2020

Tetanus Immunity Among Adults in the Democratic Republic of the Congo

Sue K. Gerber
Walden University

Walden University

College of Health Sciences

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Sue Gerber

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Review Committee
Dr. Patrick Bassey Williams, Committee Chairperson, Public Health Faculty
Dr. Hebatullah Tawfik, Committee Member, Public Health Faculty
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Chief Academic Officer and Provost
Sue Subocz, Ph.D.

Walden University
2020

Abstract

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by

Sue Gerber

MPH, Walden University, 2012

BCH, New Mexico State University, 1984

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

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April 2020

Abstract

Although tetanus is not a contagious disease, it can be deadly for people who are exposed and do not have protective immunity against tetanus, which can be acquired only through vaccination. Most studies on adult tetanus protective immunity are from high-income countries; limited studies occur in Africa, and none has been reported from the Democratic Republic of the Congo (DRC). This study sought to determine protective immunity against tetanus among adults in DRC in relation to their age, sex, place of residence, and for women, antenatal care. The primary purpose of this observational cross-sectional study using a nested serosurvey within the Demographic Health Survey was to assess protective immunity against tetanus among adults in DRC from November 2013 to February 2014. The secondary purpose was to identify characteristics of populations at greatest risk of tetanus infection. The health belief model was the theoretical foundation for this study, and multivariate logistic regression for complex samples was used to analyze data from 8,602 participants. Women in the DRC were 10 times as likely to be protected against tetanus, but both sexes ages 15–19 had lower immunity as teens, with 75% not protected against tetanus. As women were targeted for antenatal care during pregnancy, immunity against tetanus increased in women over age 20. This evidence will allow the Ministry of Health to make informed policy decisions regarding adolescent and adult immunization. There is potential for positive social change by influencing immunization policy, providing equity in preventive health services in DRC and preventing death from a disease that has an efficacious vaccine.

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Dedication

To my wonderful husband Hector who makes all things possible.

Acknowledgments

I would like to thank all the faculty, family members, and friends who have helped me reach this point in my academic career and attain my goal. Specifically, I would like to thank my committee chair, Dr. Patrick Williams, for his guidance and encouragement together with methodologist Dr. Heba Tawfik and university research reviewer Dr. Lee Caplan, whose feedback, advice, and tough questions were critical in the development of the study, presentation of the findings, and my personal growth. I would also like to thank Drs. Anne Rimion, Nicole Hoff, and Reena Doshi from UCLA for the opportunity to work on this study and to learn of serosurveys and the Democratic Republic of the Congo. For subject matter expertise and guidance, I thank Drs. Faten Kamel, Francois Gasse, Ashraf Wahdan, Arie Voorman, Vivian Larson, Victoria Gammino, and Steve Wassilak. Marge Gerber, PhtD, you and dad, Dr Robert Gerber, have been the guardians and the guides. Thank you, Dr. Lisa Gerber for your consultation and encouragement from the very first outline to the last word.

And, finally, to Dune, who led the way to the casita

with

energy and enthusiasm every day!

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Chapter 1: Introduction to the Study

Introduction

Tetanus is unique among vaccine preventable diseases because though it is not contagious, both humans and animals may be infected. This is because the bacterium that causes tetanus, *Clostridium tetani,* is found in the environment. The bacteria generally enter the body through a puncture wound and produce a potent neurotoxin that targets the central nervous system. The symptoms of tetanus often develop within 7 days of infection. The clinical symptoms of tetanus are distinct. A spasm of the person's jaw muscles cause "lockjaw" and any stimulus, such as light or movement, will produce muscle spasms. These muscle spasms cause the back to arch and the arms to clench, often resulting in a full body spastic arch. Without swift medical attention, the symptoms of tetanus can result in death.

Several decades ago, tetanus infection leading to death was common. With the increased use of an effective vaccine in the 1940s, there was a gradual reduction in the number of cases and deaths worldwide. The vaccine first reduced tetanus on the battlefields of major wars, and later the vaccine reduced cases of tetanus in neonates and children. However, as recently as 1980, there were an estimated 1 million deaths due to tetanus.

Countries worldwide follow World Health Organization (WHO) recommendations and provide immunization for children and pregnant women against tetanus. Many countries in Europe and the Americas provide booster immunization for adolescents and adults. Some countries have surveillance systems that assess tetanus

immunity through serosurveys to ensure the population is protected. However, some countries do not routinely provide tetanus immunization for adolescents or men, and this leads to susceptibility of tetanus infection in this population. In developed countries, there are gaps in immunity to tetanus in elderly people and in women. In developing countries, there are limited retrospective studies and few serosurveys, but immunity gaps do exist and morbidity is higher in men (Aliyu et al., 2017; Chalya et al., 2011; Muteya, Kabey a Kabey, Lubanga, Tshamba, & Tambwe a Nkoy, 2013; Scobie et al., 2017). In the Democratic Republic of the Congo (DRC), vaccination is provided to children under 1 year of age and to pregnant women. The level of protective immunity against tetanus in DRC was unknown prior to this research. This research filled a critical gap in knowledge about the actual level of protective immunity against tetanus among the adult population in DRC. This study addressed a significant public health problem given the high morbidity and mortality associated with tetanus.

Background

In 1967, DRC, at that time Zaire, began its first large scale immunization activity to eradicate smallpox by vaccinating every person in the country (Muyembe-Tamfum et al., 2011). DRC's smallpox eradication program was effective. Smallpox vaccination together with smallpox surveillance and containment led to interruption of transmission. The last case of smallpox in DRC was in 1971, and globally, the last case was in Somalia in 1977. Global public health leaders used the momentum from the successful eradication of smallpox to establish an expanded program for immunization in the mid-1970s with

the realization that universal access to immunization was possible (Okwo-Bele & Cherian, 2011; WHO, 2014).

Against this backdrop, DRC established its Expanded Programme on Immunization (EPI) that included the following vaccines: Bacille Calemette Guerin (BCG) against tuberculosis; the combined vaccine of diphtheria, tetanus, and pertussis (DPT); oral polio (OPV); and measles and tetanus toxoid for pregnant women in 1977 (Mambu, 1989). Most vaccinations were administered through a mobile strategy in urban areas, but this shifted to fixed vaccination at health facilities in 1981 with outreach where facilities were not available. In 1985, to serve DRC's population of 30 million people, the 11 provinces of DRC were divided into 306 health zones; only 250 zones had the infrastructure to provide the EPI with substantial support from nongovernmental organizations in remote areas (Mambu, 1989).

EPI was, and remains, an essential piece of primary health care in DRC. The health infrastructure now serves an estimated population of 78 million people in 26 provinces, divided into 516 health zones. EPI is presently being provided in many of the 8,264 primary government health centers and through private providers in urban settings (Ministry of Planning and Implementation of the Modern Revolution, Ministry of Public Health, & ICF International, 2014). There are now 10 vaccinations provided to children under 1 year of age. DTP has been replaced with a pentavalent vaccine, a combination of five vaccines—diphtheria, tetanus, pertussis, hepatitis B, and haemophilus influenza b (Hib)—in addition to pneumococcal vaccine (PCV), oral polio, inactivated polio (IPV), measles, and yellow fever with tetanus toxoid for pregnant women (Mwamba et al.,

2017). Even with this expansion, documented improvements in national immunization coverage remain slow. For example, the 2013–2014 Demographic Health Survey (DHS) reported that 45% of DRC children 12–23 months of age completed a full vaccination series, and 66% of women had been adequately immunized with tetanus toxoid according to WHO recommendations prior to delivery, thus protecting their newborns against tetanus (Ministry of Planning and Implementation of the Modern Revolution et al., 2014).

In 1986, there were an estimated 1 million global deaths due to tetanus (Roper, Wassilak, Scobie, Ridpath, & Orenstein, 2017), and in that year, DRC reported only 198 cases (WHO/United Nations Children's Fund [UNICEF], 2018). Roper et al. (2017) stated that most cases of tetanus occur in rural areas and are often unreported; when advanced medical care is not available, the fatality rate can reach 100% (Roper, Vandelaer, & Gasse, 2007). At the time of the 1984 census, 70% of the population of DRC resided in rural communities, but there has been a shift as more people moved to urban areas due to economic opportunity and to conflict in some parts of the country (Ministry of Planning and Implementation of the Modern Revolution et al., 2014).

DRC continues to rely heavily on the donor community for financing EPI; most funds come from the Global Alliance for Vaccines Initiative. Little funding comes from the DRC national budget for routine vaccination, which has a complex process with less than transparent disbursement and expenditure practices (Le Gargasson, Breugelmans, Mibulumukini, Da Silva, & Colombini, 2013; Le Gargasson, Mibulumukini, Gessner, & Colombini, 2014). This practice remains despite increased donor engagement and new government leadership that has expressed a willingness to improve routine immunization

coverage in DRC. With a new president, there is a new minister of health, who may lead change in 2020. Domestic funding for EPI has increased over the past decade from $300,000 in 2002 to $2.5 million in 2010, and EPI has a permanent budget line in the national budget (Le Gargasson et al., 2013).

Problem Statement

In 1980, an estimated over 1 million cases of tetanus worldwide resulted in death (Roper et al., 2017). WHO maintains that only immunization can provide protection against tetanus and recommends that everyone should be provided lifelong protection against tetanus using the following vaccination regimen: three doses in the first year of life, with one booster at 12–23 months of age, one booster between 4 and 7 years, and one booster between 9 and 15 years (WHO, 2017). WHO (WHO, 2006, 2017) also recommends that women should receive up to five doses of tetanus vaccine beginning with their first pregnancy. Even with this long-standing recommendation, few developing countries, including DRC, have immunization programs that provide tetanus vaccine for adolescents and adults (Mwamba et al., 2017; WHO, 2018).

Although tetanus is not infectious, it is deadly for those who do not have protective antibodies that confer immunity against the disease. Retrospective studies in Nigeria, Tanzania, and DRC found that 40% to 54% of adults admitted to the hospital with tetanus die (Chalya et al., 2011; Chukwubike & God'spower, 2009; Muteya et al., 2013).

Studies have been conducted predominately in high-income countries that measure the levels of antibody protection against tetanus. The findings of these studies

have been used to document levels of protective immunity and, together with programmatic data, support changes in immunization policy so that all citizens have the opportunity to maintain high levels of protective immunity against tetanus (Chen & Orenstein, 1996; Ölander, Auranen, Härkänen, & Leino, 2009; WHO, 2017).

There are, however, limited studies on adult protective immunity against tetanus in Africa, and none has been reported in DRC. In East Africa, researchers found that in Tanzania and Kenya between 36% and 53% of individuals tested, respectively, did not have antibodies that would protect them against tetanus (Aboud, Lyamuya, Kristoffersen, & Matre, 2002; Scobie et al., 2017). In this study, I used data collected in the 2013–2014 DHS in DRC together with nested serosurvey results to assess the level of protective immunity against tetanus among the adult population in DRC. The results of this study provide the first nationally representative evidence of protective immunity against tetanus by age, sex, and geographic location in DRC.

Purpose of the Study

The primary purpose of this study was to use a serosurvey to assess protective immunity against tetanus among adult populations in DRC between November 2013 and April 2014. The secondary purpose was to identify the characteristics of populations at greatest risk of tetanus infection based on lowered immunity levels. The dependent variable was protective immunity against tetanus and the independent covariables included age, sex, place of residence, wealth, education, and antenatal care during pregnancy in women.

Research Questions and Hypotheses

The following are the research questions and hypotheses for this study:

RQ1: Is there an association between age and protective immunity against tetanus among adults in DRC?

$H_0$1: There is no association between age and protective immunity against tetanus among adults in DRC.

H_a1: There is an association between age and protective immunity against tetanus among adults in DRC.

RQ2: Is there an association between sex and protective immunity against tetanus among adults in DRC?

$H_0$2: There is no association between sex and protective immunity against tetanus among adults in DRC.

H_a2: There is an association between sex and protective immunity against tetanus among adults in DRC.

RQ3: Is there an association between province and place of residence (urban vs. rural) and protective immunity against tetanus among adults in DRC?

$H_0$3: There is no association between province and place of residence (urban vs. rural) and protective immunity against tetanus among adults in DRC.

H_a3: There is association between province and place of residence (urban vs. rural) and protective immunity against tetanus among adults in DRC.

RQ4: Is there an association between antenatal care and protective immunity against tetanus among women in DRC?

$H_0$4: There is no association between antenatal care and protective immunity against tetanus among women in DRC.

H_a4: There is an association between antenatal care and protective immunity against tetanus among women in DRC.

Theoretical Framework

The health belief model (HBM) explains and predicts behavior based on the beliefs of individuals. HBM has been used in public health research and practice as the theoretical framework for many studies and public health interventions because it provides an explanation for health-seeking and preventive health behavior. Although this study was not specifically about changing health behavior, HBM was the theoretical framework for this study because the model has been used repeatedly to counsel patients, promote understanding, and change knowledge and attitudes of adults about different diseases, including tetanus (Glanz, Rimer, & Viswanath, 2008). HBM provided six constructs that support the decision of individuals to take preventive action against a defined health problem.

In this study, the presence of antibodies against tetanus provided evidence of preventive action taken. The serology results provided quantitative evidence because antibodies against tetanus can only be acquired through vaccination. Preventive action occurs when a person acts from the following perceptions: (a) susceptibility to acquiring the disease, (b) severity of illness if acquired, (c) perceived personal benefits, (d) perceived barriers or negative aspects of action, (e) cues that may trigger action, and (f) a conviction that the individual can change behavior to achieve the desired outcome. HBM

continues to be widely used to support public health intervention programs, such as immunization and cancer prevention where the individual may be asymptomatic at the time of screening, and this model frames the key issues to address related to health-seeking behavior (Glanz & Bishop, 2010). This theory suggests that if the risk of the disease is known, then an individual will make the choice to prevent the disease if health-related interventions, such as immunization or screening, are available to them. Findings from this research study suggest this is evident for women. HBM constructs are effective in understanding heath behavior, and this study showed evidence of health-seeking behavior resulting in protective immunity against tetanus. The study results provided later show immunologic evidence of protection against tetanus that can only be achieved through immunization.

Nature of the Study

The nature of this study was observational, cross-sectional, and quantitative. In this study, I used secondary data from the DHS conducted in DRC between November 2013 and February 2014 and data from a serosurvey nested in the DHS. Surveyors collected information from 19,681 households nationwide. From these households, interviewers gathered detailed health information from over 18,827 women ages 15 to 49 and 8,656 men ages 15 to 59. In addition, blood samples were collected to test for tetanus antibody levels as well as polio, measles, mumps, and other vaccine-preventable diseases. I analyzed secondary data from the DHS questionnaires matched with the positive or negative results of the tetanus antibody assays to determine the characteristics associated with immunity against tetanus among adults in DRC.

Definition of Terms

The following definitions are used in public health to describe terms related to tetanus and tetanus vaccination:

Antenatal care: Self-reported recent service for the current pregnancy or most recent pregnancy from the list of possible antenatal care services, which include, but are not limited to, visits with skilled professionals, iron supplements, treatment for intestinal parasites, blood pressure screening, and vaccination with tetanus toxoid (DHS, 2013).

Ethnicity: The self-selected tribal affiliation in DRC from those listed in the survey (Ministry of Planning and Implementation of the Modern Revolution, 2014).

Immunity produced by tetanus vaccination: Vaccine-induced antibodies produced by b lymphocytes that can bind to a toxin that then inhibits the disease from presenting or developing (Roper et al., 2017).

Immunization: A process by which a person becomes protected against a disease through vaccination (Centers for Disease Control and Prevention, 2018).

Neonatal tetanus: An illness occurring in a child who has the normal ability to suck and cry in the first 2 days of life, loses this ability between 3 and 28 days of life, and becomes rigid or has spasms (WHO, 2017). It is caused by *Clostridium tetani*.

Non-neonatal tetanus: An illness occurring in someone over 1 month of age with one or more of the following signs: trismus or lockjaw (the inability to open the mouth), risus sardonicus (sustained spasm of the facial muscles), or painful muscle contractions with a history of a wound or injury; however, tetanus may also occur in persons who are unable to recall a wound or injury (WHO, 2017).

Rural cluster: The smallest geographical survey statistical unit in the countryside with adjacent households selected (DHS, 2013).

Serosurvey: The collection and testing of blood specimens from a defined population over a specified period to determine antibodies against a given etiologic agent as a direct measure of the population's immunity. Routinely conducted serosurveys are referred to as serosurveillance (WHO, 2013).

Tetanus toxoid: The vaccine administered that has a toxin produced by *Clostridium tetani* that has been rendered harmless by chemical treatment while retaining its antigenic activity (WHO, 2018).

Vaccination: The process or art of introduction of a vaccine into the body to produce immunity to a specific disease (Centers for Disease Control and Prevention, 2018).

Urban clusters: The smallest geographical survey areas selected for survey, drawn from capital cities, large cities with populations over 1 million, small cities with populations over 50,000, and towns (DHS, 2013).

Wealth: A summary of household assets documented during the DHS. Wealth is measured by type of flooring, water source, electricity, and possession of durable goods. The assets are combined to form a single index that is then divided into one of five ranked quintiles based on the World Bank household wealth index (DHS, 2013).

Assumptions

Several assumptions were made regarding the use of secondary data and nested serosurvey results. I assumed that the survey was conducted with the rigor afforded all

DHS surveys, which is well documented in the DHS manual and in the 2013–2014 DHS report. I assumed the participants answered honestly. I assumed the laboratory staff conducted the serosurvey used the assay and methodology as recommended by the manufacturer. I assumed the presence of tetanus antibody beyond the cut-off value for immunity was evidence of vaccination and protection against tetanus. Based on the DHS sampling and weights, I assumed that those included in the DHS and the serosurvey were a nationally representative sample of adults in DRC, with the few mentioned limitations. Based on serosurvey sample split, I assumed the samples selected for CDC's polio study and the samples selected for this tetanus study were representative of the population. Therefore, the findings of this study can be generalized to the DHS study population and the population of adults of DRC.

Scope and Delimitations

The scope of this study was men 15 to 59 years of age and women 15 to 49 years of age who resided in DRC during the 2013–2014 DHS. These individuals were available in a household at the time of the interview and were willing to participate in the survey. The focus was on adult men and women because adults, except for pregnant women, are a neglected population regarding immunization against tetanus.

The study was delimited in two ways: (a) limited to individuals 15 years and above and (b) restricted to the demographic variables listed in Table 3. The 2013–2014 DHS for DRC contained over 17 pages of variables; I included those variables deemed most relevant based on the existing literature.

Limitations

A limitation in using secondary data is that the investigator of the secondary study must work with the variables operationalized for the primary study or variables derived through recoding or reclassification. There are also limitations in that the DHS questions related to antenatal care and to receiving tetanus vaccination were based on recall (Cutts, Claquin, Danovaro-Holliday, & Rhoda, 2016). Men and nonpregnant women were not asked questions on receiving tetanus vaccination. In addition, the DHS was limited to only those living in a house, so adults living on military bases or in hotels or adults who were homeless were not included in this survey (DHS, 2013).

Significance of the Study

This research was designed to fill a critical gap in knowledge about protective immunity against tetanus among the adult population in DRC, thus addressing a problem of public health significance given the high morbidity and mortality associated with tetanus disease. This study was the first nationally and provincially representative serosurvey for tetanus in the country and in the African subregion of WHO. This survey measured protective immunity against tetanus in adults across age, sex, and geographic location of residence. In addition, because this serosurvey was nested into the 2013–2014 DHS, information on socioeconomic status, occupation, and additional health indicators examined may affect adult susceptibility to tetanus. The outcome of this study provides public health practitioners much needed evidence about the impact of the immunization program as well as immunity gaps.

Positive Social Change

Currently, DRC provides tetanus vaccination for children under 1 year of age through the EPI program and for pregnant women during antenatal visits (Mwamba et al., 2017). Although WHO (2017) recommendations for tetanus vaccination include one booster at 12–23 months of age, one booster between 4 and 7 years, one booster between 9 and 15 years, and catch-up boosters for adults who have not been vaccinated, DRC's schedule does not include vaccination for children over 1 year of age, adolescents, men, or nonpregnant women. This study promotes positive social change because quantitative data show gaps in protective immunity against tetanus and provide information on inequity in preventive immunization against tetanus by age and sex. This study provides scientific evidence to inform and shape immunization policy, support requests to government and donors for public health financing for immunization, and focused health education efforts on the susceptible populations. Finally, this study will support the immunization program to assess progress toward the goal of maternal and neonatal tetanus elimination (MNTE) because aspects of this study methodology can be incorporated in MNTE assessments in DRC and other countries.

Summary

Tetanus infections have serious consequences and are often fatal for those not protected by immunization. In DRC, the relatively young immunization program reached a small proportion of the urban population in early 1977 and expanded further in 1981. Data collected in the 2013–2014 DRC DHS in major urban areas, such as Kinshasa, include adults 36 years or younger who may have received tetanus vaccination during

routine immunization as an infant. Similarly, people living in the countryside and in rural communities who were 32 or younger may have received tetanus vaccination during routine immunization as an infant. People older than 36 years in urban areas and older than 32 in rural areas are likely never to have had the opportunity for tetanus vaccination as an infant. Additionally, there is no vaccination policy for older women or men in DRC.

HBM was the theoretical framework for this study and has been used widely to support public health interventions. HBM is effective for understanding health-seeking behavior for preventive services, and in this study, there was evidence of health-seeking behavior, such as prenatal care during pregnancy. Over 85% of women who were pregnant within the past 5 years sought some type of prenatal care, and women seeking prenatal care were twice as likely to have protective immunity against tetanus than pregnant women who did not seek prenatal care.

This observational, cross-sectional study assessed the tetanus immunity of adults using a nested serosurvey in DRC DHS and secondary data collected in DHS. This study provides scientific evidence of immunity against tetanus in adults in DRC that will allow the Ministry of Health and development partners to make informed policy decisions regarding adolescent and adult immunization. The positive social change outcome of this study is an influence on immunization policy providing equity in preventive health services in DRC, preventing death from a disease that has an efficacious vaccine.

Chapter 2 is a review of the literature and provides an overview of the theoretical framework, clinical epidemiology, tetanus prevention, and measures and assessments of immunity. In addition, evidence of the morbidity and mortality of tetanus, through

surveillance and retrospective studies, is discussed. Evidence linked to serosurveys, study methodology, and serosurvey data from a range of countries and the contrast of immunity between men and women by age is reviewed. In Chapter 3, the methodology for the analysis of secondary data from the observational cross-sectional serosurvey is discussed in detail with a review of the research questions, the sampling procedures, and the sample size. In Chapter 4, I review each research question, the hypotheses, and the detailed statistical analyses. Chapter 5 is a discussion of this study's findings and ideas for social change and further research, and it closes with a conclusion.

Chapter 2: Literature Review

Introduction

In 1980, over 1 million cases of tetanus worldwide resulted in death (Roper et al., 2017). An effective vaccine can provide protection against this deadly noncommunicable disease. WHO maintains that only immunization with tetanus toxoid containing vaccine (TTCV) can provide immunological protection against tetanus. In 2006, WHO recommended that everyone should be provided lifelong protection against tetanus using the following vaccination regimens: three doses in the first year of life, one dose at four, one dose at 12, and a booster dose in adulthood (WHO, 2006). In 2017, WHO revised the global recommendations on the timing of vaccination doses to allow countries to provide lifelong protection against tetanus with six doses of TTCV through a routine immunization schedule. These revised recommendations will help reach the global goal of MNTE (WHO, 2017). The immunization schedule still includes three doses of TTCV before the age of 1, the first booster between 1 and 2 years, the second booster between 4 and 7 years, and the third booster between 9 and 15 years of age. This is a total of six doses for children. In addition, WHO recommends that countries provide opportunities to complete the full series for those people who are not fully vaccinated during childhood. WHO also recommends that women, regardless of their childhood immunization status, receive up to five doses of TTCV beginning with their first pregnancy to protect newborns against tetanus because tetanus can be acquired during or following birth. However, few developing countries, including DRC, presently have immunization

programs that provide tetanus vaccine for adolescents, nonpregnant women, and adult men (Mwamba et al., 2017; Nanni et al., 2017; WHO, 2018).

Although tetanus is not infectious, it is deadly for those who do not have protective immunity against the disease. WHO uses evidence from multiple studies that measure antibody levels in populations to document protective immunity against tetanus and to provide evidence for immunization policy changes (WHO, 2006, 2017). However, many of these studies are found predominately in high-income countries. There are limited studies on adult protective immunity against tetanus in Africa and no reported studies in DRC (Bourée, 2003; WHO, 2018).

In this study, I used 2013–2014 DRC DHS data together with nested serosurvey results to measure protective immunity against tetanus in a nationally representative sample of adults in DRC between November 2013 and February 2014. For secondary purpose, I identified populations at greatest risk of tetanus infection (based on negative antibody test results) and characterized these results by age, sex, and geographic location. In this literature review, I examined evidence from peer-reviewed literature, book chapters, technical reports, and meetings proceedings that provided the theoretical foundation and the methodological framework for the research.

Review Related to Content

In this chapter I review the research literature pertaining to the content of this dissertation. This includes references and studies focused on HBM, the theoretical framework for this study; the clinical features of tetanus, including the outcomes of tetanus infection; and the level of protection required that forms the framework for the

study. I also review research studies that informed the study design and methods, including the operational definitions of immunity used in previous research. The review next considered surveillance methods that inform recent estimates of tetanus morbidity and mortality. These estimates provided evidence for the independent variables (age, sex, residence, and use of antenatal care) that were the focus of this research. The review also highlighted gaps in the literature regarding documented levels of protective immunity against tetanus among adults in Africa generally and within DRC specifically, where tetanus poses a significant public health problem.

Literature Search Strategy

For the literature search, I used electronic databases in the Walden University Library, including the multidata base Thoreau and CINAH & Medline, ScienceDirect, and SAGE Journals. I searched these databases to identify relevant books, scientific articles, dissertations, and other references applicable to this study. I reviewed the websites of the CDC, the WHO, and UNICEF for disease specific data, disease updates, and peer-reviewed journal articles. I also used the web search engine Google Scholar to identify relevant resource materials. The following key word terms were used for the searches: *tetanus immunity, immunization for tetanus, serosurveillance, serosurvey for tetanus, tetanus morbidity, tetanus mortality, tetanus in adults, tetanus and immunization policy, the Democratic Republic of the Congo, tetanus epidemiology, tetanus surveillance, non-neonatal tetanus,* and *the health belief model*.

In the initial search, I identified papers that included these terms in the title or the abstract, and the search was initially restricted to papers published between 2010 and

2018. I amended the search to include publications prior to 2010 because a rich seminal body of work provided the knowledge base for tetanus vaccinology. The search also revealed several technical reports, meeting proceedings, abstracts, theses, and other published data over the past 5 years. I reviewed all relevant articles for content and for additional references that may have been omitted in the electronic search. I also used Walden library and ProQuest to search for relevant dissertations published between 2013 and 2018 that included HBM, studies related to tetanus, or the methods used for this research.

Theoretical Foundation

The theoretical framework for this study was HBM, which provided a foundation for individual preventive health action. Although I did not focus the study on changing health behavior per se, the HBM theoretical framework has been widely used to counsel patients, to promote understanding, and to change knowledge and attitudes of adults about many conditions and diseases including tetanus (Champion & Skinner, 2008; Cutts, Orenstein, & Bernier, 1992). HBM was developed by Rosenstock, Kegeles, Leventhal, and Hochbaum, psychologists who worked with the U.S. Public Health Service in the 1950s (Rosenstock, 1974) and focused on understanding individual behavior related to uptake of screening and prevention activities. Rosenstock et al.'s primary work focused on tuberculosis, cervical cancer, and dental diseases. Their later work focused on poliomyelitis (*polio*) and influenza. Based on their public health research, their theory provided insights on why healthy individuals engage in health-promoting and prevention behavior. Their theory was also informed by the polio outbreaks in the 1950s, and they

sought to determine why some individuals failed to protect themselves when a safe and effective vaccine was available (Rosenstock, Derryberry, & Carriger, 1959). Their research showed that perceived susceptibility to disease and the perceived severity of the disease outcome were key factors in seeking immunization. The early model asserted that the primary drivers of decision-making were based on perceived susceptibility to disease, severity of the outcome, and the belief that the recommended intervention would be beneficial. Early HBM focused on taking preventive actions, such as vaccination.

Contemporary HBM contains six constructs that predict the decision of individuals to take preventive action against a defined health problem. The six constructs are based on perceived personal benefits, barriers, and threats. The relevant beliefs of the individual include (a) susceptibility to acquiring the disease, (b) severity of the disease, (c) personal benefits of intervention, (d) barriers, (e) cues which may trigger action, and (e) personal conviction that preventive action will lead to the desired outcome (Champion & Skinner, 2008). HBM has been widely used to design public health intervention programs such as vaccination of healthy individuals and cancer prevention where the individual may be asymptomatic at the time of screening (Glanz & Bishop, 2010). I used HBM for this study because it suggests that if the disease risk is known, then an individual will make a rational choice to prevent disease if the health-related interventions, such as vaccination or screening, are available.

Tetanus disease has characteristics that make it amenable to the HBM framework given the presence of tetanus spores in both urban and rural environments; tetanus spores are in soil, dust, and animal intestines and feces throughout the world. This means that

susceptibility to tetanus spore exposure is a universal risk to all humans. The severity of tetanus infection is well documented by the high rates of morbidity and mortality where these data are captured. TTCV has been shown to be safe and effective, and most countries provide it for no or low cost to citizens. HBM is effective in understanding health-seeking behaviors, including decisions about and acquisition of immunization. In this study, the presence of tetanus antibodies provided evidence of preventive action, primarily in women of childbearing age.

Literature Review

Clinical Epidemiology of Tetanus

Tetanus is caused by the toxin produced by the bacterium *Clostridium tetani*. According to CDC, tetanus spores live in the soil, and for humans, the risk of exposure is universal (Roper et al., 2017). In addition to soil, tetanus spores are found in the feces of domesticated animals, such as cows, horses, sheep, pigs, chickens, dogs, cats, and rats—all which act as disease reservoirs. Manure used to fertilize crops or as fuel for cook fires potentially exposes people at risk of infection, as the spores are hardy and heat resistant. These spores are not susceptible to many antiseptics, including ethanol and formalin, but they can be neutralized by hydrogen peroxide or iodine. Tetanus spores generally enter the body through a break in the skin from a wound, injury, or animal bite. In neonates, this is generally through the umbilical cord stump following unhygienic delivery practices (Roper et al., 2007; Thwaites, Beeching, & Newton, 2015). Exposure has also been known to take place while undergoing medical or dental procedures when there are poor sterilization procedures. Infections with tetanus from the reuse of needles in the case

of injecting drug users have been well documented (Hahné et al., 2006; Roper et al., 2017). Soil displacement coupled with injury following floods and earthquakes may also increase potential exposure to spores and likelihood of increasing risk of infection (Afshar, Raju, Ansell, & Bleck, 2011; Sutiono, Qiantori, Suwa, & Ohta, 2009; Thwaites et al., 2015). Given that tetanus is in the environment, anyone can be exposed to tetanus.

The incubation period between exposure and onset of symptoms ranges from 3 to 21 days depending on age and susceptibility of the individual. Individuals who have not achieved full protection from TTCV or those who have waning immunity are susceptible to infection if exposed (CDC, 2015; Roper et al., 2017).

The gram-negative anaerobic spores produce a potent neurotoxin and grow well in oxygen-deprived environments, such as necrotic tissue. The toxin enters the nervous system and disinhibits motor neuron discharge causing generalized spasms. Tetanus diagnoses are made based on clinical findings such as history, signs, and symptoms. The recommendation is to begin treatment immediately. It is possible to culture Clostridium tetani on oxygen-reduced blood agar or meat broth, however this process takes a significant amount of time (Joyce, 2008).

There are three clinical types of tetanus: generalized, cephalic, and localized tetanus. Symptoms of generalized tetanus include rigid jaw muscles and stiff shoulders and can also include adduction of arms, clenching of fists, and generalized spasms. In neonates, tetanus can occur following *Clostridium tetani* introduction through the umbilical cord stump. The first symptoms are often excess crying and difficulty sucking in infants who previously had a normal ability to suck. This is followed by trismus,

rigidity, and spasms. Neonatal tetanus is generalized tetanus. Cephalic tetanus generally follows infection during a head injury; symptoms will include lockjaw as well as eye- and face-related complications (Roper et al., 2007). Localized tetanus causes fixed spasms of muscles at the site of the injury. Most cases of tetanus are generalized (CDC, 2015).

Tetanus Prevention

Although tetanus toxoid was developed in 1924, the vaccine was not widely used in developed countries until the 1940s, when soldiers were vaccinated during World War II. The American Academy of Pediatrics recommended use of tetanus toxoid for infants in 1944. Tetanus toxoid was later combined with diphtheria toxoid and pertussis vaccine (CDC, 2015). The use of the combined DPT vaccine was approved in 1951 and was widely adopted in developed countries. After the success of the smallpox eradication program, in 1974 the World Health Assembly passed a resolution to establish the EPI intended to provide access to six critical antigens, including tetanus, for all children under 1 year (Keja, Chan, Hayden, & Henderson, 1988).

WHO currently recommends that people receive six doses of TTCV in their lifetime to provide lifelong protection against the disease. Most countries provide a series of three vaccinations before age 1, the first booster between 1 and 2 years, the second booster between 4 and 7 years, and the third booster between 9 and 15 years. Although policies have been developed and boosters recommended, most African countries do not have school-based immunization or booster programs in place, and vaccinations given to children above the age of 2 are not generally reported (Mihigo, Okeibunor, Anya, Mkanda, & Zawaira, 2017).

Tetanus toxoid will cause the formation of specific antitoxins instrumental in protecting against tetanus. Immunity to tetanus is antibody mediated with tetanus antitoxins belonging to the immunoglobin G (IgG) class. These antitoxins are found in the blood and extra vascular spaces; if tetanus toxins do enter the tissue, the antitoxins are well placed to neutralize the toxin produced in an infected wound. When a mother is actively immunized, the antitoxin can pass though the placenta to the fetus. This can prevent neonatal tetanus (Roper et al., 2017).

Measures of Immunity

Traditionally, immunization programs have measured population immunity by proxy using *administrative data* for a given vaccine. Administrative coverage is calculated as the number of doses provided to children within an assigned age range in a geographic area over a period against the estimated age-specific target population. The EPI program established procedures to document vaccine administration (*routine coverage*) through the maintenance of clinic-level registries and provision of vaccination cards to parents that record the name, date, and batch number of all vaccinations received. Each medical provider or clinic reports the number of vaccinations administered to the appropriate administrative unit, such as the district, province, or region, where the data are aggregated up to the national level. These administrative data measure routine immunization coverage and are used to estimate population immunity against vaccine preventable diseases for infants under the age of 1 and for women receiving TTCV during antenatal care. There are limitations to administrative coverage

both in the estimated numerator or doses given and the denominator which, in many countries like DRC, is a figure estimated from the last census (Sodha & Dietz, 2015).

Country programs have also used coverage surveys to estimate routine immunization. During these surveys, individual households are randomly selected within systematically sampled (multistage or clustered) enumeration areas and the vaccination records of children between ages of 12–24 months and women 15–49 years of age are reviewed. In the absence of clinic records or vaccination cards, maternal recall is also used to document the number and timing of vaccinations. Maternal recall may also be problematic as a parent may not remember a child's vaccination and feel pressured to give a positive answer which may lead to information bias resulting in increased coverage (Cutts, Izurieta, & Rhoda, 2013). A coverage survey may be independent and limited to immunization or embedded into larger population-based surveys such as the DHS or UNICEF's Multiple Indicator Cluster Survey. Large coverage surveys such as the DHS and the Multiple Indicator Cluster Survey provide nationally representative data and information from children and women in both the public and private sector (Cutts et al., 2016; Cutts et al., 2013; UNICEF, 2015).

Although coverage surveys are believed to offer more reliable estimates than routine administrative data, both survey methods share an inherent limitation where card retention is low (Cutts et al., 2013). The reliability of the administrative data is routinely questioned as the officially reported immunization rates in some regions of the world can be significantly higher than standardized nationally representative surveys. An analysis of DHS data from 45 countries compared country reports versus documented administration

of three doses of DPT and immunization survey findings and discovered that the official DPT3 coverage reported by 25 of 45 of the countries was at least 20% higher than the DHS while only three countries were found to be lower (Murray et al., 2003). Another analysis of data from 2001 found that the DRC reported coverage for DPT3 at more than 10 million children, 1 million more than the previous year; however, data from a household coverage survey of the same period estimated coverage to be 500,000 less than what was reported (Lim, Stein, Charrow, & Murray, 2008).

Levine suggested that biological markers are the most robust method to document population immunity provided that the survey methods used to collect these data are valid. In addition, Levine noted that underserved, high-risk communities may be identified through serosurveys and appropriate services can be targeted to these communities (Levine & Pasetti, 2016). Other researchers promote the use of seroprevalence surveys to monitor the effectiveness of immunization programs for tetanus (Cutts & Hanson, 2016; MacNeil, Dietz, & Cherian, 2014). In Ethiopia researchers conducted an immunization coverage survey in 3 districts reviewing clinic records and vaccination cards, interviewing mothers, and collecting serum from over 1,100 children under 2 years of age. They found that traditional data sources, clinic records, daily vaccination sheets, individual vaccination cards, and maternal recall provided conflicting information and did not correlate with the objective measure of the serology. For example, in one district the Pentavalent-3 coverage was documented as 85%, the coverage survey was 35%, and serosurvey results were 53% (Travassos et al., 2016).

Assessment of Immune Response

Two testing techniques are used to measure immunity to tetanus: in vivo, those tests that take place in a living organism, and in vitro, tests that take place in test tubes or machines. The in vivo assay is considered the gold standard because it measures active antitoxin in serum. Neutralization assays are developed by injecting mice with a series of dilutions of sera of deadly doses of tetanus toxin. These assays are the most reliable, are very sensitive, and can detect neutralizing antibody at levels as low as 0.001 units per milliliter (IU/mL). In the 1930s, Sneath's work led to the determination that the accepted level of antitoxin required for protection against tetanus is ≥ 0.01 IU/mL (WHO, 2018; Sneath, Kerslake, & Scruby, 1937).

There are several in vitro assays that are in use to detect anti-tetanus antibodies; these are simple and relatively inexpensive, but they vary in terms of sensitivity and in what they measure. The passive hemagglutination test measures Immunoglobulin M (IgM) in red blood cells. IgM will not neutralize tetanus toxin, but there is a high correlation when an individual has a high level of protection. This test is rarely used today. The radioimmunoassay is cited but there is no evidence of use for field studies (WHO, 2018).

The enzyme-linked immunosorbent assay (ELISA) is used to identify Immunoglobulin G (IgG) antibodies produced following immunization. This assay is one of the most widely used. As commercial assays do not separate active antibody and non-neutralizing antibody, they are unable to distinguish antibodies at low levels. The resulting low specificity of these tests is a significant limitation in their use. The

threshold of current commercial tests is set between 0.1 and 0.2 IU/ml, rather than at 0.01 IU/ml. At the higher levels the non-neutralizing antibodies are included in what is measured. When the cut offs are increased, there is a risk of underestimating the seroimmunity in populations.

There are three in vitro tests that correlate well with in vivo testing at low titers and are only available for research: the competition ELISA and the Double Antigen ELISA (DAE)—both variations of the Elisa— and the Multi Bead Assay(MBA) described by Scobie (Scobie et al., 2016). There are other tests that have been recently approved or are in development. Dynex Technologies Multiplier chemiluminescent-immunoassay platform is a bead assay with tetanus validated against the DAE and the MBA (in press). This "for research use only" approved assay, has a conservative protective cut off at 0.20 ≥IU/m with 80% sensitivity and 84% specificity, was used in this study. The Dynex measles, mumps, rubella assay was validated and the findings published (Higgins et al., 2019).

Methodology for Passive Surveillance Systems for Global, Regional, and National Reporting

This section describes the process of tetanus surveillance and a review of the global morbidity and mortality associated with tetanus. There is an examination of tetanus morbidity and mortality reports' estimates from retrospective hospital reviews and serosurveys that focus on data found in Africa more broadly and from the DRC specifically. Many African countries have passive surveillance systems which rely upon data from clinicians in health facilities to create disease reports on a weekly or monthly

basis, typically as part of WHO's Integrated Disease Surveillance System (Kasolo et al., 2013). Unlike other vaccine preventable diseases such as measles, there is no confirmatory laboratory test for tetanus and so case definitions, rather than objective laboratory diagnostics, are used to define a case (Roper et al., 2017). In many developing countries where women may deliver their children at home, deaths due to neonatal tetanus and maternal tetanus can go unrecorded (Khan, Vandelaer, Yakubu, Raza, & Zulu, 2015; Thwaites et al., 2015). This is also true for adolescents and adults who may not have financial or geographic access to clinics. In addition, since many countries do not have a birth or death registry, adolescent and adult deaths due to tetanus may never be confirmed. Reliance of clinicians for passive reporting, the lack of a confirmatory test, and unreported deaths all contribute to an underrepresentation of both tetanus morbidity and mortality estimates. Thwaites (2015) suggested that many cases of tetanus occur in remote areas with limited access to health facilities resulting in both high morality of untreated tetanus cases and low chance of the death due to tetanus being reported. Under the current system in the African Region as few as 10% of cases may be reported (Mihigo et al., 2018). Although there is insufficient epidemiological data available to conclusively attribute risk of mortality by age and sex, it is well recognized that many children and men are unprotected against tetanus due to the lack of booster immunizations available in low- and middle-income countries (Thwaites & Loan, 2015).

In 1980, WHO began collecting and publishing surveillance and vaccination coverage data from 195 countries on 11 vaccine preventable diseases (polio, measles, neonatal tetanus, non-neonatal tetanus, diphtheria, rubella, congenital rubella syndrome,

yellow fever, pertussis, Japanese encephalitis, and mumps). In that first year, 137 of 194 (71%) of WHO member nations reported at least one tetanus case. In 2016, 87 countries (45%) reported tetanus cases, however 52 (27%) countries (including the United States) did not report at all (WHO, 2018). The WHO/UNICEF report does not report the number of morbid cases. WHO does note that the data provided is incomplete, however these inconsistencies highlight the challenge of working with tetanus morbidity and mortality data.

Despite the discrepancies, it is generally acknowledged that substantial progress in reducing tetanus morbidity and mortality globally has been made. Tetanus moved from the 19th leading cause of death in 1990 to the 67th cause of death in 2013 (Abubakar, Tillmann, & Banerjee, 2015). This reduction is attributed, in part, to improved routine immunization coverage and childbirth practices implemented during the past two decades.

This seems encouraging, yet due to underreporting these model-derived rankings likely underestimate the true picture. Using data collected for the global burden of disease study and the cause of death ensemble modeling strategy known as CODEm for global disease specific death estimates, researchers postulate there may have been over 56,743 deaths globally (range 48,199 to 80,042) in 2015. Of these, approximately 19,937 occurred in neonates, and 36,806 occurred in older children and adults (Kyu et al., 2017; Naghavi, Wang, & Lozano, 2015). The discrepancy between these estimates and those of WHO, which notified only 10,337 cases from member states in the same year, is approximately 140%. Even accounting for the 48 countries that did not report, the

difference is greater than the sum of all cases reported from India which has had the highest disease burden over the last decade (WHO, 2018).

Developed countries are also still at risk for tetanus. A review of 2015 tetanus data published by the European Centers for Disease Control reported 117 cases from 26 European countries whereas WHO reported 104 for the same countries that year (European Centre for Disease Prevention and Control, 2016). Of 117 cases 72% (84/117) were ≥ 65 years of age and 71% were women. Most cases occurred between the months of June and October, corresponding with increased outdoor activities and thus with the likelihood of exposure. Of the 67 cases with outcome data, there were 17 tetanus fatalities in individuals ranging from 5–90 years of age (European Centre for Disease Prevention and Control, 2016).

In the United States between 2000 and 2008 there were 233 reported cases of non-neonatal tetanus, 30% occurred in adults ≥ 65 with the highest mortality in this age group. Men accounted for 59% of all cases (CDC, 2011). In 2015, of 29 cases of tetanus in the United States 79% were men and 10% were over the age of 65. The majority of the cases were White, non-Hispanic, and between the ages of 25 and 64 (CDC, 2017).

In 2016, there were 5,771 tetanus cases reported in the African Region of the WHO. The totality of cases were from 25 of the 47 AFRO member states; 11 countries reported zero cases and 11 did not report at all. These missing (or “silent”) areas can have a significant impact, particularly when the countries failing to report have a high or even moderately high burden of disease. For example, Angola and Central African Republic did not report in 2016 however they reported consistently in the five preceding years an

average of 498 and 62 cases respectively. These unreported cases (based on the average) would have contributed an additional 560 cases or 9% of the burden in the region (WHO, 2018).

Between 2014 and 2016, the DRC reported only neonatal tetanus cases to WHO. DRC reported 80, 330, 201 cases of neonatal tetanus in 2016, 2015, and respectively with no report for 2013. Data from the National Integrated Disease Surveillance and Response program in DRC suggest that in 2013 and 2014 there were approximately 1,300 and 1,400 total cases during that period (Hoff, 2014), further underscoring the challenge presented by missing data. Nonetheless, findings from the global burden of disease study estimated non-neonatal tetanus death rates in 1990 and 2015 based on data from birth and death registries; verbal autopsy studies; and mortality surveillance data. The authors estimated a net change in mortality rates of -49.13 between 1990 to 2015. The decline in tetanus mortality was attributed to the increase in tetanus immunization during this period (Kyu et al., 2017). Overall, there is a reduction in the number of tetanus cases over the last several decades, however the inconsistency in tetanus reporting across countries may lead to under reporting. The unintended consequence is that many think the disease is not an issue when in fact it will continue to affect those who are most exposed due to livelihood.

Surveillance Methodology: Retrospective Hospital Reviews

In addition to country surveillance data reported to WHO, there are several retrospective hospital reviews that provide evidence of morbidity and mortality across the globe and report on factors associated with tetanus susceptibility in adult populations.

The methodology for these studies includes reviews of the final diagnosis classifications International Classification of Disease (ICD) in hospital record systems or reviews of hospital inpatient and outpatient registry books. Several retrospective hospital studies document the presence of tetanus cases in Africa.

A retrospective hospital-based study in southern Nigeria reviewed the outcomes of 86 adult tetanus cases over a 10-year period from 1996 to 2005 (Chukwubike & God'spower, 2009). The age of cases ranged from 16–90 years; 75% were less than 40 years of age, with a mean age of 30 years. Men accounted for 58% of the cases and 44% of deaths. Overall the case fatality rate was 43%. For cases above the age of 40, the case fatality rate (CFR) was 76%. Of the 86 cases only 10 people (eight men and two women) reported that they had received vaccination against tetanus.

Similarly, in northern Nigeria a retrospective study was conducted over a 14-year period between 2001 and 2014 (Aliyu et al., 2017). During this time 91 tetanus cases were admitted to and evaluated at the Ahmadu Bello Hospital in Zaria. Of these 75% were men, with a median age of 14 years and 62% of the cases were under 20 years of age. Over 95% of cases had generalized tetanus with a CFR of 48%. There was no documentation of previous tetanus immunization for any of the patients. In the case notes there were strong recommendations given to clinicians to take every opportunity to review immunization records and to provide immunization to infants and boosters to preschool children and young adults.

In northwestern Tanzania researchers evaluated records of cases of tetanus that presented to the Gilyoma teaching hospital between 2001 and 2010 (Chalya et al., 2011).

Of the 102 cases of tetanus during this 10-year period, 92% were men. The median age was 34 years and 74% of tetanus cases were less than 40 years of age. Again, the case fatality rate was high at 43%. This study provided information on occupation finding 51% were farmers and 22% laborers; this is consistent with data provided by Roper et al. (2017) who reported higher prevalence of tetanus in occupations associated with agriculture. In this study 24% of cases had reported they had received tetanus vaccination in the past, however as tetanus vaccination is given at an early age, a booster in adolescence or as an adult is required to maintain protective immunity (WHO, 2017; WHO, 2018).

There is limited published data on tetanus from the DRC; however, a retrospective hospital review of tetanus found high mortality from patients admitted to a major hospital in southern DRC (Muteya et al., 2013). Between 2005 and 2009, 22 patients were admitted with tetanus of which 95% were men. The median age was 39 and the CFR was 52% with a majority of patients from semi urban areas. This study was limited to patients admitted to the intensive care unit and the researchers thought that this was an under representation of the total cases seen at the hospital during the period.

Recent tetanus deaths in adolescent boys and men between the ages of 11 and 47 following voluntary male circumcision have highlighted the lack of tetanus immunity of men. Between 2012 and 2016, 13 cases of tetanus with 9 deaths were reported from 6 countries in sub-Saharan Africa (CDC, 2016; Dalal et al., 2016). The WHO Report on male circumcision and tetanus in 2015 noted that many African countries focused on

improving infant immunization and achieving MNTE, but the goal of childhood tetanus booster doses for adolescents and men had not been a priority (Farley, 2015).

In the four retrospective studies reviewed from the African region on hospital admissions for tetanus, men constitute most cases and deaths. These studies provide evidence of both morbidity and mortality from large facilities based in urban areas. What these studies do not provide is evidence from facilities in rural areas and there is little data on adolescent girls and younger women. In the next section there is a review of studies that provide objective quantitative evidence of protective levels of immunity against tetanus using serosurveys.

Literature Related to Methods

Developed countries, such as the United States, the Netherlands, United Kingdom, and Australia, have comprehensive surveillance systems for vaccine preventable diseases and conduct population-based surveys that use banked residual sera to provide evidence of immunity against vaccine preventable diseases (Wilson, Deeks, Hatchette, & Crowcroft, 2012). Serosurveillance has also been conducted using methods such as cross-sectional serosurveys or ad-hoc surveys to provide estimates of protective immunity or to address specific questions about disease and variables such as age and sex. In this section population-based surveys that have used serosurveys to determine immunity levels for tetanus are reviewed as this will inform the methodology for the research proposal.

Surveillance Methodology: Serosurveys

This section will review the serosurvey data that document tetanus antibody levels and factors such as age, sex, place of residence, and antenatal care. These studies provided the methodological foundation for this research. The studies are summarized in Tables 1 and 2 and listed in the order they are mentioned in the literature review. Each study summary includes the name of the first author, the year the study was published, the country in which the study was conducted, the study size, the target population, and the method of survey design. As there are several different assays used to measure tetanus immunity, the assay with the cut-off for protective immunity, key age groups of analysis, and prevalence findings are also presented in Tables 1 and 2. The studies are divided between high- to medium-income countries and medium- to low-income countries based on findings from the literature. The findings are discussed in the section that follows Tables 1 and 2.

Table 1

Seroprevalence Studies: High- to Middle-Income Countries

Author	Year	Country	Sample size	Target population	Survey design sample type	Assay/cut-off protective immunity	Age group	Sero-prevalence in adults
Olander	2009	Finland	900	Adults >30	Cross-sectional, population-based	DAE ≥0.1IU/ml	30-39 40-49 50-59 60-69 ≥70	99% M, 99% F 98% M, 90% F 90% M, 70% F 77% M, 64% F 46% M, 35% F
Filia	2014	Italy	3,604	Pop.-based Ages 0-95	Cross-sectional	DA-DELFIA ≥0.1IU/ml	15-24 25-44 45-64 65-74 75-84 ≥70	87% adults 78% adults 26% adults 28% adults 17% adults 37% M, 20% F
Wu	2009	Taiwan	326	Adults 16-70	Prospective study of booster impact, convenience sample	ELISA ≥0.1IU/ml	16-59 ≥ 60	95% adults 89% adults
Thuy	2017	Vietnam	180	Adults 30-45	Random sample national population study	ELISA ≥0.1IU/ml	30-45	74% F, 24% M
Black	2014	Laos	1,128	Adults 15-70	Cross-sectional survey health care workers	ELISA ≥0.1IU/ml	15-70	78% adults 89% F, 38% M

A serosurvey of adults in Finland ≥ 30 years of age found a high level of protective immunity in both sexes up to 50 years of age, however immunity was higher among men aged 50–69 (Ölander et al., 2009). Adults ≥ 70 years were found to be susceptible to tetanus infection. The authors suggested that military service and higher rate of injury may have increased the number of tetanus immunizations among adult men, thus explaining the sex differential in levels of immunity between ages 50 and 70 as shown in Table 1.

In Italy, a national population-based serosurvey of banked sera from routine laboratory testing estimated the prevalence of immunity at 71% across all age groups (Filia et al., 2014). However, the authors found that protective antitoxin levels declined steadily over time beginning in mid-life and this confirms the importance of a booster with waning immunity. This serosurvey confirms the low population immunity of Italian adults over 45 years and may explain the high incidence of tetanus reported annually.

A prospective serosurvey on the requirement of tetanus boosters conducted in Taiwan also examined factors that influenced immunity (Wu et al., 2009) and found that anti-toxin levels declined with age. As with the Finnish study, Wu et. al. (2009) discovered that protective immunity levels were significantly associated with being male or being born after 1955, although they found no association with military service or working with soil or gardening.

In Vietnam, sera were provided from a long-term general population serosurvey in Ho Chi Minh City (Thuy et al., 2017). A random sample of 180 men and women born in or before 1984 during the early years of the immunization program were selected. Of

the women, 73% had protective antibodies compared with 24% of the men. This cohort would not have had the opportunity for vaccination as infants, however women could have been vaccinated at antenatal clinics when pregnant or through tetanus immunization campaigns for women of childbearing age.

A cross-sectional serosurvey to evaluate heath care workers immunity to 6 vaccine preventable diseases and hepatitis C in Laos was conducted at thirteen hospitals throughout the country (Black et al., 2015). A majority (78%) of the participants were women and under 40 years of age. Serology demonstrated that 90% of women versus 38% of men were protected against tetanus. The investigators also noted a decline in immunity after age 40. They attributed this decline to a "cohort effect"; the tetanus vaccine was introduced in 1979 so adults 34 or older were unlikely to be immunized against diphtheria, tetanus, and pertussis unless through a catch-up campaign. Laos has offered tetanus vaccine to pregnant mothers since 1991 and this practice has been effective as 95% of women with children had higher antibody levels.

There are several factors that may be linked with low immunity and age in older adults. First, adults born before the EPI program became well established may never have received tetanus vaccine, and second, immunity wanes with age as has been documented in studies of adults (Aboud et al., 2002; Roper et al., 2017). Finally, immunization programs were designed to provide vaccination in most African countries during the first year of life and for women during pregnancy with very few countries providing adolescent or school-based vaccination and limited catch up immunization for those that were not fully immunized (Dalal et al., 2016; Mihigo et al., 2017).

Table 2

Seroprevalence Studies: Middle- to Low-Income Countries

Author	Year	Country	Sample size	Target population	Survey design sample type	Assay/cut-off protective immunity	Age group	Sero-prevalence in adults
Oncu	2011	Turkey	293	Adults ≥ 50	Cross-sectional	ELISA ≥0.1IU/ml	50-59 60-69 ≥70	52%adults 36%adults 10%adults
Aboud	1999	Tanzania	200	Males 18-70	Cross-sectional	ELISA ≥0.1IU/ml	18-27 28-37 38-47 48+	83% M 86% M 90% M 47% M
Scobie	2017	Kenya	192	Adults ≥ 15	Convenience sample	MBA ≥0.1IU/ml	≥15	45% M, 96% F
		Tanzania	425	Adults ≥ 15	Convenience sample	MBA ≥0.1IU/ml	≥15	28% M, 94% F
		Mozambique	626	Adults ≥ 15	Convenience sample	MBA ≥0.1IU/ml	≥15	64% M, 90% F
Scobie	2016	Cambodia	2,150	Females 21-50	Multistage cluster	MBA ≥0.1IU/ml	15-19 20-24 ≥25	63% F 87% F 95% F
Orimadegun	2017	Nigeria	244	Females 20-34	Cross-sectional	TQS ≥0.1IU/ml	20-24 25-29 30-34	88% F 80% F 53% F

A serosurvey conducted in western Turkey among farmers aged 50 to 71 found protective immunity of 34% in this high exposure group (Öncü, Önde, Öncü, Ergin, & Öztürk, 2011). Overall 45% of men had protective immunity compared with 25% of women and immunity significantly declined with increasing age. Öncü et. al. (2011) attributed the higher immunity in men to increased injury secondary to greater physical activity and to risks in men leading to a higher rate of medical follow up that included tetanus injection.

In a 1999 cross-sectional study of male blood donors in Dar es Salaam, Tanzania researchers found that 36% of men did not have protective immunity against tetanus. As with previous studies, middle-aged men ≥48 years of age had lower immunity levels. The Tanzania EPI began in 1975, therefore only individuals 18−24-years of age at the time of the study would have been eligible for immunization as an infant. Other people could have received tetanus toxoid following an injury. Overall 128 of 200 men in this study had protective antibodies against tetanus. The age of 47 men who were excluded from the analysis is unclear because they did not report a vaccination history.

Following the cases of tetanus in men after voluntary male circumcision in sub-Saharan Africa between 2013 and 2016 described earlier (Dalal et al., 2016), researchers assessed immunity gaps in a convenience sample of children, adolescents, and women and men ≥ 15 years in Kenya, Tanzania, and Mozambique (Scobie et al., 2017). The methodology for this study was archived data and banked serum specimens or dried blood spots from a 2012 neglected tropical disease study. This review focused on those adults over 15 years of age, illustrated in Table 2. In Kenya samples were collected from

participants of villages in Mbita District, Homa Bay County. Overall, 91% of participants were seroprotected; 96% of women were seroprotected compared to 45% of men. In Tanzania, participants were from villages of Kongwa District, Dodoma Region. Overall 73% of participants were seroprotected with 94% of women and 28% of men with protective antibodies against tetanus. Mozambique samples were collected from participants in 2 villages in Morrupula District, Nampula Region with 79% of individuals seroprotected; this included 90% of women and 64% of men. Although, this was a convenience sample and not generalizable to each country or to the sub-region, the study does provide evidence of the success of the efforts to immunize women of childbearing age through outreach vaccination and antenatal clinics. It is also a testament to the inequity of booster vaccination for the nonchildbearing sex faced with waning immunity. The findings do not note the protection by age to determine the immunity of adolescents, but for all three cohorts aged 4 to 14 overall immunity ranged from 66% to 91%, the highest being in Mozambique.

In 1989, the WHO established a neonatal tetanus elimination goal, which was defined as < 1 case per 1000 live births, and in 1999 this initiative expanded to include maternal tetanus (Thwaites et al., 2015; WHO, 2017). A nationwide serosurvey was conducted in Cambodia in 2012 to assess the status of tetanus immunity in women of reproductive age aged 15 to 39 (Scobie et al., 2016). The methodology of the study was a cross-sectional nationally representative multistage cluster survey with a nested serosurvey. Of the 2,150 women tested, 88% of women were seroprotected (Table 2). There was no significance difference between living in a rural or urban area, however the

western region had 82% protection and all other regions had seroprotection of 89%. In women 15−19-years of age seroprotection was 63%. Although Cambodia has an immunization schedule that includes a booster in later childhood for adolescent girls and boys, uptake is suboptimal. Scobie et. al. (2016) suggests that low seroprotection in women 15−19 could be due to not receiving tetanus toxoid during their first pregnancy as 97% of the parous women were seroprotected compared with 71% of the nulliparous women.

In Ibadan, Nigeria, a rapid diagnostic test was used to evaluate immunity against tetanus in a cross-section of mothers following delivery of their first child (Orimadegun, Orimadegun, & Bamgboye, 2017). The methodology used for the study was a cross-sectional design limited in geographic scope. Overall, of the 244 women aged 20−34 who participated in the study, 71% had protective immunity against tetanus; however, the older mothers aged 30−34 had the greatest immunity gap with 37% of the cohort unprotected. There was no significant difference in protective immunity between urban and rural residents and 80% of the participants were middle class with access to similar health facilities and reported at least 2 tetanus vaccinations during antenatal care visits in the current pregnancy. All participants in the study attended antenatal clinics so health-seeking behavior was high, this study highlights missed opportunities to vaccinate.

In summary the literature review related to methods provided evidence of several studies that used serology to provide evidence of immunity against tetanus. The method that provided the answers to this study's research questions was an observational, cross-

sectional, nationally representative, multistage cluster survey as described by Scobie et. al. (2016).

Summary and Conclusions

Overall, the reported morbidity and mortality from tetanus has declined globally with the increased use of vaccine for infants, safe delivery practices, and vaccination of women of childbearing age and in antenatal clinics. The data shows there are still additional lives to be saved and there is a safe effective vaccine that can be used for that purpose. In general, in high-income countries and in older age groups, women have lower immunity against tetanus. This is reversed in middle- and low-income countries where women have higher immunity than men in the older age groups. There are no studies comparable to Finland and Italy (Filia et al., 2014; Ölander et al., 2009) which reviewed cohorts of people in their 70s, but several studies did review immunity against tetanus in adults in middle- and low-income countries. These studies identified a greater proportion of women having protective immunity against tetanus than men, which was attributed to vaccination of women during antenatal care beginning in the mid 1970s. (Black et al., 2015; Scobie et al., 2017; Thuy et al., 2017).

What is striking is the high morbidity found in the retrospective studies from Nigeria, Tanzania, and DRC where the case fatality rate was on average 50% and men had the highest CFR (Aliyu et al., 2017; Chalya et al., 2011; Muteya et al., 2013). The limited serosurveys from Africa show that immunity against tetanus is much lower in men (Scobie et al., 2017). Finally, the death of 12 men in six African countries due to tetanus after a medical circumcision procedure to reduce their risk of HIV demonstrates

decreased immunity and higher risk of tetanus infection and death if exposed (Dalal et al., 2016; Farley, 2015).

There are limited studies on adult protective immunity levels against tetanus in Africa. What was unknown was the immunity to tetanus in the rural areas for younger women and men, and for older women who have not had children. For those groups, this was the first study reported in DRC. This study bridged the gap in the literature and in our knowledge of tetanus immunity in adults in DRC.

Chapter 3: Research Methods

In this study, I used findings from the DRC DHS and nested serosurvey data collected between November 2013 and February 2014, to assess protective immunity against tetanus among adult populations in DRC. The secondary purpose of this study was to identify characteristics of populations at greatest risk for tetanus infection based on immunity against tetanus among adults in DRC. This research was designed to fill a critical gap in knowledge about protective immunity against tetanus among adults in DRC, thus addressing a significant public health problem, given the high morbidity and mortality associated with tetanus disease.

This chapter includes a rationale for the study and a detailed review of the research design. I also address the sampling strategy and procedures for the nested tetanus serosurvey and the methodology for using DHS data from DRC. The chapter concludes with a description of potential threats to study validity and a summary of the ethical procedures.

Research Design and Rationale

In this quantitative study, I used individual-level DHS data from DRC paired with individual serosurvey data collected from a subset of adult survey respondents between November 2013 and April 2014. I chose this nested, observational cross-sectional study design because a nationally representative study would provide an understanding of the protective immunity against tetanus that is generalizable to the adult population in DRC. The DHS was selected because the DHS is a survey that has been used, replicated, and

refined over the past 34 years, thus providing reliable data output (DHS, 2013; Frankfort-Nachmias, 2008).

In many low-income countries, the DHS is conducted at regular intervals allowing for comparisons across time (Aschengrau & Seage, 2013; Creswell, 2009). The DHS includes questions on multiple health indicators, making it both comprehensive and cost-effective for the countries to evaluate issues of interest across multiple sectors simultaneously. The nested serosurvey was designed to take advantage of the methodological rigor of the DHS while also reducing the costs of conducting a separate large survey (Manual for Serosurveys, UCLA in press).

There is evidence that nested serosurveys have provided detailed information for preventable diseases in DRC. To gain insight on malaria in DRC, Taylor et. al. (2011) used the DHS to conduct a serosurvey in adults to gather information on the type of malaria present and the prevalence of each type, and Levitz et. al. (2018) evaluated the use of bed nets and prevalence of malaria in children. The method for gathering information on vaccine preventable diseases is efficient and cost effective when combined with a large cross-sectional study. The 2013–2014 survey was the second DHS carried out in DRC, so some comparisons between surveys is possible. The first DHS was conducted in 2007, but it did not include a nested serosurvey for immunity against vaccine preventable diseases (Ministry of Planning and Implementation of the Modern Revolution et al., 2014).

Research designs were reviewed against the goal of this research, which was to use a serosurvey to assess protective immunity against tetanus among adults in DRC and

to identify the characteristics of those at greatest risk of tetanus infection based on immunity against tetanus. When considering study designs, I ruled out an experimental research design because the purpose of this study was not to test the impact or causal effect of the intervention of vaccination on the adult population. A quasi-experimental research design was also not appropriate because a quasi-experimental design would necessitate an intervention that would require the assignment of adults to a group where vaccination would be withheld and others to a group where vaccination would take place (Frankfort-Nachmias, 2008; White & Sabarwal, 2014). The efficacy of tetanus vaccine is well understood as well as the consequence of exposure to tetanus; therefore, it would be unethical to knowingly withhold vaccine for research purposes (WHO, 2018).

By contrast, the observational cross-sectional survey design is nonexperimental; the benefit of this approach was that it did not require random assignment of individuals to an intervention. This was a significant advantage because withholding an effective intervention such as tetanus vaccine would be unethical (Frankfort-Nachmias, 2008). Therefore, the use of secondary data gathered from an observational cross-sectional survey design was both ethical and efficient.

The variables were selected for this study based on the research questions and the literature review. The dependent variable for this study was protective immunity against tetanus. The independent covariables were age, sex, province, place of residence, wealth, education, antenatal visits during pregnancy, and tetanus vaccination when pregnant. The following were the research questions and hypotheses for this study:

RQ1: Is there an association between age and protective immunity against tetanus among adults in DRC?

$H_0$1: There is no association between age and protective immunity against tetanus among adults in DRC.

H_a1: There is an association between age and protective immunity against tetanus among adults in DRC.

RQ2: Is there an association between sex and protective immunity against tetanus among adults in DRC?

$H_0$2: There is no association between sex and protective immunity against tetanus among adults in DRC.

H_a2: There is an association between sex and protective immunity against tetanus among adults in DRC.

RQ3: Is there an association between province and place of residence (urban vs. rural) and protective immunity against tetanus among adults in DRC?

$H_0$3: There is no association between province and place of residence (urban vs. rural) and protective immunity against tetanus among adults in DRC.

H_a3: There is association between province and place of residence (urban vs. rural) and protective immunity against tetanus among adults in DRC.

RQ4: Is there an association between antenatal care and protective immunity against tetanus among women in DRC?

$H_0$4: There is no association between antenatal care and protective immunity against tetanus among women in DRC.

H_a4: There is an association between antenatal care and protective immunity against tetanus among women in DRC.

Methodology

The survey population for this research was drawn from all women ages 15 to 49 and men ages 15 to 59 in DRC (Ministry of Planning and Implementation of the Modern Revolution et al., 2014). The most recent population-based census was conducted in DRC in 1984, and the population was estimated at 30.7 million with 70% of DRC inhabitants residing in rural areas. Using data from 1984 census and an estimated population growth rate of 3%, the DRC National Institute of Statistics prepares annual population projections. At the time of DHS preparations in 2012, the National Institute of Statistics (2011) estimated the size of the population of DRC to be 77.8 million people.

In 2006, DRC's constitution was amended and the number of administrative provinces was slated for increase from 11 to 26 with further subdivisions into cities and districts. The cities are urban and further divided into communities, and the districts are rural divided into territories. The implementation of this division began in 2015, and the DHS technical team designed the survey with sufficient power for all questions in the provinces, ensuring the DRC DHS was both provincially and nationally representative. (Ministry of Planning and Implementation of the Modern Revolution et al., 2014).

Sample and Sampling Procedures

The DHS is well-established with a sampling manual developed by the firm ICF International to promote rigor, precision, and replicability in the survey methodology (DHS, 2013). This cross-sectional survey uses a stratified multistage cluster design,

where stratification reduces variations in estimates for specified subgroups (Frankfort-Nachmias, 2008). The DRC DHS used probability sampling to allow for accurate estimates of population parameters giving each sampling unit in the survey a known probability of being included (DHS, 2013). The sampling frame is the comprehensive list of all units or subjects that are included in the target population (DHS, 2013; Frankfort-Nachmias, 2008). According to the DHS manual, an up-to-date and comprehensive sampling frame is critical for an unbiased assessment. The DRC conducted the Multiple Indicator Cluster Survey in 2010 was considered to have a reliable sampling frame (National Institute of Statistics, 2011) and data gathered during the 2012 1-2-3 Survey (Employment, Informal Sector, and Household Consumption) provided additional information on the sampling frame in province and rural areas (Ministry of Planning and Implementation of the Modern Revolution, 2014). The sampling frame used for the DHS combined previous survey data with 2013 projected National Institute of Statistics population figures and government administrative and electoral census data (DHS, 2013).

This survey had a two-stage stratified cluster design. In the first stage, each province had a cluster of samples selected independently in each district with the probability of the cluster being selected proportional to its population size; then all households within each selected cluster were listed. During the second stage, households for interview were selected by equal probability through systematic sampling at the cluster level. Substitute clusters were preselected, in case of violence, war, civil disturbance, or natural disaster.

The sample size for the DRC DHS was calculated based on the estimated number of households required for the desired precision of the key indicators required for the women's questionnaires for both fertility and mortality and HIV. For the 11 provinces, this was 1,000 women per province, and for the 26 new provinces, this was 500 women per province. The final sample size ranged from 500 to 1,500 women per province (Ministry of Planning and Implementation of the Modern Revolution et al., 2014).

In this study 540 clusters were selected nationwide with 379 rural clusters and 161 urban clusters (Figure 1). Teams visited 34 households in each cluster. Due to insecurity, four clusters were not visited, two in Katanga, one in Kasai Orientale, and one in North Kivu, these clusters were not replaced because of ongoing security challenges (Ministry of Planning and Implementation of the Modern Revolution et al., 2014).

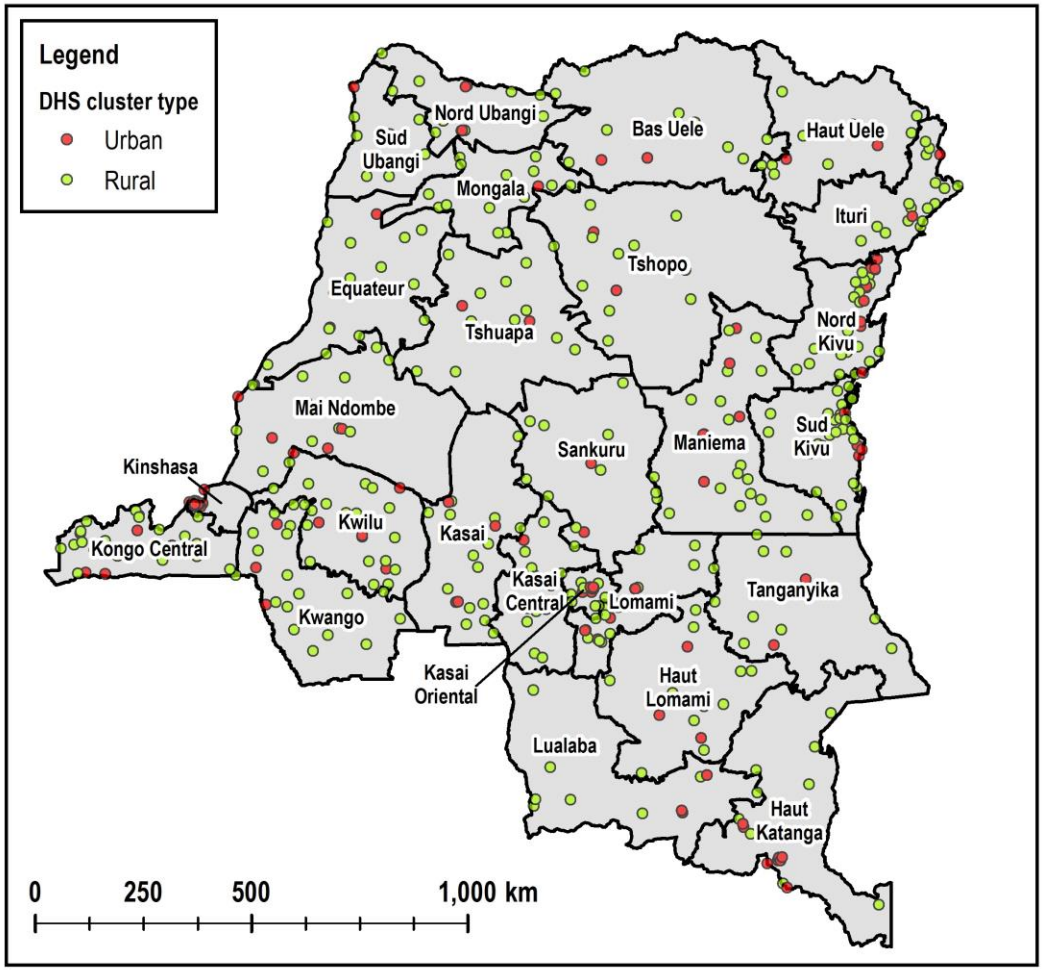

Figure 1. Map of DRC: Provinces with 2013–2014 DHS urban and rural clusters.

Nationwide, 19,681 households were visited, and 18,827 women aged 15 to 49 and 8,656 men aged 15 to 59 were interviewed. Men were only eligible to be selected into the sample in half the selected households whereas women were able to be interviewed in every household which could be the rational for the increasing the age in men. The men and women in households in which men were selected for interview were eligible for all serologic testing including HIV, malaria, and immunity against vaccine preventable diseases. Of the serologic samples collected, approximately 50% were selected for polio testing at the CDC and the other 50% of samples were tested in the

DRC for immunity against tetanus, measles, rubella, mumps, and chicken pox. The available samples were split into two equally representative sub-sets of the sample and of the population from which it was drawn as follows: within each cluster and province, samples were sorted by age and sex to form pairs of men and pairs of women, with one half of each pair being randomly selected for polio neutralizing antibodies at CDC. Singletons were assigned with 50% probability of assignment to CDC or DRC. In correspondence with researchers at the Kinshasa School of Public Health (KSPH) and the University of California at Los Angeles (UCLA), 8,713 samples were sent to CDC for testing for polio 8,706 remained for the tetanus serosurvey; statistician Dale Rhoda provided guidance on the sample split (personal correspondence, May 2018). Among the 8,706 samples listed as available for testing 132 were duplicate records, 305 had no corresponding interview data and 149 had insufficient blood for testing leaving 8,120 samples with corresponding interview records for the study (Figure 2).

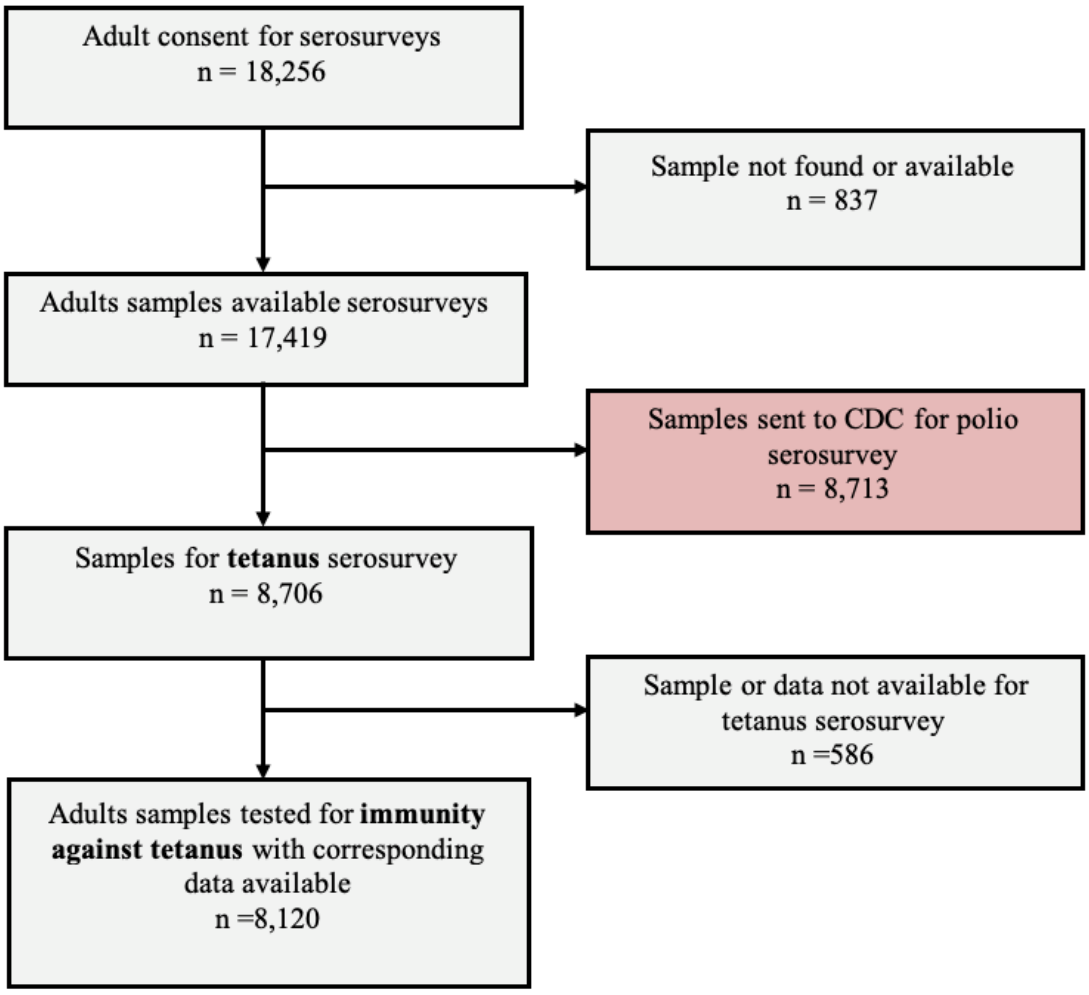

Figure 2. Flow diagram of samples available for tetanus serology.

The sample for the observational cross-sectional study on tetanus immunity was drawn from a subpopulation of the adults who were available for testing from the DRC DHS. The appropriate sample size was identified for the study on the factors associated with tetanus immunity among the adult population in the DRC using G*Power software version 3.1.9.2 (Faul, Erdfelder, Lang, & Buchner, 2007). In initial sample size calculation, attention was given to (a) alpha (α), the risk of making a type I error and

incorrectly rejecting the null hypothesis of significance, (b) beta (β), statistical power, the probability of rejecting the null hypothesis when it should be, and (c) effect size.

The effect size is the standardized measure of the extent of the observed effect which can be set at 0.10 for a small effect, 0.30 for a medium effect or 0.50 for a large effect size which would account for 25% of the effect (Cohen, 1992; Field, 2013). G*Power was used to calculate the initial sample size with a medium effect size of 0.3, α of 0.05 and statistical power of 0.80 (Type II error rate β = 0.20). The sample size for the study was 143 and the sample size was doubled per province to answer the primary research question of the association between immunity against tetanus and sex. At the time of the DHS there were 11 provinces, therefore the national sample required would be 3,146 participants for this study. After the data were received and cleaned, there were 8,120 samples with associated information available for the study. At this time the administrative structure changed there were 26 provinces and I made the decision to conduct the final analysis for the 26 new provinces. The final provincial unweighted sample size ranged from 201 to 701 participants. G*Power was used to calculate the statistical power, with a minimum sample size of 201particapants per province, α of 0.05, and effect size of 0.3. The statistical power was 0.9122 and the study sample size was sufficient for generalization.

Procedures for Archival Data

I followed the procedures outlined for archival data. To access the adult DRC DHS data, I received permission from the DHS program through the website https://dhsprogram.com/data/new-user-registration.cfm. To access the tetanus serology

data, I received permission from UCLA following a written request and agreement to share all analysis.

Operationalization of Variables

The dependent variable for this study was protective immunity against tetanus. Independent demographic variables were age, sex, province, and urban or rural residence. Age was recorded as the age at the time of interview as a continuous variable and re-coded in 5–year increments beginning at age 15. Additional covariates such as marriage, education, literacy, ethnicity, occupation, having health insurance, and religion were included in the analysis. There were specific variables for women that included antenatal visits within the past 5 years, number of children ever born, births in last 5 years, and number of tetanus vaccinations before last pregnancy. Table 3 summarizes the variables, the type of variable, and the definition.

Table 3

Variables

Variable	Type	Definition	Measure/code
Dependent variable			
Tetanus immunity	Binary	Not protected	0
		Protected	1
Independent variables			
Sex	Binary	Male	0
		Female	1
Age (years)	Categorical ordinal	15-19	1
		20-24	2
		25-29	3
		30-34	4
		35-39	5
		40-44	6
		45-49	7
		50-54	8
		55-59	9
	Continuous	15-59	15-59
Province	Categorical nominal	26 provinces	1 to 26
Type of residence	Categorical nominal	Urban	1
		Rural	2
Education	Categorical ordinal	No education	0
		Primary	1
		Secondary	2
		Higher	3
Wealth index	Categorical ordinal	Lowest	1
		Low	2
		Middle	3
		High	4
		Highest	5
Ethnicity	Categorical nominal	BasKasai & KwiluKwngo	12
		Cuvette Central	13
		Ubangi & Itimbiri	14
		Uele Lac Albert	15
		Basele-K, Man. & Kivu	16
		Kasai, Katanga, Tanganika	17
		Lunda, Pygme, other	18
Occupation	Categorical nominal	Not working	0
		Pro/tech/managerial	1
		Sales/clerical	3
		Agriculture	4
		HH/domestic service	6
		Laborer	9
		Army	10

Variable	Type	Definition	Measure/code
Health insurance status	Binary	No	0
		Yes	1
Literacy	Categorical ordinal	Cannot read at all	0
		Read part of sentence	1
		Read whole sentence	2
		Not assessed	3
Marital status	Categorical nominal	Not Married	0
		Married	1
		Living with Partner	2
		Widowed/divorced	3
		Not Living with Partner	4
Religion	Categorical nominal	No religion	0
		Catholic	1
		Protestant	2
		Local Religion	3
		Other - Christian	4
		Muslim	5
Women's births in the last 5 years	Categorical ordinal	No births	0
		1	1
		2	2
		3	3
		4-5	4
Women's prenatal visit to any provider	Binary	No	0
		Yes	1
Women's visit to prenatal provider	Categorical nominal	No visit	0
		Facility based staff	1
		Others	2
		Combined	3
Women's age at first child's birth	Categorical ordinal	10-14	1
		15-19	2
		20-24	3
		25-29	4
		30-42	5
Women's reported # TT before last child's birth	Categorical ordinal	0	0
		1-2	1
		3-4	2
		5+	3
		Do not know	8
Women's total children born	Categorical ordinal	0	0
		1-2	1
		3-4	2
		5-9	3
		10+	4

Data Analysis Plan

Data received from the DHS program was transformed into a SPSS dataset and a codebook was developed with variables named and each variable coded. The data were reviewed for missing variables and decisions were made on values that were missing, small in number, or that contained inconsistent data. Following this process literacy, religion, and occupation were recoded and the variable for the number of years since a tetanus vaccination was not used. One variable response had missing data, 0.2% of the variable “literacy “was missing. The missing data for literacy was imputed as “not assessed” to allow the variable to be included in the analysis. Each variable was named and codes updated as modifications were made.

The data from this study was analyzed using IBM SPSS version 25. Sample weights and strata were obtained from the 2013–2014 DRC DHS data set from which the data were drawn and incorporated into the SPSS complex sample design module. The sample design module incorporated the strata, clustering, and sample weight of the survey and was then used for all the analysis.

Univariate analysis was conducted to understand the distribution, central tendency, and dispersion of the variables. Categorical variable analysis included the percentage and 95% confidence intervals (CI ; binary, ordinal, nominal). For the continuous variable age shown on Table 3, the mean, median, range, and 95% confidence intervals are described. Immunity against tetanus was stratified by all variables to review susceptibility and identify trends and gaps in immunity among subgroups. In this study Rao-Scott chi square tests, which account for the DHS study design, were used to identify

demographic characteristics that were significantly associated with protective immunity against tetanus at $p < 0.05$ (Rao & Scott, 1984 ; Appendix A, Tables A1-A3). These variables were considered for inclusion in the multivariate logistic regression model. Variables that were not statistically significantly associated with the dependent variable were reviewed and removed from the model.

Covariates were tested using Pearson's rho to identify covariates that are correlated and then one of the pairs that were found to be correlated were not entered in the final model. Literacy was significantly correlated to education rho = .803, p = .001 and literacy was excluded from the model. Descriptive analysis was conducted to describe all variables and their features.

Variables with the strongest association described above were included in the multivariate model. The logistic regression model was created using the complex sample module recommended by DHS statisticians (Croft, Marshall, & Allen, 2018). The final models contained predictors that were significant at the threshold of $p < 0.05$. Results from the multiple logistic regression model are displayed using adjusted odds ratios (AOR) with p values and confidence intervals in Tables 8 and 9.

Threats to Validity

In this study, archived data from a large cross-sectional nationally representative survey that used a stratified cluster sampling design were analyzed. Threats to internal validity are more common in studies with experimental designs where researchers are faced with various threats to intervention groups such as death, extreme scores, and familiarity with test instruments that may affect the outcome; all or some of them may

call into question the cause and effect of the design. For experimental research, measures can be built into the study design to counter these threats (Creswell, 2009; Trochim, Donnelly, & Arora, 2015).

This study did not try to establish a causal effect because immunity against tetanus following the recommended vaccination schedule has already been established (Roper et al., 2017). A nonexperimental, quantitative, observational cross-sectional survey using probability sampling did not have the experimental design threats to internal validity. Based on DHS sampling methods of the study design, this study avoided the internal validity threats of selection and information bias. The updated sampling frame minimizes the risk of selection bias which can happen if the sampling frame is outdated and nonrepresentative of the current population. The use of probability sampling ensured random selection and preselection of clusters at the central coordination unit minimized selection bias, which can happen when field staff select easily accessible clusters or households (Cutts et al., 2013). It is also a general health and household survey so participants may be more likely to take part than if this were for a specified health outcome, due to rumors, misconceptions, or myths. Finally, the serosurvey sample split was conducted to avoid selection bias through the rigorous randomized division process. Information bias may occur as information gathered on adult immunization against tetanus was based on recall; however, survey enumerators were well trained and spoke the local language to enable the respondent to feel at ease and not pressured for an assumed answer (Cutts et al., 2013; DHS, 2013).

External validity threats occur when researchers generalize study findings well beyond the study to other groups, places, or times that were not intended. This happens when there are unique characteristics of the sample or the setting and unreproducible features in the study design (Creswell, 2009; Trochim et al., 2015). External validity had the potential to be a legitimate threat to the study. However, to minimize threats to external validity, the study had a representative sample at national and provincial levels, and the stratified selection of clusters were incorporated into the study design and analysis. While this study may not be generalizable to all countries in central Africa, the DHS has been replicated in over 90 countries improving the comparability of immunization data; recent serosurveys and the literature review suggest a pattern found in other countries in sub-Saharan Africa (Scobie et al., 2017). The DHS began in 1984 and since then over 300 surveys have been conducted (ICF International, 2018). The DHS is a nationally representative survey providing data on health and populations using specific modules or questionnaires on child health, family planning, reproductive health, nutrition, and more. These large surveys are conducted about every 5 years in low- and middle-income countries and generally include 5,000 to 30,000 households per country based on population size. Modifications are allowed, but they are kept to a minimum to maintain comparability and to limit the complexity of an already lengthy questionnaire. The data collected by DHS can be comparable across countries. This is because DHS uses standard model questionnaires including the DHS Model Household Questionnaire, the DHS Model Woman's Questionnaire, and the DHS Model Men's Questionnaire (DHS, 2013).

To ensure data were gathered correctly, consistently, and with continuous quality supervision, the DHS had an extensive training program for supervisors on survey implementation and intensive training for interviewers (Hancioglu & Arnold, 2013; Ministry of Planning and Implementation of the Modern Revolution et al., 2014). Training materials were continuously reviewed and updated by subject matter experts supported by ICF and international partners. Data were double entered into a database designed for the questionnaire that had consistency checks and computation of sampling errors. Data were checked while the teams were in the field so errors could be corrected. The DHS used handheld devices for many questionnaires to reduce transcription error and to collect GPS points for cluster locations (Cutts et al., 2016; DHS, 2013; Ministry of Planning and Implementation of the Modern Revolution et al., 2014).

Given the history, the rigor in the survey sampling process, the degree of standardization, and the quality control the DHS has been cited as one of the best data sources to use to compare health indicators and immunization data from middle- and low-income countries (Cutts et al., 2013; Murray et al., 2003). This provides support for the survey design and the potential to reproduce the nested serosurvey because the results will provide concise data for programmatic change required by the Ministry of Health and the development partners. This is important because this is the first DHS in Africa to include biomarkers for vaccine preventable diseases for adults and children nationwide.

The study is generalizable to the DRC with the exceptions of the limitations that have been previously mentioned and the mobile and remote subpopulations that were not included in the study population. To avoid threats to construct validity precise definitions

and measure of variables were included in the study design. Threats to statistical conclusions can occur because a study either did not have the appropriate statistical power or violated statistical assumptions regarding the data because of poor statistical power (Creswell, 2009). The appropriate statistical analyses were conducted to test the hypotheses that are put forward in this proposal prior to the onset of the study and the statistical power was evaluated once the study sample was finalized.

Ethical Procedures

The DRC DHS and the nested serosurvey for tetanus antibodies were approved by the DRC Ethical Review Board in 2012. All participants were given an explanation of the reasons for the survey, the ways the collected data would be used and disseminated, and the reasons why blood specimens were being collected. This information was fully disclosed and interviewers obtained consent from the participants. Rules for data confidentiality and data integrity were applied to the DHS. The data are de-identified, and geospatial data points (GPS) are kept in a separate file. Linkages can be made between DHS data and serology through a unique barcode assigned to each individual. For the DHS, ICF International has reviewed ethical concerns associated with the survey, and a plan to address them is included in the handbook for the DHS (DHS, 2013). As required by the Walden Internal Review Board, I completed the course on Human Research Protections Training offered by the National Institutes of Health Office of Extramural Research. All DRC DHS data were kept confidential. All data were stored on a password-protected computer located in a locked office when not in use. All pertinent information generated from the study was shared with the DRC Ministry of Health for programmatic

decision-making. All initial analyses will be reviewed with the Ministry prior to any presentation in an international conference or publication.

Summary

In chapter three I provided a detailed review of the research methods for this quantitative, observational cross-sectional study using secondary data. The nationally representative stratified sample has an advantage because it allows DRC Ministry of Health to review key demographic and health indicators and trends. In addition, the nested serosurvey for immunity against tetanus allows for analysis and generalization at the national and provincial levels. The first DHS was conducted in the DRC in 2007, though without a nested serosurvey.

This research used archival data from the 2013–2014 DRC DHS and anonymized laboratory results from the serosurvey nested within the DHS. Following the precise and standardized data cleaning procedures, the appropriate univariate and bivariate analyses were conducted to identify the independent variables potentially related to immunity against tetanus by their statistical significance. These were followed by tests of collinearity to inform the inclusion of independent variables in the multivariate analysis. Potentially significant threats to internal and external validity have been addressed by DHS investigators in their methodology. All procedures to assure ethical assurances and considerations have been addressed through DHS procedures.

The study had a sufficient sample size, with results from 8,120 sero samples with corresponding survey data included in the study. The study sample was determined sufficient for generalization with the provincial sample size ranging from 201 to 701 per

province with statistical power of 0.9122 calculated for the minimum provincial sample size of 201. Therefore, the final analysis was done for the 26 new provinces to allow for programmatic use by the Ministry of Health.

The data were requested from both the DHS and from UCLA following the receipt of Walden IRB approval. The Walden IRB approval number for this study was 01-15-19-07769. The data analysis plan included univariate analysis, bivariate analysis using Rao-Scott chi-square test, and multiple logistic regression using a complex sample model to account for the study design. Variables with the strongest associations were retained in the model.

Threats to validity of this study were assessed and proper steps were taken to ensure appropriate statistical power to avoid statistical conclusion validity which may be the largest threat. Threats to external validity were acknowledged. Although findings from the DRC DHS may not be generalizable to all countries, there will be components that are comparable to other countries in the region due to immunization practice.

To summarize, Chapter 3 described the research methodology that was used to conduct the study, and Chapter Four contains the detailed summary of the analysis.

Chapter 4: Results

This chapter provides the results of the observational cross-sectional study using data from the 2013–2014 DHS, coupled with the results from a nested serosurvey to assess the protective immunity against tetanus among the adult population in DRC. The research questions examined the association between protective immunity against tetanus and independent variables of age, sex, province, place of residence (urban, rural), and women reporting to have at least one visit to a provider of antenatal care services in the past 5 years. Other important factors, such as education, occupation, ethnicity, religion, literacy, and health insurance, were also explored. This chapter provides an overview of the demographic and socioeconomic characteristics of the study sample, reviews the research questions and hypotheses, reports the statistical analyses, and summarizes the findings.

Characteristics of the Sample

In total, 8,120 adults were included in the unweighted sample. Due to the complex sampling of the survey, the sample was weighted as described in the methodology, and these weights were used for all analyses, figures, and tables. The weighted sample size for this study was 8,169.

Demographic Characteristics and Results of Bivariate Analysis

This study included women from 15 to 49 years of age, reflecting women of childbearing age, and men from 15 to 59 years. In this study, 40% of the population were 15–24 years of age, and a third were between ages 25 and 34. Overall, 53% of the study population was women and comprised 58% of 20–24-year-olds, 61% of 25–29-year-olds,

and 57% of 35–39-year-olds. Women had a mean age of 28.4 years ± a standard deviation of 9.5 years, compared to men, who had a mean age of 31.6 years ± a standard deviation of 12.2 years.

DRC is a predominately rural country. Therefore 64% of the sample was drawn from rural areas and only 36% from urban areas. Every province had participants from both rural and urban areas except for Kinshasa, which is the only all-urban province (see Figure 1). The 14 major ethnicities of DRC were represented (see Table 2 and Figure 3), with Kasai, Katanga, and Tanganyika ethnicities making up 26% of the study population; followed by 19% for Basele-K, Man, and Kivu ethnicities from North and South Kivu and Maniema; and 17% for Bas-Kasai and Kwilu-Kwango ethnicities primarily found in Kwilu province. Kinshasa province had the most diverse number of ethnic groups.

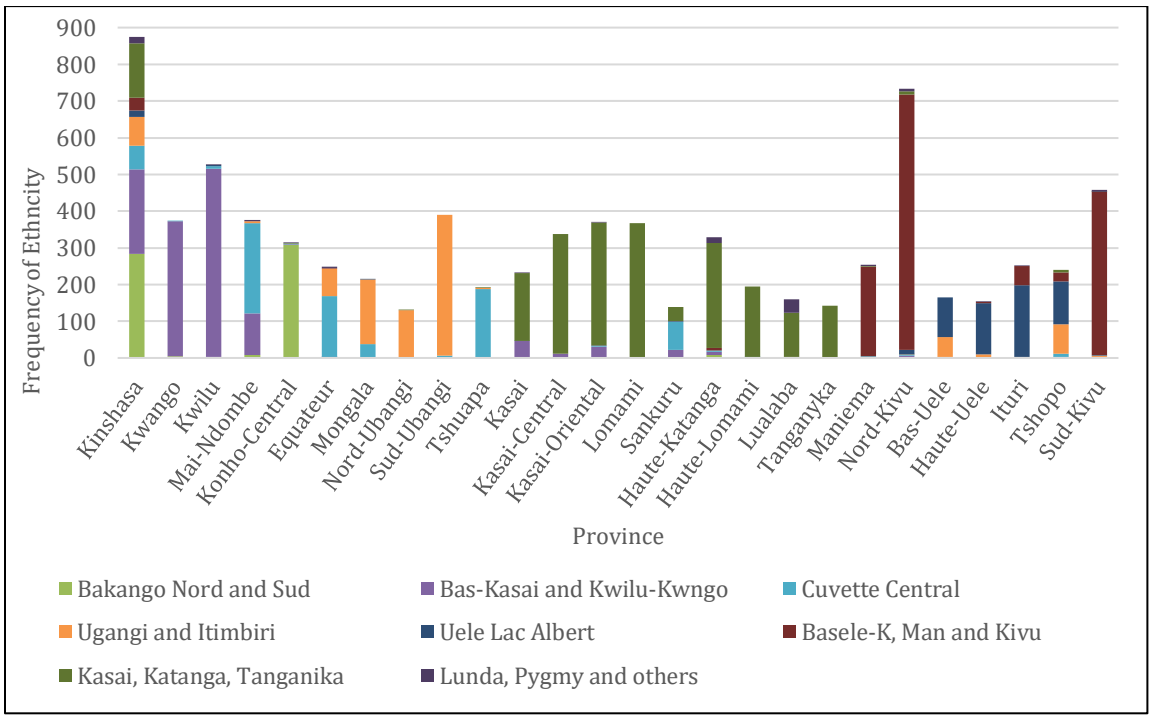

Figure 3. Ethnicity by province in DRC 2013–2014.

Among all participants, 11% had no formal education, 31% completed primary school, 53% completed some or all secondary education, and 5% had gone on for further studies. Overall 40% of men and women of the study population continued to secondary school, while 16% of women and 4% of men did not have a formal education (see Figure 4). Most adolescents in this study, both boys and girls, attended at least some primary school.

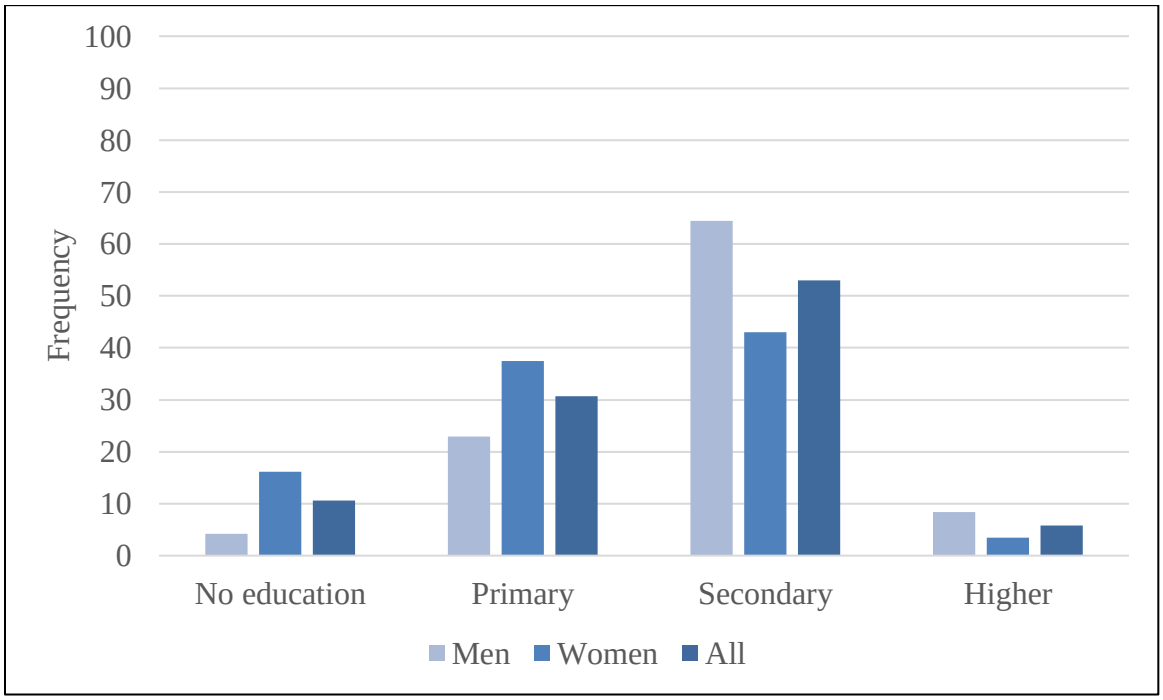

Figure 4. Education in DRC, 2013–2014.

In this study, 76% of participants were working; and of those working, 43% worked in agriculture where a majority were self-employed, 15% worked in sales or clerical positions, and 6% held professional or managerial positions (Appendix A, Table A1). Of the working study population, 64% were women who made up 53% of the agriculture workers, while men made up 92% of the laborer work force and 80% of professional and managerial positions.

Regarding wealth, close to one third of the participants were in the poorest or poor categories of the wealth index, 22% in the richest category, and the remaining 39% in the middle and high wealth categories. The wealth index represented family wealth, documenting assets such as housing type, access to safe water supply, and transportation and was not reflective of individual wealth. Regarding marital status, 62% were married or living with their partner, whereas 31% were never married, and 7% were widowed, divorced, or separated. Predominant religions included Catholic (29%), Protestant (28%) and other Christian religions (36%), while Islam and local religions accounted for 6% and a small minority had no faith.

In the 4 months prior to the DHS, 16% of women and 14% of men went to a health facility for medical treatment. The primary reason was malaria or fever; however, 5% of men went to a health facility because of an injury (Ministry of Planning and Implementation of the Modern Revolution et al., 2014). Health insurance was limited in DRC; only 4% of respondents had health insurance (Appendix A, Table A1). Of the 4,349 women in this study, 25% never had children, 25% had one to two children, 20% had three to four, and 30% had over five. Of the women who had been pregnant, the range of age at first birth was 10 years to 42 years.

Of the 2,581 women in this study who were currently pregnant or had a pregnancy within the past 5 years, 87% had been seen by a provider of antenatal care at least once. By age group, 94% of those 15–19 years old and 91% of 20–24-year-olds made at least one antenatal visit (Appendix A, Table A2). These percentages were much lower in women who were pregnant in older age groups, where only 79% of those 40–44

and 77% of those 45–49 years of age made a visit during their pregnancy. This disparity in antenatal visits by age was probably responsible at least in part for the finding that only 57% of women 40–49 compared to 74% of those 15 to 39 received at least one dose of TTCV (see Figure 5).

Vaccination data were self-reported with no documentation submitted for verification. Without documentation and a review of the immunization record, the verity of the self-report cannot be ascertained. However, in this study the blood samples were collected and tested to serve as a proxy and provide evidence of tetanus vaccination and seroprotection among women (Appendix A, Table A2).

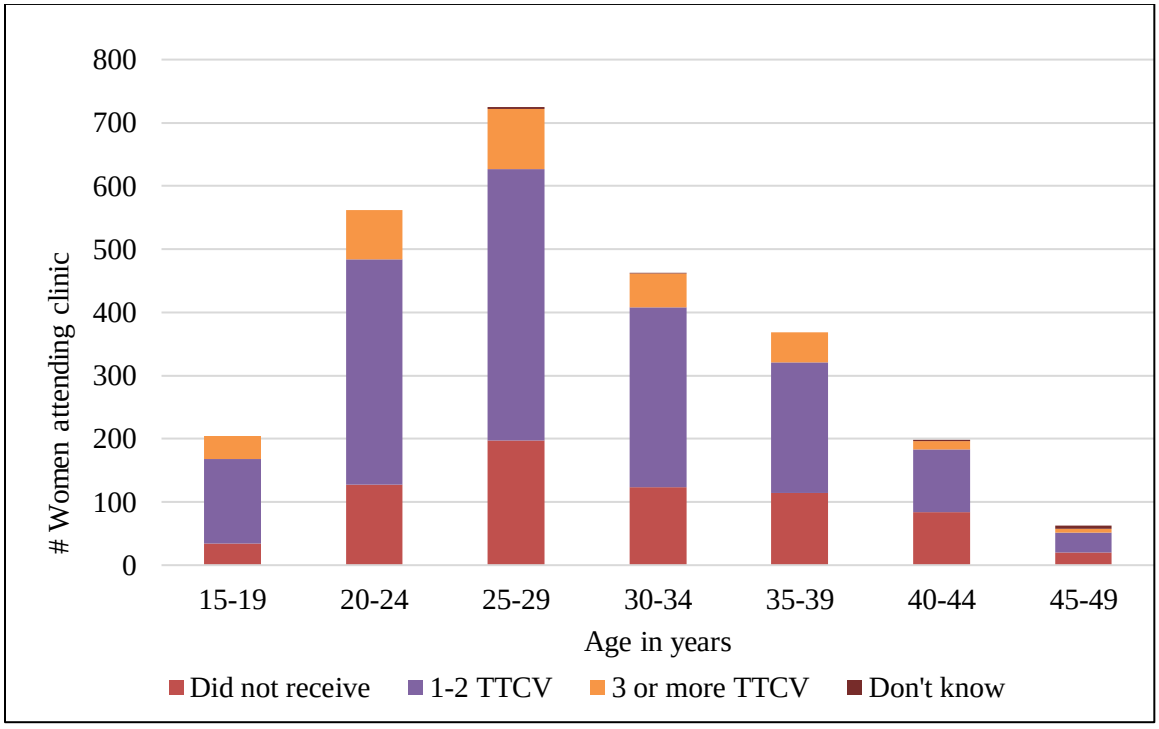

Figure 5. Women attending antenatal clinic with receipt of TTCV by age, 2013–2014.

Many variables in the bivariate analysis had a significant association with protective immunity in the combined analysis of men and women (see Appendix A,

Table A1), including sex, age, province, urban/rural, education, ethnicity, occupation, literacy, marital status, and religion. Several variables were not included in the final model including literacy due to collinearity and marital status because it was not significant for men p = 0.638 or women p = 0.236 (see Appendix A, Tables A2 and A3). Wealth index (p = 0.715) and health insurance (p = 0.938) were not found to be associated with protective immunity against tetanus and were also not included in the model (see Appendix A, Table A1). The variables included in the final model were sex, age, province, urban/rural, education, ethnicity, occupation, and religion.

Research Questions and Hypotheses Testing

To answer the four research questions, each hypothesis was tested to assess potential associations between the independent variable and dependent variables. For each of the hypotheses, Rao-Scott chi square and complex sample multiple logistic regression models were used while controlling for covariates.

RQ1: Is there an association between age and protective immunity against tetanus among adults in DRC?

$H_0$1: There is no association between age and protective immunity against tetanus among adults in DRC.

H_a1: There is an association between age and protective immunity against tetanus among adults in DRC.

The results showed that age was significantly associated with protective immunity (χ^2 = 485.6 with p = 0.001). The odds of protection among the 20–24-year-olds was over twice as high (OR 2.43), while the odds of protection in the 25–34-year-old group was

three and a half times as high (OR 3.54), as in those aged 15–19, and the odds of protection showed a decrease in those aged 35 and older (see Table 4). The covariates sex, province, religion, occupation, and education were entered into the model. Age remained significant in the multivariate model (p = 0.001). A similar result was observed in the adjusted analyses for 20–25-year-olds (AOR 2.94, 95% CI 2.29 – 3.77). However, the odds of protection among the age group 30–34 was much higher (AOR 5.24, 95% CI 3.92 - 7.00). The odds of protection then declined with increasing age as it did in the unadjusted analyses, with the exception being men 50–59 (AOR 2.85, 95% CI 1.88 – 4.31). Therefore, I rejected the null hypothesis that stated there is no association between age and protective immunity against tetanus among adults in DRC.

Table 4

Bivariate and Multivariate Regression Model for Association of Protective Immunity Against Tetanus and Age

Independent Variables										
	χ^2	p	UOR	95% CI		Wald	p	AOR	95% CI	
Characteristics				LL	UL				LL	UL
Age (years)										
15-19	485.6	0.05	1			39.6	0.001	1		
20-24			**2.43***	1.99	2.97			**2.94***	2.29	3.77
25-29			**3.54***	2.84	4.41			**4.40***	3.34	5.81
30-34			**3.54***	2.77	4.54			**5.24***	3.92	7.00
35-39			**2.92***	2.31	3.68			**3.69***	2.72	5.02
40-44			**2.05***	1.65	2.55			**2.77***	2.15	3.57
45-49			**1.96***	1.53	2.52			**2.53***	1.88	3.39
50-59			0.57	0.39	0.81			**2.85***	1.88	4.31

Note. * p < 0.05 bold is considered significant in model. Variables adjusted for in the model: sex, province, education, occupation, religion. 50-59 age group is only men. UOR is the unadjusted odds ratio, LL is the lower limit and UL is the upper limit of the 95% confidence interval.

RQ2: Is there an association between sex and protective immunity against tetanus among adults in DRC?

$H_0$2: There is no association between sex and protective immunity against tetanus among adults in DRC.

H_a2: There is an association between sex and protective immunity against tetanus among adults in DRC.

Sex was the most significant predictor of protective immunity (χ^2 = 1917.86, p = 0.001). In the bivariate model, the odds of women being protected against tetanus were nine times as high as in men (OR = 9.10, 95% CI 7.86–10.53). All significant covariates from the bivariate analysis (sex, age, province, education, religion, and occupation) were entered into the multivariate model, and after controlling for them, sex and nothing else remained significant (p = 0.001) with an AOR 10.46 (95% CI 8.88-12.33; see Table 5).

Table 5

Bivariate and Multivariate Logistic Regression Models for Association of Protective Immunity and Sex

Independent Variables										
	χ^2	p	UOR	95% CI		Wald	p	AOR	95% CI	
Characteristics				LL	UL				LL	UL
Sex										
Men	1919	0.1	1			794	0	1		
Women			**9.10***	7.86	10.53			**10.46***	8.88	12.33

Note. * p < 0.05 bold is considered significant in the model. Variables adjusted in the model: age, province, education, occupation, religion.

As shown in Figure 6 the percentage of women protected, compared to men of the same age, was higher in every age category with the adjusted odds of women being more than 10 times as likely to be protected.

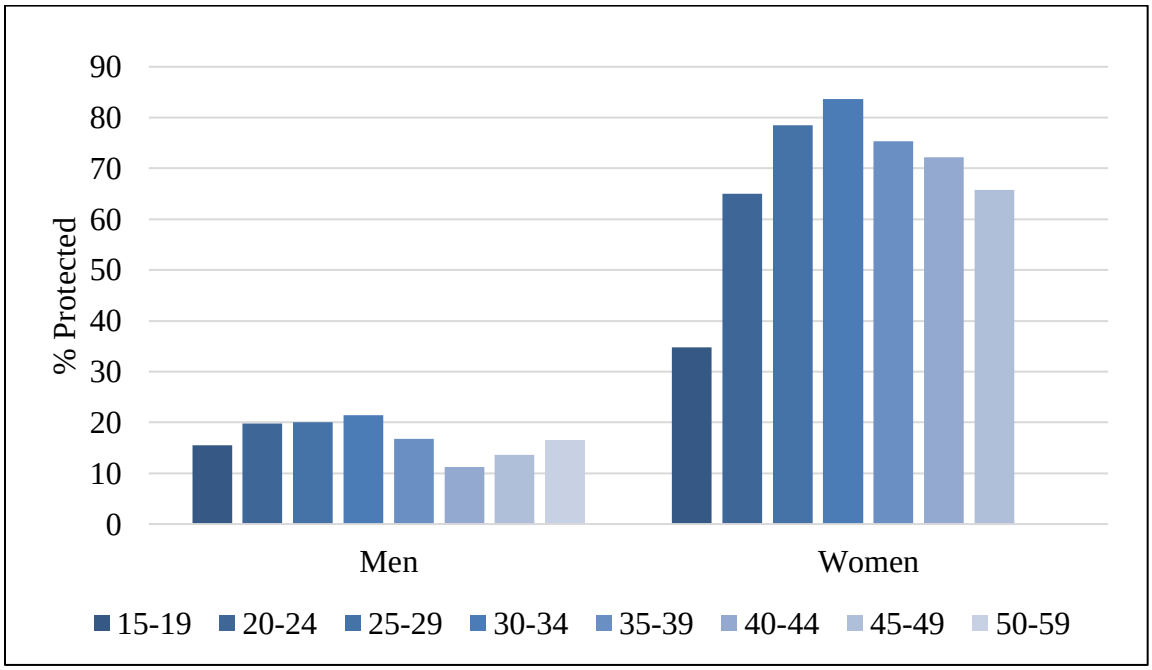

Figure 6. Men and women by age with protective immunity against tetanus in the DRC 2013–2014.

After controlling for confounders, the strongest predictor for protective immunity against tetanus was a woman. Therefore, I rejected the $H_0$2 null hypothesis that stated there is no association between sex and protective immunity against tetanus.

RQ3: Is there an association between province and place of residence (urban/rural) and protective immunity against tetanus among adults in DRC?

$H_0$3 – There is no association between province and place of residence (urban/rural) and protective immunity against tetanus among adults in the DRC.

H_a3 – There is an association between province and place of residence (urban/rural) and protective immunity against tetanus among adults in the DRC.

In the initial bivariate analysis, urban/rural area of residence was not found to be significant ($\chi^2 = 0.3$, $p = 0.676$; see Appendix A, Table A1). In stratified analyses by sex, the association between place of residence and protective immunity was significant for

women ($\chi^2 = 9.3$, p = 0.002, see Appendix A, Table A2) but not for men ($\chi^2 = 0.64$, p = 0.424, see Appendix A, Table A3).

For men, the odds of men being protected were slightly higher, but not significant, in an urban environment (AOR 1.1, 95% CI 0.87-1.40 ; see Appendix A, Table A2) than in rural areas. The odds of women being protected were 1.5 times as high in an urban area than in a rural environment (see Appendix A, Table A3 ; AOR 1.50, 95% CI 1.18-2.14).

Province was found to be significantly associated with protective immunity ($\chi^2 = 74.7$, p = 0.007) after adjusting for age, sex, occupation, and religion (Table 6). In Kwilu and Equateur provinces, the odds of protection for adults were one and a half times as high as in Kinshasa (AOR 1.57, 95%, CI 1.1-2.25) and (AOR 1.67, 95% CI 1.12-2.51). In Tanganika the odds of protective immunity against tetanus were slightly more than half that of Kinshasa (AOR 0.58, 95% CI 0.43-0.78) making living in Tanganyika a potential risk factor for lack of protective immunity. Therefore, I rejected the null hypotheses $H_0$3 that stated there is no association between province and protective immunity against tetanus among adults in DRC, while I did not reject the null hypotheses that stated there is no association between place of residence (urban/rural) and protective immunity against adults in the DRC.

Table 6

Bivariate and Multivariate Logistic Regression Results for the Association of Protective Immunity and Province

Independent Variables	χ^2	p	UOR	95% CI		Wald	p	AOR	95% CI	
Characteristics				LL	UL				LL	UL
Province										
Kinshasa	74.5	0.05	1			39.6	0.001	1		
Kwango			1.19	0.94	1.51			1.34	0.96	1.87
Kwilu			**1.49***	1.13	1.96			**1.67***	1.12	2.51
Mai-Ndombe			0.98	0.70	1.38			1.04	0.65	1.68
Konho-Central			1.07	0.83	1.37			1.14	0.83	1.57
Equateur			1.17	0.94	1.46			**1.57***	1.10	2.25
Mongala			1.17	0.84	1.62			1.30	0.89	1.89
Nord-Ubangi			**1.36***	1.01	1.82			**1.57***	1.09	2.27
Sud-Ubangi			1.21	0.82	1.79			1.42	0.83	2.41
Tshuapa			0.77	0.57	1.04			0.74	0.46	1.19
Kasai			0.64	0.35	1.16			0.61	0.31	1.19
Kasai-Central			1.17	0.82	1.68			1.34	0.80	2.23
Kasai-Oriental			1.19	0.98	1.44			**1.42***	1.05	1.92
Lomami			1.07	0.89	1.28			1.19	0.94	1.51
Sankuru			0.74	0.44	1.25			0.66	0.32	1.36
Haute-Katanga			1.00	0.71	1.42			1.07	0.64	1.78
Haute-Lomami			0.97	0.72	1.30			1.11	0.72	1.72
Lualaba			1.22	0.93	1.61			**1.41***	1.01	1.98
Tanganika			0.58	0.43	0.78			0.48	0.34	0.70
Maniema			0.95	0.70	1.28			0.94	0.62	1.43
Nord-Kivu			0.84	0.62	1.13			0.81	0.54	1.22
Bas-Uele			1.19	0.80	1.77			1.39	0.94	2.06
Haute-Uele			1.09	0.82	1.44			1.18	0.81	1.74
Ituri			1.03	0.68	1.58			1.15	0.65	2.02
Tshopo			0.77	0.48	1.22			0.76	0.42	1.38
Sud-Kivu			0.98	0.72	1.33			0.97	0.67	1.40

Note. * $p < 0.05$ bold is considered significant in the model. Variables adjusted for in the model: age, sex, education, occupation, religion.

RQ4: Is there an association between antenatal care and protective immunity against tetanus among adult women in the DRC?

$H_0$4 - There is no association between antenatal care and protective immunity against tetanus among adult women in the DRC.

H_a4 - There is an association between antenatal care and protective immunity against tetanus among adult women in the DRC.

Only women who reported a live birth within the past five years were asked if they had made a visit to a provider of antenatal care. There was a statistically significant association in the unadjusted analysis between antenatal care and protective immunity against tetanus among women in the DRC, ($\chi^2 = 18.35$, $p = 0.001$). Women receiving antenatal care had twice the odds of protective immunity as those that did not receive antenatal care (OR 2.15, 95% CI 1.51-3.05).

Figure 7 shows the proportion of men, nulliparous and parous women in each five-year birth cohort with protective immunity. While all individuals born between 1984 and 1999 had the opportunity for childhood immunization, it is unlikely there was an opportunity for a booster dose until women were of childbearing age and pregnant or until either men or women were injured. The percentage of men that were protected rarely exceeded 20%, while among nulliparous women, the percentage was high in the early birth cohorts but fell dramatically with later birth cohorts. These results in teenage men and nulliparous women reflected missed opportunities for immunization and waning immunity in these cohorts.

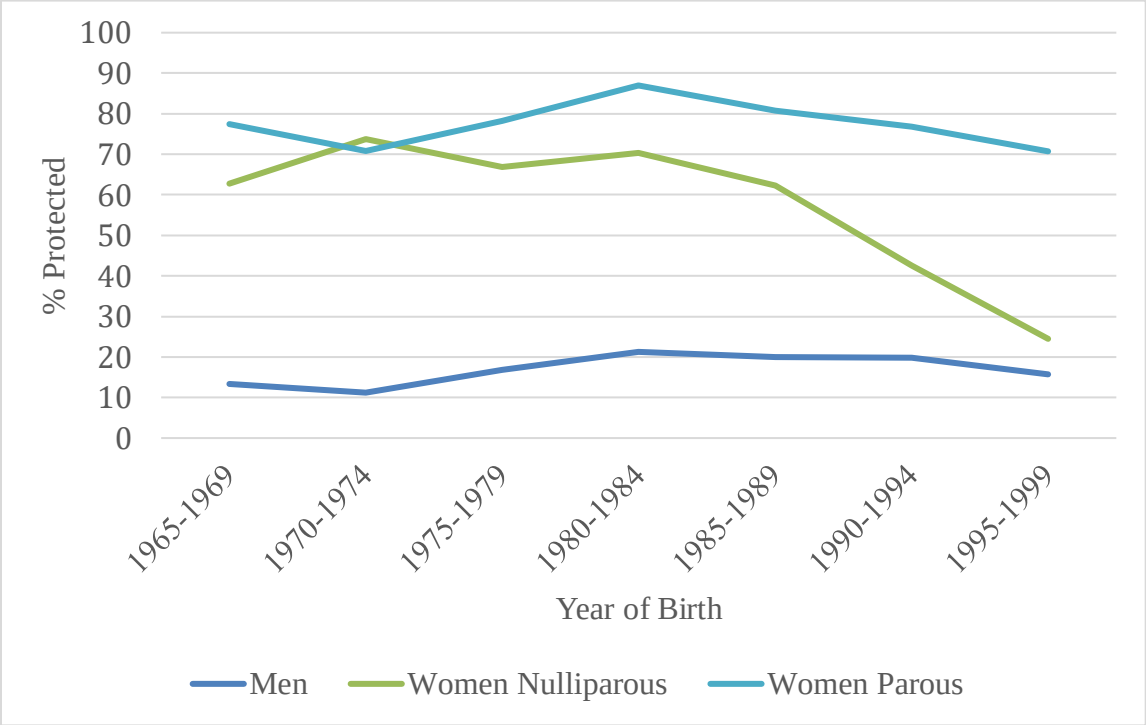

Figure 7. Protective immunity against tetanus in men, nulliparous and parous women by year of birth in 5-year increments in DRC 2013–2014.

In the multivariate model shown in Table 7 receiving antenatal care remained significantly associated with protective immunity (AOR 2.18, 95% CI 1.53 - 3.09) after adjusting for covariates age, province, urban/rural, education, ethnicity, wealth, and number of TTCV before the last birth. Those who received antenatal care were a little more than twice as likely to have protective immunity than those that did not report such care. Therefore, I rejected the null hypotheses H04 stating there is no association between antenatal care and the level of protective immunity against tetanus among adult women in the DRC.

Table 7

Bivariate and Multivariate Logistic Regression Results for the Association of Protective Immunity and Women

Independent Variables	χ^2	p	UOR	95% CI		Wald	p	AOR	95% CI	
Prenatal visit to any provider				LL	UL				LL	UL
No	18.35	0.05	1			20.8	0	1		
Yes			**2.15***	1.51	3.05			**2.18***	1.53	3.09

Note. * $p < 0.05$ bold is considered significant in the model. Cox and Snell $R^2 = 0.27$. Variables adjusted for in the model: age, province, urban/rural, education, prenatal care, ethnicity, wealth, # TTCV before last birth

The final multiple regression model for men and women identified sex, age and province as the three factors that were associated with protective immunity against tetanus (Table 8). The three independent variables, sex, age, and province, together account for 27% of the explanation of why adults had protective immunity against tetanus.

Table 8

Multiple Regression Model of Factors Associated with Protective Immunity Total Sample

Independent Variables Characteristics	SE	Wald	p	AOR	95% CI	
					LL	UL
Sex			0.001	1		
Men						
Women	0.083	794.1		**10.46***	8.88	12.33
Age (years)						
15-19		156.7	0.001	1		
20-24	0.13			**2.94***	2.29	3.77
25-29	0.14			**4.40***	3.34	5.81
30-34	0.15			**5.24***	3.92	7
35-39	0.16			**3.69***	2.72	5.02
40-44	0.13			**2.77***	2.15	3.57
45-49	0.15			**2.53***	1.88	3.39
50-59	0.21			**2.85***	1.88	4.31
Province						
Kinshasa		74.7	0.001	1		
Kwango	0.17			**1.34**	0.96	1.87
Kwilu	0.21			**1.67***	1.12	2.51
Mai-Ndombe	0.24			1.04	0.65	1.68
Konho-Central	0.16			1.14	0.83	1.57
Equateur	0.18			**1.57***	1.1	2.25
Mongala	0.19			1.3	0.89	1.89
Nord-Ubangi	0.19			**1.57***	1.09	2.27
Sud-Ubangi	0.27			1.42	0.83	2.41
Tshuapa	0.24			0.74	0.46	1.19
Kasai	0.34			0.61	0.31	1.19
Kasai-Central	0.26			1.34	0.8	2.23
Kasai-Oriental	0.15			**1.42***	1.05	1.92
Lomami	0.12			1.19	0.94	1.51
Sankuru	0.37			0.66	0.32	1.36
Haute-Katanga	0.26			1.07	0.64	1.78
Haute-Lomami	0.22			1.11	0.72	1.72
Lualaba	0.17			**1.41***	1.01	1.98
Tanganika	0.19			0.48	0.34	0.7
Maniema	0.21			0.94	0.62	1.43
Nord-Kivu	0.21			0.81	0.54	1.22
Bas-Uele	0.2			1.39	0.94	2.06
Haute-Uele	0.2			1.18	0.81	1.74
Ituri	0.29			1.15	0.65	2.02
Tshopo	0.3			0.76	0.42	1.38
Sud-Kivu	0.19			0.97	0.67	1.4

Note. * $p < 0.05$ bold is considered significant. Cox and Snell $R^2 = 0.26$. Variables adjusted for in the model: age, sex, province, education, occupation, religion. SE refers to standard error.

In summary, the final multiple regression model for women identified two factors, age and prenatal visits, that were associated with protective immunity against tetanus (Table 9). The six independent variables, age, province, education, occupation, religion, and prenatal visit to any provider, together account for 26% of the explanation of why women pregnant within the past five years had protective immunity against tetanus.

Table 9

Multiple Regression Model of Factors Associated with Protective Immunity for Women

Independent Variables Characteristics	SE	Wald	p	AOR	95% CI	
					LL	UL
Age (years)						
15-19		20.94	0.002	1		
20-24	0.25			1.40	0.86	2.27
25-29	0.24			**1.79***	1.11	2.90
30-34	0.29			**2.86***	1.62	5.05
35-39	0.29			1.57	0.89	2.75
40-44	0.28			1.14	0.65	1.99
45-49	0.46			1.66	0.67	4.12
Prenatal visit to any provider						
No		18.89	0.000	1		
Yes	0.18			**2.18***	1.53	3.09

Note. * $p < 0.05$ bold is considered significant. Variables adjusted for in the model: age, province, education, occupation, religion, prenatal visit to any provider.

Summary and Transition

The study findings were presented in this chapter. These included a summary of demographic, socioeconomic, and other characteristics of the study population. The statistical analyses that were conducted to answer the research questions were reviewed, and each of the four research questions was assessed to determine the variables significantly associated with immunity against tetanus in adults in the DRC. The

variables age, sex, and province were found to be significant in the all subjects model, while age and antenatal care were found to be significant in the women's model.

Sex was the most significant finding as women were more than 10 times as likely to have protective immunity against tetanus as men with an AOR of 10.46. Age was significant in the multivariate model, as 30–34-year-olds were more than 5 times as likely to be protected (AOR 5.24) as those 15 to 19 years of age. Province was also significant with multiple provinces having about half the protective immunity than Kinshasa. Finally, women that attended antenatal clinics were twice as likely to be protected against tetanus as pregnant women that did not attend them. Chapter 5 provides a discussion of the findings, the limitations of the study, recommendations for future research and action, and implications for positive social change.

Chapter 5: Summary, Conclusion, and Recommendations

Summary of the Study

This study had two major purposes. The first was to demonstrate that a serosurvey could be used to assess protective immunity against tetanus among adult populations in DRC. The second purpose was to identify the characteristics of populations at greatest risk of tetanus infection based on lowered immunity levels. In this chapter, I describe the results of the study to demonstrate that the purpose was achieved. Social change should occur because of the scientific evidence provided by the study findings adding to the body of evidence from the findings of serosurveys and immunity against tetanus in adults in DRC and in Africa. Chapter 5 includes a discussion of the study findings, recommendations for further research, ideas for social change, recommendations for action, and the conclusion to this study.

Discussion and Interpretation of Findings

One of the most significant findings from this study was the association between protective immunity and sex. The likelihood of women having protective immunity against tetanus was 10 times higher than in men. Overall, 65% of women and 17% of men were protected. These findings were similar to those of other studies in sub-Saharan Africa, including in Kenya where 96% of women were seroprotected compared to 45% of men; in Tanzania, where 94% of women were protected compared to 28% of men; and in Mozambique, where 90% of women and 64% of men were protected (Scobie et al., 2017). Women may receive TTCV at prenatal care visits and during MNTE campaigns while men may only be immunized as infants or if injured, and in the case of

Mozambique, in school. Immunization programs in most African countries were initially designed to provide vaccination during the first year of life and for women during pregnancy. Except Mozambique, few African countries provided immunization after the age of 1 year, adolescent or school-based vaccination, or catch-up immunization for those who were not fully immunized (Dalal et al., 2016; Mihigo et al., 2017).

In a recent DHS study on childhood immunization in DRC, Acharya Kismul, Mapatano, and Hatløy (2018) found that 4 of 5 children in DRC were from households headed by a man. When looking at risk factors, there are professions that result in more exposure to tetanus for men and women, including agriculture, manual labor, and military (Roper et al., 2017). In the current study in DRC, 59% of men and 14% of women were named as heads of their respective households. Of the female heads of households, 73% were protected against tetanus; only 13% of men in men-led households were. Being unprotected puts families at risk. This lack of protection against tetanus in men was also shown in a serosurvey in Uganda (Makumbi et al., 2019). Makumbi found 43% of men and boys between 10–49 years of age were unprotected against tetanus. In the Uganda study, participants were given two doses of TTCV 28 days apart, and 100% of the study population was protected against tetanus at the 42-day assessment. Tetanus mortality is high for those unprotected, as demonstrated by a retrospective record review conducted in Lubumbashi hospital in Haute Katanga Province, DRC (Muteya et al., 2013). Of the patients admitted to the intensive care unit with tetanus, 95% were men 30–50 years old, and 52% died from tetanus while in the hospital. The lower level of protection against tetanus in men was also highlighted by 13 cases of tetanus in men resulting in nine deaths

in six countries over a period of 6 years (CDC, 2016; Dalal et al., 2016). These deaths followed voluntary male circumcision procedures, and the subsequent investigations led to a change in WHO immunization policy that men should now be evaluated and vaccinated against tetanus prior to circumcision procedures (WHO, 2017).

The results in this study demonstrated an association between age and protective immunity with the odds of protection peaking at 2.5 to 3.5 times in those aged 20 to 35 compared to 15–19-year-olds. This age variation was largely driven by the increase in protective immunity in women. In a serosurvey in Laos to evaluate heath care workers' immunity to vaccine-preventable diseases, 90% of women versus 38% of men were protected against tetanus (Black et al., 2015). In Laos, like DRC, TTCV was not introduced until the late 1970s, so adults 34 or older were unlikely to be immunized against tetanus unless they received antenatal care or were injured. In Cambodia, Scobie et al. (2016) found that 63% of women aged 15–19 had protective immunity against tetanus, as compared to 87% of 20–24-year-olds. These percentages were much higher than those of this study; I found that 35% of women aged 15–19 were protected, compared to 65% of 20–24-year-olds in DRC. In contrast, among men in the DRC, only 15% of those 15–19 years of age and 20% of those 20–24 were protected against tetanus. As DRC has no school booster program, the current level of immunity could have been due to TTCV following an injury or vaccination for employment. The decline in immunity in adults with increasing age that was seen in this study was not surprising because adults born before the EPI program became well established may never have had

the opportunity to receive TTCV. Waning immunity with increasing age has also been documented in other studies of adults (Aboud et al., 2002; Roper et al., 2017).

The findings from this study demonstrated differences in protection against tetanus in the population across the provinces when compared with Kinshasa. Areas of low protective immunity in some provinces were also areas known for armed conflict, including Equator, North Kivu, and Tanganika (Grundy & Biggs, 2019).

Attending an antenatal care clinic was found to be associated with protection against tetanus. Women attending antenatal care were twice as likely to be protected against tetanus. These findings support the effectiveness of booster doses in pregnancy, which was also shown in a recent study in Cambodia (Scobie et al., 2016). The researchers found that in women who had received at least one dose of TTCV during their last pregnancy, as doses of TTCV increased, protection also increased, with 98% of women with two or more doses being protected. I found in the current study, of women that were currently pregnant or had given birth in the last five years, 81% of women reporting 1-2 doses of TTCV and 87% of women with 3-4 doses were protected against tetanus. Similar to this study, Cambodia women 15–19 years of age were the most likely to be unprotected against tetanus, and this was attributed to missing TTCV campaign doses and, if pregnant, the first booster.

In a study using DHS data in Kenya researchers reviewed antenatal visits and receipt of TTCV of women that had been pregnant within 12 months of the survey (Haile, Chertok, & Teweldeberhan, 2012). They found that 90% of pregnant women attended at least one antenatal clinic, and they estimated based on self-reports that 69% of the

women that had been pregnant in the past year had enough vaccinations to provide protection against tetanus at the birth of their child, although no serologic tests were conducted. In this study, among women who gave birth in the 12 months prior to the DRC DHS, 80% had serologic evidence of protection against tetanus.

Support for the Theoretical Framework

The results of this work indicate that a majority of women are immunized against tetanus, and that the presence of antibodies against tetanus provides immunological evidence of preventive action taken by the individual. For women of childbearing age and especially pregnant women, this evidence is important because antibodies against tetanus can only be acquired through vaccination. Preventive action occurs when a person acts from the following perceptions: (a)susceptibility to acquiring the disease, (b)severity of illness if acquired, (c)perceived personal benefits, (d)perceived barriers or negative aspects of action, (e)cues that may trigger action, and (f) a conviction that the individual can change behavior to achieve the desired outcome. HBM continues to be widely used to support public health intervention programs such as immunization and antenatal care (Glanz & Bishop, 2010). This model frames the key issues to address related to health-seeking behavior. HBM suggests that if the risk of the disease is known, then an individual will make the choice to prevent the disease if health-related interventions, such as immunization are made available to them and findings of high levels of protective immunity against tetanus from this study suggest this is the case for women during pregnancy.

This study has shown evidence of health-seeking behavior resulting in protective immunity against tetanus. The study results provide evidence of protection against tetanus that can only be achieved through immunization. The current DHS data provide evidence of health-seeking behavior of men and women. Given this information, the DRC should avail adults the opportunity for booster immunization against tetanus for men and women.

Limitations

This study had limitations in that I used secondary data from the DHS in which the variables were operationalized for the primary study and were not developed specifically for this study.

Another limitation was that the responses to the DHS questions related to antenatal care and to receiving tetanus vaccination were based on recall (Cutts et al., 2016). In addition, the DHS was only administered to those living in a house; adults living on military bases and in hotels, as well as homeless adults, were not included in this survey (DHS, 2013). Furthermore, mobile and other difficult to reach populations and people living in active conflict zones may not have been represented.

Although great care was taken to split original survey sample matched pairs evenly between the polio and tetanus serosurveys by province, cluster, sex, and age-strata, there is a probability the samples may have differed due to singletons of men or women. Finally, because men were not asked about immunization, there could not have been any comparison made between sex and vaccination history by recall or record (Corsi, Neuman, Finlay, & Subramanian, 2012).

Recommendations for Future Research

From this study there are several areas that would be appropriate for future research. The use of periodic serosurveys to support MNTE goals should be further evaluated as well as the extent that campaigns for women 15–49 years of age are effective in improving immunity in poorly performing areas (Eshetu et al., 2018). Further, the current study provided evidence of waning immunity and lack of immunity in adolescents. In contrast, data from Mozambique revealed that school-based programs had a substantial impact on boosting immunity in adolescents (Scobie et al., 2017). Therefore, operational research and innovative actions by the MOH partnering with the education department are recommended to reach primary school children in DRC for booster vaccination, as the current study found that a majority of children attended some primary school.

As the current study showed evidence of low immunity in men and adolescents and evidence of health-seeking behavior in the population in DRC, operational research should also be conducted within the health facilities to study the inclusion of TTCV for adults. Finally, the MOH in DRC should examine the feasibility of providing vaccination services within the outpatient setting and seek opportunities to educate clinicians to increase their knowledge, as was done by research in Turkey (Egici, Taş, Özkarafakılı, & Öztürk, 2018).

Recommendations for Action

Perhaps the most important recommendation for DRC would be to consider full implementation of the WHO recommendations for tetanus immunization (WHO, 2018).

This implementation would provide booster doses for all children over the age of 1 year and require school age immunization. To implement the WHO recommendations, the MOH in the DRC, together with supportive partners, would need to review the total cost needs for operations and vaccines, and the feasibility of implementing the recommendations for children over 9 months, school age children, and adults. This implementation should show a clear value as the cost to protect an individual against tetanus is estimated to be less than $1 for the vaccine and supplies (Burgess et al., 2017). These WHO recommendations should be reviewed within the context of the health systems that support the immunization program in the development of the overall plan (Kamadjeu, 2017). Serosurveys should be used to evaluate the immunity levels of children under 5, children 5–15-years of age and adults on a periodic basis.

Implications for Social Change

There have been several examples of outcomes of a DHS making contributions to health policy making (Nolan, Lucas, Choi, Fabic, & Adetunji, 2017). The current study provides scientific evidence for positive social change as the quantitative data showed gaps in protective immunity against tetanus and provided information on inequity in preventive immunization against tetanus by sex. Women were 10 times as likely to be protected against tetanus, and overall 17% of the men in the study had evidence of protective immunity. This study also showed the gap by age, specifically in young adults and provided evidence that both sexes have lower immunity as teens until women are targeted for MNTE services. These data can inform and shape immunization policy, support requests to government and donors for public health financing for immunization,

and focus health education efforts on the susceptible populations. Finally, this study provided data which the immunization program requires in assessing progress of MNTE, because using serology can be incorporated in MNTE assessments in the DRC and other countries.

Conclusions

There are many people who have spent their lifetimes working to close the immunity gap in maternal, neonatal and non-neonatal tetanus. The current study provides evidence of the efforts that the DRC has put into MNTE, but there is a gap in services provided to children over the age of 1 year through the age of 15 and then for men of all ages. As Burgess and Gasse emphasized, all individuals need to be protected against tetanus and for that they must be immunized (Burgess et al., 2017). The current study has identified the inequity in immunization services for adolescents and adults, specifically men and nonchildbearing women, and with this evidence it will hopefully provide the impetus for immunization policy to be addressed.

In 1986, on the steps of a clinic in Liberia, I watched as a child died in the arms of her mother from neonatal tetanus implanting an image that would remain with me for years. Whether in Liberia or the DRC, whether a neonate, adolescent, or adult¬, whether male or female, surely now with years of research, a safe, inexpensive effective vaccine, global guidance documents that support protection against tetanus across the lifespan and the quantitative evidence on limits of protective immunity against tetanus in the DRC, the DRC can and should take action.

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Appendix A: Descriptive Analysis & Bivariate Regression Models

Table A1

Descriptive Analysis & Bivariate Regression Model, Adults

Independent Variables	Weighted Adults		Unprotected		Protected							
Characteristics	n	%	n	%	n	%	χ^2	df	p-value	UOR	LL	UL
Sex												
Men	3820	46.8	3161	82.7	659	17.3	1918.7	1	0.000	1		
Women	4349	53.2	1502	34.5	2847	65.5				9.10	7.86	10.53
Age (years)												
15-19	1698	20.8	1258	74.1	440	25.9	485.6	7	0.000	1		
20-24	1477	18.1	798	54.0	679	46.0				2.43	1.99	2.97
25-29	1371	16.8	612	44.6	759	55.4				3.54	2.84	4.41
30-34	1045	12.8	466	44.6	579	55.4				3.54	2.77	4.54
35-39	856	10.5	423	49.4	433	50.6				2.92	2.31	3.68
40-44	716	8.8	417	58.2	299	41.8				2.05	1.65	2.55
45-49	624	7.6	370	59.3	254	40.7				1.96	1.53	2.52
50-59	382	4.7	319	83.5	63	16.5				0.57	0.39	0.81
Province												
Kinshasa	875	10.7	506	57.8	369	42.2	74.7	25	0.007	1		
Kwango	374	4.6	200	53.5	174	46.5				1.19	0.94	1.51
Kwilu	526	6.4	252	47.9	274	52.1				1.49	1.13	1.96

Independent Variables	Weighted Adults		Unprotected		Protected							
Characteristics	n	%	n	%	n	%	χ^2	df	p-value	UOR	LL	UL
Mai-Ndombe	376	4.6	219	58.2	157	41.8				0.98	0.70	1.38
Konho-Central	314	3.8	176	56.0	138	44.0				1.07	0.83	1.37
Equateur	249	3.0	134	53.8	115	46.2				1.17	0.94	1.46
Mongala	216	2.6	116	53.7	100	46.3				1.17	0.84	1.62
Nord-Ubangi	131	1.6	66	50.4	65	49.6				1.36	1.01	1.82
Sud-Ubangi	390	4.8	207	53.1	183	46.9				1.21	0.82	1.79
Tshuapa	192	2.4	123	64.1	69	35.9				0.77	0.57	1.04
Kasai	232	2.8	158	68.1	74	31.9				0.64	0.35	1.16
Kasai-Central	338	4.1	182	53.8	156	46.2				1.17	0.82	1.68
Kasai-Oriental	370	4.5	198	53.5	172	46.5				1.19	0.98	1.44
Lomami	366	4.5	206	56.3	160	43.7				1.07	0.89	1.28
Sankuru	139	1.7	90	64.7	49	35.3				0.74	0.44	1.25
Haute-Katanga	329	4.0	190	57.7	139	42.3				1.00	0.71	1.42
Haute-Lomami	196	2.4	115	58.7	81	41.3				0.97	0.72	1.30
Lualaba	159	1.9	84	52.8	75	47.2				1.22	0.93	1.61
Tanganika	143	1.8	100	69.9	43	30.1				0.58	0.43	0.78
Maniema	254	3.1	150	59.1	104	40.9				0.95	0.70	1.28
Nord-Kivu	733	9.0	455	62.1	278	37.9				0.84	0.62	1.13
Bas-Uele	165	2.0	88	53.3	77	46.7				1.19	0.80	1.77
Haute-Uele	155	1.9	86	55.5	69	44.5				1.09	0.82	1.44
Ituri	251	3.1	143	57.0	108	43.0				1.03	0.68	1.58
Tshopo	239	2.9	153	64.0	86	36.0				0.77	0.48	1.22
Sud-Kivu	457	5.6	266	58.2	191	41.8				0.98	0.72	1.33

Independent Variables	Weighted Adults		Unprotected		Protected							
Characteristics	n	%	n	%	n	%	χ^2	df	p-value	UOR	LL	UL
Type of Residence												
Rural	5198	63.6	2978	57.3	2220	42.7	0.3	1	0.676	1		
Urban	2971	36.4	1685	56.7	1286	43.3				1.02	0.92	1.15
Education												
No education	865	10.6	368	42.5	497	57.5	177.1	3	0.001	1		
Primary	2505	30.7	1284	51.3	1221	48.7				0.70	0.58	0.85
Secondary	4330	53.0	2693	62.2	1637	37.8				0.45	0.36	0.55
Higher	469	5.7	318	67.8	151	32.2				0.35	0.26	0.47
Wealth Index												
Lowest	1517	18.6	898	59.2	619	40.8	3.7	4	0.715	1		
Low	1614	19.8	912	56.5	702	43.5				1.12	0.94	1.33
Middle	1722	21.1	981	57.0	741	43.0				1.10	0.90	1.35
High	1517	18.6	852	56.2	665	43.8				1.14	0.92	1.40
Highest	1799	22.0	1020	56.7	779	43.3				1.11	0.94	1.31
Ethnicity												
Bakongo Nord & Sud	625	7.7	349	55.9	276	44.1	29.4	7	0.023	1		
BasKasai & KwiluKwngo	1357	16.6	704	51.9	653	48.1				1.17	0.97	1.42
Cuvette Central	818	10.0	484	59.1	334	40.9				0.87	0.69	1.10
Ubangi & Itimbiri	1002	12.3	543	54.2	459	45.8				1.07	0.86	1.33
Uele Lac Albert	601	7.4	349	58.1	252	41.9				0.91	0.66	1.25
Basele-K, Man. & Kivu	1511	18.5	909	60.1	602	39.9				0.84	0.67	1.05

Independent Variables	Weighted Adults		Unprotected		Protected							
Characteristics	n	%	n	%	n	%	χ^2	df	p-value	UOR	LL	UL
Kasai, Katanga, Tanganika	2157	26.4	1272	59.0	885	41.0				0.88	0.73	1.06
Lunda, Pygme, Other	98	1.2	53	54.3	45	45.7				1.06	0.66	1.72
Occupation												
Not Working	1926	23.6	1166	60.5	760	39.5	139.4	6	0.001	1		
Pro/tech/managerial	478	5.9	328	68.6	150	31.4				0.70	0.53	0.93
Sales/clerical	1236	15.1	525	42.5	711	57.5				2.08	1.71	2.52
Agriculture	3553	43.5	1962	55.2	1591	44.8				1.24	1.04	1.48
HH/Domestic Service	583	7.1	411	70.5	172	29.5				0.64	0.48	0.85
Manual laborer	231	2.8	179	77.5	52	22.5				0.44	0.28	0.70
Army	162	1.9	92	56.8	70	43.2				1.15	0.71	1.87
Health Insurance												
No Insurance	7799	95.5	4451	95.4	3348	95.5	0.0	1	0.938	1		
Insurance	370	4.5	212	4.6	158	4.5				0.99	0.74	1.33
Literacy												
Cannot read at all	2016	24.7	898	44.6	1118	55.4	189.9	3	0.000	1		
Read part of sentence	741	9.1	397	53.6	344	46.4				0.70	0.54	0.89
Read whole sentence	5375	65.8	3343	62.2	2032	37.8				0.49	0.43	0.56
Not Assessed	37	0.5	24	64.9	13	35.1				0.44	0.19	0.98
Marital Status												
Not Married	2510	30.7	1863	74.2	647	25.8	454.0	4	0.000	1		

Independent Variables	Weighted Adults		Unprotected		Protected							
Characteristics	n	%	n	%	n	%	χ^2	df	p-value	UOR	LL	UL
Married	3857	47.2	1988	51.5	1869	48.5				2.71	2.34	3.13
Living with Partner	1223	15.0	559	45.7	664	54.3				3.42	2.75	4.27
Widowed/divorced	279	3.4	128	45.9	151	54.1				3.38	2.30	4.95
Not Living with Partner	300	3.7	125	41.8	175	58.2				4.01	2.91	5.53
Religion												
No Religion	118	1.4	80	67.7	38	32.3	16.8	5	0.001	1		
Catholic	2352	28.8	1368	58.1	984	41.9				1.51	0.96	2.37
Protestant	2255	27.6	1292	57.3	963	42.7				1.56	0.98	2.49
Local Religion	371	4.5	214	57.7	157	42.3				1.54	0.89	2.64
Other - Christian	2976	36.4	1643	55.2	1333	44.8				1.70	1.07	2.70
Muslim	97	1.2	67	69.4	30	30.6				0.92	0.52	1.64

Table A2

Descriptive Analysis & Bivariate Regression Model, Women

Independent Variables	Weighted Women		Unprotected		Protected						95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	p	UOR	LCL	UCL
Women	4349	53.2	1502	34.5	2847	65.5						
Age (years)												
15-19	919	21.1	599	65.2	320	34.8	21.7	6	0.001	1		
20-24	854	19.6	299	35.0	555	65.0				1.35	0.83	2.2
25-29	831	19.1	179	21.5	652	78.5				1.72	1.06	2.78
30-34	570	13.1	93	16.3	477	83.7				2.70	1.53	4.77
35-39	492	11.3	121	24.6	371	75.4				1.48	0.84	2.61
40-44	359	8.3	100	27.9	259	72.1				1.01	0.58	1.76
45-49	324	7.6	111	34.3	213	65.7				1.44	0.6	3.42
Province												
Kinshasa	480	11.0	189	39.4	291	60.6	58.1	26	0.000	1		
Kwango	199	4.6	45	22.6	154	77.4				1.20	0.55	2.61
Kwilu	293	6.7	80	27.3	213	72.7				0.91	0.48	1.73
Mai-Ndombe	193	4.4	63	32.6	130	67.4				0.51	0.23	1.12
Konho-Central	173	4.0	51	29.5	122	70.5				0.58	0.26	1.27
Equateur	121	2.8	28	23.1	93	76.9				0.98	0.27	3.59
Mongala	116	2.7	38	32.8	78	67.2				0.64	0.31	1.29

Independent Variables	Weighted Women		Unprotected		Protected						95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	p	UOR	LCL	UCL
Nord-Ubangi	67	1.5	17	25.4	50	74.6				0.34	0.13	0.92
Sud-Ubangi	207	4.8	70	33.8	137	66.2				0.45	0.23	0.87
Tshuapa	99	2.3	44	44.4	55	55.6				0.35	0.15	0.84
Kasai	123	2.8	59	48.0	64	52.0				0.37	0.16	0.87
Kasai-Central	181	4.2	56	30.9	125	69.1				0.80	0.36	1.78
Kasai-Oriental	194	4.5	62	32.0	132	68.0				0.51	0.24	1.06
Lomami	197	4.5	59	29.9	138	70.1				1.04	0.5	2.16
Sankuru	72	1.7	29	40.3	43	59.7				0.41	0.14	1.18
Haute-Katanga	176	4.0	68	38.6	108	61.4				0.45	0.17	1.2
Haute-Lomami	98	2.3	30	30.6	68	69.4				0.62	0.29	1.31
Lualaba	84	1.9	29	34.5	55	65.5				0.40	0.2	0.79
Tanganika	76	1.7	40	52.6	36	47.4				0.20	0.1	0.38
Maniema	136	3.1	53	39.0	83	61.0				0.35	0.17	0.75
Nord-Kivu	406	9.3	158	38.9	248	61.1				0.46	0.23	0.91
Bas-Uele	81	1.9	17	21.0	64	79.0				1.05	0.3	3.74
Haute-Uele	77	1.8	27	35.1	50	64.9				0.46	0.22	0.95
Ituri	134	3.1	57	42.5	77	57.5				0.31	0.1	0.94
Tshopo	118	2.7	50	42.4	68	57.6				0.33	0.15	0.73
Sud-Kivu	248	5.7	83	33.5	165	66.5				0.68	0.3	1.52
Type of Residence												
Rural	2731	62.8	924	33.8	1807	66.2	9.3	1	0.002	1		
Urban	1618	37.2	578	35.7	1040	64.3				1.59	1.18	2.14

Independent Variables	Weighted Women		Unprotected		Protected						95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	p	UOR	LCL	UCL
Education												
No education	702	16.1	234	33.3	468	66.7	19.6	3	0.000	1		
Primary	1630	37.5	532	32.6	1098	67.4				1.13	0.83	1.54
Secondary	1868	43	660	35.3	1208	64.7				1.90	1.34	2.67
Higher	149	3.4	76	51.0	73	49.0				5.00	0.96	26.1
Wealth Index												
Lowest	818	18.8	315	38.5	503	61.5	18.7	4	0.001	1		
Low	863	19.8	279	32.3	584	67.7				1.59	1.09	2.32
Middle	880	20.2	274	31.1	606	68.9				1.69	1.15	2.49
High	791	18.2	254	32.1	537	67.9				1.92	1.19	3.09
Highest	997	22.9	380	38.1	617	61.9				2.34	1.52	3.58
Ethnicity												
Bakongo Nord & Sud	348	8	124	35.6	224	64.4	21.8	7	0.003	1		
BasKasai & KwiluKwngo	731	16.8	196	26.8	535	73.2				1.31	0.71	2.41
Cuvette Central	414	9.5	146	35.3	268	64.7				0.73	0.34	1.55
Ubangi & Itimbiri	514	11.8	158	30.7	356	69.3				0.66	0.35	1.26
Uele Lac Albert	320	7.4	124	38.8	196	61.3				0.51	0.23	1.13
Basele-K, Man. & Kivu	821	18.9	309	37.6	512	62.4				0.65	0.34	1.24
Kasai, Katanga, Tanganika	1144	26.3	423	37.0	721	63.0				0.71	0.39	1.31
Lunda, Pygme, Other	57	1.3	22	38.6	35	61.4				0.42	0.18	0.98

Independent Variables	Weighted Women		Unprotected		Protected						95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	p	UOR	LCL	UCL
Occupation												
Not Working	1226	28.2	583	47.5	643	52.5	7.6	6	0.272	1		
Pro/tech/managerial	94	2.2	26	28.4	68	71.6				1.12	0.41	3.07
Sales/clerical	921	21.2	273	29.6	648	70.4				0.99	0.63	1.53
Agriculture	1875	43.1	532	28.4	1343	71.6				0.78	0.54	1.14
HH/Domestic Service	123	2.8	36	29.3	87	70.7				1.58	0.74	3.37
Manual laborer	18	0.4	6	33.3	12	66.7				0.27	0.06	1.27
Army	92	2.1	46	50.0	46	50.0				0.80	0.2	3.21
Health Insurance												
No Insurance	4143	95.3	1412	34.08	2731	65.9	29.8	1	0.281	1		
Insurance	206	4.7	90	43.69	116	56.3				1.55	0.7	3.43
Literacy												
Cannot read at all	1575	36.2	516	32.8	1059	67.2	108	3	0.000	1		
Read part of sentence	431	9.9	144	33.4	287	66.6				0.70	0.54	0.89
Read whole sentence	2324	53.4	835	35.9	1489	64.1				0.49	0.43	0.56
Not Assessed	19	0.4	7	36.8	12	63.2				0.44	0.19	0.98
Marital Status												
Not Married	1093	25.1	707	64.7	386	35.3	5.5	4	0.236	1		
Married	2049	47.1	477	23.3	1572	76.7				1.68	0.95	2.99

Independent Variables	Weighted Women		Unprotected		Protected						95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	p	UOR	LCL	UCL
Living with Partner	783	18	191	24.4	592	75.6				1.73	0.92	3.24
Widowed/divorced	220	5.1	78	35.5	142	64.5				1.00	0.43	2.3
Not Living with Partner	204	4.69	49	24.0	155	76.0				1.48	0.75	2.94
Religion												
No Religion	36	0.8	9	25	27	75	9.7	5	0.084	1		
Catholic	1204	27.7	418	34.7	786	65.3				0.75	0.3	1.87
Protestant	1193	27.4	402	33.7	791	66.3				0.77	0.28	2.08
Local Religion	205	4.7	76	37.1	129	62.9				0.50	0.18	1.43
Other - Christian	1669	38.4	580	34.8	1089	65.2				0.89	0.35	2.29
Muslim	42	1.0	17	40.5	25	59.5				0.45	0.15	1.32
Women births in the last 5 years												
No births	1767	40.6	960	54.3	807	45.7	269	4	0.000	1		
1	1153	26.5	289	25.1	864	74.9				3.55	2.84	4.44
2	1135	26.1	211	18.6	924	81.4				5.21	4.11	6.61
3	265	6.1	35	13.2	230	86.8				7.94	5.16	12.2
4	29	0.7	7	24.1	22	75.9				3.51	1.21	10.2
Prenatal visit to any provider												
No	294	11.4	100	34.01	194	66.0	18.4	1	0.000	1		
Yes	2287	88.6	441	19.28	1846	80.7				2.15	1.51	3.05

Independent Variables	Weighted Women		Unprotected		Protected						95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	p	UOR	LCL	UCL
Prenatal provider												
No visit	294	11	100	34.0	194	66.0	18.4	3	0.000	1		
Facility Based Staff	2204	85	428	19.4	1776	80.6				2.13	1.5	3.02
Others	42	2	8	19.0	34	81.0				2.37	0.92	6.12
Combined	41	2	6	14.6	35	85.4				3.32	0.93	11.9
Women: Age at first child's birth												
10-14	227	7.0	54	23.8	173	76.2	4.6	4	0.325	1		
15-19	1716	52.8	406	23.7	1310	76.3				0.76	0.45	1.29
20-24	1015	31.3	226	22.3	789	77.7				0.83	0.47	1.48
25-29	235	7.2	43	18.3	192	81.7				1.19	0.66	2.14
30-42	54	1.7	17	31.5	37	68.5				0.49	0.19	1.28
Women: Reported # TT before last child's birth												
0	700	27	194	27.7	506	72.3	21.7	4	0.001	1		
1 to 2	1542	60	298	19.3	1244	80.7				1.60	1.23	2.07
3 to 4	311	12	44	14.1	267	85.9				2.34	1.5	3.65
5 +	19	1	5	26.3	14	73.7				1.08	0.38	3.08
Do not know	11	0	2	18.2	9	81.8				1.66	0.17	16.2
Women: # of TT before current preg												
0	747	51.2	205	27.4	542	72.6	9.5	4	0.049	1		
1 to 2	359	24.6	70	19.5	289	80.5				1.56	1.08	3.27

Independent Variables	Weighted Women		Unprotected		Protected						95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	p	UOR	LCL	UCL
3 to 4	188	12.9	32	17.0	156	83.0				1.85	0.98	3.48
5 +	147	10.1	25	17.0	122	83.0				1.85	1.01	3.4
Do not know	19	1.3	2	10.5	17	89.5				3.03	0.36	25.6
Women: # years last received TT												
0-2 Years	241	33.8	31	12.9	210	87.1	4.5	2	0.105	1		
3-4 Years	245	34.3	47	19.2	198	80.8				1.98	1.05	3.74
5+ years	228	31.9	52	22.8	176	77.2				1.26	0.67	2.35
Women: Total children born												
0	1101	25.3	755	68.6	346	31.4	343	4	0.000	1		
1 to 2	1074	24.7	285	26.5	789	73.5				3.62	3.02	4.34
3 to 4	860	19.8	164	19.1	696	80.9				4.35	3.58	5.29
5 to 9	1164	26.8	251	21.6	913	78.4				3.45	2.92	4.06
10+	149	3.4	46	30.9	103	69.1				1.19	0.93	1.53

Table A3

Descriptive Analysis & Bivariate Regression Model, Men

Independent Variables	Men	Weighted	Unprotected		Protected				p		95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	value	UOR	LL	UL
Men	3820	46.8	3161	82.7	659	17.3						
Age (years)												
15-19	779	20.4	658	84.5	121	15.5	14.85	7	0.038	1		
20-24	623	16.3	500	80.3	123	19.7				1.35	0.93	1.97
25-29	540	14.1	432	80.0	108	20.0				1.36	0.90	2.04
30-34	475	12.4	373	78.5	102	21.5				1.49	0.96	2.30
35-39	364	9.5	303	83.2	61	16.8				1.11	0.72	1.70
40-44	357	9.3	317	88.8	40	11.2				0.69	0.43	1.12
45-49	300	7.9	259	86.3	41	13.7				0.86	0.53	1.41
50-59	382	10.0	319	83.5	63	16.5				1.08	0.71	1.65
Province												
Kinshasa	395	10.3	317	80.3	78	19.7	56.59	25	0.001	1		
Kwango	175	4.6	155	88.6	20	11.4				0.51	0.25	1.02
Kwilu	233	6.1	172	73.8	61	26.2				1.41	0.64	3.11
Mai-Ndombe	183	4.8	156	85.2	27	14.8				0.72	0.42	1.23
Konho-Central	141	3.7	125	88.7	16	11.3				0.50	0.25	1.02
Equateur	128	3.4	106	82.8	22	17.2				0.82	0.56	1.20
Mongala	100	2.6	78	78.0	22	22.0				1.13	0.68	1.87

Independent Variables	Men	Weighted	Unprotected		Protected				p		95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	value	UOR	LL	UL
Nord-Ubangi	64	1.7	49	76.6	15	23.4				1.35	0.76	2.37
Sud-Ubangi	183	4.8	137	74.9	46	25.1				1.37	0.73	2.59
Tshuapa	93	2.4	79	84.9	14	15.1				0.73	0.35	1.51
Kasai	109	2.9	99	90.8	10	9.2				0.38	0.13	1.12
Kasai-Central	157	4.1	126	80.3	31	19.7				1.00	0.46	2.16
Kasai-Oriental	176	4.6	136	77.3	40	22.7				1.17	0.60	2.28
Lomami	169	4.4	147	87.0	22	13.0				0.60	0.30	1.18
Sankuru	67	1.8	61	91.0	6	9.0				0.36	0.13	0.97
Haute-Katanga	153	4.0	122	79.7	31	20.3				1.05	0.61	1.82
Haute-Lomami	98	2.6	85	86.7	13	13.3				0.62	0.31	1.22
Lualaba	75	2.0	55	73.3	20	26.7				1.49	0.84	2.64
Tanganika	67	1.8	60	89.6	7	10.4				0.38	0.16	0.90
Maniema	118	3.1	97	82.2	21	17.8				0.87	0.44	1.74
Nord-Kivu	327	8.6	297	90.8	30	9.2				0.40	0.22	0.74
Bas-Uele	84	2.2	71	84.5	13	15.5				0.71	0.47	1.07
Haute-Uele	78	2.0	59	75.6	19	24.4				1.28	0.72	2.27
Ituri	117	3.1	86	73.5	31	26.5				1.45	0.76	2.73
Tshopo	121	3.2	103	85.1	18	14.9				0.70	0.34	1.41
Sud-Kivu	209	5.5	183	87.6	26	12.4				0.58	0.33	1.03
Type of Residence												
Rural	2467	64.6	2054	83.3	413	16.7	0.64	1	0.424	1		
Urban	1353	35.4	1107	81.8	246	18.2				1.10	0.87	1.40

Independent Variables	Weighted Men		Unprotected		Protected				p	95% CI		
Characteristics	n	%	n	%	n	%	χ^2	df	value	UOR	LL	UL
Education												
No education	163	4.3	134	82.2	29	17.8	11.86	3	0.008	1		
Primary	875	22.9	752	85.9	123	14.1				0.74	0.35	1.55
Secondary	2462	64.5	2033	82.6	429	17.4				0.96	0.48	1.91
Higher	320	8.4	242	75.6	78	24.4				1.45	0.64	3.31
Wealth Index												
Lowest	699	18.3	583	83.4	116	16.6	4.30	3	0.366	1		
Low	751	19.7	633	84.3	118	15.7				0.95	0.64	1.39
Middle	842	22.0	707	84.0	135	16.0				0.96	0.62	1.49
High	726	19.0	598	82.4	128	17.6				1.09	0.76	1.55
Highest	802	21.0	640	79.8	162	20.2				1.28	0.91	1.80
Ethnicity												
Bakongo Nord & Sud	277	7.3	225	81.2	52	18.8	13.25	7	0.066	1		
BasKasai & KwiluKwngo	626	16.4	508	81.2	118	18.8				1.03	0.57	1.87
Cuvette Central	404	10.6	338	83.7	66	16.3				0.87	0.53	1.44
Ubangi & Itimbiri	488	12.8	385	78.9	103	21.1				1.19	0.72	1.96
Uele Lac Albert	281	7.4	225	80.1	56	19.9				1.11	0.61	2.04
Basele-K, Man. & Kivu	690	18.1	600	87.0	90	13.0				0.66	0.40	1.09
Kasai, Katanga, Tanganika	1013	26.5	849	83.8	164	16.2				0.86	0.52	1.41
Lunda, Pygme, Other	41	1.1	31	75.6	10	24.4				1.40	0.55	3.54
Occupation												

Independent Variables	Men	Weighted	Unprotected		Protected				p		95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	value	UOR	LL	UL
Not Working	700	18.3	583	83.3	117	16.7	14.87	6	0.021	1		
Pro/tech/managerial	384	10.1	302	78.6	82	21.4				1.36	0.89	2.10
Sales/clerical	315	8.2	252	80.0	63	20.0				1.24	0.76	2.02
Agriculture	1678	43.9	1430	85.2	248	14.8				0.87	0.63	1.19
HH/Domestic Service	460	12.0	375	81.5	85	18.5				1.13	0.75	1.71
Laborer	213	5.6	173	81.2	40	18.8				1.16	0.69	1.95
Army	70	1.8	46	65.7	24	34.3				2.55	1.13	5.77
Health Insurance												
No Insurance	3656	95.7	3039	83.1	617	16.9	5.63	1	0.018	1		
Insurance	164	4.3	122	74.4	42	25.6				1.70	1.09	2.62
Literacy												
Cannot read at all	441	11.5	383	86.8	58	13.2	8.55	3	0.036	1		
Read part of sentence	310	8.1	253	81.6	57	18.4				1.47	0.76	2.86
Read whole sentence	3051	79.9	2508	82.2	543	17.8				1.42	0.93	2.15
Not Assessed	18	0.5	17	94.4	1	5.6				0.22	0.05	0.96
Marital Status												
Not Married	1417	37.1	1156	81.6	261	18.4	2.53	4	0.638	1		
Married	1808	47.3	1511	83.6	297	16.4				0.87	0.71	1.07
Living with Partner	440	11.5	368	83.6	72	16.4				0.87	0.59	1.27
Widowed/divorced	59	1.5	50	84.7	9	15.3				0.73	0.30	1.75
Not Living with Partner	96	2.5	76	79.2	20	20.8				1.15	0.60	2.20

Independent Variables	Men	Weighted	Unprotected		Protected				p		95% CI	
Characteristics	n	%	n	%	n	%	χ^2	df	value	UOR	LL	UL
Religion												
No Religion	82	2.1	71	86.6	11	13.4	4.47	5	0.484	1		
Catholic	1148	30.1	950	82.8	198	17.2				1.35	0.69	2.67
Protestant	1062	27.8	890	83.8	172	16.2				1.26	0.63	2.54
Local Religion	166	4.3	138	83.1	28	16.9				1.28	0.48	3.41
Other - Christian	1307	34.2	1062	81.3	245	18.7				1.50	0.78	2.91
Muslim	55	1.4	50	90.9	5	9.1				0.71	0.26	1.99