes)	Lower levels of education and access to antenatal care services associated with hospital delivery	Kitu et. al., 2013
Factors associated with loss to follow up (LTFU)	Place of birth (home) Infant birth weight Maternal occupation (farming, salaried worker) Housing type (mud) Number of children mother has of her own (≤ 3)	Low income mothers had increased LTFU Younger mothers had increased LTFU	Olusegun et. al., 2007 Beneri et. al., 2013
Factors associated with attendance of unscheduled visits	Residency (HDSS) Mother education (none and primary) HIV status (positive) Mother Age	Mothers with lower educational levels sought medical care for their sick babies	Ndugwa and Zulu, 2008
Morbidity burden – symptoms associated with unscheduled visits	Leading signs and symptoms during inpatient visits were fever, fast breathing and vomiting compared to breathing difficulty, fever and fast breathing for outpatient visits.	Fevers were associated with sick visits Breathing difficulty was associated with medical care visits	Nnedu et. al., 2010 Chintu et. al., 2002

7. Conclusion

We demonstrated the capacity to recruit, enroll and retain the infant cohort. Utilization of VRs was pivotal in successful accrual of infants into the tuberculosis cohort study. The study managed to improve BCG vaccination in the county tremendously. Further qualitative research is needed to understand reasons for withdrawal and choice of place of delivery. Consideration of protectors and predictors of LTFU in planning and implementation of future pediatric studies is recommended. However, these need to be validated as findings are not generalizable. Additionally, assessment of recruitment and retention strategies is important in measuring efforts and monies put in versus outcomes. The utility of unscheduled visits is better understood to inform planning and management of free medical care in the study. There are benefits associated with unscheduled visits in terms of maintaining contact with research participants thereby increasing retention and identification of study endpoints that may be missed in routine study visits. There are costs involved in provision of free medical services that sites need to negotiate with sponsors as financial agreements are signed. This study has availed information that is useful for those planning to recruit infants in large phase III trials in developing countries to avoid pitfalls that come with high loss to follow up which affect measurement of trial endpoints.

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Annexes

Curriculum Vitae

Education:

Year	Degree	University
October 2010 - 2015	PhD candidate in International Health	Ludwig Maximillians University, Muenchen, Germany
September 2007-September 2009	Master of Arts in Project Planning and Management	University of Nairobi, Kenya
September 1994 – September 1998	Bachelor of Science	University of Nairobi, Kenya

Work Experience:

Year	Position	Institution
June 2012	Management Chief TB Branch	KEMRI/CGHR
December 2009-2012	Communications, Education and Training Manager	KEMRI/CDC
October 2007-2009	Professional Development Programme Manager	KEMRI/CDC
November 2004-2007	Assistant Research Officer	KEMRI-RCTP

Professional Interests:

- Soon will be following a TB/HIV patient cohort as part of intensified case finding (ICF) activity under the EURIPRED project with funding from European Commission in FP7 grant.
- Principal Investigator of TB Indepth Study looking at TB suspects with health and demographic surveillance system (HDSS) area.
- Member of Clinical Trials/Stakeholder and Community Engagement working groups within Critical Path to TB drug Regimen (CPTR)
- KEMRI/CDC lead in East African Consortium for Clinical Research (EACCR) activities covering TB, HIV, Malaria and Training.
- Expert Reviewer of continuing professional development scheme in the Global Health Trials Network

List of Publications

1. Predictors of retention and loss to follow up (LFTU) in an infant tuberculosis (TB) epidemiological cohort study in Siaya District, Nyanza Province, Kenya (In Press).

KEMRI Ethical Review Committee Initial Approval

KENYA MEDICAL RESEARCH INSTITUTE

P. O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541 , 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: kemri-hq@nairobi.mimcom.net: director @ kemri. org; website: www.kemri.org

KEMRI/RES/7/3/1 **August 3, 2009**

TO: **VIDELIS NDUBA, (PRINCIPAL INVESTIGATOR)**
TB-R-VAC PROJECT
Email: vnduba@ke.cdc.gov

THRO': **DR. EVANS AMUKOYE,**
CENTRE DIRECTOR, CRDR,
NAIROBI

RE: **SSC NO. 1465 (NEW): A PROSPECTIVE EPIDEMIOLOGICAL COHORT STUDY TO EVALUATE THE INCIDENCE OF TUBERCULOSIS IN INFANTS IN WESTERN KENYA (version 1.3 dated March 13, 2008)**

This is to inform you that during the 168th meeting of KEMRI/National Ethics Review Committee held on Tuesday 14th July 2009, the suggested amendment to the approved study was considered.

We acknowledge receipt of the revised protocol version 1.3 dated March 13, 2008
The Committee made the following observations:

1. The amendments have been occasioned by the review of the Chesapeake IRB and are:
   a. The inclusion of confidentiality information in the oral consent from and in the focus group scripts earlier before the group begins
   b. The inclusion of the exit interview which is given as Appendix T (page 113)

The Committee was of the view that the proposed amendments do not alter the risk/benefit status of the study and are granted approval for implementation.

You are required to submit any further amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

Respectfully,

R.C. Kithinji

R. C. KITHINJI
FOR: SECRETARY,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE

KEMRI Ethical Review Continuation of Approval

KENYA MEDICAL RESEARCH INSTITUTE
CENTRE FOR GLOBAL HEALTH RESEARCH
08 APR 2010
P.O. Box 1578, KISUMU

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **April 2, 2010**

TO: **DR. VIDELIS NDUBA (CRDR)**
TB_R_VAC PROJECT,
PRINCIPAL INVESTIGATOR

THRO': **DR. JOHN VULULE,**
THE DIRECTOR, CGHR,
KISUMU

FORWARDED
8/4/2010
DIRECTOR
CENTRE FOR GLOBAL HEALTH RESEARCH

RE: **SSC NO. 1465 (*REQUEST FOR ANNUAL RENWAL*): A PROSPECTIVE EPIDEMIOLOGICAL COHORT STUDY TO EVALUATE THE INCIDENCE OF TUBERCULOSIS IN INFANTS IN WESTERN KENYA**

This is to inform you that during the 176th meeting of the KEMRI/ERC meeting held on 30th March 2010, the request for continuation with the above mentioned study was considered.

The Committee is satisfied that sufficient progress has been made in the review period, and therefore grants the study **approval** for continuation.

Please note that authorization to conduct this study will automatically expire on **1st April 2011.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **18th February 2011**.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

Yours sincerely,

RCTKithinji

R. C. KITHINJI,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

Amendment approval from KEMRI Ethics Review Committee

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **December 21, 2009**

TO: **DR. VIDELIS NDUBA**
PRINCIPAL INVESTIGATOR, TB_R_VAC PROJECT
RESEARCH OFFICER, CRDR

THROUGH: **DR. JOHN VULULE,**
THE DIRECTOR, CGHR,
KISUMU

RE: **SSC PROTOCOL NO. 1465 (*REQUEST FOR 3RD AMENDMENT*): A PROSPECTIVE EPIDEMIOLOGICAL COHORT STUDY TO EVALUATE THE INCIDENCE OF TUBERCULOSIS IN INFANTS IN WESTERN KENYA**

This is to inform you that during the 173rd meeting of KEMRI/National Ethics Review Committee held on Tuesday 15th December 2009, the suggested amendment to the approved study was considered.

The Committee was of the view that the proposed amendment to expand your projected recruitment area to contiguous areas in order to achieve your enrollment goals within a year of the commencement of the trial which will be May 25, 2010 does not alter the risk/benefit status of the study and is granted approval for implementation.

You are required to submit any further amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

Yours sincerely,

RCKithinji

R. C. KITHINJI,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

Informed Consent Form

Protocol Title: **A Prospective Epidemiological Cohort Study to Evaluate the Incidence of TB in Infants in Western Kenya**

Introduction

We are requesting you to allow your child to take part in medical research. Research is also called a study. A medical study can look at what causes a disease. It can also look at ways to prevent or control a disease.

The following information will tell you about the study and your child's part in it. Please listen carefully. Feel free to ask any questions. This is a research study being done by KEMRI/CDC.

Voluntary Participation

You may decide not to have your child be in this study. No health care benefit will be taken away from you or your child if you decide to say no. Your child can leave the study at any time. There will be no penalty or loss of benefits if you do not participate.

Background

Experts believe that BCG vaccination helps prevent severe tuberculosis (TB) in children. BCG is one of the most widely used vaccines in the world. It has been given to almost all newborns in Kenya for many years. Still, TB is a common cause of infant, child and adult deaths in Kenya. TB is caused by a germ that is inhaled into the lungs. Most people do not get disease but the germ lives in them for many years. This is called TB infection. Some people may feel ill and have symptoms very quickly; others develop signs and symptoms much later. This is called TB disease. Finding germs in the sputum confirms TB disease. New vaccines for TB are being created. These will most likely be given to infants.

Reason for the Study

The researchers conducting this studywant to find out the amount of TB infection and TB disease in infants in this community. They also want to find out if a TB vaccine study could be done in infants in this community and whether it is possible to vaccinate all newborns with the regular BCG within 4 days. They would also like to find out the clinical predictors for TB in a place where HIV is also common. HIV is the virus that causes the Acquired Immune Deficiency Syndrome (AIDS). HIV infection occurs in some infants because the virus is carried from the mother to the baby during the pregnancy or during delivery or during breast-feeding. If an infant is infected with HIV, the mother is almost always also infected. HIV is commonly spread by sexual intercourse in adults.

Since HIV infection will affect the immune system, it is important to know if an infant is infected with HIV before trying to understand your child's response to TB. You will be informed of the results

of your child's HIV test. No one else (for example, your sexual partner) will be informed of your child's status, unless you give the counselors your permission to inform them. If the results are positive you, your child, and others in your family will be referred to the best management that our health care system provides, including pre- and post-test counseling and HIV tests for family members who desire it. The counselors who will see you are very aware that being known to be HIV positive can lead to people being unfairly shunned or otherwise victimized by members of their community, and will support you and advise you on how best to cope with this possibility.

Procedures to be followed

This study does not use an experimental drug or vaccine. The study is being done in Karemo division **and contiguous areas e.g Boro, Gem etc**. Study staff are aiming to enroll all infants born in this area. Permission to enroll the infants will be obtained from the infants' mother, as soon as possible after delivery, either at the mothers' homes (if the infant was born at home) or at a health facility (if the infant was born at a health facility). To enroll in the study, an infant must receive the regular BCG used in the public clinics but given by study staff, and must be available for follow up for at least one year after birth. When we enroll your infant into this study, we would also like to know the mother's HIV status. This information will enable us to take steps that will prevent mother to child transmission of HIV. It will also enable us to link both the mother and the infant to HIV treatment and care services near them. If we enter you into the study while pregnant, we will seek permission to access your HIV test result if already tested, if you are not tested, we will encourage you to get tested at the nearest HIV testing center. If the result is positive and you are enrolled for HIV care, you will be given a drug during labor to prevent the transmission of the HIV virus to your child. Your child will also be given a drug within 3 days of delivery and another one to take everyday for one month. You will also be advised on safe infant feeding practices to prevent your child from getting infected with the HIV virus. We will also ask you to come back for another HIV test when your baby is six weeks old. This test will inform us if the baby has the HIV infection or not. If we enter you into the study after the birth of the baby, and you have not had an HIV test in the preceding 2 months, or were tested more than 2 months ago and had a negative result, we you will request you to be tested again. If you were or are tested and the result was/ is positive, you will be given a drug known as Nevirapine to take at the onset of labor and your baby child will also be given some drugs: Nevirapine for three days and zidovudine for 1 month. These drugs prevent transmission of the virus from mother to the child. In addition, your baby will be given daily septrin and multivitamins. until we do another HIV test when the baby is six weeks old. This test at the age of six weeks will be done on all babies. This particular test will inform us whether your infant is infected with the HIV virus or not. We would like to test your child for the HIV virus when they are admitted to hospital. The HIV testing in this study is entirely voluntary. You may choose to participate in this study without undergoing HIV testing.

Follow up

Your infant will be followed up once every four months for up to 2 but at least one year. This means that a study staff member will **arrange to meet you at the health facility you routinely attend,** every fourth month to examine and weigh your infant and to ask you questions about your infant's health, including questions about signs and symptoms of TB in your infant and possible exposure to TB**. we will give you Ksh 150 as reimbursement for your transport costs.If you don't make it to the health facility, a study nurse will visit you at your home, and therefore for this, there shall be no reimbursement.** Study staff may also look at your infant's clinic and/or hospital records to see if s/he has been examined for TB or diseases that might be TB. If TB is suspected in your infant, you will be asked to bring him/her to the Siaya District Hospital for special tests. These are described in a

later section of this document on TB diagnosis. The study staff will also offer HIV testing and counseling if your baby was / you were negative or was / were not tested at the beginning of the study.

TB Diagnosis

If study staff suspect your infant may have TB or that s/he has been exposed to TB, they will ask you to bring your infant to the Case Verification Ward at Siaya District Hospital. Transportation to the ward will be provided by the study. You and your infant will be admitted in the afternoon and you will spend two nights together in the ward. Each morning a study nurse will use a small tube to remove some contents from your infant's stomach. A spray to make your infant cough and then a soft suction catheter will be used to remove the material coughed up from your infant's throat. This material will be tested for the germ that causes TB. These procedures are normal tests used to diagnose TB in infants but are not normally available in our public health facilities. Your infant will also receive a skin test that shows if your child has come into contact with the germ that causes TB, and will also have a **chest X-rays** done

If your infant is admitted to the case verification ward for TB tests, you will be given and asked to sign another consent form with more detail about the tests, including the risks and benefits of these tests.

Mother's Finger Print and Baby's Toe print

As part of your participation in this study, we wish to scan your finger print and your baby's toe print. The fingerprint reader will not store the image of your finger print or your baby's toe- print, but will use the skin lines and ridges to create a number that is unique to each person. No one can re-create a copy of your finger print or baby's toe print from this number. It is of no use to anyone, including the police. At every clinic visit, your finger print or baby's toe print will be scanned. This will confirm that it is you who has come for that particular visit. This will enable us to better identify you so that we can link your residency information with your health records with ease. All the pieces of information about you and your family, including the fingerprint code will be kept in strict confidence and will not be subjected to any other use other than for identification purposes during study visits.

Risks finger print or toe print scanning:

We do not believe there are any serious risks involved in having your finger- or toe- print scanned.

You are free to choose for you and your baby to have your finger- or toe- prints scanned respectively. You have the right to refuse. You can still participate in this study even if you refuse to have your finger print or baby's toe print scanned.

Study Close Out

At the end of follow-up (at least 1 year, maximum 2 years) you) we will ask you about TB signs and symptoms concerning your infant and yourself. Study staff will also ask you about people suffering from TB who might live in your home. If your infant has signs or symptoms of TB, s/he will be referred to the case verification ward at Siaya District Hospital as mentioned previously for further TB investigations.

Linkage with DSS Data

Your family may be part of the KEMRI/CDC Demographic Surveillance System (DSS). That is a different study from this study. The DSS collects information on:

1. The number of births and deaths in the ccommunity
2. How much education people have
3. How wealthy or poor people are
4. How much community members use health services and why
5. Other factors which are important in controlling infectious diseases and promoting health in the community.

If **you allow your** child **to** take part in this study and your family is also part of the KEMRI/CDC DSS, **we** will link some information from the **DSS to this study**. This would link some information about your household with your child's information from this TB study, such as:

1. the number of people living in your house
2. The characteristics of your household, and so on.
3. Any visits made to the health facilities within and outside Karemo **and contiguous areas e.g Boro, Gem etc**.

Should your child die, the DSS may collect information about your child's death which they will share with us.but we shall not be giving any information to the DSS about you or your involvement in the study.

If your family is not part of the KEMRI/CDC DSS, it will be impossible to link to information about your family's household unless you provide this information to the study staff.

Cause of Death Investigations:

To prepare for a vaccine study it is important to know the cause of death if someone passes away. If your child was to pass away, we would want to know if TB or a related condition has something to do with the death. This will require further investigations. We will approach you to discuss these with you in more detail should your child pass away.

Exclusions

Your child will not be eligible for the study if you do not plan to stay in the study area for at least one year after the birth of your infant. There may be other reasons why your infant cannot participate. The study staff will discuss these with you.

Risks and Benefits to Your Child

Since your infant will be under active surveillance for TB, s/he will benefit by receiving an earlier diagnosis of TB and have less severe disease. The TB tests used in the study are usually not available in the public health facilities.

You and your child will also receive early referral for HIV treatment and care if found to be HIV positive. If you are infected with HIV, we will also advise you on way to prevent your baby from getting infected

There may be a small risk to your child from the tests used to diagnose TB. These will be explained if you are asked to bring your child in for testing.

Refund

If you have to pay for transportation to the Siaya District Hospital when you come to our clinic or when your child is admitted to the case verification ward, you will be given back the amount of Kshs 100-400, depending on the distance travelled. If you and your child are admitted to the CVW in SDH, you will be given some baby products whose value shall not be more than Kshs **500.at the time of discharge.**

If your child suffers any adverse vaccine effects as a direct result of the BCG vaccine, the government health services will provide medical care.

Privacy

You will receive a copy of this consent form. The researchers conducting this study will keep your child's study records strictly private. Nobody else but they and their staff will see your records unless you give them permission. Your child's name will not appear in any publication written from this study.

Contact Information for Questions or Concerns

The study has been approved by:

1. The National Ethical Review Committee (ERC), Kenya Medical Research Institute (KEMRI)
2. The Institutional Review Board of the Centres for Disease Control and Prevention (CDC) in the United States of America.
3. The Institutional Review Board used by the Aeras Global TB Vaccine Foundation.

If you have questions about your child's rights being in research, you should contact:

The Secretary of KEMRI NERC on (020) 2722541 or 0722205901, fax (254) (020) 2720030, P.O.Box 54840-00200 Nairobi-Kenya.

If at any time, you have questions or concerns about this study or your child is injured as a result of being in this study you should contact:

Dr Videlis Nduba or Dr. Anja Van't Hoog at KEMRI/CDC Field Research station at Kisian, Kisumu. (P.O. Box 1578, Kisumu) at telephone 057-20-22902/59/83.

By signing Box A below, I agree that:

- I have read this consent form or had it read to me.
- I have had the chance to ask questions and they have been answered.
- I understand that my child taking part in this study is my free choice
- I give permission to use and share my child's health and research data as described in this form.
- I have the ability to withdraw my child at any time with no penalties or consequences

I will receive a signed copy of this consent form.

Authorization to Participate in This Study

Name of study participant/ Child *(please print)*
_____ Name of parent / guardian*(please print)*
_____ Signature/Thumbprint of parent/ guardian Date
_____ Name of study personnel taking consent *(please print)*
_____ Signature of study personnel taking consent Date
[ ] Interpreter used ***(tick only if applicable)*** [ ] Witness used ***(tick only if applicable; tick one below to describe witness)*** [ ]
_____ Name of witness *(please print)* _____ Signature of witness

Consent for finger print or toe print scanning

Name of parent/guardian	Name:.....	Signature:	Date ☐☐/☐☐/☐☐
Witness	Name:.....	Signature:	Date ☐☐/☐☐/☐☐

Case Report Form Content for Enrollment

Study Day 0: (Birth) - Visit 1

Verify eligibility criteria

Written informed consent from parent / guardian

- **Entry** and assignment of SIN

History from parent/guardian, including:

- Demographic characteristics of parent(s) and infant
- Details of infant's BCG immunization if already given at hospital or administer BCG for infants delivered at home and exact date after birth.
- History of close contact with a person with confirmed TB
- Maternal HIV/PMTCT status Plus referral if not HIV tested or tested > 2 months ago
- Infant's medical history
  - Birth history (e.g. birth weight, complications)
  - History of hospitalizations since birth
  - Diagnoses made of acute or chronic diseases since birth
  - Current signs or symptoms of TB

Physical examination of the infant, including

- Weight / head circumference/ height
- Congenital anomalies of the face and/or central nervous system.
- Evaluation of BCG injection site (BCG scar) and axillary lymph nodes of injection arm
- Parent/guardian given information on BCG side effects

Case Report Form Content for Follow Up Visit (1)

Study Day 42 [±7]: (6 weeks) - Visit 2

HIV counseling and testing (PCR) of infant

History from parent/guardian, including:

- History of close contact with an adult with confirmed TB during last 4 months
- Infant's medical history, including assessment of adverse events
  - History of hospitalizations during last 4 months
  - Diagnoses made of acute or chronic diseases during last 4 months
  - Current signs or symptoms of TB

Physical examination of the infant, including

- Weight
- Evaluation of BCG injection site and all nodes.

Case Report Form Content for Follow Up Visit (2)

Study Day 119 [±14]: (4 months) - Visit 3

Study Day 245 [±14]: (8 months) - Visit 4

Study Day 483 [±14]: (16 months) - Visit 6

Study Day 609 [±14]: (20 months) - Visit 7

Study Day 728 [±14]: (24 months) - Visit 8

History from parent/guardian, including:

- History of close contact with an adult with confirmed TB during last 4 months
- Infant's medical history, including assessment of Adverse Events
  - History of hospitalizations during last 4 months
  - Diagnoses made of acute or chronic diseases during last 4 months
  - Current signs or symptoms of TB
  -

Physical examination of the infant, including

- Weight

Evaluation of BCG injection site and all nodes

Case Report Form Content for Follow Up Visit (3)

Study Day 364 [±14]: (1 year) - Visit 4

History from parent/guardian, including:

- History of close contact with an adult with confirmed TB during last 4 months
- Infant's medical history, including assessment of Adverse Events
  - History of hospitalizations during last 4 months
  - Diagnoses made of acute or chronic diseases during last 4 months
  - Current signs or symptoms of TB

Physical examination of the infant, including

- Weight, (height needed for percentile charting)
- BCG scar

KEMRI Publications committee approval of manuscript

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2724036
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/PUB/9/18 4th March 2015

Steve Wanliga

Thro The Director
CGHR, Kisumu

11/3/2015

KEMRI/PUB/3584 – Predictors of loss to follow up in an infant tuberculosis Cohort study in Siaya County, Kenya by Steve Wanliga, CGHR

This is to inform you that during the 188th Publications Committee meeting held on 2nd February 2015, the above manuscript was approved for publication.

Thank you.

DR. CECILIA MBAE
SECRETARY,
PUBLICATIONS COMMITTEE

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In Search of Better Health

Table 20. Variables description, codes, values and names

Description	Codes/values	Name
Mother's occupation	1=Farming	momoccupation
	2=Salaried worker	
	3=Business	
	4=Labor	
	5=Fishing	
Mother HIV status	1=Negative	rechivresultsas
	2=Positive	
Lost to follow-up status	1=Lost	retained
	2=Retained	
Type of Housing	1=Mud	housingtype
	2=Semi-permanent	
	3=Permanent	
	4=Other	
Mother received antenatal care	1=Yes	recreceivedantentlcare
	2=No	
	3=Unknown	
Number of children mother own	1=<3 children	mothersown
	2=>=3 children	
Mother's level of education	1=None	momeducationlevelf
	2=Primary	
	3=Secondary	
	4=Tertiary	
	5=Unknown	
Residency	1=HDSS	demdssidstatus
	2=Non-HDSS	
Infant's sex	1=male	enrsex
	2=female	
Place of birth	1=home	enrplaceofbirthf
	2=Health facility	
Mother's age	Years	mumage
Infant's age	years	age
Number of infants delivered	1=Singleton	enrinfantsdelivered
	2=Twins	
Birth weight	Kg	

Statistical Analyses

Univariate analysis

Descriptive statistics were used to describe the efforts and outcomes of recruitment, enrollment, follow up and retention using a flow chart where counts and percentages are presented. Recruitment strategies and BCG profiles were described using table of frequencies, percentages and pie charts. A line graph was used to illustrate the accrual of infants into the tuberculosis cohort by month and year. A histogram was used to describe the age distribution of the infant cohort. Infant and maternal characteristics collected at enrollment were summarized in pie charts using percentages. Table of count and frequencies were used to summarize infant and maternal characateristics. Infants and maternal characteristics are summarized in table of percentiles.

Bivariate analysis and logistic regression

Maternal factors for place of delivery were tabulated in crude odds ratio and p-values for bivariate characteristics considering whereas for logistic regression was presented in adjusted odds ratio to control for confounding using p=0.05 as the rest for statistical significance. Graphical illustration was used to depict the multivariate analyses pictorially.

Infant and maternal factors associated with LTFU versus retained and unscheduled visits (yes versus no) were tabulated in crude odds ratio and p-values for bivariate characteristics considering Chi-square test for 2 X 2 tables and Mantel Haenszel test for greater that 2 X 2 tables whereas for logistic regression was presented in adjusted odds ratio to control for confounding using p=0.05. Graphical illustrations for infant and maternal characteristics were used to depict the multivariate analyses for infant and maternal factors associated with LTFU versus retained and unscheduled visits (yes versus no) pictorially.

Kaplan Meier Estimation was used to explore predictors of LTFU through exploratory analysis using graphical curves with p=0.05 as the test for statistical significance.

Cox Proportional Hazards model was used to assess for factors associated with LTFU only for both infant and maternal characteristics. Log rank test was used to assess binary variables where Generalized Wilcoxon test was used for variables > 2.

Signs and symptoms among infants attending unscheduled visits were tabulated in order of higher frequency and percentage divided into inpatient and out patient categories. Leading clinical impressions were tabulated and split between inpatient and outpatient visits. Laboratory investigations requested by clinicians during inpatient and outpatient unscheduled visits were tabulated accordingly. Prescriptions dispensed during unscheduled visits were tabulated by medication, class, count and formulation. A pie chart was used to pictorially represent the prescriptions. An association between unscheduled visit days and market day was illustrated graphically. TB suspects with unscheduled visits history were tabulated to depict the source of identification followed by pie chart. TB cases and deaths with unscheduled visits history were tabulated.