CHAPTER 1

GENERAL INTRODUCTION

Background

In sub-Saharan Africa, human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS) is the leading contributor to life-years lost (1), causing 1.1 million (73%) of the 1.5 million deaths worldwide due to HIV/AIDS in 2014 (2). In addition, the Joint United Nations Programme on HIV/AIDS (UNAIDS) reported that 70% of the 1.5 million new HIV infections worldwide occurred in sub-Saharan Africa (2). Only 37% of HIV-infected individuals in sub-Saharan Africa are receiving life-saving antiretroviral therapy (ART) (3). These numbers document the major disproportionate burden of disease inflicted by HIV in sub-Saharan Africa, as well as the substantial potential for improvement if HIV prevention, diagnosis, and management can be optimized.

In patients who have, or are at risk for, HIV infection, challenges are further exacerbated in the setting of overlapping co-infections. For example, sexually-transmitted infections (STIs) in women not only cause direct morbidity and mortality (e.g., chronic pelvic inflammatory disease leading to infertility in women and congenital infection in their babies), but they additionally increase susceptibility to HIV acquisition (4). Tuberculosis patients who are co-infected with HIV have a two-fold higher mortality rate than tuberculosis patients without HIV infection (5). Tuberculosis, STIs, and a variety of other co-infections in HIV-infected patients increase the plasma concentration of HIV RNA (viral load), which is well-recognized as a primary marker of HIV transmissibility and disease progression. A recent systematic review summarized 18 different studies, all of which demonstrated that the viral load could be reduced through appropriate treatment of tuberculosis, STIs, malaria, or helminth infections (6). Taken together, these examples clearly document the impact of multiple infections on the health of an individual, and the importance of investigating ways in which co-infections can be managed to improve morbidity and mortality.

This thesis will focus on HIV and parasitic infections caused by *Schistosoma mansoni* and *Schistosoma haematobium* in Tanzania. It will begin by investigating the overlap and interactions between these two highly prevalent infections (**Chapters 2 and 3**). It will then describe the chronic complications related to schistosomiasis of the urogenital tract that may increase a woman's susceptibility to HIV acquisition even after treatment (**Chapter 4**), and will document an innovative laboratory technique by which additional studies of the relationship between schistosome infection and HIV may be explored using dried blood spots (**Chapter 5**). After this, the focus will turn to a study of the interactions of schistosome infection and HIV in HIV-infected individuals who are taking antiretroviral medications (**Chapter 6**). Finally, the thesis will investigate the

implementation of better care to improve early infant diagnosis of HIV infection in infants born to HIV-infected mothers (**Chapter 7**). The discussion will focus on the knowledge gained through studies to this point, as well as the recommended next steps for further research to improve diagnosis, treatment, and disease prevention in individuals living in sub-Saharan Africa.

Overview of Schistosomiasis

Schistosomiasis is a parasitic infection caused by helminthic worms of the *Schistosoma* genus. The three main species of schistosomes that cause human disease are *S. haematobium*, *S. mansoni,* and *S. japonicum*. The World Health Organization (WHO) estimates that, in 2013, over 260 million individuals required treatment for schistosomiasis, and that an additional 700 million were at risk of infection, with over 90% of these individuals living in Africa where both *S. mansoni* and *S. haematobium* are endemic (7,8). Schistosomiasis is a disease of poverty acquired by contact with fresh water that has been contaminated by parasite larvae and is classified as a neglected tropical disease by the WHO (9). Schistosomiasis causes a large global burden of morbidity, with an estimated 3.31 million disability-adjusted life years in 2010. This was second only to leishmaniasis (3.32 million) among the neglected tropical diseases (10).

Schistosomiasis has a complicated life cycle that is propagated when the following requirements are met: (1) human excrement containing schistosome ova must contaminate fresh water; (2) specific intermediate snail hosts must reside in the contaminated water; and (3) humans must come into contact with contaminated water. Schistosome eggs, excreted into fresh water, hatch to release miracidium larvae that seek snail hosts, where they replicate asexually to produce multiple generations of sporocysts (11,12). Sporocysts mature into cercariae with a characteristic bifurcated tail, and are then released from snails into fresh water to seek their definitive host. Cercariae typically live for 24 hours and can penetrate unbroken skin, resulting in human infection (11,13). During skin penetration, cercariae lose their bifurcated tail and become schistosomulae, which migrate through tissue into the host blood stream, the lung, and ultimately the portal vein (11,12). Schistosomulae mature in the portal vein for 4-6 weeks into adult worms and, once mature, migrate preferentially to venules of the urinary and genital tracts (*S. haematobium*) or the gastrointestinal tract (*S. mansoni, S. japonicum*).

Adult schistosome worms living in venules form permanent reproductive male-female pairs, producing hundreds to thousands of eggs per day and living an average of 3-5 years (11,14). Schistosome miracidia within eggs secrete

proteolytic enzymes that facilitate the migration of the egg through the vessel wall and into the surrounding tissues toward the mucosal tissue and lumen of the genitourinary tract or intestine (15). Approximately one-third of eggs produced by worms ultimately arrive at the lumen of the bladder or intestine and are excreted in urine or stool (14); the remaining eggs are trapped in the tissues or are embolized to distant organs. Repeated exposure to schistosome-infected fresh water progressively increases the number of worms and eggs and the severity of the infection.

Schistosomiasis causes a plethora of immune alterations in the immune system of the host, with effects on T-helper (Th) 1, Th2, Th17, and T-regulatory (T-reg) immune responses. The initial immune response to schistosome infection is T-helper 1 (Th1)-mediated, directed against worm antigens. Once adult worms have matured and egg production begins, the immune response rapidly becomes T-helper 2 (Th-2)-mediated due to the high antigenicity of eggs as they migrate through tissue (16). This Th2-type response leads to granulomatous inflammation that surrounds eggs trapped in tissue, and, ultimately, to tissue fibrosis and chronic morbidity (17). This granulomatous response is paradoxical in its effect: though it does incite tissue fibrosis, it also protects the host tissue by sequestering toxic secretions of schistosome eggs that can otherwise result in widespread host tissue necrosis (16,18,19). Prolonged infection with granulomatous changes lead to late-stage tissue obstructive changes. Interestingly, such obstructive changes have been associated with increased tissue burdens of eggs in autopsy studies, suggesting that late-stage fibrosis may decrease transmission of the parasite from the index case (20,21).

A natural history study of morbidity caused by *S. haematobium* in an endemic region of Kenya supports this progression of tissue pathology over time (20). Investigators demonstrated that, in the initial years of infection, urinary tract lesions were inflammatory granulomata of the ureters or bladder. Over time, these lesions progressed to "established" or obstructive tissue changes such as hydronephrosis, hydroureter, and bladder fibrosis. The study investigators suggested that, because hydronephrosis predominantly affected adults and appeared to be independent of intensity of schistosome infection, the duration of parasite infection more accurately predicts risk of chronic tissue disease than the intensity of infection. Multiple studies have also demonstrated the importance of other factors including treatment, reinfection, and a complicated interplay of host genetics influencing the balance of Th1, Th2, Th17, and T-reg-type reactions to the parasite eggs in determining the degree of tissue damage and host pathology (11,17,22,23).

The major morbidity experienced in chronic schistosomiasis is attributable to the host's egg-induced granulomatous response and subsequent fibrosis in the bladder, intestine, and liver (24). Prominent clinical sequelae of various species of schistosomes depend largely on where adult worms reside. *S. haematobium* worms live in venules surrounding the urinary and genital tracts, and thus their migrating eggs typically cause bladder/ureter fibrosis leading to hydronephrosis, bladder cancer, and genital tract damage including erosions, edema, sandy patches, and neovascularization of the cervix, as well as alterations in immune cell populations in the genital mucosal tissue (25–28). Major sequelae of *S. mansoni* infection include diarrhea, intestinal inflammation, and liver fibrosis leading to esophageal varices and hematemesis. A 2003 paper estimated the annual global mortality from renal failure secondary to *S. haematobium* to be 150,000 and from hematemesis induced by *S. mansoni* to be 130,000 (29). Morbidity is also significant in those with chronic schistosome infection, who suffer from significantly higher burdens of anemia, poor nutrition, pain, and impaired academic performance and have been estimated to function at a disability level of 2-15% (30).

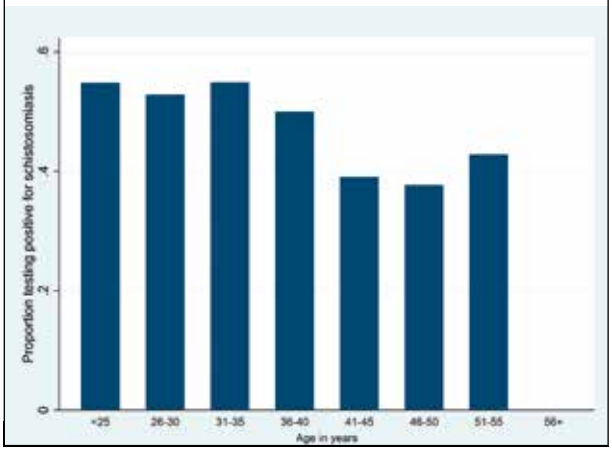
The gold standard for diagnosis, recommended by the WHO, is microscopic visualization of parasite eggs in urine or stool (8). Urine is filtered through a porous membrane filter through which schistosome eggs cannot pass, and the filter is examined directly under the microscope. Urinary infection intensity is reported as eggs per 10 milliliters (ml) of urine. Eggs in stool can be quantified using the Kato Katz technique, in which a thick fecal smear of known mass is prepared, stained, and examined for schistosome ova. Stool infection intensity is reported as eggs per gram (epg). It is well-recognized that egg excretion varies, particularly in those with light infections, and that this gold standard may be less sensitive than other diagnostic strategies including antigen testing and polymerase chain reaction (PCR) (31–33).

A highly promising diagnostic strategy is the detection of schistosome circulating antigens, which is used for much of the work done in this thesis. Circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) are produced in the gut of schistosome worms and regurgitated by the worm into the host bloodstream during active infection with either *S. mansoni* or *S. haematobium* (34). CAA becomes detectable in serum approximately 3 weeks after infection (35). It is heat-resistant and extremely stable, remaining detectable in tissue isolated from Egyptian mummies (36) and in dried blood spots (37). CAA levels fall rapidly post-treatment, with a serum half-life of 2 days, becoming undetectable within 3-6 weeks (38,39) and become positive again with reinfection (40). The CAA assay is recommended by the WHO for serologic screening programs in

which repeated parasitologic examinations of urine and stool are logistically not possible (36,41,42). CAA testing has been optimized using up-converter phosphor technology with dry reagents that are robust and straightforward for use (43,44). CCA testing has been developed into a commercially available point-of-care rapid test with high sensitivity and specificity for *S. mansoni*, though its performance for *S. haematobium* diagnosis is less reliable (44–47).

Schistosome infections occur often and early in endemic areas, beginning with babies and preschoolers and typically increasing in both prevalence and intensity throughout childhood, peaking during adolescence, then diminishing slowly as adults acquire partial but incomplete immunity (11,48–50). Our prior work has demonstrated that significant proportions of both young and middle-aged adults remain chronically infected in endemic areas (**Figure 1**). The WHO schistosomiasis treatment guidelines focus primarily on children, recommending that all children in high-risk communities should treated annually with the antihelminthic drug praziquantel, at an estimated cost of USD $0.32 per dose (51,52). Treatment is additionally extended to adults at special risk (such as those occupationally exposed to water or women using water for domestic chores) and to entire communities in endemic areas (51). In the real world, such treatment is a challenge, and the usual practice in Tanzania is to provide school-based praziquantel treatment for children annually, with no routine treatment after that. The WHO estimates that, in 2013, only 13% of those who needed treatment for schistosomiasis actually received praziquantel (8).

Figure 1. Proportion of schistosome infections by age among 477 rural Tanzanian adults living in villages highly endemic for schistosomiasis.



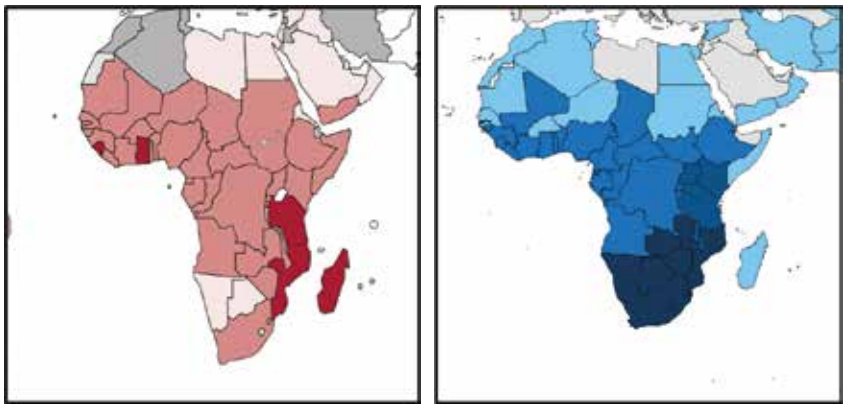
Thus it is clear that major implementation gaps remain and millions of children and adults continue to live with the chronic burden of untreated schistosomiasis. Moreover, in the absence of improved access to clean water, many individuals living in schistosome-endemic areas have no choice but to continue to use unclean water in order to sustain their livelihood. The result is a lifetime of chronic

schistosome infection and suboptimal health endured by millions of individuals living in sub-Saharan Africa.

The Confluence of Schistosomiasis and HIV Infection in sub-Saharan Africa

Approximately 78 million individuals have been infected by HIV since the epidemic began, and 35 million people are currently living with HIV worldwide (53). The HIV epidemic arose in sub-Saharan Africa and over 70% of global HIV infections (over 24 million) remain in this region (2,54). Epidemiologic mapping of HIV and schistosomiasis reveals a substantial overlap between areas in which both schistosomiasis and HIV are epidemic (**Figure 2**). In Tanzania, the prevalence of schistosomiasis in many areas is >50%, and the prevalence of HIV is 5.1% (55).

Figure 2. Schistosomiasis and HIV epidemics in Africa.



Schistosomiasis
World Health Organization, 2012

HIV Prevalence in Adults Aged 15-49
UNAIDS and Kaiser Family Foundation, 2014

Country-level epidemiologic data also suggests a relationship between schistosomiasis and HIV infection. A recent regression analysis used publicly-available data to determine the relationships between HIV infection, schistosome infection, and other epidemiologic and demographic data. Investigators reported that, after adjusting for other factors associated with HIV prevalence including male circumcision prevalence, years since the HIV epidemic began in a country, and immunization coverage, each one additional case of *S. haematobium* per 100 people was associated with a 2.9% (95% confidence interval (CI), 0.2% - 5.8%)

relative increase in the prevalence of HIV infection (56). While this data is cross-sectional and therefore not able to determine causality or to exclude potential confounders, it does lend plausibility to the existence of a relationship between the two infections.

In further support of the HIV-schistosomiasis association, the first cross-sectional study performed in individual patients was published in 2006 by Kjetland and colleagues. The study, which enrolled 527 women living in a region in Zimbabwe in which *S. haematobium* was highly endemic (with 43% of the overall study population having *S. haematobium* ova detected in urine or genital specimen), demonstrated that women who had *S. haematobium* ova found in a cervical Pap smear had a higher prevalence of HIV infection than those without ova in a Pap smear, with an adjusted odds ratio (OR) of 2.9 [95% CI, 1.1-7.5] (p=0.030) (57). Other measured factors significantly associated with HIV on multivariable analysis included Herpes Simplex Virus-Type 2 (HSV-2) infection, widowhood, infertility, and age. Investigators subsequently followed 224 women for 12 months and documented 7 new HIV seroconversions; all 7 of these occurred in women who had had signs of urogenital schistosomiasis at baseline, compared with 142/217 who had had signs of urogenital schistosomiasis at baseline but remained negative (p=0.098).

Though others had previously speculated that genital *S. haematobium* infection may predispose to HIV due to its ability to cause genital ulcer disease (25,58), the study from Zimbabwe was the first and, at the time of its publication, the only clinical study to demonstrate a clear association between the two infections. While the study design did not permit determination of causality, investigators postulated that *S. haematobium* genital infection, typically acquired during childhood prior to HIV exposure, could increase a woman's susceptibility to HIV infection. **Chapters 2 and 3** of this thesis document additional clinical studies from Tanzania that were conducted to quantitate the relationship between schistosomiasis and HIV infection in women.

Urogenital Schistosomiasis: a Risk Factor for HIV Acquisition?

Formerly termed "urinary schistosomiasis," *S. haematobium* infection was renamed "urogenital schistosomiasis" in 2009 by the WHO in recognition that up to 75% of individuals with *S. haematobium* infection have both bladder and genital tract involvement (59). *S. haematobium* worms reside in venules surrounding the urinary bladder and pelvic organs, and schistosome ova become sequestered in the not only the bladder and ureters, but also throughout both the female and male reproductive tracts (**Figure 3**). Post-mortem and surgical studies have confirmed

that, in women, the cervix is the most common site of ova sequestration, followed by vagina and Fallopian tubes (60). Eggs lodged in genital sites provoke signs and symptoms including pelvic discomfort, postcoital bleeding, itching, and vaginal discharge in women and painful ejaculation, hematospermia, and leukocytospermia in men (25,26,61–63). Findings on clinical examination in women include edema of the cervical or vaginal mucosae, erosions, ulcerations, neovascularization, and mucosal friability (26,28). These breaches in the epithelial integrity of the female genital tract, similar to those caused by trauma or STIs, have been suggested to enhance the ease of viral entry beneath the surface of the genital epithelium, thereby facilitating HIV acquisition in women with schistosomal cervical damage (25,64).

Figure 3. *Schistosoma haematobium* ova obtained in a crushed cervical biopsy (performed to exclude cervical cancer) from a 24-year-old woman in rural Tanzania.

Schistosome ova sequestered in genital tissue cause a spectrum of clinical pathology, which are best visualized using a colposcope and are used by some to make a clinical diagnosis of urogenital schistosomiasis since detection of ova or parasite DNA in genital secretions and tissues can be challenging (65,66). Classic schistosome-induced lesions of the genital mucosa have recently been photographically detailed in a "Colposcopic Atlas of Schistosomiasis" that is intended to serve as a diagnostic guidebook for clinicians and researchers (67). "Grainy sandy patches," which are rough grains located superficially within the genital mucosa that represent calcified ova beneath an atrophic epithelium, are pathognomonic for urogenital schistosomiasis (26). Another lesion, the "homogeneous yellow sandy patch," can be identified both in women with *S. haematobium* infection and in those with STIs, making this finding less specific. Sandy patches are often surrounded by abnormal blood vessels with diffuse inflammation and contact bleeding (67). The contact bleeding may provide an additional mechanism—direct access of the virus to fresh blood—by which HIV risk could be increased in the presence of *S. haematobium* infection. Importantly, sandy patches and vascular abnormalities appeared not to be completely reversible following praziquantel treatment in women (68,69). This

suggests that schistosome ova in tissue could cause longstanding and possibly permanent effects, particularly in women who are not treated until they are older than age 20 (70).

Finally, at the cellular level, schistosome ova in tissue induce a complex immune response that is characterized by infiltration of plasma cells, lymphocytes, macrophages, and eosinophils to the genital tract (71). Jourdan and colleagues quantified HIV-susceptible cells in cervicovaginal biopsies from Malawian women with and without *S. haematobium* ova and found that biopsies containing ova had higher densities of $CD4^+$ T-lymphocytes and macrophages than biopsies without ova (27). These cellular changes at the tissue level also appear to be reflected systemically in the peripheral blood of infected individuals. In Kenya, the monocytes and $CD4^+$ T-cells of car washers with *S. mansoni* infection exhibited higher densities of the chemokine receptors CCR5 and CXCR4 than the monocytes and $CD4^+$ T-cells of those who had been treated for schistosomiasis (72). Because CCR5 and CXCR4 are also HIV co-receptors, investigators postulated that the monocytes and $CD4^+$ T-cells from individuals with schistosome infection may be more susceptible to HIV infection than these same cells in uninfected or previously-treated individuals. Further, the tendency of schistosome infection to incite a Th2-type response, with an accompanying shift away from the cytotoxic Th1-type immunity that is critical to the early antiviral defense against HIV, may also promote an immune environment that is more permissive to HIV acquisition (64,73).

Taken together, the apparent interactions between HIV and schistosomiasis across a breadth of lines of investigation, and the biologic plausibility of such an association, lends credence to the hypothesis that schistosomiasis may increase susceptibility to HIV acquisition. Furthermore, the effects of schistosomiasis may not be reversible with treatment, leading to chronic enhanced HIV susceptibility. The two studies that explored response of urogenital schistosomiasis to praziquantel treatment documented clinical and parasitological findings, but did not use molecular techniques and therefore relied on tests with poorer negative predictive value to indicate whether the parasite had been eradicated or whether infection persisted. Confirming and extending these prior studies using molecular diagnostics was the goal of the longitudinal work described in **Chapter 4**.

The other clear need that emerges from this review of the relationship between HIV and schistosomiasis is the need for prospective studies that are able to document conclusively that pre-existing schistosome infection is a risk factor for incident HIV infection. Such longitudinal studies are complex and fraught with ethical issues, including the gynecological examination of pre-adolescent and adolescent

girls and/or the need to follow a group of individuals with known, untreated schistosome infection to determine whether the incidence of HIV is higher than in those without schistosome infection. One possible idea to circumvent these ethical challenges would be to use serum schistosome antigen tests in order to analyze banked serum from prior HIV-seroincidence cohorts. We have access to stored serum samples from an HIV-seroincidence cohort of 30,000 individuals in rural Tanzania who have been followed since 1994. The serum was stored in the form of dried blood spots (DBS) on Whatman 903 ProteinSaver cards. Two prior papers sought to elute CAA from two other types of filter paper and demonstrated that CAA was indeed detectable, but that available concentrations were low (37,74). Therefore, the goal of the work described in **Chapter 5** was to optimize detection of CAA in Whatman 903 cards, which are the most widely-used cards for dried blood spot collection worldwide. This would facilitate diagnosis of schistosomiasis in a variety of studies in which DBS were collected, and would pave the way for analysis of the incidence of HIV among individuals who were and were not CAA-positive prior to their HIV-seroconversion in our Tanzanian cohort.

Schistosomiasis and HIV Disease Progression in Co-Infected Individuals

Studies of HIV-infected individuals with schistosome co-infection demonstrate that schistosomiasis may exert some effect on HIV disease progression and outcomes. In particular, meta-analyses published in 2008 and 2010 suggested, though not conclusively, that the treatment of helminthic infections (75) and of helminthic plus other co-infections (6) may decrease HIV viral load. These analyses were limited by the dearth of randomized controlled trials and other methodological problems in the existing studies on this issue.

Kallestrup and colleagues conducted the single randomized trial that explored the effect of schistosomiasis treatment on viral loads in Zimbabwe, and they reported significant viral load differences in treated and untreated groups. In this study, antiretroviral therapy (ART)-naïve HIV-infected patients with schistosome co-infection were randomized to receive single-dose praziquantel either at enrollment or after three months. Those randomized to delayed praziquantel treatment had greater increases in HIV viral loads and greater decreases in CD4 counts than those treated immediately (76). Treatment effectiveness was not reported in this population. More recently, a nonblinded, randomized trial of empiric albendazole and praziquantel in over 900 Kenyan HIV-infected patients found no effect of antihelminthic treatment on CD4 counts, viral loads, time to initiation of antiretroviral therapy (ART), or mortality (77). Of note, the prevalence of schistosomiasis in this study population was only ~5%, and the study was not

designed or powered to detect the effect of treating individual helminth species. In the wake of these conflicting findings, unanswered questions remain about the impact, or lack of impact, of schistosome co-infection on HIV disease progression. Moreover, both the Zimbabwean and the Kenyan trials enrolled only HIV-infected patients who had not yet initiated ART. To the best of our knowledge, no studies had been done to assess the impact of schistosomiasis on the response to ART, which is becoming increasingly relevant as ART coverage in sub-Saharan Africa continues to rise, recently reaching above 35% (3). Therefore, the work in **Chapter 6** was conducted to investigate, for the first time, the effect of schistosome infection among HIV-infected individuals who were receiving ART in Tanzania.

The Need for Implementation Science Research to Promote Early HIV Diagnosis in Tanzania

The 1.4 million new HIV diagnoses occurring annually in sub-Saharan Africa, together with the fact that less than 50% of people living with HIV in the region are aware of their infection status (3), demonstrate the ongoing need for major population-based interventions to prevent HIV altogether and to diagnose it expeditiously. Even when cost and logistics are not limiting factors, the journey from awareness of evidence-based best practices to implementation of these practices can be challenging. Religion, cultural values, education levels, health beliefs, and social norms all impact a person's, or a population's, willingness to adopt practices, even those that have been scientifically proven to be beneficial for health. Challenges are often exacerbated in resource-poor settings.

The growing field of implementation science strives to address these issues. Implementation science seeks to determine effective strategies by which research findings can be incorporated into healthcare policy and practice in specific settings in order to improve population health (78). The final chapter of this dissertation, inspired by the experience of spending years living and working in a country in which healthcare providers struggle to implement evidence-based guidelines, is such an implementation science study. This chapter focuses on issues related to implementing practices that, although known to be effective in early infant diagnosis of HIV, have faced implementation obstacles in Tanzania.

Chapter 7 is an implementation science project, conducted in rural Tanzania, that focused on streamlining early infant diagnosis (EID) of HIV infection in infants who were exposed perinatally to HIV. Early diagnosis is urgent because infants who become HIV-infected before, during, or shortly after birth have a mortality rate of approximately 50% before 12 months of age, with 20% dying before they

reach 3 months of age (79). In resource-poor areas without local laboratory facilities able to perform the PCR necessary to detect HIV RNA or DNA in an infant's blood, the WHO recommends collecting blood as DBS and shipping them to a reference laboratory with PCR capacity (80). Bugando Medical Centre in Mwanza, Tanzania houses the reference laboratory for 13 million people, and receives DBS for EID from 96 clinics in the region. Returning an HIV test result to an infant's mother typically takes weeks because the testing system depends on a multi-step process in which DBS are collected, transported to the laboratory, tested at the laboratory, returned to the clinic, and then communicated to the mother. This implementation science work was prompted by the observation that many mothers were never receiving their infants' test results, and those who did were receiving them extremely late. The implementation science research described in this chapter used a systems improvement methodology to make sequential interventions in this multi-step process and to monitor the effects of these interventions.

Finally, in the general discussion in **Chapter 8,** the key findings from the preceding chapters will be analyzed, and the contribution that these findings have made to the knowledge base will be summarized. We will close each section of the discussion by outlining logical next research steps to explore research questions that remain.

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