EMERGING INFECTIOUS DISEASES WITH VACCINATION AS A MAJOR CONTROL APPROACH: THE CASE OF THE AFRICAN 2 (Af2) CLONAL COMPLEX OF *MYCOBACTERIUM BOVIS* IN UGANDA AND PNEUMOCOCCAL VACCINE FOR *STREPTOCOCCUS PNEUMONIAE* IN THE UNITED STATES

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MASTER OF SCIENCE

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ABSTRACT

Respiratory infectious diseases are among the leading cause of morbidity and mortality in the world. This paper presents two respiratory diseases, *Mycobacterium bovis* and *Streptococcus pneumoniae*, which cause significant global health issues despite the advancement of vaccines.

In Uganda, *M. bovis* isolates (n=27) were PCR-tested for a specific chromosomal deletion (RDAf2) and spoligotyped for specific characterization of the Af2 clonal complex. Seventeen (63%) isolates contained the Af2 clonal complex. The high prevalence indicates Af2 clonal complex is of epidemiological interest and a likely cause of bovine tuberculosis in Kampala, Uganda.

Ten vervet serum (varying age groups) levels were measured (Luminex) for the immunoglobulin G (IgG) antibody response against the 23-valent, Pneumovax 23® (PN23), pneumococcal capsular polysaccharide serotypes when simultaneously administered with F1/V plague vaccine and Influenza vaccine. Subjects 9-11 years and 25-26 years have shown a slightly better immune response (11%) to PN23 serotypes compared to 19-23 year subjects.

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Last but not least, I would like to thank my family: my parents Thomas Miller and Dr. Rita Miller, to my siblings Darby A. Miller and Anthony T.R. Miller, and to my nephew Beau Miller for their persistent encouragement, support, love, and overall kindness.

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CHAPTER 1. INTRODUCTION

The 'One Health - One World' concept is described as the synergistic relationship between the health of the environment, livestock, and humans (15). In recent years, many multidisciplinary health professionals have collaborated to address the 'One Health – One Word' concept in an effort to understand the multitude of infectious disease (15). These include emerging infectious diseases (EIDs) and re-emerging (resurging) infectious diseases (RIDS) (15). With a vast amount of genetic microbial diversity associated with infectious disease (i.e. zoonotic, vector-borne, water-borne, air-borne), it is imperative to understand the complex interplay between host structure(s), pathogen, and environment in order to raise awareness, to sustain, and to prevent EIDs, and RIDs (10, 25, 31). An estimated 15 million deaths result annually from infectious diseases worldwide, rivaling war and famine, not including mortalities from progressive and chronic diseases associated with infections (10, 25). Despite best efforts, EIDs and RIDs continue to challenge public health systems throughout the world as the dynamic host-pathogen interplay continues to evolve into new and advantageous ecological conditions (10, 25, 31).

Emerging and resurging infectious diseases are frequent and more devastating in developing countries, causing significant public health, as well as hindering the economic growth and increasing the likelihood of a poverty trap (7, 25). Many factors that vary with poor surveillance and treatment may lead to epidemics and pandemics (13, 25). These factors include environmental change (i.e. earthquakes, floods, droughts), climate anomalies, poor sanitation, host-agent interactions, overcrowding and urbanization, diet and nutrition, failure of social cohesion, genetic mutations, the genetic composition of the population, drug resistance, and zoonosis (13, 25, 31). The resources to monitor, control, and prevent EID and RID outbreaks are limited or nonexistent in much of the developing world (3, 4, 7, 11, 25).

My project at the Department of Medical Microbiology, College of Health and Sciences, Makerere University in Kampala, Uganda allowed me to study the significance of zoonotic *Mycobacterium bovis* and its impact upon everyday life. Zoonotic infections alone account for over 60% of the world's EIDs and play a major role in RIDs (13, 25, 31). With limited awareness, surveillance, and diagnosis in developing countries, *M. bovis* contributes to an unknown prevalence of zoonotic tuberculosis globally.

My second project was at the United States (US) Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. This experience provided me with the opportunity to study the immunological effects of simultaneous vaccine (F1/V plague vaccine and Influenza [Flu] vaccine) administration upon multivalent pneumococcal vaccine, Pneumovax 23® (PN23), using a simian model. PN23 is one of the most important and frequently administered vaccines for *Streptococcus pneumoniae* in high-risk populations (children, the immunodeficient, and the elderly) worldwide. It is pertinent to understand the effect of multiple vaccines and treatments when developing and enhancing disease surveillance and control methods.

Tuberculosis and *S. pneumoniae* are among the most common and life threatening respiratory diseases throughout the developing world. Within the last couple decades, respiratory diseases have become more frequent in developing countries. International trade and travel possibly contribute to the more frequent infections. Further research of disease surveillance and vaccine development is pertinent to successfully manage and treat EIDs and RIDs throughout the world.

CHAPTER 2. PREVALENCE OF AFRICAN 2 CLONAL COMPLEX (Af2) IN *MYCOBACTERIUM BOVIS* ISOLATES FROM BOVINE TISSUE IN KAMPALA-UGANDA, AFRICA

Abstract

A collection of twenty-seven *Mycobacterium bovis* samples from bovine tissues was characterized to determine the prevalence of the African 2 (Af2) clonal complex of *Mycobacterium* in Kampala, Uganda to further determine strain pathogenicity, communicability, and infectivity. Samples were analyzed using PCR for specific chromosomal deletion (RDAf2) and typed by spoligotyping (spacer oligonucleotide typing), a PCR and hybridization technique, for specific characterization. Af2 clonal complex is characterized by a deleted RDAf2 locus and absence of spoligotype spacers 3 to 7. Of all isolates, 63% contained the Af2 clonal complex; of which, 53% were characterized by the spoligotype strain SB0133 and 47% SB1407. Of the 37% of isolates negative for Af2, the largest cluster (60%) was characterized by the spoligotype strain SB1405. The high prevalence of the Af2 clonal complex within the *M. bovis* samples is a likely cause of increased transmission and infection within cattle in Kampala, Uganda.

Introduction

Mycobacterium bovis is the causative agent of bovine tuberculosis (BTB). BTB is prevalent in cattle among developing countries such as Uganda, Tanzania, and much of western Africa (3, 4, 11). *M. bovis* causes zoonotic infections in humans from the ingestion of unpasteurized milk from infected cattle or the inhalation of the organism from infected aerosolized droplets (11, 38).

Animal BTB surveillance and treatment programs in developing countries are insufficient or nonexistent which contributes to the poor amount of data regarding BTB-infected cattle and zoonotic infections to humans (11). It is important to identify the strains of *M. bovis* in order to

determine if a specific strain has increased infectivity, communicability, and virulence between and in host populations. This study characterized 27 *M. bovis* samples to determine the proportion of the Af2 clonal complex and spoligotype strains within the collection.

A recent report in eastern Africa indicates the presence of a unique clonal complex of *M. bovis* named African 2 (Af2) from cattle in Uganda, Burundi, Tanzania, and Ethiopia (3, 4). The *Mycobacterium tuberculosis* complex (MTC) is made up of five species including *M. bovis*, *M. africanum*, *M. microti*, *M. canetti* and *M. tuberculosis* (3, 4, 11, 17). The genomes within the MTC are highly homologous due to the nonexistent chromosomal DNA exchange across the species within the complex (3, 4, 11, 17). The study found 11 isolates of *M. bovis* from a collection of 87 MTC samples, six of which belonged to the Af2 clonal complex (3, 4). The high incidence rate of BTB and the high levels of genetic exchange between strains are likely due to Kampala's close proximity to the Ugandan Cattle Corridor (UCC), the large number of dairy cattle (cross/grade cattle) within the city, and the high human density (Figure 1).

Literature Review

Mycobacterium bovis

Mycobacterium is rod shaped, aerobic, infectious, and pathogenic bacteria (14). As with all *Mycobacterium* species, *M. bovis* is characterized by a cell wall composed in part by mycolic acid. The high-lipid mycolic acid results in a positive acid-fast reaction. The organism is the causative agent for BTB in cattle as well as zoonotic TB in humans (3, 14). *M. bovis* has a slow generation time (16-24 hours) and infects the host through latent infection (3, 4, 20). *M. bovis* also is known to infect other warm-blooded mammals (i.e. deer, bison, lions, badgers, and swine), causing an approximated annual loss of $3 billion to Agriculture worldwide (14). The actual number is estimated to be higher due to the limited BTB surveillance and treatment programs throughout the world (3, 14).

Clinically, *M. bovis* cause symptoms similar to *M. tuberculosis* (MTB) and include night-sweats, coughing, chronic invigoration, emaciation, granulomas, bacteremia, and vertebral osteomyelitis (Pott's Disease) (1, 14). Additionally, the genome of *M. bovis* is >99.52% identical to *M. tuberculosis* (14). Extrapulmonary infection occurs in humans when an infected animal, with a mycobacterial mastitis infection, sheds the bacteria in the milk that is then consumed by humans without pasteurization (3, 14). Pulmonary infection can also occur through the inhalation of infected aerosol droplets (3, 14). Due to the small number of documented cases of BTB throughout the world, frequently do not receive the correct treatment because of misdiagnosis, increasing the risk of sever disease (3, 14). A recent study in the United States found that BTB-infected individuals have a higher death rate compared to MTB-infected individuals (20). Additionally, the study indicated that individuals co-infected with both HIV and BTB have a significantly higher death rate compared to individuals infected with only HIV or BTB (20). The high death rates associated with BTB may be attributed to the organism's resistance to pyrazinamide, a short-term antibiotic regimen (6 months) used along with other antibiotics to treat MTB (20). However, since *M. bovis* is resistant to pyrazinamide, the therapy regimen must be lengthened (approximately 94 days longer) (20). Without the correct regime, BTB-infected humans have an increased likelihood of developing sever complications and disease (20).

To date, the *M. bovis* bacilli Calmette-Guérin (BCG) vaccine has been the most widely used prophylactic throughout the world to prevent BTB and MTB (19). Despite the advances in vaccine technology and administration of the BCG vaccine, TB still remains one of the leading causes of death from infectious diseases worldwide (19). The high mortality rate from TB is in part due to the inconsistent vaccine efficacy and diagnostic limitations in developing countries. BCG efficacy varies from 0% to 80%, which is dependent on geographical location, climate, causative agent, strain variance, administration, and the age of the individual upon vaccination

(8, 19). Unfortunately, vaccine efficacy is low and supplies scarce in populations that need the BCG vaccine and treatment the most (i.e. developing countries, tropical regions, immunocompromised people, and the elderly) (8, 19). The low vaccine efficacy within these populations is likely due to frequent exposure to nontuberculosis *Mycobacterium* species (8). Furthermore, *M. bovis* is less responsive to the BCG vaccine compared to *M. tuberculosis* (8). With such a wide range in efficacy and the high levels of morbidity and mortality due to TB in the world, additional research is vital for the prevention and treatment of zoonotic and non-zoonotic TB.

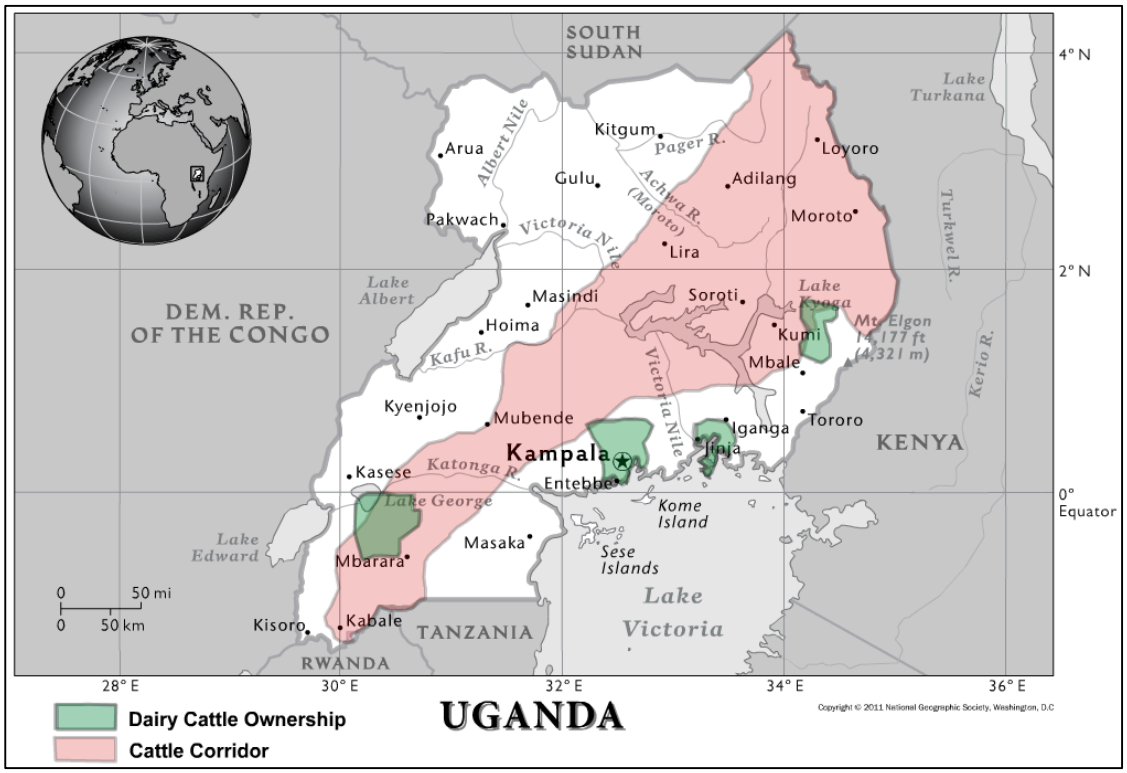


Figure 1. Map of the Ugandan Cattle Corridor and Dairy Cattle Ownership in Uganda. Dairy cattle ownership is characterized by the number of households with cross/grade dairy cattle (30% - 45%) (3**,** 22**)**. Map courtesy of National Geographic Education. National Geographic does not review or endorse content added to this background by others.

Spoligotyping Assay

Spacer oligonucleotide typing (spoligotyping), first described by Kamberbeek et al. (1997) is a rapid polymerase chain reaction (PCR)-based method used to differentiate and characterize *Mycobacterium* species from collected samples without having to grow the bacteria (3, 4, 17). This method uses 43 unique spacer sequences, specific to the MTC, in the repetitive direct repeat (DR) region of the mycobacterium locus to differentiate and characterize collected samples by the hybridization of biotinylated denatured DNA (3, 4, 17). The DR region of the locus contains conserved 36bp DRs with non-conserved 34 to 41bp spacer sequences located between the DRs (17). These unique non-conserved spacer sequences between the DRs are hybridized to the membrane of the miniblotter, yielding a positive spacer sequence if hybridized to the corresponding covalently, membrane-bound oligonucleotide sequence (3, 17). The spoligotype patterns are crosschecked against an online spoligotype database using binary code to characterize the sample; sample SB0133 yields the binary code [1100000101111110111111111111111111111100000] (3, 17). *M. bovis* samples are characterized with spacers 39 to 43 absent (17).

The Af2 clonal complex is characterized by the absence of spoligotype spacers 3 to 7 as well as the presence of the chromosomal RDAf2 deletion (3, 4). A multiplex PCR method was used to determine the RDAf2 deletion. The multiplex PCR uses three primers (RDAf2_Fw, RDAf2_Rev, and RDAf2_IntRev), which yields either the presence of the RDAf2 (458bp) or deletion of the RDAf2 (707bp) between the Mb0599 and Mb0610 of the chromosome (3, 4). The RDAf2 method is similar to Nested PCR where the target DNA sequence is internally reversed synthesized with the first product produced resulting in a smaller bandwidth (458bp) (3, 4).

Materials and Methods

Mycobacterium Isolates

The Department of Medical Microbiology, College of Health Sciences at Makerere University in Kampala-Uganda, collected, processed, and supplied the twenty-seven purified DNA samples from various cattle lesions. The International Foundation for Sciences (IFS) provided funds for the *Mycobacterium* sample collection.

PCR Parameters

Initial denaturation step was 15 min at 96°C, 35 cycles of 30 s at 96°C, 30 s at 55°C, and 1 min at 72°C, followed by a final elongation step of 10 min at 72°C (3). PCR products were separated on an ethidium bromide-stained 1% agarose gel at 120V for one hour against a Hi-Lo DNA marker (3). The gel was analyzed using a BioDoc-IT 220UV Gel Imaging Workstation (Ultra Violet Products, Upland, CA) transilluminator.

RDAf2 Screening

PCR mixture contained 2µl of supernatant of heat-killed mycobacteria, 10µl of 1X HotStartTaq master mix (Qiagen, Valencia, CA), 0.3µl primers RDAf2_FW (5'-ACTGGACCGGCAACGACCTGG-3'), RDAf2_Rev (5'-CGGGTGACCGTGAACTGCGAC-3'), and RDAf2_IntRev (5'-CGGATCGCGGTGATCGTCGA-3') (primer set Af2) (provided by Mulago Hospital, Kampala, Uganda), and sterile distilled water to a final volume of 20µl (3).

Spoligotyping

Processed samples were spoligotyped according to the method of Kamerbeek et al. (1997) using a commercialized kit with Drb (5'-CCGAGAGGGGACGGAAAC-3') and biotinylated-Dra primers (5'-GGTTTTGGGTCTGACGAC-3') (Isogen Bioscience BV, Maarssen, The Netherlands) according to thermal cycler parameters (3, 17). Denatured biotin-labeled *M. bovis* PCR samples were hybridized onto a membrane containing 43 covalently bound

spacer sequences in a miniblotter system (Miniblotter 45; Immunetics, Cambridge, Mass.) (3). Spoligotype patterns were exposed using chemiluminescence (3).

Data Analysis

Spoligotype patterns were compared with those existing in the International *Mycobacterium bovis* database at www.mbovis.org/spoligodatabase/singlpattern.php

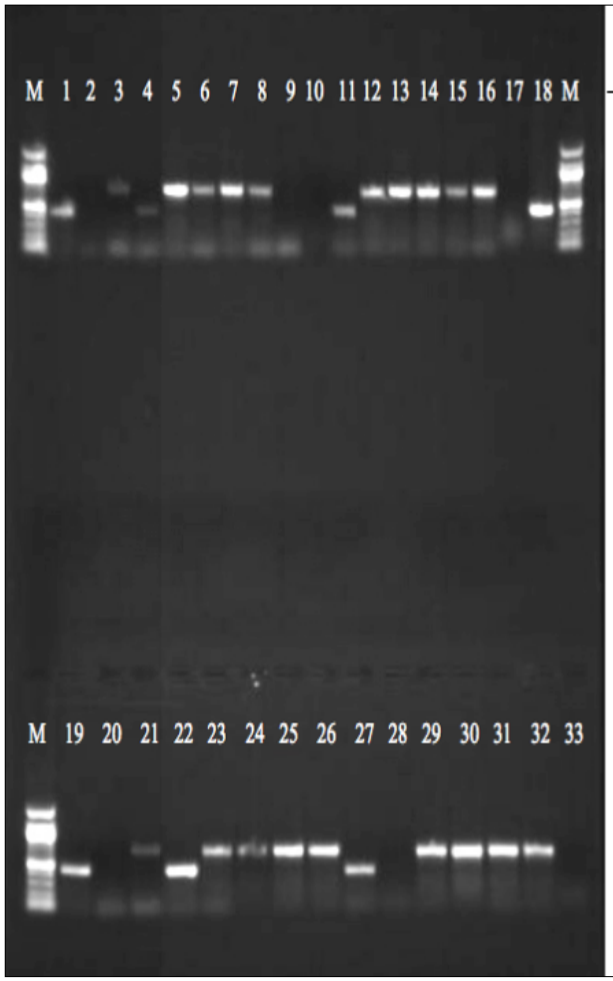
Results

Distribution of RDAf2 Among Cattle Isolates in Kampala

Multiplex PCR was used to analyze all 27 *M. bovis* isolates for the presence of RDAf2 deletion (3). The RDAf2 deletion was identified by a 707 bp band in the PCR gel and missing spacers 3 to 7 in the spoligotype pattern as shown in Fig 1 and Fig 4, respectively (3). SB1033 and SB1407 spoligotype patterns accounted for 17 out of 27 (63%) of the sample (3).

Spoligotyping Results

Five different spoligotype patterns were identified among the 27 *M. bovis* samples collected from cattle lesions (Figure 4). All spoligotype patterns were previously documented in the online spoligotype database at www.mbovis.org/spoligodatabase/singlpattern.php. The identified spoligotypes were strain SB1033, observed for nine samples (33%); strain SB1407 observed for eight samples (29%); strain SB1405 observed for six samples (22%); strain SB1404 observed for two samples (7%); and strain SB0134 for one sample (3%). The most prevalent spoligotype strains SB0133 and SB1407 (63%) had spacers 3 to 7, 9, 16, and 39-43 absent, with strain SB1407 with additional spacers 19 to 22 absent.



Lane	Isolate	RDAf2	Lane	Isolate	RDAf2
M	Ladder		19	*M.bovis* Af2122/97	Intact
1	*M.bovis* Af2122/97	Intact	20	H20 Control	
2	H20 Control		21	*M.bovis* Burundi	Deleted
3	*M.bovis* Burundi	Deleted	22	4014	Intact
4	4013	Intact	23	4015	Deleted
5	3099	Deleted	24	3677	Deleted
6	4511	Deleted	25	3649	Deleted
7	4510	Deleted	26	4318	Deleted
8	4012	Deleted	27	3121	Intact
9	5323	Intact	28	3548	Intact
10	6592	Intact	29	4512	Deleted
11	4016	Intact	30	4316	Deleted
12	4888	Deleted	31	3678	Deleted
13	3648	Deleted	32	3679	Deleted
14	4509	Deleted	33	4514	Intact
15	4578	Deleted			
16	4581	Deleted			
17	6618	Intact			
18	3205	Intact			

Figure 2. RDAf2 Deletion Multiplex PCR.
Multiplex PCR with primers specific for *Mycobacterium bovis* RDAF2 deletion (707bp) or insertion (458bp) detection. Lane M, is the molecular weight marker. *M. bovis* Af2122/97 (RDAf2 Intact), *M. bovis* Burundi (RDAf2 Deleted), and negative water used as Controls. The presence of the deletion of RDAf2 (14.1 kb between Mb0599 and Mb0610) is the definition of Af2 clonal complex (3**,** 4).

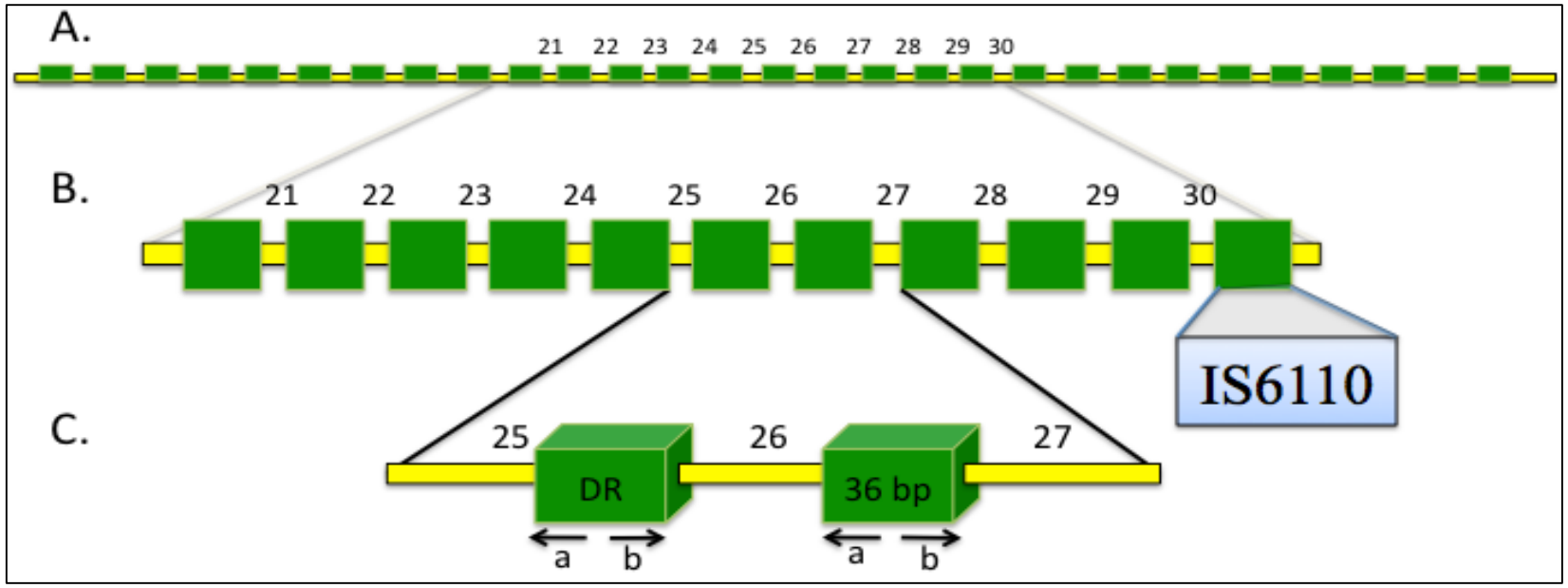


Figure 3. Schematic Representation of Direct Repeat Region of Mycobacterium Locus.
(A) Structure of the Direct Repeat region of the mycobacterium locus. (B) DR region depicted as rectangles with corresponding unique spacer oligonucleotides numbered 21-30. The IS6110 copy is inserted within the 36-bp DR locus of the majority of strains. (C) Principle in vivo amplification of fragments using Dra (a) and Drb (b) primers. The ordered spacers correspond to the spoligotype pattern.

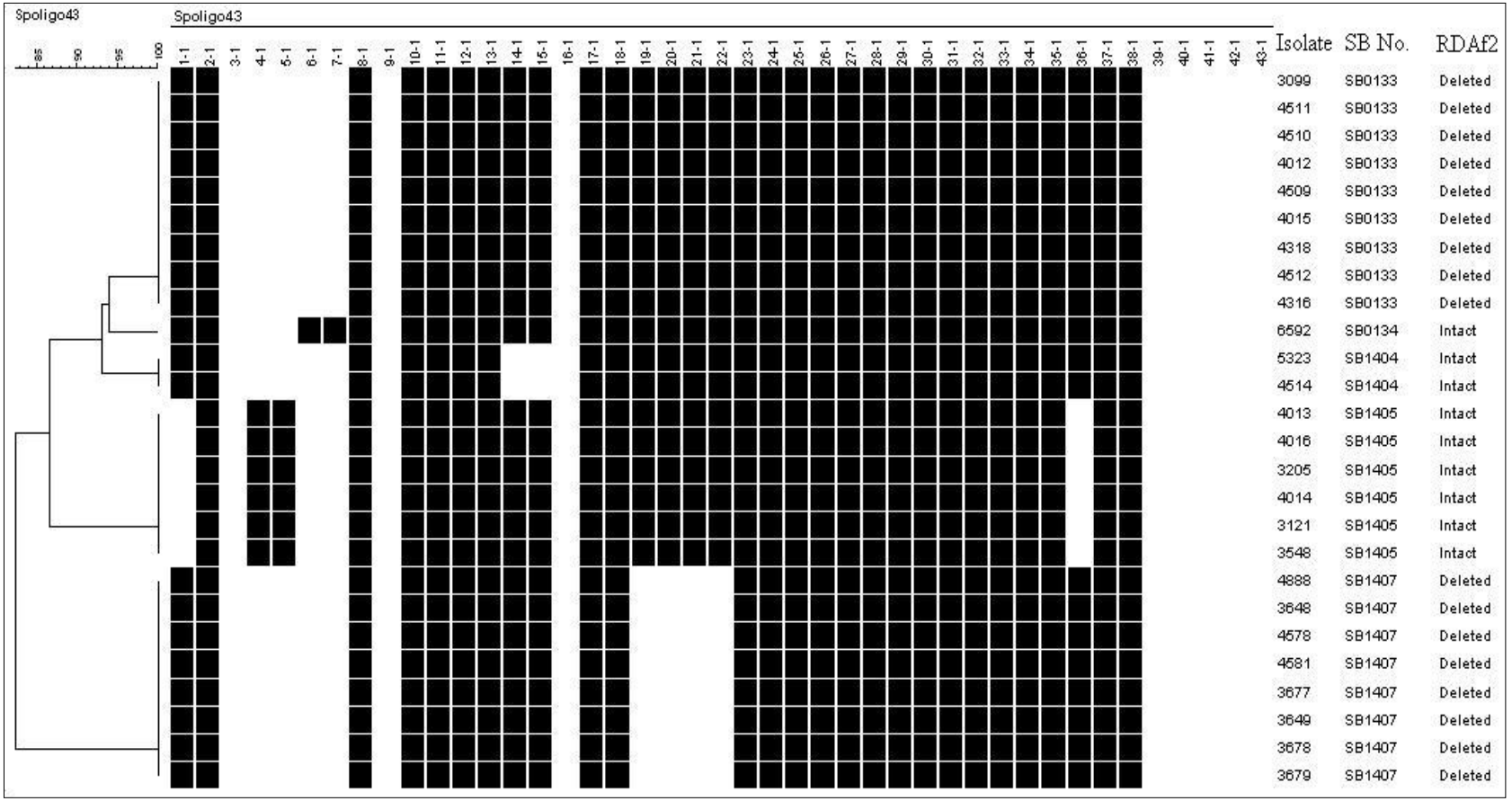


Figure 4. Schematic Representation of Spoligotype of *Mycobacterium bovis* Isolates.
Schematic representation of the spoligotypes of 26 clustered *M. bovis* strains. Spoligotype pattern is shown as a series of 43 spacer sequences. Filled rectangles representing positive hybridization of biotinylated-PCR samples, and empty rectangles indicating lack of hybridization (3**,** 4). Absence of spacers 3-7 represents Af2 clonal complex (3**,** 4). Isolate 6618 is not shown.

Discussion

Developing countries have limited disease surveillance and identification strategies to differentiate *M. tuberculosis* and *M. bovis* causing human TB (3). Identification and characterization of samples collected from cattle lesions can attribute to disease surveillance as well as help identify zoonotic public health and agricultural concerns among the population, leading to increased awareness (3**,** 11). In Kampala-Uganda, one of the most densely populated cities, the interface between cattle and humans is high due to the agriculturally dominant way of living, traditional practices, and human social life (3, 11). These factors are in part increasing the risk of BTB exposure among host populations (3, 11). Additionally, occupational hazards also contribute to the unknown prevalence of zoonotic BTB among developing countries. Ranchers, traditional farmers, veterinarians, and butchers are among some of the most common occupations that are at increased risk for developing BTB as well as other zoonotic diseases (3**,** 11).

In Kampala, a study of 27 *M. bovis* samples from cattle lesions revealed five different spoligotype strains, which may be due to the genetic diversity of *M. bovis* or the exposure to various strains in the cattle corridor (3). Of the five strains, we have found two Af2 strains SB1033 and SB1407 yielding sixty-three percent of the samples. We have determined that the high prevalence of Af2 strains (SB1033 and SB1407) indicate increased strain pathogenicity. The remaining three non-Af2 strains consist of SB0134, SB1404, and SB1405.

With the high prevalence of the Af2 clonal complex among BTB, we can conclude that Af2 is of epidemiological importance in the disease surveillance of zoonotic infectious diseases within Kampala, Uganda. Future studies might seek to further understand the prevalence of specific *M. bovis* strains between human and cattle zoonotic tuberculosis cases in Kampala, Uganda as well as determine the infectivity rate of Af2 strains for the advancement of tuberculosis vaccine and treatment.

CHAPTER 3. THE MEASUREMENT OF IMMUNOGLOBULIN G ANTIBODY PRODUCTION OF A 23-VALENT, PNEUMOVAX 23®, PNEUMOCOCCAL CAPSULAR POLYSACCHARIDE VACCINE SIMULTANEOUSLY ADMINISTERED WITH F1/V PLAGUE VACCINE AND INFLUENZA VACCINE: USING A NON-HUMAN (VERVET MONKEY) MODEL

Abstract

Streptococcus pneumoniae is one of the leading causes of respiratory-associated death in the world among high-risk populations. A 23-valent, PN23, pneumococcal capsular polysaccharide (PnPs) vaccine is one of the most predominately administered anti-capsular pneumococcal immunoglobulin G (IgG) antibody vaccines in the world. The efficacy and immune response to individual PN23 serotypes (ST) vary significantly. We simultaneously (multiplexed Luminex immunoassay) measured (pre- and post-vaccination) the IgG immune response to each 23 PnPs STs (i.e. ST 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20 22F, 23F, and 33F) when simultaneously vaccinated with two high efficacy vaccines; F1/V plague vaccine and Flu vaccine. Using simian models with varying age distributions 9-11 years (n=5), 19-23 years (n=3), and 25-26 years (n=2). All animals had an increase in IgG response to ≥3 PnPs STs after vaccination. Three animals (30%) mounted an immune response to ≥3 - ≥10 PnPs; seven (70%) mounted an immune response to >10 PnPs STs; and one (10%) mounted an immune response to all 23 PnPs serotypes. Of all the serotypes, nine (39.1%) PnPs STs (ST4, ST7F, ST8, ST9N, ST11A, ST15B, ST17F, ST19F, and ST20) mounted a robust immune response in five or more subjects. One or more subjects in the 9-11 age group responded to all 23 (100%) PnPs STs, subjects in the 19-23 age group responded to 17 (73.9%)

PnPs STs, and subjects in the 25-26 age group responded to 18 (78.2%) PnPs STs. Age groups 9-11 and 25-26 displayed slightly higher immune responses compared to the 19-23 age group.

Introduction

Streptococcus pneumoniae (pneumococcus) is a Gram-Positive pathogen that asymptomatically colonizes in the nasopharynx of humans, the only known natural reservoir (36, 37). Pneumococcus is an opportunistic pathogen the can cause severe disease once it enters into the blood stream (24, 27, 36). Pneumococcus is the causative agent of Invasive Pneumococcal Diseases (IPDs) such as pneumonia, otitis media, meningitis, bacteremia, and sepsis (24, 27, 36). Each year, an estimated 40,000 deaths in the United States and 5 million deaths globally result from pneumococcal-associated diseases (24, 27, 36). In 2000, over 800,000 (~11%) deaths and 14.5 million cases of IPDs occurred in children (27). Primarily effecting children (<5 years of age), the elderly population (>65 years of age), and immunocompromised individuals, pneumococcus is one of the leading causes of morbidity and mortality worldwide despite recent advances in antibiotic therapy and vaccinations (24, 27, 36).

Pneumococcus produces a variety of virulence factors that contribute to its infectivity and colonization including toxin pneumolysin (PLY), surface antigens, and the capsular polysaccharide (23, 36). The distinctive structure of the capsular polysaccharide differentiates the various STs among *S. pneumoniae*, as well as stimulates a specific host antibody response (23, 36). The pneumococcal capsule is one of the most important virulent factors associated with IPDs (23, 36). The role of the capsule provides antiphagocytic activity, prevents degradation from the host complementary defense mechanism, initiates colonization onto mucosal surfaces, and provides antibiotic resistance (23, 36). People in high-risk groups (i.e. children <5 years, the elderly >65 years, immunocompromised individuals, and those with specific polysaccharide antibody deficiency syndrome) do not produce antibodies against the PnPs antigens (30, 36). Of

the ≥90 different PnP STs identified, only 20 STs account for nearly 90% of IPDs that lead to hospitalization (12, 21, 36). Encapsulated pneumococcus has shown to be 10^5 times more virulent compared to unencapsulated strains (2).

Pneumovax 23 (PN23; Merck, Whitehouse Station, NJ) is a multivalent PnPs vaccine comprised from 23 highly purified PnPs of *S. pneumoniae* STs. PN23 PnP STs; STs 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20 22F, 23F, and 33F account for 88% of the most prevalent and invasive *S. pneumoniae* STs known to cause IPDs (6, 21). The Advisory Committee on Immunization Practices (ACIP) recommends PN23 vaccinations to individual's aged ≥65 years and ≤5 years of age (21). In clinical studies, PN23 has shown to be effective against *S. pneumoniae* hospital-acquired IPDs and penicillin-resistant IPDs (21). However, immune responses for each pneumococcal STs vary extensively among individuals, with age being a major factor (6, 21, 30). Individuals may exhibit different immune responses, if at all, to specific PN23 pneumococcal STs (6, 21, 30). Children ≤5 years of age respond to approximately 50% of the PN23 STs, whereas individual's ≥6 years of age respond to about 70% of the PN23 STs (6, 21, 30). Thus far, no studies have evaluated the immunological antibody (IgG) response of the 23-valent, PN23, PnP STs vaccine when simultaneously administered with F1/V plague vaccine and influenza (Flu) vaccine, two vaccines known to elicit high IgG responses. This study is to determine the host IgG immune response of the 23-valent, PN23, pneumococcal capsular polysaccharide serotypes using a simian (vervet monkey) model with various age groups (9-11 years, 19-23 years, and 25-26 years). This study will also analyze and determine which age group elicits a stronger immune response towards the 23-valent PnPs STs by comparing pre- and post-vaccination serum samples.

Literature Review

Streptococcus pneumoniae

Streptococcus pneumoniae is a cocci-cell shaped pathogen that grows in pairs or chains (2, 33, 36). A thick peptidoglycan layer characterizes *S. pneumoniae*, resulting in Gram-Positive staining reaction (2**,** 36). Pneumococcus is alpha-hemolytic, causing partial lyses of red blood cells (RBCs), useful for diagnosis and identification of clinical and research samples. Clinically relevant pneumococcal organisms are non-motile, non-spore forming, non-acid fast, and possess a capsular polysaccharide (33**,** 36). IPDs occur when pneumococcus crosses the mucosal tissues in the nasopharynx and enter the blood stream causing infections such as bacteraemia and sepsis (2, 26, 36). Meningitis may also occur when the pathogen enters the blood stream and crosses the blood brain barrier (2, 26, 36). Pneumonia may follow inhalation of *S. pneumoniae*-containing respiratory droplets (2, 26, 36).

Luminex Immunoassay

The Luminex immunoassay, first described by Lal et al. (2005) is an Enzyme-linked Immuno Sorbent Assay (ELISA)-based flow cytometric system used to simultaneously measure all antibody production for all antigens or analytes. The Luminex immunoassay uses minimal volume (≤10 µl) of serum samples and dilutions, without the requirements of growing the bacteria, in a single assay (5, 6, 28, 29, 30). The ELISA was adopted as the standard assay for PnPs-antibody testing by the World Health Organization (WHO) Advisory Committee (30). The WHO Advisory Committee requires 12-14 individual ELISA assay for each PnPs STs and serum sample (30**)**. Unlike an ELISA, the Luminex immunoassay can evaluate and analyze the possible antibody-analyte interactions as well as the cross-reactivity of desired antibodies within in the serum (5, 6, 28, 29, 30). The Luminex immunoassay overall is less time-consuming, less

laborious, less expensive, and offers a wider dynamic range then compared to ELISA methods (30**).** Figure 5 depicts the schematic representation of the Luminex immunoassay principle.

Materials and Methods

Vervet Serum Samples

The Meningitis and Vaccine Preventable Diseases Branch (MVPDB), Division of Bacterial Diseases (DBD) at the CDC in Atlanta, GA, in collaboration with Wake Forest University, supplied the pre- and post-vaccinated vervet serum samples with pre-categorized age groups (9-11 years, 19-23 years, and 25-26 years).

Study Design

The purpose of this study was to analyze the antibody production of the 23-valent, PN23, PnPs vaccine using a vervet (n=10) model with varied age distribution; 9-11 years (n=5), 19-23 years (n=3), and 25-26 years (n=2). The vervet model was used due to immunological similarities to humans. Each subject was vaccinated simultaneously with three, single dose, individual vaccines; F1/V plague vaccine, Flu vaccine, and Pneumovax 23 vaccine. Serum was collected prior and post vaccinations. IgG serum production was measured using the Luminex system (Luminex 200, Luminex, Austin, TX).

Multiplexed Luminex Pneumococcal Anti-Capsular IgG Capture Assay

Multiplex (23-plex) pneumococcal anti-capsular IgG capture assay was performed based on previous methods with modifications (Lal et al., 2005). Twenty-three purified PnPs STs (i.e. ST 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20 22F, 23F, and 33F) were purchased from the American Type Culture Collection (ATCC) (Manassas, Va.). Briefly, PnPs (Ps, ATCC, Manassas, VA) were conjugated to poly-L-lysine (PLL, Sigma, St. Louis, MO) (30). The PLL-Ps was covalently conjugated to carboxylate-modified microspheres (Luminex, Austin, TX) using a two-step carbodiimide reaction (18, 30). PLL-Ps beads for 23

PnP ST (2500 beads/serotype in 25µl volume) and sera samples (25µl /well) were transferred to 96-well MV multi-screen filter plate and incubated at room temperature (RT) for 30 minutes with 150 rpm agitation. Plates were rinsed three times with wash buffer (1X PBS containing 0.05% Tween 20 and 0.2% newborn bovine serum albumin; NBBS, Sigma, St. Louis, MO), 50µl /well of biotin-anti monkey-IgG (43R-IG021BT; Fitzgerald, Acton, MA) was added to each well, and the plates incubated at RT for 30 minutes with 150 rpm agitation. Plates were then washed three times in wash buffer, streptavidin-PE (50µl/well) was added to each well, and the plates incubated at RT for 30 minutes with 150 rpm agitation. Plates were washed three times in wash buffer, the beads were resuspended in 130µl /well, and data acquired in a Luminex reader (Luminex 200, Luminex, Austin, TX) with Masterplex CT software suite (MiraiBio, South San Francisco, CA) and expressed as AU/ml (Median Fluorescent Intensity (MFI) x standard dilution factor).

Three post-vaccination sera (1171, 1232 and 1414) that had a high AU/ml signal (>50% increase over baseline) for PnPs ST specific IgG (STs 3, 4, 6B, 7F, 9V, 14, 18C, 19F, and 23F) of a previous study were combined to generate the vervet reference standard (VV2011). The VV2011 dilution curve was generated by plotting median fluorescence intensity (MFI) against the dilution factor. Arbitrary units (AU) for PnPs ST specific IgG values was calculated (AU = MFI × dilution factor) and assigned to VV2011 for each ST. The VV2011 reference standard was included on each assay plate in duplicate and the PnP ST specific IgG values (AU/ml) for vervet serum samples were computed using Masterplex QT (MiraiBio, South San Francisco, CA).

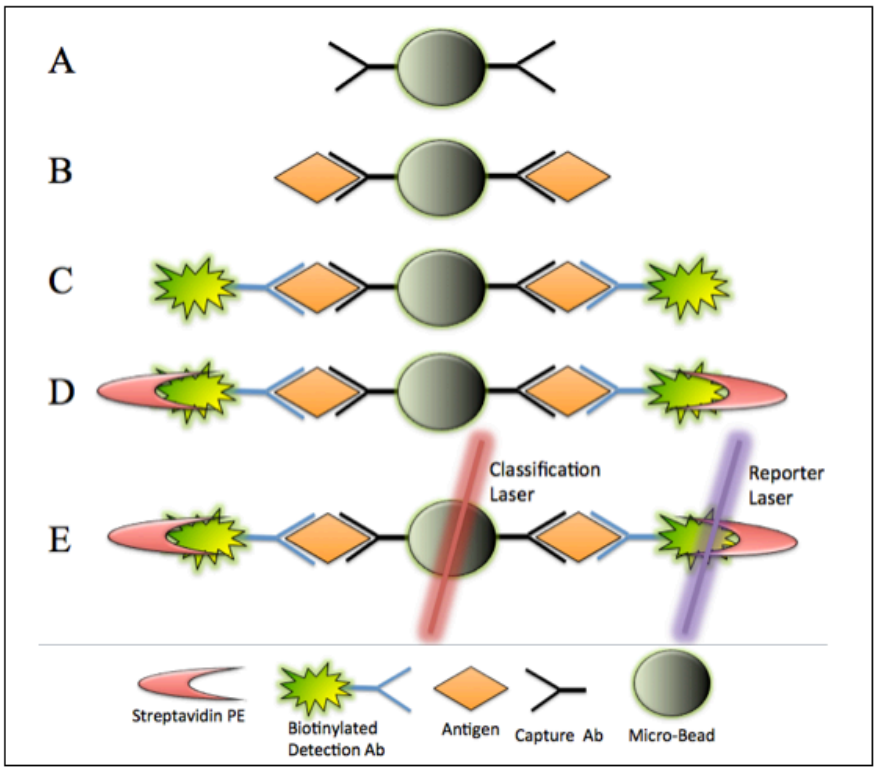


Figure 5. Pneumococcal Polysaccharide Multiplexed Luminex Immunoassay Principle. Luminex Assay Principle; (A) Microsphere with capture antibody, (B) Capture antibody binds to antigen, (C) Biotinylated detection antibody binds to capture antibody, (D) Streptavidin-PE binds to biotinylated detection antibody, (E) Microsphere identification and reporter quantity determined by microsphere and detection antibody specific laser detector using a Luminex Instrument.

Results

Vervet Immune Response

Table 1 shows the antibody levels specific for the 23 PnP serotypes in serum samples obtained from the vervets, n=10, pre- and post-vaccination. The IgG antibody response to the STs varied considerably among the vervets; however, all vervet samples (n=10) did have an increased IgG response to at least three or more of the PnPs STs after vaccination (Table 2.) One subject (vervet serum sample 1414) had an antibody response (≥50% increase over baseline) to all 23 PnPs STs in the vaccine. Thirty percent (n=3) of the animals had an antibody response to >10 (43.5%) PnPs STs. Seventy percent (n=7) animals had an antibody response to ≥3 - ≥10

(≥13.0 - ≥43.5%) PnPs STs. A robust (≥5/10 animals) antibody response was seen in nine (39.1%) PnPs STs; (ST4, ST7F, ST8, ST9N, ST11A, ST15B, ST17F, ST19F, and ST20).

Age-Wise Distribution Analysis

Age is often one of the most important factors for the efficacy of vaccines or treatments, which applies to the PN23 vaccine (2, 26, 36). The age-wise analysis of the PnPs is shown in Table 3. One or more vervets the 9-11 age group mounted an antibody response to all 23 PnPs ST tested. The average IgG response in PnPs STs for age group 9-11 years (n=5) was 9.8 (42.6%). Seventeen (73.9%) of the PnPs STs elicited an antibody response in all subjects in the 19-23 age group (n=3), while failing to respond to PnPs; ST5, ST6B, ST9V, ST14, ST22F, and ST23F. Subjects within the 25-26 age group (n=9) produced antibody to 18 (78.2%) of the 23 PnPs STs (ST1, 2, 3, 4, 7F, 9N, 9V, 10A, 11A, 12F, 15B, 17F, 18C, 19A, 19F, 20 22F, and 23F).

Table 1. Immunoglobulin G Concentrations in Vervet Serum Samples Before and After Pneumococcal Vaccine for 23 Pneumococcal Polysaccharide Serotypes Determined by Multiplexed Luminex Assay.*

PnPs STs	Vervet Serum Sample Number														
	1099			1171			1202			1220			1232		
	Before	After	*P*[a]	Before	After	*P*[a]	Before	After	*P*[a]	Before	After	*P*[a]	Before	After	*P*[a]
1	5.68	24.93	≤.001[b]	0.62	0.60	.621	0.867	2.69	≤.001[b]	8.35	6.85	≤.001[b c]	4.81	2.69	≤.001[b c]
2	3.45	17.43	.024[b]	1.83	1.17	.081	1.54	1.25	.638	6.89	4.21	.246	137.09	101.81	.011[b c]
3	28.38	35.55	.032[b]	1.08	1.08	>.99	.89	0.95	.345	6.03	3.53	≤.001[b c]	6.87	6.19	.270
4	1.81	6.13	≤.001[b]	2.37	7.42	≤.001[b]	1.37	3.43	.012[b]	6.00	3.63	.014[b c]	24.01	15.49	.01[b c]
5	39.50	6.65	≤.001[b c]	22.17	18.38	≤.001[b c]	6.60	0.54	≤.001[b c]	58.38	3.81	≤.001[b c]	85.40	5.25	≤.001[b c]
6B	254.01	177.03	≤.001[b c]	3.69	3.50	0.525	2.31	7.88	≤.001[b]	15.70	16.20	.498	89.92	36.29	≤.001[b c]
7F	216.86	997.41	≤.001[b]	15.87	683.33	≤.001[b]	81.14	204.80	≤.001[b]	25.57	80.31	≤.001[b]	62.69	138.15	≤.001[b]
8	7.90	23.45	≤.001[b]	4.73	7.49	.012[b]	1.50	9.73	≤.001[b]	288.26	135.43	.004[b c]	93.53	67.58	.021
9N	5.37	14.83	≤.001[b]	2.72	67.51	≤.001[b]	2.77	3.56	.096	6.68	5.34	.024[b c]	29.96	20.68	≤.001[b c]
9V	6.85	10.06	.025[b]	3.31	1.25	≤.001[b c]	3.31	1.13	.002[b c]	5.87	1.52	≤.001[b c]	92.56	34.96	≤.001[b c]
10A	9.50	15.89	≤.001[b]	2.62	1.49	≤.001[b c]	140.42	36.17	≤.001[b c]	114.48	93.37	.003[b c]	22.99	11.24	.002[b c]
11A	1035.05	1318.71	.021[b]	163.68	407.46	≤.001[b]	6.94	2.63	≤.001[b c]	9.60	11.02	.020[b]	36.16	38.84	0.15
12F	1.00	1.96	≤.001[b]	0.25	0	>.99	0.12	0	>.99	3.25	3.36	.477	3.74	1.42	≤.001[b c]
14	2.75	2.88	.478	2.37	1.38	≤.001[b c]	2.62	1.50	≤.001[b c]	2.87	1.63	≤.001[b c]	105.54	48.04	≤.001[b c]
15B	3.95	74.08	≤.001[b]	0.87	7.64	≤.001[b]	1.31	7.97	≤.001[b]	5.02	6.22	.038[b]	14.05	24.40	≤.001[b]
17F	4.15	14.83	≤.001[b]	2.62	7.19	≤.001[b]	1.63	21.36	≤.001[b]	10.41	35.67	≤.001[b]	12.62	19.71	≤.001[b]
18C	677.47	842.55	≤.001[b]	14.68	14.75	>.99	9.62	7.13	.021[b c]	29.68	15.45	≤.001[b c]	108.07	61.95	.002[b c]
19A	141.22	186.33	≤.001[b]	9.71	15.17	≤.001[b]	10.53	8.39	≤.001[b c]	84.17	40.83	≤.001[b c]	33.10	35.03	.417
19F	32.61	178.63	≤.001[b]	7.25	25.31	≤.001[b]	6.56	80.80	≤.001[b]	25.83	52.21	≤.001[b]	148.20	98.88	.004[b c]
20	6.00	7.41	.016[b]	14.49	22.17	≤.001[b]	2.61	1.69	.004[b c]	11.64	11.23	.630	24.52	16.93	≤.001[b c]
22F	1.50	1.88	0.20	2.56	0.38	≤.001[b c]	2.68	1.38	.018[b c]	5.50	1.67	≤.001[b c]	8.06	1.81	≤.001[b c]
23F	6.30	9.68	≤.001[b]	2.12	0.38	≤.001[b c]	2.10	0.38	≤.001[b c]	3.93	0.63	≤.001[b c]	42.10	24.10	≤.001[b c]
33F	16.02	12.02	≤.001[b c]	3.22	3.61	.122	1.48	0.35	.006[b c]	18.85	8.75	≤.001[b c]	30.35	13.42	≤.001[b c]

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Table 1. Immunoglobulin G Concentrations in Vervet Serum Samples Before and After Pneumococcal Vaccine for 23 Pneumococcal Polysaccharide Serotypes Determined by Multiplexed Luminex Assay* (continued).

PnPs STs	Vervet Serum Sample Number														
	1305			1311			1414			1417			1444		
	Before	After	P^a	Before	After	P^a	Before	After	P^a	Before	After	P^a	Before	After	P^a
1	2.719	1.60	.015 [b c]	7.63	17.57	≤.001 [b]	4.12	66.44	≤.001 [b]	3.45	2.00	.032 [b c]	27.95	12.21	≤.001 [b c]
2	71.71	32.31	.016 [b c]	15.84	15.43	.789	91.32	1463.81	≤.001 [b]	1.63	2.35	.205	1.04	1.70	.021 [b]
3	2.00	1.85	.475	3.17	2.65	.022 [b c]	6.20	191.37	≤.001 [b]	2.36	3.39	.006 [b]	4.62	2.38	≤.001 [b c]
4	14.13	11.27	.329	2.37	5.54	≤.001 [b]	102.25	3244.28	≤.001 [b]	1.31	3.21	≤.001 [b]	6.71	5.29	.044 [b c]
5	48.44	3.92	≤.001 [b c]	54.74	13.58	≤.001 [b c]	99.97	232.88	≤.001 [b]	45.48	7.60	≤.001 [b c]	150.86	8.82	≤.001 [b c]
6B	38.29	16.08	≤.001 [b c]	97.56	45.44	≤.001 [b c]	44.59	1278.26	≤.001 [b]	6.31	4.72	.013 [b c]	23.16	8.73	≤.001 [b c]
7F	27.90	112.69	≤.001 [b]	447.10	526.16	≤.001 [b]	63.41	1461.20	≤.001 [b]	37.17	93.83	≤.001 [b]	47.45	67.69	≤.001 [b]
8	37.15	68.35	≤.001 [b]	3.12	3.32	.366	26.76	1362.25	≤.001 [b]	1.62	1.42	.649	30.53	11.54	≤.001 [b c]
9N	7.43	7.47	.973	4.56	5.28	.071 [b]	57.20	2097.60	≤.001 [b]	8.38	11.48	≤.001 [b]	421.32	2950.44	≤.001 [b]
9V	33.84	14.61	.011 [b c]	5.30	5.55	.693	65.95	1888.59	≤.001 [b]	6.37	3.42	.008 [b c]	9.35	5.56	≤.001 [b c]
10A	30.17	26.36	.007 [b c]	8.28	9.02	.280	40.89	1098.40	≤.001 [b]	66.26	153.46	≤.001 [b]	560.84	1060.89	≤.001 [b]
11A	14.77	42.70	≤.001 [b]	1041.56	576.45	≤.001 [b c]	1080.58	1551.47	≤.001 [b]	509.36	716.40	≤.001 [b]	146.86	233.68	≤.001 [b]
12F	1.85	1.42	.10	2.15	5.65	≤.001 [b]	10.04	74.73	≤.001 [b]	0.70	0.45	.055	7.98	3.55	≤.001 [b c]
14	39.76	23.60	≤.001 [b c]	3.00	1.38	.031 [b c]	55.86	2058.26	≤.001 [b]	2.37	1.25	≤.001 [b c]	3.18	1.75	.055
15B	7.27	77.96	≤.001 [b]	3.52	8.94	≤.001 [b]	13.81	578.89	≤.001 [b]	3.55	9.90	≤.001 [b]	16.79	15.10	.039 [b c]
17F	29.02	553.11	≤.001 [b]	13.43	19.98	≤.001 [b]	30.92	367.25	≤.001 [b]	125.60	124.08	.843	25.28	17.44	≤.001 [b c]
18C	57.37	115.02	≤.001 [b]	33.81	28.78	.026 [b c]	142.14	1976.83	≤.001 [b]	8.49	42.30	≤.001 [b]	21.31	16.19	≤.001 [b c]
19A	23.14	20.54	.184	120.55	239.13	≤.001 [b]	63.10	359.71	≤.001 [b]	82.75	74.08	.023 [b c]	327.95	141.50	≤.001 [b c]
19F	66.41	50.19	≤.001 [b c]	363.54	767.33	≤.001 [b]	94.64	1773.04	≤.001 [b]	17.29	46.23	≤.001 [b]	22.54	46.29	≤.001 [b]
20	5.75	2.42	≤.001 [b c]	7.47	13.78	≤.001 [b]	250.83	1676.80	≤.001 [b]	75.86	128.73	≤.001 [b]	17.21	8.63	≤.001 [b c]
22F	2.50	0.25	.004 [b c]	2.25	0.25	≤.001 [b c]	94.18	3098.31	≤.001 [b]	1.37	0.25	≤.001 [b c]	2.10	0.38	≤.001 [b c]
23F	15.54	7.54	≤.001 [b c]	2.75	2.00	.181	54.47	1585.88	≤.001 [b]	2.95	0.38	≤.001 [b c]	5.27	2.43	≤.001 [b c]
33F	13.83	5.56	≤.001 [b c]	125.76	105.68	.020 [b c]	22.13	469.21	≤.001 [b]	36.95	56.97	≤.001 [b]	53.45	27.18	≤.001 [b c]

[a] *P* values for the comparisons of vervet serum samples before and after PN23 vaccine for 23 PnPs STs statistically significantly difference between before and after vaccination ($P<.05$). [c] Statistically significantly difference between pre- and post vaccination that did not produce immunological response to PnPs. [*] IgG concentrations are given as Arbitrary Units (median fluorescence intensity x dilution factor) per milliliter.

Table 2. Vervet Immune Response to 23 Pneumococcal Polysaccharide Serotypes Determined by Multiplexed Luminex Assay.

Vervet Serum Sample No.	PnPs Serotype																						Total (%)
	1	2	3	4	5	6B	7F	8	9N	9V	10A	11A	12F	14	15B	17F	18C	19A	19F	20	22F	23F	33F	
1099 [b]	++	++	+	++	-	-	++	++	++	+	++	+	++	-	++	++	+	+	++	+	+	++	-	19 (82.6)
1171 [d]	-	-	-	++	-	-	++	++	++	-	-	++	-	-	++	++	-	++	++	++	-	-	-	10 (43.5)
1202 [d]	++	-	-	++	-	++	++	++	+	-	-	-	-	-	++	++	-	-	++	-	-	-	-	9 (39.1)
1220 [d]	-	-	-	-	-	-	++	-	-	-	-	-	-	-	+	++	-	-	++	-	-	-	-	4 (17.4)
1232 [d]	-	-	-	-	-	-	++	-	-	-	-	-	-	-	++	++	-	-	-	-	-	-	-	3 (13.0)
1305 [c]	-	-	-	-	-	-	++	++	-	-	-	++	-	-	++	++	++	-	-	-	-	-	-	6 (26.0)
1311 [c]	++	-	-	++	-	-	-	-	-	-	-	-	++	-	++	+	-	++	++	++	-	-	-	8 (34.8)
1414 [d]	++	++	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++	++	++	++	++	++	23 (100)
1417 [c]	-	+	+	++	-	-	++	-	+	-	++	+	-	-	++	-	++	-	++	++	-	-	++	12 (52.2)
1444 [b]	-	++	-	-	-	-	+	-	++	-	++	++	-	-	-	-	-	-	++	-	-	-	-	6 (26.1)
All (10)	4	4	3	6[a]	1	2	9[a]	5[a]	6[a]	2	4	6[a]	3	1	9[a]	8[a]	4	4	8[a]	5[a]	2	2	2	100 (43.5)

(++) Increase in immune response (≤50%). (+) Increase in immune response (20% - 49%). (-) Increase in immune response (≤19%). [a] Robust Immune response to pneumococcal polysaccharide (PnPs) serotype in vervet serum samples (≥5/10 animals). [b] Age group 25-26 years. [c] Age group 19-23 years. [d] Age group 9-11 years.

Table 3. Age-Wise Analysis for Vervet Serum Samples.

Age (Years)	No. of Subjects	PnPs STs Count: (%)*																						Total (%)**
		1	**2**	**3**	**4**	**5**	**6B**	**7F**	**8**	**9N**	**9V**	**10A**	**11A**	**12F**	**14**	**15B**	**17F**	**18C**	**19A**	**19F**	**20**	**22F**	**23F**	**33F**	
9-11[b]	5	2	1	1	3	1	2	5	3	3	1	1	2	1	1	5	5	1	2	4	2	1	1	1	49
		(4.08)	(2.04)	(2.04)	(6.12)	(2.04)	(4.08)	(10.2)	(6.12)	(6.12)	(2.04)	(2.04)	(4.08)	(2.04)	(2.04)	(10.2)	(10.2)	(2.04)	(4.08)	(8.16)	(4.08)	(2.04)	(2.04)	(2.04)	(42.6)
19-23[c]	3	1	1	1	2	0	0	2	1	1	0	1	2	1	0	3	2	2	1	2	2	0	0	1	26
		(3.85)	(3.85)	(3.85)	(7.69)	-	-	(7.69)	(3.85)	(3.85)	-	(3.85)	(7.69)	(3.85)	-	(11.54)	(7.69)	(7.69)	(3.85)	(7.69)	(7.69)	-	-	(3.85)	(37.7)
25-26[d]	2	1	2	1	1	0	0	2	1	2	1	2	2	1	0	1	1	1	1	2	1	1	1	0	25
		(4)	(8)	(4)	(4)	-	-	(8)	(4)	(8)	(4)	(8)	(8)	(4)	-	(4)	(4)	(4)	(4)	(8)	(4)	(4)	(4)	-	(54.3)
All	10	4	4	3	6[a]	1	2	9[a]	5[a]	6[a]	2	4	6[a]	3	1	9[a]	8[a]	4	4	8[a]	5[a]	2	2	2	100
		(4)	(4)	(3)	(6)	(1)	(2)	(9)	(5)	(6)	(2)	(4)	(6)	(3)	(1)	(9)	(8)	(4)	(4)	(8)	(5)	(2)	(2)	(2)	(43.5)

[a] Immune response to PN23, PnPs STs in vervet serum samples (≥50%). [b] Age group 9-11 year vervet samples (serum 1171, serum 1202, serum 1220, serum 1232, and serum 1414). [c] Age group 19-23 year vervet samples (serum 1305, serum 1311, and serum 1417). [d] Age group 25-26 year vervet samples (serum 1099 and serum 1444). (-) indicates no response. [*] Percent given for specific serotype within age group. [**] Percent given for total serotype in age group.

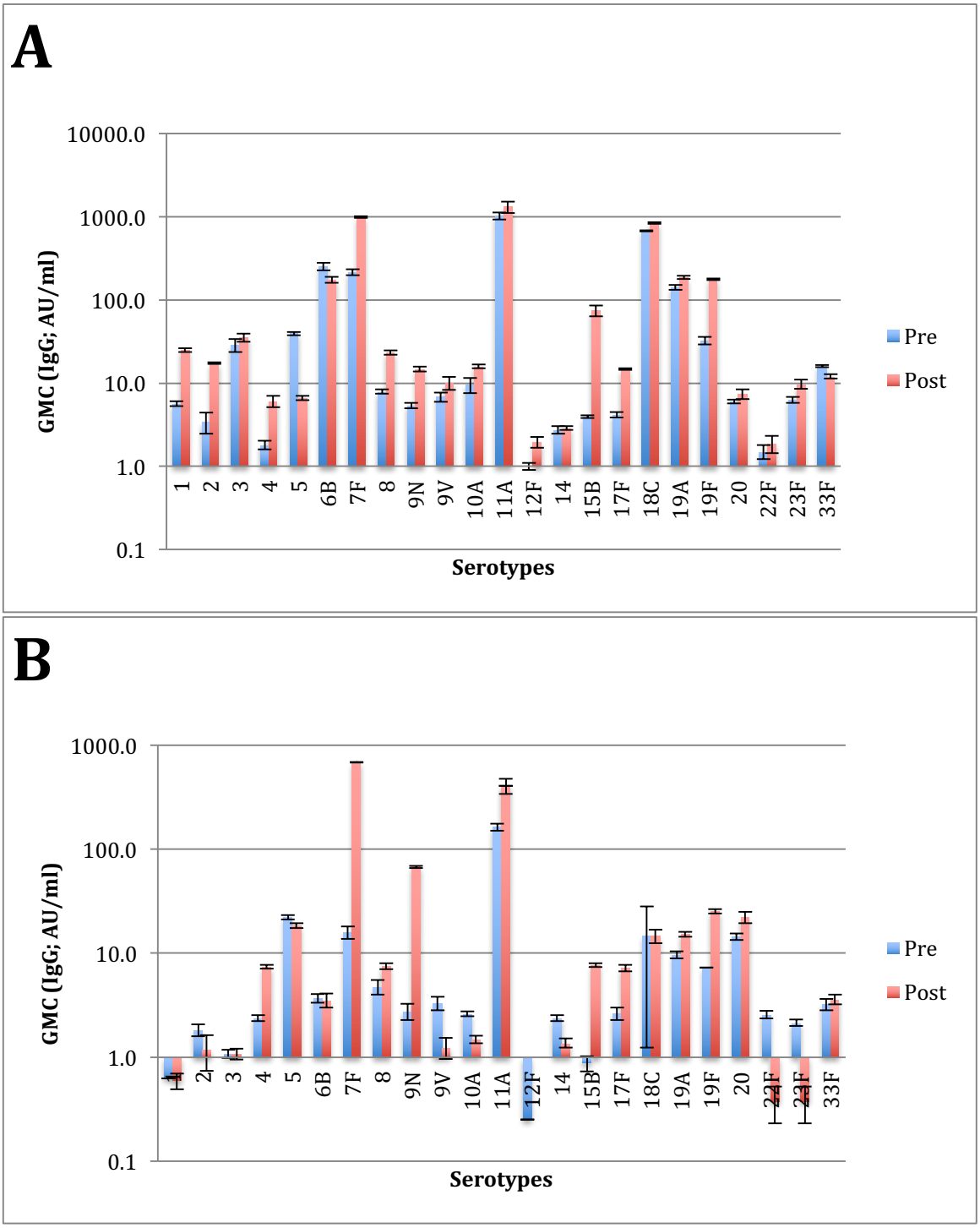

Figure 6. Geometric Mean Concentrations of Vervet Immune Response to 23 Pneumococcal Polysaccharide Serotypes Determined by Multiplexed Luminex Assay.

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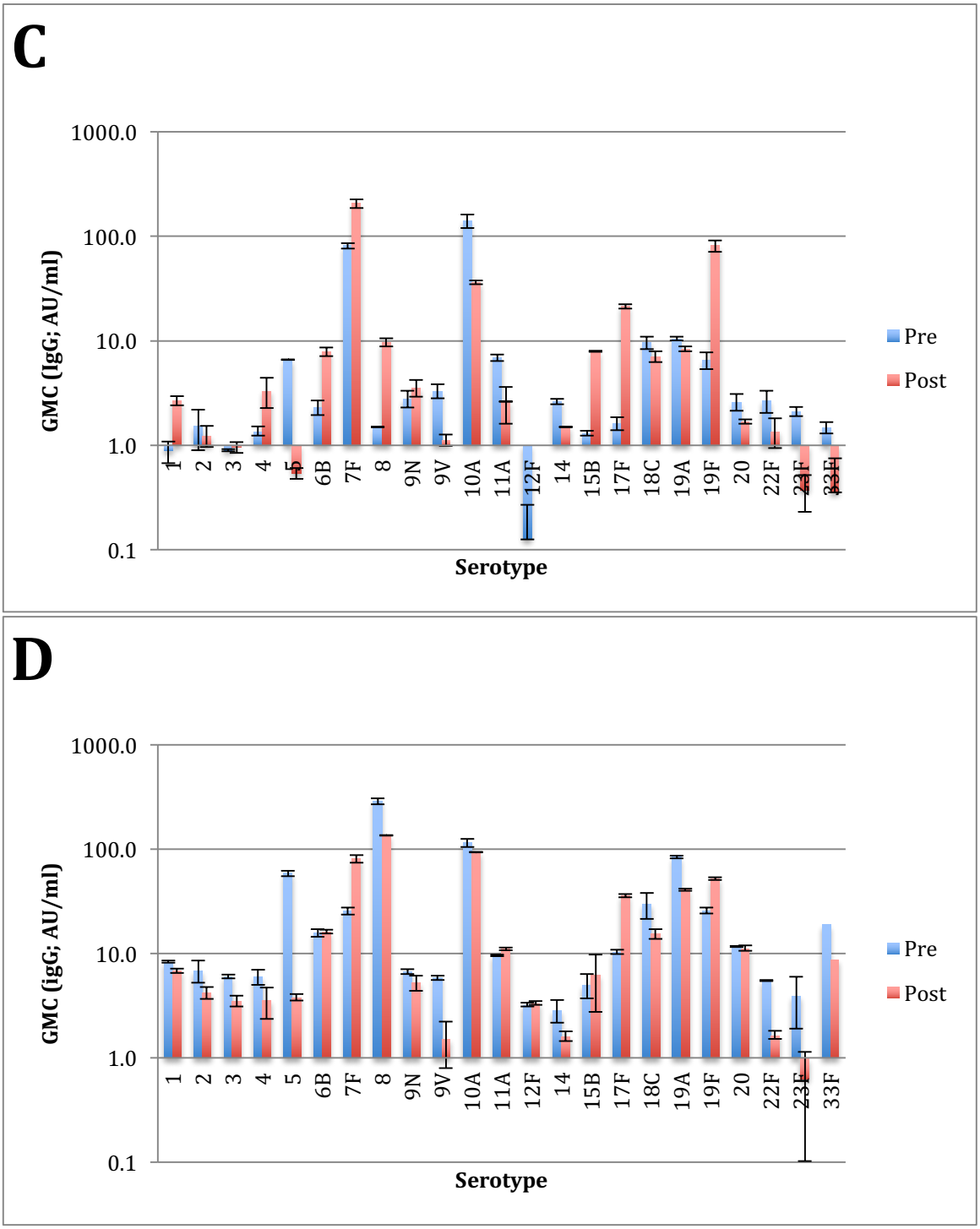

Figure 6. Geometric Mean Concentrations of Vervet Immune Response to 23 Pneumococcal Polysaccharide Serotypes Determined by Multiplexed Luminex Assay (continued).

Continue Overleaf

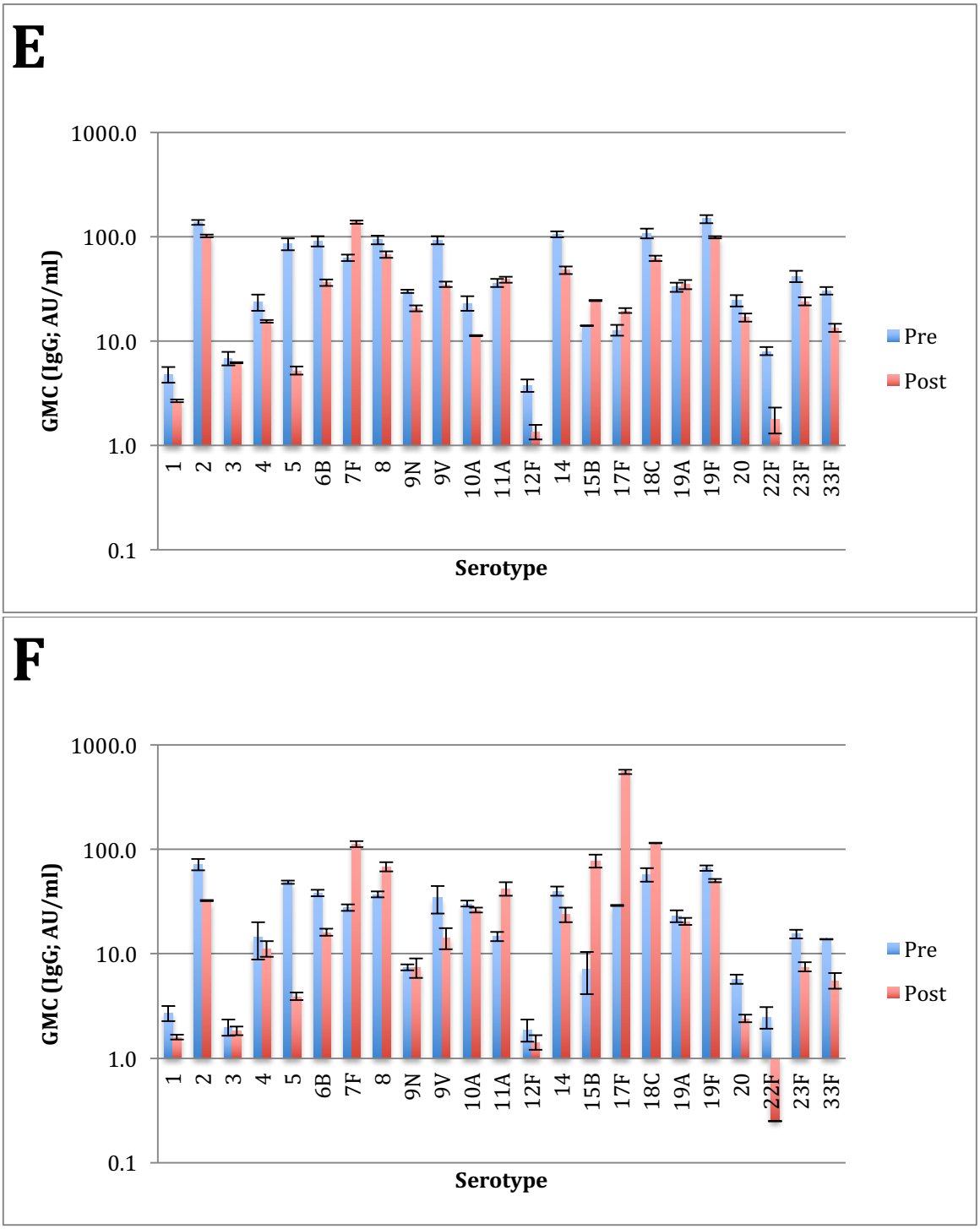

Figure 6. Geometric Mean Concentrations of Vervet Immune Response to 23 Pneumococcal Polysaccharide Serotypes Determined by Multiplexed Luminex Assay (continued).

Continue Overleaf

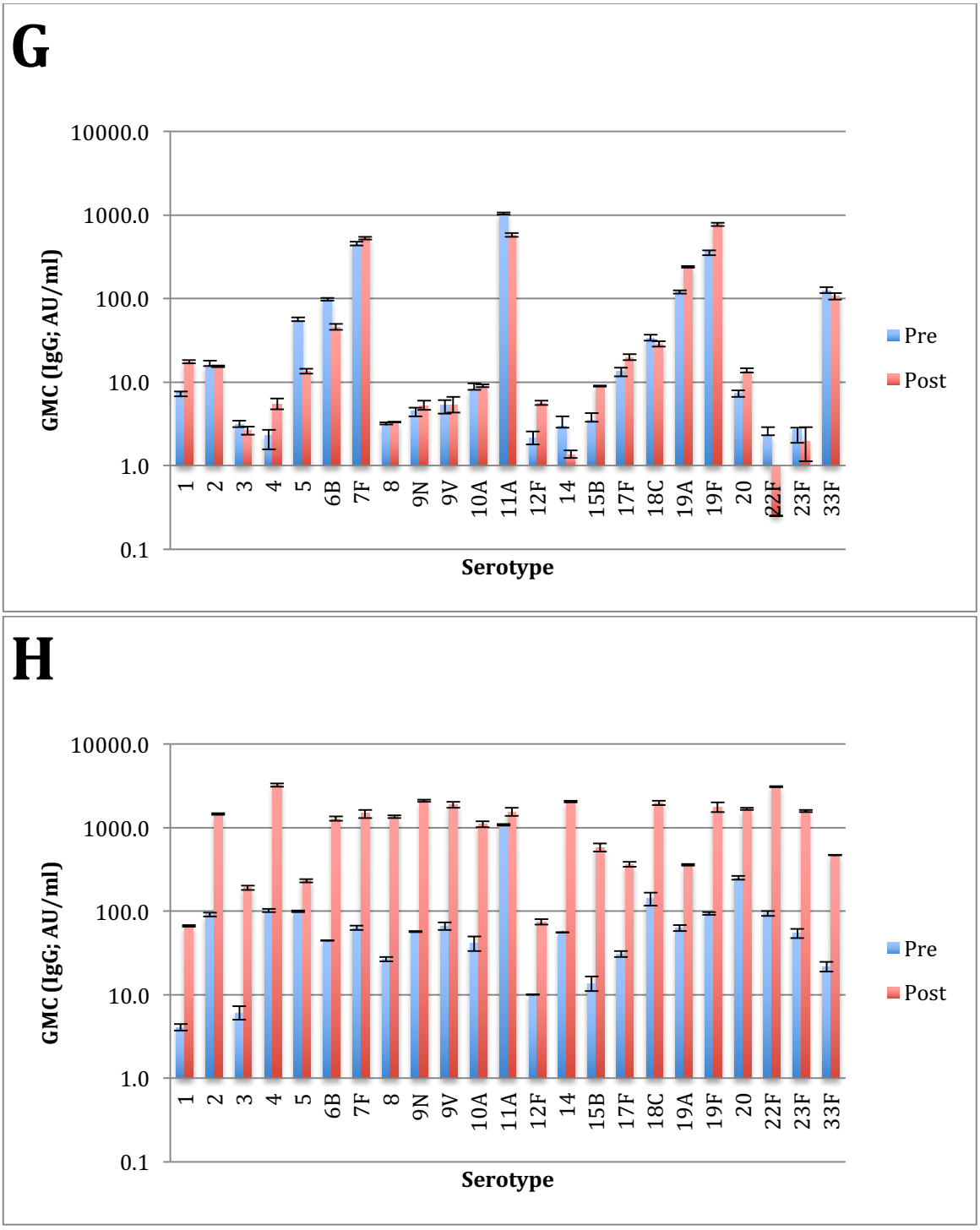

Figure 6. Geometric Mean Concentrations of Vervet Immune Response to 23 Pneumococcal Polysaccharide Serotypes Determined by Multiplexed Luminex Assay (continued).

Continue Overleaf

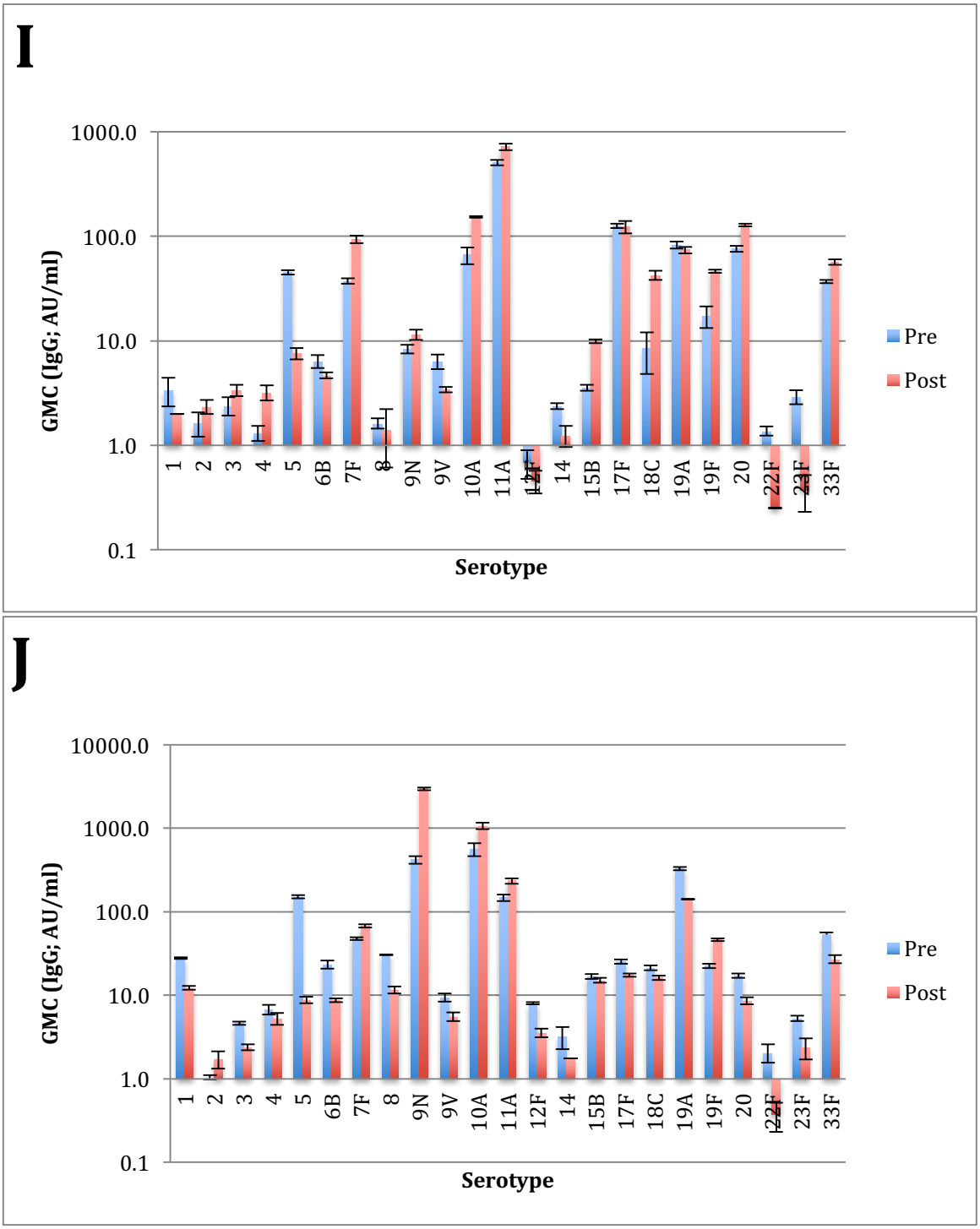

Figure 6. Geometric Mean Concentrations of Vervet Immune Response to 23 Pneumococcal Polysaccharide Serotypes Determined by Multiplexed Luminex Assay (continued). Geometric mean concentrations (GMC) of pre- and post-vaccination of PN23 pneumococcal polysaccharide serotypes for Vervet Serum Samples: (A) 1099, (B) 1171, (C) 1202, (D) 1220, (E) 1232, (F) 1305, (G) 1311, (H) 1414, (I) 1417, and (J) 1444. IgG concentrations are given as Arbitrary Units (median fluorescence intensity x dilution factor) per milliliter. All experiments were performed in triplicate.

Discussion

Responsible for over one million deaths in children under the age of five alone, *S. pneumoniae*-associated diseases remains one of the leading causes of morbidity and mortality throughout the world **(**37). The high morbidity and mortality rates among high-risk populations (i.e. children ≤five years of age; immunocompromised, asthmatic, or asplenic individuals; the elderly ≥65 years; and individuals in the developing world) are due to the inability to produce effective host antibodies against the PnPs (24, 27, 36, 37). Pneumococcus is an opportunistic pathogen that colonizes the mucosal epithelium in the nasopharynx of humans (37). The organism becomes pathogenic under host stress where it crosses the mucosal epithelium and enters the blood stream (37). External colonization and internal penetration by pneumococcus are determined by a multitude of factors including receptor-ligand interactions, receptor-ligand avidity and affinity, bacterial strain or ST, presence of a capsule, and previous exposure (37). The pneumococcal capsular polysaccharide interferes with the host's ability to effectively carry out complement-opsonization activity, therefore, protecting pneumococcus against phagocytosis (9**,** 36). Additionally, the pneumococcal surface protein A (PspA) binds to the C3 complement factor, inhibiting the host's complement immune response and block opsonization (9, 36). PspA also protects the pneumococcus from the bactericidal properties of the apolactoferrin enzyme (36).

Recent studies indicated that approximately thirty-seven percent of children under the age of five years are colonized with pneumococcus, with rates increased (57%) in crowded conditions (i.e. as schools, parks, and hospitals) where exposure to contaminated aerosolized respiratory droplets occurs more frequency (37). Exposure to pneumococcus in immunocompetent individuals induces type-specific anticapsular antibodies to PnPs STs, whereas high-risk populations lack the ability to elicit effective T-cell independent responses

against individual PnPs STs (9). The unresponsiveness to PnPs results in local infections and IPDs such as otitis media, bacteremia, meningitis, pneumonia, and sepsis, contributing to the high mortality rate around the world (9, 36, 37). Although there have been advancement in the development of anticapsular polysaccharide vaccines (PN23) against *S. pneumoniae*, the mortality rate is still a global issue especially among the developing world (9, 37). This high mortality rate may be due to the unpredictable host immune response against individual PnPs STs of the 23-valent, PN23 vaccine (5, 6, 21, 37). PN23 primarily benefits healthy individuals, who can produce effective type-specific antibodies, but does not elicit high efficacy, if at all, among the high-risk populations (21, 36, 37). Furthermore, the anticapsular antibody response among the very young and very old, differ dramatically due to naïve and weak immune systems, respectively (6, 37). The STs within PN23 account for approximately 90% of the most common IPDs; therefore, a consistent high-efficacy vaccine is needed (12, 21, 36).

The present study was conducted to determine if subjects, vervet monkeys, administered simultaneously with F1/V and Flu vaccine, known to elicit high IgG immune responses, would increase the efficacy rate as well as induce higher PnPs ST-specific IgG antibodies against the given PN23 vaccine. Additionally, this study analyzed and compared the PN23 ST-specific immune response against different age groups ranging; 9-11 years, 19-23 years, and 25-26 years. Serum sample levels were collected pre- and post-vaccination and the total IgG antibody responses were analyzed. All subjects measured displayed an increased response to at least three or more PnPs STs but did not respond equally to a specific ST. We observed a significant immune response, ≥50% increase over baseline, among the STs in the majority (≥5) of subjects, subject 1414 responded to all serotypes. These results suggest that simultaneous administration of the F1/V, Flu, and PN23 vaccines does not necessarily elicit an overall increased response to

the total amount of STs among hosts. However, the STs that elicited an immune response were significantly increased, over the baseline, after vaccination.

The age group, 9-11 years, had one or more subjects respond to all 23, PN23, PnPs STs. The age-wise analysis of vervets in the 19-23 age range did not produce an immune response to PnPs STs; ST5, ST6B, ST9V, ST14, ST22F, and ST23F. Subjects within the age group 25-26 years produced an immune response towards PnPs STs; ST1, 2, 3, 4, 7F, 9N, 9V, 10A, 11A, 12F, 15B, 17F, 18C, 19A, 19F, 20 22F, and 23F, failing to respond to five STs. These results indicate that the subjects, 9-11 years, and subjects, 25-26 years, displayed slightly higher (11%) immune responses then the age group 19-23 years. Furthermore, the 25-26 year subjects had a 17% increase over the age group 19-23 years (Table 3). The antibody titers among high-risk populations are lower and therefore are at a greater risk for developing *S. pneumoniae*-associated diseases, whereas healthy population antibody titers are higher and more protective (21).

The information gathered in this study is important for understanding the importance of anti-capsular polysaccharide vaccines among age distributions as well as determining if multiple vaccines will affect the immune response against *S. pneumoniae*. Although the immune response towards individual PnPs STs remains inconsistent among individual subjects, the overall baseline antibody response increased significantly toward some STs. The youngest (9-11 years) and oldest (25-26 years) age groups displayed stronger immune responses, which is essential for protection against pneumococcal infections. Therefore, the combination of F1/V, Flu, and PN23 vaccine is a good model for developing a vaccine treatment that elicits high-efficacy and long-lasting immunity in high-risk populations. Future studies may seek to further understand the molecular immunology interplay between the host and pathogen following the administration of PN23, F1/V, and Flu vaccines, for the advancement in vaccine technology and pneumococcal prevention.

CHAPTER 4. CONCLUSION

My involvement at the CDC in Atlanta, GA, and Mulago Hospital in Kampala, Uganda, provided me with further insight and appreciation into the dynamic nature of respiratory infectious diseases, especially their ability to advantageously adapt and survive in different host species. Seeing firsthand how a single disease can cause devastation from individual health to an entire economy, I was able to more fully appreciate the efforts of multi-disciplinary health professionals and researchers.

My time spent at Mulago hospital allowed me to study the significance of zoonotic tuberculosis in a densely populated city where the interactions between cattle and humans occurs frequently. The high interface between hosts contributes to *Mycobacterium bovis* diversity, and its impact on everyday life, especially among dairy cattle owners. Due to the limited disease surveillance in developing countries, the exact number of *M. bovis* associated tuberculosis in livestock and humans is unknown and often misdiagnosed. Because livestock plays such a vital economic and social role to the livelihood of individuals in developing countries, it is imperative to increase the awareness and control of zoonotic tuberculosis.

I was also able to appreciate the implications and importance of vaccine development against respiratory infectious diseases, specifically *S. pneumoniae* in different age groups. The experience at the CDC allowed me to study the immunological effect of multiple vaccines, F1/V plague vaccine, Flu vaccine, and PN23, against the 23 PnPs STs in the given PN23 vaccine. We observed that younger (9-11 years) and older (25-26 years) vervet monkeys display a slightly better host immune response compared to subjects in the age range of 19-23 years. This is particularly important in high-risk individuals (i.e. children ≤5 years, the immunocompromised, and the elderly ≤65 years) that are unable to produce effective antibodies (IgG) against

pneumococcal serotypes. Additionally, due to the high morbidity and mortality rates in high-risk populations, especially children, advancement in vaccine technology is desirable.

The current vaccines against zoonotic BTB (BCG) and pneumococcus (PN23) lack the ability to induce high efficacy rates and lifelong immunity. Because different strains of STs of bacteria within a species induce the immune response differently, the research conducted in my presented studies will provide further insight into the important of microbial diversity and its ability to independently affect the host immune system.

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