Meningococcal and Pneumococcal Meningitis in Northern Ghana

INAUGURALDISSERTATION

Zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät der

Universität Basel

Von

Abudulai Adams Forgor

Aus

Sawla, (Ghana)

Basel 2007

Genehmigt von der Philosophisch- Naturwissenschaftlichen Fakultät

Der Universität Basel auf Antrag der

Herren Prof. Dr. M. Tanner, Prof. Dr. T. Smith, Prof. Dr. G. Pluschke und Prof. F. Binka

Basel, 24th October 2006

Prof. Dr. Hans-Peter Hauri

Dekan

Dedicated to my mother
and my family

TABLE OF CONTENTS

TABLE OF CONTENTS I

ACKNOWLEDGEMENTS IVII

SUMMARY V

ZUSAMMENFASSUNG VIII

LIST OF TABLES XI

LIST OF FIGURES XII

ABBREVIATIONS XIV

CHAPTER 1 INTRODUCTION 1

1.1 EPIDEMIOLOGY OF MENINGOCOCCAL MENINGITIS 2
1.2 EPIDEMIOLOGY OF PNEUMOCOCCAL AND *H. INFLUENZAE* TYPE B MENINGITIS 3
1.3 PATHOGENESIS AND PATHOPHYSIOLOGY 5
1.4 EPIDEMICS OF MENINGOCOCCAL MENINGITIS 6
1.5 FACTORS FAVOURING EPIDEMICS OF MENINGOCOCCAL MENINGITIS 8
1.6 CHANGING EPIDEMIOLOGY OF ACUTE BACTERIAL MENINGITIS 9
1.7 CLINICAL FEATURES AND DIAGNOSIS 11
1.8 MANAGEMENT, CONTROL AND PREVENTION 13
1.9 BACTERIAL MENINGITIS IN GHANA 16
1.10 RATIONALE AND RESEARCH FRAME WORK 17

CHAPTER 2 GOAL AND OBJECTIVES 19

2.1 GOAL 20
2.2 OBJECTIVES 20

CHAPTER 3 METHODS 21

3.1 STUDY AREA. 22
3.2 STUDY DESIGN 23

CHAPTER 4 CLONAL WAVES OF COLONIZATION AND DISEASE OF *NEISSERIA MENINGITIDIS* IN THE AFRICAN MENINGITIS BELT. AN EIGHT-YEAR LONGITUDINAL STUDY IN NORTHERN GHANA 24

4.1 ABSTRACT 26
4.2 INTRODUCTION 26
4.3 MATERIALS AND METHODS 28
4.4 RESULTS 29
4.5 DISCUSSION 36
4.6 ACKNOWLEDGEMENTS 39

CHAPTER 5 EMERGENCE OF W135 MENINGOCOCCAL MENINGITIS IN GHANA 40

5.1 SUMMARY 42
5.2 INTRODUCTION 42
5.3 MATERIALS AND METHODS 43
5.4 RESULTS 45
5.5 DISCUSSION 50
5.6 ACKNOWLEDGEMENTS 52

CHAPTER 6 AN OUTBREAK OF SEROTYPE 1 *STREPTOCOCCUS PNEUMONIAE* MENINGITIS IN NORTHERN GHANA WITH FEATURES CHARACTERISTIC OF EPIDEMIC MENINGOCOCCAL MENINGITIS 53

6.1 ABSTRACT 55
6.2 INTRODUCTION 55
6.3 METHODS 57
6.4 RESULTS 58
6.5 DISCUSSION 66
6.6 ACKNOWLEDGEMENTS 69

CHAPTER 7 SURVIVAL AND SEQUELAE OF PNEUMOCOCCAL MENINGITIS IN NORTHERN GHANA 70

7.1 ABSTRACT 72
7.2 INTRODUCTION 73
7.3 MATERIALS AND METHODS 73
7.4 RESULTS 76
7.5 DISCUSSION 81
7.6 ACKNOWLEDGEMENTS 84

CHAPTER 8 INFLUENCE OF CLIMATIC FACTORS ON THE INCIDENCE OF MENINGOCOCCAL AND PNEUMOCOCCAL MENINGITIS IN NORTHERN GHANA .. 91

8.1 ABSTRACT 93
8.2 INTRODUCTION 94
8.3 METHODS 95
8.4 RESULTS 97
8.5 DISCUSSION 106
8.6 ACKNOWLEDGEMENTS 109

CHAPTER 9 DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS 110

9.1 DISCUSSION OF MAIN FINDINGS AND RECOMMENDATIONS 111
9.2 SUGGESTIONS FOR FURTHER RESEARCH 113
9.3 CONTROL OF PNEUMOCOCCAL MENINGITIS IN AFRICA 115
9.4 CONTROL OF MENINGOCOCCAL MENINGITIS IN THE AFRICAN MENINGITIS BELT 120
9.5 CONTROL OF MENINGOCOCCAL AND PNEUMOCOCCAL MENINGITIS IN NORTHERN GHANA 122
9.6 CONCLUSIONS 127

REFERENCE 129

APPENDIX PROCEDURE FOR PERFORMING LUMBAR PUNCTURE. 151

CURRICULCUM VITAE 154

ACKNOWLEDGEMENTS

This thesis forms part of a collaborative research project on bacterial meningitis between the Navrongo Health Research Centre (NHRC), Navrongo, Ghana and the Swiss Tropical Institute (STI), Basel, Switzerland. I am very grateful to Prof. Fred Binka and Prof. M. Tanner, initiators of this collaboration, for their foresight.

My sincerest gratitude goes to Prof. Fred Binka and Prof. Fred Wurapa for their advice, keen interest in my work and encouragement. My profound thanks go to my supervisors Prof. Tom Smith and Prof. Gerd Pluschke at STI and Dr Abraham Hodgson (Director NHRC) for their guidance, concern and useful discussions during my field work and throughout the period of analysis of the data and writing of this thesis. I wish to thank Dr. Penelope Vounosou, Martin Adjuik and Sama Wilson for their statistical support during the analysis of the data collected.

At STI, I wish to thank Julia Leimkugel (my counterpart), Valentin Pflüger and Jean Pierre Dangy for their fruitful discussions, support and excellent collaboration. I sincerely appreciate the team work and friendly atmosphere offered to me by the Molecular Immunology Group members, both current and past: Claudia Daubenberger, Daniela Schütte, Diana Diaz, Dorothy Yeboah-Manu, Denise Vogel, Charlotte Huber, Elizabetta Peduzzi, Marija Curcic, Markus Müller, Martin Naegeli, Marco Tamborrini, Max Bastian, Michael Kësar, Shinji Okitsu and Simona Rondini. I would also like to thank Christian Walliser, Eliane Ghilardi, Margrit Slaoui for all the assistance offered to me during my stay in Basel. My heartfelt thanks go to all the members of the Epidemiology group of STI. I wish to express my sincere gratitude to Mrs Juliana Smith for her editorial work.

I wish to thank the chiefs and people of the Kassena Nankana District, Kpalkpalgbeni, Alhassan Kura and Bolgatanga for their cooperation and willing participation in the research work.

I am very grateful to Dr Erasmus Agongo and Dr Cornelius Debpuur for the fruitful discussions and encouragement at the start of the PhD programme. I thank Dr Joseph Amankwah, Dr Lawson Ahadzi, Dr Teye Agyasi, Dr Seth Owusu-Agyei, Dr Ali Francis Soah, Dramani Ekwesi, the Municipal Health Management Team, Bolgatanga, and staff of the Regional Hospital laboratory, Bolgatanga, for the assistance offered to me on the field.

I wish to thank all the staff of NHRC for their contribution in diverse ways to making this thesis a success. My special thanks go to Christiana Amalba, Stanley Welaga, Akalifa Bugri, Abdul-Wahab Hamid, Raymond Allou, Mathilda Tipura, Elizabeth Awine, Joseph Asampana, Cletus Tindana, John Krugu, Oscar Bangre, Keneth Akabigre, James Danzumah, Ernest Atutiga, Maxwell Naab, for the wonderful and adventurous field experience. I greatly acknowledge the services of Peter Wuntuo of the Computer Centre and his team for the cooperation and assistance offered to me. My gratitude goes to Dr Bawa Ayaga and George Wak and their team at the Navrongo Demographic Surveillance Unit of NHRC for their support and expertise. I would like to thank Isaac Akumah, Andriana Sumboh, Diana Abagale, Jacob Anabia, Margaret Bugase, for the various ways they assisted me during my fieldwork at Navrongo. Special thanks go to Fred Ayamgba (May He Rest in Perfect Peace) for his profound assistance and making transport available even during very difficult times.

I wish to thank all staff of the War Memorial Hospital, (WMH) Navrongo, all staff of the Kassena Nankana District Health Directorate and all staff and in-charges of the Health Centres in the KND for their assistance and cooperation. My special thanks go to all members of staff of the Sampa Government Hospital, Sampa, Nana Kofi Sono II, Omanhene of Sampa Traditional Council, the people of Sampa and the Jaman Districts for making it possible for me to persue this programme.

My thanks go to my mother, Achulo Jeduah, mother-in-law, Mrs Cecilia Adu-Fokuo, brothers- and sisters-in-law, brothers and sisters for all their prayers and support. My sincere gratitude goes to my family for the patience they had with me during the period of this PhD programme.

I wish to acknowledge with thanks the financial support given me, for my studies in Basel, by the Amt für Ausbildungsbeiträge des Kantons Basel-Stadt, Switzerland. I am very grateful for this support.

The Meningitis Research Foundation, UK, the Meningitis Vaccine Project and the Volkswagen foundation financially supported this work.

SUMMARY

Despite improvements in technology, treatments and understanding of how bacterial meningitis develops, the disease remains a potentially life-threatening emergency capable of causing significant morbidity and mortality. *N. meningitidis*, *S. pneumoniae* and *H. influenzae* type b, which are commensally normal human nasopharyngeal flora, are the most important and common causes of bacterial meningitis. *N. meningitidis* (especially, serogroup A) is well known for its association with epidemics in the meningitis belt of sub-Saharan Africa. This nearly always starts during the dry season and stops during the onset of the rains and occurs every 8-12 years in the "meningitis belt" with attack rates sometimes exceeding 1% during these epidemics. *H. influenzae* type b and *S. pneumoniae* are mostly endemic affecting certain risk groups. *N. meningitidis* serogroup W135, traditionally known to cause isolated cases, has raised general concern in recent years due to outbreaks in Burkina Faso since 2002 attributed to it.

Following a major meningococcal meningitis epidemic in Northern Ghana in 1996/7 the Navrongo Health Research Centre in collaboration with the Swiss Tropical Institute in 1998 initiated a long-term colonization and disease study in the Kassena Nankana District (KND), with the aim of contributing to the understanding of the epidemiology, pathogenesis, improved intervention and early detection of bacterial meningitis epidemics in the "meningitis belt". As part of this long term study, this thesis focuses on meningococcal colonization and invasive disease surveillance (pneumococcal and meningococcal), burden of pneumococcal meningitis and the relationship between environmental factors and the risk of meningococcal and pneumococcal meningitis.

From 1998 to 2005 clonal waves of nasopharyngeal colonization with pathogenic and non-pathogenic meningococcal genoclouds were observed in the KND through the longitudinal meningococcal colonization study of residents of 37 randomly selected compounds. These meningococci were not only less diverse and unstable in composition with rare non-groupable strains, but they were also mostly made up of predominantly hyperinvasive strains (up 71%) with constant microevolution. In 1998 serogroup A meningococci ST5 caused an outbreak of meningococcal meningitis in the KND with persistent carriage up to 1999, disappearing in 2001. In 2000 serogroup X ST571 meningococci emerged with high carriage rates and few cases. Carriage of this serotype persisted until 2001 when it was replaced by serogroup A ST7 which only disappeared at the latter part of 2005 after causing outbreaks between 2002 and 2004.

Although *N. meningitidis* serogroup W135 has been the cause of epidemics in neighbouring Burkina Faso since 2002, only sporadic cases (4) were reported in Ghana from 2003 to 2004. The disease isolates were very similar to the Burkinabe epidemic strains by Pulse Field Gel Electrophoresis analysis. Colonization surveys over a one-year period in one of the patient home communities (which has semi-closed features) showed an initial high carriage rate of 17.5% and persistence of carriage with rapid microevolution.

Between 2000 and 2004 there was an outbreak of pneumococcal meningitis (PCM) caused by a *S. pneumoniae* serotype 1 clonal complex in the KND with features (seasonality, clonality and broad age spectrum of the patients) characteristic of meningococcal meningitis (MCM). This hypervirulent serotype is repeatedly being isolated in various parts of sub-Saharan Africa.

A two-year survival analysis comparing 67 PCM cases recorded at the War Memorial Hospital (WMH), Navrongo, Ghana, identifiable on a demographic surveillance system, with equal numbers of MCM and community controls, showed profound excess mortality of the PCM compared with both MCM and community controls. A case-control study of sequelae (using a structured disability questionnaire, neuropsychological and audiometric examinations of both cases and controls), matching for age, sex and geographical location, including 46 traceable survivors of PCM (cases), 46 community controls (CC) and 34 survivors of MCM, showed that hearing and speech impairment as well as psychiatric disorders are much more frequent and severe in PCM than MCM.

Epidemics of MCM and PCM are closely related to climate. A time series analysis of weekly meteorological data (humidity, rain fall, dust, wind speed, temperature and sunshine) from the local weather station and the corresponding reported epidemiological data (confirmed meningococcal and pneumococcal cases) from 1998 - 2004 from the WMH microbiology database was carried out using negative binomial regression and Bayesian methods. The aim of these micro epidemiological analyses was to describe as well as provide an early warning system for the short-term prediction of likely meningococcal and pneumococcal meningitis outbreaks in the KND.

The environmental factors that influence the incidence of PCM and MCM were found to be similar but not always the same. The duration of a preceding absence of rainfall appears to be the best predictor of both PCM and MCM outbreaks. Outbreaks of MCM are best predicted by concurrent decrease in rainfall with increase in weekly mean maximum temperature. Those of PCM are influenced by concurrent decrease in rainfall.

The natural variations in the predominance of different pharyngeal meningococcal serotypes and serogroups over time might contribute to meningococcal meningitis epidemics in the African meningitis belt. The future epidemiological trend of meningococcal and pneumococcal meningitis will be influenced by changes in the use of antibiotics, immune status, aging of the global population and technology. The introduction of carbohydrate-conjugate or common protein vaccines to routine immunization schedules, together with maternal immunization and enhanced disease (and/or colonization) surveillance, could make pneumococcal and meningococcal diseases of less public health importance.

ZUSAMMENFASSUNG

Trotz deutlicher Fortschritte in der Diagnosetechnik, verbesserten Behandlungsmethoden und einem erweiterten Verständnis der Pathogenese der bakteriellen Meningitis, bleibt diese eine lebensbedrohliche Krankheit mit signifikanter Morbidität und hoher Letalität. *Neisseria meningitidis, Streptococcus pneumoniae* und *Haemophilus influenzae* type b, natürliche Kommensalen des menschlichen Nasen-Rachenraumes, stellen die häufigsten Erreger der bakteriellen Meningitis dar. *N. meningitidis* (insbesondere die Serogruppe A) ist bekannt für Epidemien im südlich der Sahara gelegenen Meningitis-Gürtels Afrikas. Diese treten in dieser Region typischerweise alle 8-12 Jahre auf, beginnen mit Anfang der Trockenperioden und enden mit Eintreten der Regenzeit. Sie können Inzidenzraten von über 1% der Population erreichen. Meningitis verursacht durch *H. influenzae* type b und *S. pneumoniae* tritt meistens endemisch auf und ist mit bestimmten Risikogruppen assoziiert. *N. meningitidis* Serogruppe W135 ist gemeinhin bekannt als Verursacher vereinzelter Meningitis-Fälle. Jedoch erregen seit dem Jahre 2002 W135 Meningitis Ausbrüche in Burkina Faso allgemeine Besorgnis.

Nach einer grossen Meningokokken Epidemie in den Jahren 1996/7 in Ghana hat das Navrongo Health Research Center in Kollaboration mit dem Schweizerischen Tropeninstitut 1998 eine Langzeit Kolonisations- und Fallstudie im Kassena Nankana Distrikt (KND) initiiert. Diese zielt darauf, zum Verständnis der Epidemiologie bakterieller Meningits-Epidemien beizutragen, insbesondere hinsichtlich verbesserter Früherkennung und rechtzeitiger Interventionen. Als Teil dieser Langzeitstudie fokussierte sich die vorliegende Arbeit auf die Analyse der Zusammenhänge zwischen Meningokokken-Kolonisation und invasiven Erkrankung. Ferner wurde die allgemeinen Belastung der Bevölkerung durch Pneumokokken Meningits einschliesslich der Spätfolgen untersucht und die Zusammenhängen zwischen Umweltfaktoren und dem Risiko für Meningitis-Ausbrüche analysiert.

Im Rahmen der Meningokokken Kolonisations-Studie, an der Bewohner von 37 zufällig ausgewählten Haushalten teilnahmen, wurden zwischen 1998 und 2005 im KND klonale Wellen der Kolonisation mit pathogenen und nicht-pathogenen Meningokokken beobachtet. Die Population der Meningokokken Trägerisolate zeigte eine begrenzte Diversität. Insgesamt drei hyperinvasiven Klone

dominierten. Alle Labor-bestätigten Meningitis Fälle wurden durch diese verursacht. Nicht-serogruppierbare Stämme wurden nur vereinzelt gefunden.
Obwohl seit 2002 Meningokokken der Serogruppe W135 im benachbarten Burkina Faso Meningitis-Epidemien verursacht haben, wurden zwischen 2003 und 2004 in Ghana nur vereinzelte Fälle gemeldet. Die Fallisolate aus Ghana und Burkina Faso waren nahe verwandt und mittels Pulsed-Field Gel Electrophorese Analytik nicht unterscheidbar. Bei einer Kolonisationsstudien über einen Zeitraum von einem Jahr im Heimatdorf eines Patienten wurde eine anfänglich sehr hohe Trägerrate von 17,5% und eine fortdauernde Kolonisation mit rascher Mikroevolution beobachtet.

Zwischen 2000 und 2004 kam es im KND zu einem Pneumokokken Meningitis (PKM) Ausbruch, verursacht durch einen „klonalen Komplex“ von Serotyp 1 Pneumokokken. Dieser Ausbruch wies Eigenschaften auf (Saisonalität, Klonalität und ein breites Altersspektrum der Patienten), die charakteristisch für Meningokokken Meningitis (MKM) Epidemien sind.

Bei einer über zwei Jahre hin durchgeführten Überlebensanalyse wurden Daten von 67 PKM Patienten mit denen von MKM Patienten und von gesunden Kontrollen verglichen. Dabei wiesen die PKM Patienten eine deutlich höhere Mortalität auf. Eine Fallstudie über Folgerscheinungen, die 46 überlebende PKM Patienten und 34 MKM Patienten einschloss, zeigte, dass Hör- und Sprachbeeinträchtigungen sowie psychische Störungen in Folge der Erkrankung bei PKM Patienten häufiger und schwerwigender auftreten.

MKM und PKM Ausbrüche sind eng mit klimatischen Faktoren assoziiert. Wöchentliche meteorologische Daten (Feuchtigkeit, Regenmenge, Staub, Windgeschwindigkeit, Temperatur, Sonnenscheindauer) der lokalen Wetterstation wurden unter Verwendung von Bayesian Methoden und negativer binomialer Regression mit korresespondierenden epidemiologischen Daten (Anzahl der bestätigten MKM und PKM Fälle) von 1998 bis 2004 korreliert. Das Ziel dieser mikroepidemiologischen Studie war, mögliche Zusammenhänge zwischen Klimafaktoren und MKM und PKM Epidemien zu erfassen.

Es stellte sich heraus, dass die Umweltfaktoren welche das Risiko für PCM und MCM erhöhen, zwar ähnlich sind, aber nicht immer strikt übereinstimmen. Die Dauer der vorausgehenden Trockenperiode scheint der Beste Indikator sowohl für PKM als auch für MKM Ausbrüche zu sein.

MKM Ausbrüche können am besten durch gleichzeitig abfallende Niederschlagsmengen und ansteigende Maximaltemperaturen vorhergesagt werden. Das Risiko für PCM is mit dem Rückgang der Niederschlagsmenge assoziiert.

Der weitere epidemiologische Trend der Meningokokken und Pneumokokken Meningitis wird durch Änderungen im Antibiotika-Gebrauch, Entwicklung neuer Impfstoffe, Mobilität der Bewohner des Meningitis Gürtels und dem Status der Gesundheitssysteme beeinflusst werden. Insbesondere durch die Einführung von Kapsel-Polysaccharid Konjugat-Impfstoffen wird sich vermutlich die Bedeutung dieser Erkrankungen als gravierendes öffentliches Gesundheitsproblem reduzieren lassen.

LIST OF TABLES

TABLE 4.1: CARRIAGE RATES IN % DURING 16 CARRIAGE SURVEYS IN THE KASSENA NANKANA DISTRICT 32

TABLE 5.1 W135 CASES REPORTED TO THE GHANAIAN DISEASE CONTROL AUTHORITIES IN 2003 AND 2004 46

TABLE 5.2: CARRIAGE OF DIFFERENT SEROGROUPS OF *N. MENINGITIDIS* AND OF *N. LACTAMICA* IN HOME COMMUNITIES OF THREE W135 MENINGOCOCCAL MENINGITIS PATIENTS AND IN A NEIGHBOURING CONTROL COMMUNITY. 48

TABLE 5.3: AGE DISTRIBUTION OF COLONIZATION WITH *NEISSERIA LACTAMICA* AND W135 AND NON-W135 *NEISSERIA MENINGITIDIS* IN THE PATIENT HOME COMMUNITY K1 48

TABLE 6.1: AGE DISTRIBUTION OF SEROTYPE 1 AND NON-SEROTYPE 1 ISOLATES FROM THE KND FROM 2000 TO 2003 63

TABLE 6.2: SEROTYPE DISTRIBUTION AND STS OF *S. PNEUMONIAE* STRAINS ISOLATED IN NORTHERN GHANA BETWEEN 1998 AND 2003 64

TABLE 7.1: RESULTS OF TRACING 78

TABLE 7.2 DISTRIBUTION OF STUDY SUBJECTS 79

TABLE 7.3 DISABILITY (SELF REPORTED) 85

TABLE 7.4 SELF-REPORTED PSYCHIATRIC SYMPTOMS 86

TABLE 7.5 PSYCHIATRIC SYMPTOMS REPORTED BY RELATIVES 87

TABLE 7.6 HEARING ASSESSMENT. A. LEFT EAR 88

TABLE 7.7 HEARING ASSESSMENT. B. RIGHT EAR 89

TABLE 7.8 OTHER IDENTIFIED DISABILITIES 90

TABLE 8.1 RESULTS OF MODELLED MAXIMUM LIKELIHOOD AND BAYESIAN ESTIMATES OF THE EFFECTS OF CLIMATIC COVARIATES ON REPORTED INCIDENCE OF MENINGOCOCCAL MENINGITIS IN THE KASSENA NANKANA DISTRICT OBTAINED BY FITTING BIVARIATE AND MULTIVARIATE NEGATIVE BINOMIAL MODELS. 104

TABLE 8.2 RESULTS OF MODELLED MAXIMUM LIKELIHOOD AND BAYESIAN ESTIMATES OF THE EFFECTS OF CLIMATIC COVARIATES ON REPORTED INCIDENCE OF PNEUMOCOCCAL MENINGITIS IN THE KASSENA NANKANA DISTRICT OBTAINED BY FITTING BIVARIATE AND MULTIVARIATE NEGATIVE BINOMIAL MODELS. 105

LIST OF FIGURES

FIGURE 1.THE MENINGITIS BELT _____ 8

FIGURE 4.1 A & B. WAVES OF COLONIZATION AND DISEASE IN THE KND FROM APRIL 1998 UNTIL NOVEMBER 2005. _____ 33

FIGURE 4.1 C & D. WAVES OF COLONIZATION AND DISEASE IN THE KND FROM APRIL 1998 UNTIL NOVEMBER 2005 _____ 34

FIGURE 4.2 CARRIAGE CARRIAGE OF MENINGOCOCCI AND AGE SPECTRUM OF INCIDENCE RATES OF MENINGOCOCCAL MENINGITIS _____ 35

FIGURE 5.1 MAP OF GHANA SHOWING THE LOCATION OF HOME COMMUNITIES OF W135 MENINGITIS PATIENTS _____ 46

FIGURE 5.2 PFGE PROFILE OF W135 CARRIER AND DISEASE ISOLATES _____ 47

FIGURE 6.1 NUMBER OF LABORATORY-CONFIRMED MENINGITIS CASES IN THE KASSENA-NANKANA DISTRICT OF NORTHERN GHANA BETWEEN 1998 AND 2003._____ 59

FIGURE 6.2 SEASONAL PATTERNS OF RAINFALL AND NUMBER OF PNEUMOCOCCAL AND MENINGOCOCCAL MENINGITIS IN THE KND._____ 60

FIGURE 6.3 INCIDENCE (LABORATORY CONFIRMED CASES BY LATEX AGGLUTINATION OR CULTURE) OF MENINGOCOCCAL (GREY BARS) AND PNEUMOCOCCAL (BLACK BARS) MENINGITIS IN THE KND. __ 60

FIGURE 6.4 E-BURST DIAGRAM OF THE ST217 CLONAL COMPLEX _____ 65

FIGURE 7.1 REPORTED INCIDENCE AND MORTALITY RATES OF PNEUMOCOCCAL MENINGITIS IN THE KASSENA NANKANA DISTRICT 1998 – 2004_____ 76

FIGURE 7.2 REPORTED INCIDENCE RATES OF MENINGOCOCCAL AND PNEUMOCOCCAL MENINGITIS IN THE KASSENA NANKANA DISTRICT 1998 – 2004._____ 77

FIGURE 7.3 KAPLAN-MEIER SURVIVAL CURVES COMPARING THE SURVIVAL OF PNEUMOCOCCAL MENINGITIS CASES WITH MENINGOCOCCAL MENINGITIS CASES AND COMMUNITY CONTROLS IN THE KASSENA NANKANA DISTRICT. _____ 79

FIGURE 7.4 DISABILITY OF STUDY SUBJECTS. _____ 80

FIGURE 8.1 RELATIONSHIP BETWEEN RAINFALL AND HUMIDITY IN THE KND, 1998 - 2004_____ 97

FIGURE 8.2 RELATIONSHIP BETWEEN RAINFALL AND MAXIMUM TEMPERATURE IN THE KND, 1998 – 2004._____ 98

FIGURE 8.3 RELATIONSHIP BETWEEN MINIMUM TEMPERATURE, RELATIVE HUMIDITY (RECORDED AT 15.00HRS) AND NUMBER OF REPORTED MENINGOCOCCAL MENINGITIS CASES IN THE KND, 1998 – 2004. _____ 99

FIGURE 8.3 REPORTED PNEUMOCOCCAL AND MENINGOCOCCAL MENINGITIS CASES IN THE KND 1998 – 2004. _____ 99

FIGURE 8.5 RELATIONSHIP BETWEEN DUST, RELATINVE HUMIDITY (RECORDED AT 15.00HRS) AND REPORTED MENINGOCOCCAL MENINGITIS CASES IN KND, 1998 - 2004 _____ 100

FIGURE 8.6 RELATIONSHIP BETWEEN REPORTED PNEUMOCOCCAL AND MENINGOCOCCAL MENINGITIS CASES AND DUST IN THE KND, 1998 – 2004 _____ 101

FIGURE 8.7 RELATIONSHIP BETWEEN REPORTED PNEUMOCOCCAL AND MENINGOCOCCAL MENINGITIS CASES AND MAXIMUM TEMPERATURE IN THE KND, 1998 – 2004. _____ 102

FIGURE 8.8 RELATIONSHIP BETWEEN WIND SPEED, RELATIVE HUMIDITY AND REPORTED PNEUMOCOCCAL MENINGITIS CASES IN THE KND, 1998 – 2004 _____ 103

FIGURE 8.9 CAUSAL WEB INDICATING RELATIONSHIPS OF ENVIRONMENTAL FACTORS WITH PATHOGENESIS OF MENINGITIS _____ 103

ABBREVIATIONS

CC	Community Control
CI	Confidence Interval
CSF	Cerebrospinal Fluid
CSM	Cerebrospinal Meningitis
DHMT	District Health Management Team
DIC	Deviance Information Criteria
DNA	Deoxyribonucleic acid
eBurst	Based Upon Related Sequence Types
EPI	Expanded Programme on Immunization
ET	Electrophoretic Type
EWS	Early Warning System
Hib	*Haemophilus influenzae* type b
IRR	Incidence Rate Ratio
KND	Kassena Nankana District
LR	Log Rank
LRT	Likelihood Ratio Test
MCM	Meningococcal Meningitis
MCMC	Markov Chain Monte Carlo
MIC	Minimum Inhibitory Concentration
MLST	Multilocus Sequence Typing
MOH	Ministry of Health
NADMO	National Disaster Management Organization
NDSS	Navrongo Demographic Surveillance System
NHRC	Navrongo Health Research Centre
NSOPIBMS	National Standard Operating Procedures for the Implementation of bacterial Meningitis Surveillance
PCM	Pneumococcal Meningitis
PCR	Polymerase Chain Reaction
PFGE	Pulsed-field Gel Electrophoresis
RHMT	Regional Health Management Team

ST	Sequence Type
STI	Swiss Tropical Institute
WHO	World Health Organization
WinBugs	Windows version of Bayesian inference Using Gibbs Sampling
WMH	War Memorial Hospital

CHAPTER 1

INTRODUCTION

INTRODUCTION

Bacterial meningitis is the inflammation of the meninges (the thin lining that surrounds the brain and the spinal cord) and subarachnoid space caused by bacteria. Bacterial meningitis was universally considered to be a fatal disease from the time it was first described by Vieusseux in 1806 up to the early 20th century when sulphonamides and penicillins made this disease curable. Despite this achievement mortality and morbidity from bacterial meningitis still remain very high (Schuchat et al., 1997), with up to 50% of survivors developing long term neurological and neuropsychological sequelae (Smith et al., 1988; Grimwood et al., 2000; Hodgson et al., 2001b; van de Beek et al., 2002; van de Beek and de Gans, 2004a; Schmidt et al., 2006). Bacterial meningitis is now among the top 10 infectious causes of death worldwide (Grimwood et al., 2000). Over 90% of all acute bacterial meningitis worldwide, outside the neonatal period, is caused by *Streptococcus pneumoniae* (the pneumococcus), *Hemophilus influenzae* and *Neisseria meningitidis*, the meningococcus (Hart and Cuevas, 2003). While *H. influenuae* is associated mostly with childhood meningitis, *S. pneumoniae* mostly cause invasive disease in infants, the elderly and immunocompromised, *N. meningitidis* is characterized by epidemics (Mar et al., 1979; Moore, 1992). These three bacteria are all normal nasopharyngeal inhabitants causing disease occasionally. They are all transmitted from person to person via aerosolization or by contact with respiratory secretions of infected persons.

1.1 Epidemiology of meningococcal meningitis

Bacterial meningitis occurs globally. Excluding epidemics, the World Health Organisation (WHO) estimates that at least 1.2 million cases of bacterial meningitis occur each year out of which 135,000 are fatal. Approximately 500,000 of these cases, 60,000 disabilities and 50,000 of the deaths are due to the *N. meningitidis*. Of these, 250,000 cases, 27,000 deaths (Tikhomirov et al., 1997), 16,000 (6.4%) disabilities of which 10,000 (4%) are due to impaired hearing (Hodgson et al., 2001b), are from Africa. Although effective non-toxic and affordable antibiotics are available worldwide, meningococcal disease is still associated with a very high mortality and persistent neurological defects particularly among infants and young children (Tikhomirov et al., 1997).

The highest disease rates of meningococcal meningitis are found in children 10-19 years (Hodgson et al., 2001b). During epidemics, older children, teenagers and young adults are also affected. The incubation period is 2-10 days, often 3 days. Most of the infections are sub clinical with many infected people becoming carriers without symptoms. In the interepidemic period the carriage rate of meningococcal meningitis is approximately 10% (Cartwright et al., 1987; Stephens, 1999) and the attack rate about 40 cases per 100,000 per year (Hart and Cuevas, 2003) but the attack rate may exceed 1% in some areas during epidemics (WHO, 1998). Carrier rates of meningococci can be as high as 80% in situations of overcrowding such as during the Hajj (al-Gahtani et al., 1995).

The highest burden of meningitis occurs in Sub-Saharan Africa - in the "Meningitis Belt" (figure 1.1) which extends from Senegal to Ethiopia and includes all or part of the 15 countries that lie within the belt. Epidemics of meningococcal meningitis in this region are characterised by periodicity, geographical restriction, massive size and marked seasonality. These epidemics recur approximately every 8-12 years, although recently with higher frequency, peaking during the dry season (Moore, 1992).

1.2 Epidemiology of pneumococcal and *H. influenzae* type b meningitis

Although disease occurs in only a small proportion of individuals colonized by pneumococci, the annual burden of disease currently attributed to pneumococcal disease is 700,000 to 1 million deaths (http://www.who.int).

There are at least 90 serotypes of *S. pneumoniae* based on the polysaccharide structure of the pneumococcal capsule (Henrichsen, 1995). However, only a relatively small number of serotypes cause the vast majority of pneumococcal disease, while the number of serotypes that colonize people in a given community is far greater than the "invasive" ones (Butler, 2004). The distribution of invasive serotypes depends on the age (Scott et al., 1996), immunity (Fry et al., 2003), site of infection (Hausdorff et al., 2000a) and geographic location (Brandileone et al., 2003; Eskola et al., 1992; Hausdorff et al., 2000b; Hausdorff, 2002). Some serotypes are epidemic prone (1, 2, 3 and 5) because they are rarely isolated from the naopharynges of carriers (Feikin and Klugman, 2002). These serotypes which were responsible for outbreaks of pneumococcal meningitis in the early 1920s in the USA when there was an almost 100% mortality rate of this disease are now rare there (Swartz, 2004).

Serotypes 1 and 5 account for a large proportion of invasive isolates in most developing countries: 33% in the Gambia (Adegbola et al., 2006); 54% in Mali (Campbell et al., 2004); 38% in Uruguay (Hortal et al., 2000).

The incidence of invasive pneumococcal disease in children in the developing world (O'Dempsey et al., 1996; Usen et al., 1998) is far higher than that in the industrialized countries, and approaches the levels seen in the North American Indians (Cortese et al., 1992), Alaska natives (Davidson et al., 1993) and Australian aboriginals (Torzillo et al., 1995). This has been attributed to a variety of factors such as: i) genetic (the propensity of sickle cell disease patients to pneumococcal disease (Wong et al., 1992)); ii). the presence of antecedent viral infection (Dowell et al., 2003; Kim et al., 1996); iii) age (Scott et al., 1996; Dowell et al., 2003); iv) immunity (Nuorti et al., 2000b); v) socio-economic status (Chen et al., 1998); vi) alcohol and tobacco use (Pastor et al., 1998; Nuorti et al., 2000a); vii) humidity and crowding of susceptible hosts (Dowell et al., 2003; Talbot et al., 2005); viii) HIV/AIDS children are 20 to 40 times more likely to get pneumococcal disease than children without HIV/AIDS (Mao et al., 1996).

S. pneumoniae has a very high case-fatality rate: about 20% for community-acquired meningitis in developed countries (Schuchat et al., 1997) and up to 40-75% in children who get it in the developing world (Baraff et al., 1993; Goetghebuer et al., 2000; Montefiore et al., 1978). Pneumococcal meningitis is also prevalent in the rainforest belt of West Africa (Montefiore et al., 1978). Community acquired pneumonia, bacterial meningitis, acute otitis media and acute bacterial sinusitis are the most commonly identified pneumococcal infections (Butler, 2004).

Meningococcal meningitis has overshadowed *H. influenzae* meningitis in Africa, due to the large outbreaks in the meningitis belt. The incidence of *H. influenzae* (Hib) meningitis in The Gambia is as high as it was in the USA before the introduction of the Hib vaccine, but it has a 10-fold more devastating outcome and the peak prevalence is at the age of five months (Bijlmer et al., 1990).

Pneumococcal disease outbreaks caused by a single strain of pneumococcus occur sporadically in temperate countries, with occasional reports of outbreaks of pneumoniae, meningitis and conjunctivitis in settings like nursing homes and residential care facilities (CDC, 2001; Nuorti et al., 1998), military units (Gray et al., 1999) and prisons (Hoge et al., 1994).

Pneumococcal colonization rate is highest in children <1 year, ranging between 42% and 97% and declining with age to about 4% (Gray et al., 1980; Regev-Yochay et al., 2004b; Hill et al., 2006). Colonized siblings are the strongest risk factors for pneumococcal carriage in infants in both high-income (Gray et al., 1980; Leino et al., 2001) and low-income countries (Coles et al., 2002). The main source of pneumococcal transmission seems to be children at their peak age (2-5 years) of pneumococcal carriage (Givon-Lavi et al., 2002; Leino et al., 2001). Overall, pneumococcal carriage is markedly greater in low-income countries than in high-income countries (Feikin et al., 2003; Lloyd-Evans et al., 1996; Montgomery et al., 1990).

1.3 Pathogenesis and pathophysiology

There is the need to understand the mechanisms that promote the conversion of carriage to disease in order to adopt appropriate interventions even though carriage is often, but not always, an antecedent event in invasive disease transmission in airborne, an intervention that blocks transmission of the above mentioned pathogens will greatly reduce the incidence of disease.

The initiation of infection with meningeal pathogens usually begins with host acquisition of a new organism by nasopharyngeal colonization (Stephens, 1991). The surface characteristics of the pathogens enhance mucosal colonization for example; *N. meningitidis* possesses fimbriae (pilli) which enable adherence of this organism to the nasopharynx (Tunkel and Scheld, 1993). The meningococcus is transported across the nasopharyngeal epithelial cells into the blood stream with a phagocytic vacuole via a specific cell surface receptor (Stephens, 1991).

Fimbriae also play an initial role in the adherence of Hib (Tunkel and Scheld, 1993). Invasion of the bloodstream by Hib occurs via the breakdown in tight junctions between epithelial cells (contrary to *N. meningitidis*) leading to an invasion by an intracellular mechanism (Stephens, 1991). Surface encapsulation is also an important virulence factor for nasopharyngeal colonization and systemic invasion as demonstrated by Hib (Tunkel and Scheld, 1993). The presence of surface capsule, by inhibiting neutrophil phagocytosis and resisting classic complement–mediated bactericidal activity may enhance the survival and replication of the organisms in the blood stream (Tunkel et al., 1990; Tunkel and Scheld, 1993). The process, by which the pneumococci traverse the nasopharyngeal

mucosa to other sites including the meninges, is multifactorial and can be grouped as immunological and non-immunological.

The non-immunological process consist of abnormalities of the integrity of the epithelial surface of the nasopharynx which appear acutely following viral infection and more gradually in tobacco smokers as well as people exposed to airborne pollutants like those produced by indoor fire for cooking and heating.

The immunological process is characterised by the infection of the mucosal epithelium by *S. pneumoniae*, which is facilitated by secretory IgA through secretion of IgA protease. This protease cleaves the proline-rich hinge region of IgA rendering it non-functional and allowing the pneumococcus to attach to the epithelium (Aronin and Quagliarello, 2001). *S. pneumoniae* enters the intravascular space after the mucosal attachment and invasion. Complements and cytokines are also involved in the process of invasion (Aronin and Quagliarello, 2001) leading to meningeal inflammation, brain oedema and permanent neurological damage. The cell wall component stimulate leucocyte recruitment into the subarachnoid space, induce cytokine and platelet activating factor production, enhance cerebral endothelial permeability, alter cerebral blood flow and cause direct neurological damage.

The clinical manifestation depends on the organs or tissue affected: asymptomatic (carrier) if the bacteria remain in the nasopharynx or oropharynx, bacteraemia/septicaemia (meningococcemia if the organism is *N. meningitidis,*) if the bacteria multiply in the bloodstream, arthritis (if in the joints are affected), endocarditis (if in the endocardium) and meningitis if they invade the coverings of the brain, subarachnoid space and spinal cord.

1.4 Epidemics of meningococcal meningitis

Meningococcal meningitis (cerebrospinal meningitis, CSM) is a contagious bacterial disease. The first clear account of an outbreak of CSM was given by Viesseux in 1806 following a typical epidemic in Geneva, Switzerland (Greenwood, 1999). Epidemic meningitis, as it is also known, is a very serious medical emergency with socioeconomic implications and can disrupt both public health and the community.

The meningococcus, which was first described in 1884 (Marchiafava and Celli, 1884) and first cultured from patients with CSM by Weichselbaum in Vienna (1887), is a gram negative diploccocus with thirteen serogroups based on the antigenicity of its capsular polysaccharides (Moore, 1992). These serogroups are A, B, C, D, H, I, K, L, W135, X, Y, Z, Z` with A, B and C responsible for 90% of invasive meningococcal disease. While serogroup A and C have occurred in epidemics, serogroup B is often sporadic though it may sometime cause some outbreaks (Peltola, 1983), Y and W135 were traditionally known to occasionally cause disease but since 2000, outbreaks and even epidemics of W135 are been recorded yearly (Kwara et al., 1998; Taha et al., 2000; Taha et al., 2002b; Decosas and Koama, 2002).

The bulk of disease over the past 100 years was caused by serogroup A (Greenwood, 2006). It was responsible for two pandemics in Asia throughout the 1960s, 70s and 80s spreading from China in the early 1980s to Nepal and India. In 1987, it was responsible for an outbreak involving 2000 pilgrims to the Hajj in Mecca, Saudi Arabia (Wilder-Smith and Memish, 2003).

The largest recorded epidemic of meningococcal disease in history occurred in Africa in 1996 where 250,000 cases including 25,000 deaths were reported to the WHO. Between that crisis and 2002, 223,000 meningococcal meningitis cases were reported, mainly from Burkina Faso, Chad, Ethiopia, and Niger (WHO, 2003b).

In 2002, countries further south of the meningitis belt in the Great Lakes region, such as Tanzania, Rwanda, Burundi and the Democratic Republic of Congo reported over 2200 cases of meningococcal disease, including 200 deaths; small villages and refugee camps were most affected (WHO, 2003b). There are also reports (from Côte d`Ivoire, Togo, Central African Republic and Cameroon) of smaller epidemics expanding to “new” districts southward in the Sahelian region (Savory et al., 2006). These epidemics indicate the southwards expansion of the meningitis belt probably due to reduction in rainfall and absolute humidity in these “new” epidemic districts (Molesworth et al., 2002) as a result of deforestation (Monnier, 1980) and desertification (Soro et al., 1988) in these areas.

Figure 1. The Meningitis Belt (Source: Moore, 1992)

1.5 Factors favouring epidemics of meningococcal meningitis

It is difficult to predict epidemics of meningococcal meningitis and this usually leads to the late initiation of control measures, like immunization, with a resultant poor outcome (Greenwood, 1987). Factors that facilitate epidemics include dilution of herd immunity with birth of new cohorts and migration. Extreme environmental conditions in the sub-Saharan meningitis belt during the dry season-low humidity, high temperature and the harmattan (dusty wind blowing from the Sahara), respiratory co-infections and the introduction of a new meningococcal clone into a susceptible population are thought to contribute to these epidemics (Moore, 1992). Cooking in kitchens with firewood stoves and sharing a bedroom with a case are risk factors for meningococcal meningitis (Hodgson et al., 2001a). Interactions between these factors may explain the periodicity and seasonal patterns of epidemics as well as the unusual age distribution among individuals who contract the disease during an epidemic. Peak incidence occurs generally in periods of low absolute humidity such as winter in temperate zones and the dry season in Africa.

1.6 Changing epidemiology of acute bacterial meningitis

During the past 10-15 years acute bacterial meningitis has undergone a dramatic change in epidemiology. The most significant epidemiological change is the marked decline in the incidence of bacterial meningitis due to Hib in North America, Western Europe and countries where the conjugate Hib vaccines have been introduced into routine childhood immunisation programmes (Schuchat et al., 1997). This has made *S. pneumoniae* and *N. meningitidis* the most common causes of acute bacterial meningitis in these countries with adults rather than infants and children being most affected. However, due to the high cost of the Hib vaccine, most developing countries still experience a very high case mortality and morbidity annually from acute bacterial meningitis due to Hib.

The emergence of antimicrobial resistance among causative pathogens of bacterial meningitis is another epidemiological change being witnessed, the most important of which is the resistance to penicillin and other β-lactam antibiotics (Hansman, 1978; Van Esso et al., 1987; Appelbaum, 1987b; Whitney et al., 2000). This has serious implications for the management of acute bacterial meningitis. Factors that contribute to this resistance include selective pressure, transfer of resistant genes in diverse micro organisms and mutations in common genes (Kaye et al., 2000; Kaye and Kaye, 2000). In both *S. pneumoniae* and *N. meningitidis*, humans are the only reservoir, and asymptomatic colonization is frequent. However, the natural history of colonization differs in these two bacterial species. The average colonization duration of *S. pneumoniae* is approximately 2 to 3 months (Raymond et al., 2000), whereas duration is approximately 10 months for *N. meningitidis* (Cartwright, 1995). Asymptomatic carriage of *S. pneumoniae* peaks during the first 2 years of life and then gradually declines (Butler, 2004; Hill et al., 2006). By contrast, carriage of *N. meningitidis* peaks in young adults (Cartwright, 1995), which implies a difference in antibiotic exposure and therefore in the selection pressure borne by these bacteria, as young children are treated more frequently than young adults.

The mechanism of *S. pneumoniae* resistance to penicillin and other β-lactams involves alterations in one or more penicillin-binding proteins (PBP) so as to reduce their affinity for penicillin and related antibiotics. These alterations are usually present in the transpeptidase penicillin-binding domain. In order to achieve high-level resistance among PBP variants multiple mutations take place (Charpentier

and Tuomanen, 2000). The genes that encode for the mutant PBP are called "mosaics" because they contain native pneumococcal DNA mixed with fragments of foreign DNA most likely from a commensal with more penicillin resistance. The worldwide spread of penicillin resistance among *S. pneumoniae* appears to be due to dissemination of several clones carrying altered PBP genes (Spratt, 1994). There are reports of spread of penicillin resistance among meningococci which increased from 9.6% of strains in 1997 to 34.6% of strains in 2000 in Ontario, Canada (CCDR, 2001).

High-level chloramphenicol resistance in meningococci isolates has also appeared (Galimand et al., 1998; Shultz et al., 2003). This has very serious consequences since chloramphenicol in oil (for intramuscular use) is the main drug of choice in resource-limited countries (especially in the meningitis belt) in the control of meningococcal meningitis epidemics.

There is changing time pattern of epidemics of meningococcal meningitis in the meningitis belt (the epidemics are now shorter and more frequent) while the predominant cause of epidemics is still *N. meningitidis* serogroup A. In Sudan, in the 1930s, there was an outbreak of meningococcal meningitis caused by serogroup B there has since then not been any epidemic of this in meningitis belt (Greenwood, 1999). While meningococcal meningitis epidemics between 1940 and 1960 were caused predominantly by serogorup A (Lapeyssonnie, 1963), in the 1970s there were epidemics caused by serogroup C in Nigeria and Niger (Whittle et al., 1975; Broome et al., 1983).

In the 1990s meningococcal epidemics were caused predominantly by serogroup A in the African meningitis belt (Achtman, 1995; Morelli et al., 1997; Gagneux et al., 2000) after a serogroup A subgroup III (ST5) outbreak in Mecca during the annual *Haj* pilgrimage in 1987 (Moore et al., 1988). This serogroup A subgroup III (ST5) was replaced by another serogroup A subgroup ST7 (Nicolas et al., 2001). There were reports of serogroup X outbreaks in the late 1990s (Gagneux et al., 2000; Gagneux et al., 2002a; Gagneux et al., 2002b). Since 2002 W135 has emerged as a major cause of epidemics in Burkina Faso (Decosas and Koama, 2002) though it has been in circulation for a long time in West Africa without causing epidemics (Denis et al., 1982; Kwara et al., 1998). This natural changing pattern is due to natural variations in pre-dominance of different serotypes that take place over time as evidenced by changes in the serotype of nasopharyngeal isolates in the KND over time (Gagneux et al., 2002b). During a serogroup X meningococcal meningitis outbreak there was also a high carriage of this serogroup (Gagneux et al., 2002b).

The introduction of conjugate vaccines in the routine immunization programmes in various countries has resulted in the changing patterns of vaccine related pathogens. The widespread use of 7-valent pneumococcal conjugate vaccine in the USA has led to a replacement of the vaccine-related serotypes with non-vaccine related serotypes in the nasopharynx (Ghaffar et al., 2004). There is also an increase in invasive pneumococcal disease due to non-vaccine related serotypes (Eskola et al., 2001; Kaplan et al., 2004; Byington et al., 2005). *S. pneumoiae* since the introduction of this vaccine has become the major cause of bacterial meningitis in the USA and bacterial meningitis is now a disease predominantly of adults rather than infants (Short and Tunkel, 2000).

The introduction of meningococcal serogroup C conjugate vaccine in the United Kingdom in 1999 has resulted in a sharp decline in morbidity and mortality of meningitis due to serogroup C in the target group as well as a significant reduction in the carriage of this serogroup with no significant changes in carriage of meningococci expressing other disease-associated serogroups and no capsular switching (Ramsay et al., 2001; Maiden and Stuart, 2002; Palmer, 2002).

1.7 Clinical features and diagnosis

Sudden onset of intense headache, fever, nausea, vomiting, photophobia, irritability, neck stiffness and backache are characteristics of acute bacterial meningitis. Neurological signs include lethargy, delirium, coma and/or convulsions. Kernig's and Brudzinski`s sign may be positive. Infants may have the illness without neck stiffness and a sudden onset. In infants there may be a bulging fontanel. Up to 20% of children with bacterial meningitis have convulsions but in general 26-30% of cases have convulsions (Hart and Cuevas, 2003). Generally, only about 44% of patients present with the classic triad of fever, neck stiffness and altered mental status (Glasgow coma scale <14) although almost all patients present with at least two of the signs and symptoms of headache, fever, neck stiffness and altered mental status (van de Beek et al., 2004).

Most often, respiratory tract infection precedes symptoms of meningitis. While most pneumococcal meningitis patients have underlying conditions like pneumonia, otitis, immunocompromised state (van de Beek et al., 2004; Kastenbauer and Pfister, 2003; Weisfelt et al., 2006), meningococcal meningitis patients most frequently have rashes (van de Beek et al., 2004; Attia et al., 1999). The VIII (6-10%), III (4%), IV (3%), and VII (2%) nerves (van de Beek et al., 2004) are the main cranial nerves affected

during bacterial meningitis though cranial nerve palsy is relatively rare. In about 15-23% focal cerebral deficits like aphasia, hemiparesis and monoparesis are present while ocular manifestation like papiloedema is about 4% (Durand et al., 1993; van de Beek et al., 2004).

Even with early diagnosis and adequate treatment the case fatality in pneumococcal meningitis is in the range of 19% - 37% (van de Beek et al., 2004; van de Beek et al., 2006; Weisfelt et al., 2006; Kastenbauer and Pfister, 2003). Meningococcal meningitis has lower case fatality and morbidity rates in the range of 5% to 10% respectively (WHO, 1999; Woods et al., 2000; Hodgson et al., 2001b; van de Beek et al., 2004; van de Beek et al., 2006). The most important risk factors for poor outcome in patients with bacterial meningitis are impaired consciousness, infection with *S. pneumoniae*, systemic compromise and low cerebrospinal fluid (CSF) white-cell count (van de Beek et al., 2004).

Meningococcemia is a rare but more severe (often fatal) form of meningococcal disease and is characterised by rapid circulatory collapse (septic shock) and hemorrhagic rash (coagulopathy). If untreated, it will lead to hypotension, inadequate tissue perfusion and oxygenation causing necrosis and gangrene. There can be large areas of necrosis and loss of skin that may require grafting (to speed up the healing time, protect underlying structures by reducing the chances of infection) or cause scarring. Sometimes limbs and digits are amputated as a result of gangrenous necrotic areas.

Lumbar puncture is a critical procedure in the diagnosis of bacterial meningitis and therefore mandatory in any patient in whom bacterial meningitis is suspected, although the procedure can be hazardous. It involves withdrawing CSF by the insertion of a hollow needle with a stylet into the lumbar subarachnoid space (see appendix). Depending on the presence of significant concentration of white blood cells, red blood cells, bacteria and/protein the CSF appearance may be cloudy, xanthochromic or hemorrhagic. The CSF shows pleocytosis (100 to 10000 white cells per cubic milliliter) with predominantly neutrophilia (though about 10% of patients have lymphocytosis or monocytosis), elevated protein levels (>50mg per deciliter) and decreased glucose level of <40% compared to serum glucose (Spanos et al., 1989; Durand et al., 1993; van de Beek et al., 2004)

Laboratory diagnosis of bacterial meningitis rests on CSF examination after lumber puncture. Gram staining is a simple, rapid, accurate and inexpensive method for detecting bacteria and inflammatory cells in the CSF from patients with suspected bacterial meningitis. Latex agglutination test, which

detects antigens, has a sensitivity of 50% to 100% depending on the meningeal pathogen, is simple to perform, does not require special equipment and gives rapid results (Gray and Fedorko, 1992). Multi Locus Sequence Typing, Pulse Field Gel Electrophoresis and Polymerase Chain Reaction penicillin-binding protein finger printing, ribotyping and restriction fragment end labelling are genetic typing methods used for strain characterisation in epidemiological studies. Isolation of the organism from the CSF by culture methods is the definitive diagnosis. These are expensive and also require skilled personnel.

1.8 Management, control and prevention

Bacterial meningitis should always be viewed as a medical emergency since it is potentially fatal and treatment must be initiated as quickly as possible. Sero-therapy was successfully used in the treatment of meningococcal disease (Peltola, 1983) until the discovery of sulphonamides which greatly improved the patient recovery rate. Sulphonamides were stopped in the 1970`s as a result of the emergence of sulphonamide resistant serogroup A meningococci (Greenwood, 1999). A range of drugs available currently includes penicillin G, ampicillin, chloramphenicol and ceftriaxone. Oily chloramphenicol is the drug of choice in areas with limited health facilities and during epidemics since it is less expensive and given intramuscularly as a single dose injection (WHO, 1998).

Chemoprophylaxis can be considered in endemic situations for people in close contact with patients. This is however, not effective during epidemics in view of the cost. Rifampicin (Blakebrough and Gilles, 1980), ciprofloxacin and ceftriaxone (Cuevas et al., 1995) have been shown to be effective at eradicating carriage. However, the use of rifampicin is not recommended since this is a key drug in the control of tuberculosis.

Enhanced epidemiological surveillance and prompt case management with oily chloramphenicol and mass immunization are used to control meningococcal meningitis epidemics in the African Meningitis Belt. Routine immunization is not possible with the current available vaccines as the polysaccharide vaccines provide protection for only three to five years and are not immunogenic in children under 2 years of age. It has been shown in Niger that a single-dose of ceftriaxone is a good alternative to oily chloramphenicol in the control of meningococcal epidemics (Nathan et al., 2005). This drug can be used in pregnant women and infants.

Meningococcal polysaccharide vaccines have been available for many years and proven to be protective in adults (Gotschlich et al., 1969). These vaccines are however, poorly immunogenic in young children and hypo-responsive after repeated doses in children as well as adults (Granoff et al., 1998; Richmond et al., 2000; Artenstein and Brandt, 1975). Polysaccharide vaccines are available against serogroup A, C, Y and W135 meningococci and mass immunisations of at least 80% of the entire population can arrest an epidemic (Greenwood, 1999).

Capsular polysaccharide–conjugate vaccines have been shown to induce salivary antibody, reduce nasopharyngeal colonization and are immunogenic in infants (Borrow et al., 1999; Dagan et al., 1996). Meningococcal serogroup C polysaccharide-conjugate vaccine is now in use in the United Kingdom, Spain and other developed countries. Hib conjugate vaccine is also available.

There are three arms of pneumococcal vaccines being explored. These are polysaccharide vaccines, polysaccharide-protein conjugate vaccines and common protein vaccines. While the former two are in use successfully (in the developed countries), the latter is still at the trial stage.

There is a 23-valent polysaccharide pneumococcal vaccine that contains the 23 most common serotypes responsible for 90% of serious pneumococcal disease in the developed countries. This vaccine is not available in most developing countries, especially in the African meningitis belt, where the burden of pneumococcal disease is highest. This vaccine has been shown to have no effect on HIV patients in Uganda (French et al., 2000).

By conjugating polysaccharide vaccine antigens to a protein carrier the antigen is converted from a T cell-independent one to a T cell-dependent one. Polysaccharide-protein conjugate vaccines include the 9-valent vaccine which contains the serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F, a 7-valent vaccine containing the above serotypes excluding 1 and 5, and an 11-valent containing serotype 3 and 7F in addition to all the 9 serotypes in the 9-valent.

In contrast to pure carbohydrate vaccines, conjugate vaccines confer immunity in children less than 2 years, reduce rate of colonization of vaccine serotypes, including antibiotic-resistant strains and confer herd immunity (Whitney et al., 2003; Talbot et al., 2004; McEllistrem et al., 2005; Poehling et al.,

2006). These characteristics are very promising for public health use of these vaccines in developing countries.

Conjugate vaccines are very expensive, have limited protection due to serotype specificity and not available in developing countries due to the high cost of the vaccine. The other problem with these vaccines is the effect on carriage (Huang et al., 2005) since they may cause an ecological imbalance in the ecological niche of vaccine serotypes in the nasopharynx leading to serotype replacement (Eskola et al., 2001; Poehling et al., 2006) with a substantial increase in non vaccine serotypes like 11, 15, and 19A. These strains have been shown to also carry antibiotic resistance (Kyaw et al., 2006; Huang et al., 2005) a situation very unfortunate and disturbing. Through genetic transformation, pneumococci have the capability of capsule switching with original strains like 6B, 9V and 23F having the propensity for global spread for reasons not well understood (Crook and Spratt, 1998). This indicates that, new strains can emerge that can both escape the influence of the vaccine and spread worldwide should these three strains acquire genes of non-vaccine capsules.

There is also the possibility of different bacteria like *Staphylococcus aureus* replacing (Regev-Yochay et al., 2004a; Regev-Yochay et al., 2006) *S. pneumoniae* since the latter will no longer be there to inhibit growth of the former through the production of hydrogen peroxide by its catalase (Regev-Yochay et al., 2006).

Common protein vaccines (which are not serotype specific) are being developed from conserved protein epitopes. Currently, there are 3 candidate vaccines namely: pneumococcal surface protein A, pneumococcal surface adhesion A and pneumolysoid (a mutant pneumolysin-like molecule). Pneumococcal surface protein A has been shown to protect animal models against *S. pneumoniae* infection after either oral or parenteral administration (Yamamoto et al., 1997; Briles et al., 1996).

Common protein vaccines are less expensive to manufacture than the current polyvalent vaccines (which use the capsular polysaccharide as the immunizing antigen) since they can be produced in large amounts using inexpensive recombinant technology. They are therefore ideal candidate pneumococcal vaccines for use in developing countries with high burden of disease and limited resources.

The challenge to be faced by common protein vaccines is antigenic polymorphism of the candidates and species replacement in the nasopharynx.

For mass immunization WHO proposes a weekly incidence of 15 cases per 100 000 inhabitants, averaged over 2 consecutive weeks, as a threshold to confirm the onset of a meningococcal meningitis epidemic for areas of population 30 000 to 100 000 in the African meningitis belt, and 5 per 100 000 per week was proposed to initiate vaccination when an epidemic is underway nearby (WHO, 2000). This has been criticized for its failure, under field conditions, to detect many epidemics earlier (Moore, 1992; Kaninda et al., 2000; Lewis et al., 2001) and can be effective (Woods et al., 2000) only under a very good surveillance system. This is lacking in many areas of the meningitis belt making epidemics often far ahead of logistical support including vaccines.

1.9 Bacterial meningitis in Ghana

The first recorded outbreak of CSM in Ghana was at Cape Coast in 1900 among East African labourers who were brought to the Gold Coast to support the British campaign against the Ashanti (Waddy, 1957). This outbreak died out rapidly without causing an epidemic in the local population. The next epidemic of CSM in the Gold Coast started in 1906 from the north west and spread through the northern territory during the following dry season claiming 8000 lives by 1908 (Horn, 1908). Since then there have been epidemics every 8-12 years. Epidemics occurred in 1919/21, 1939, 1944/45, 1948/50 (Waddy, 1957), 1960/61, 1972/73 (Belcher et al., 1977) and 1984. In 1996/97 Ghana experienced the biggest epidemic which recorded 18703 cases and 1356 deaths (Woods et al., 2000). The Kassena Nankana District (KND) recorded 1396 cases with 69 deaths (Enos, 1997). It was caused by serogroup A ST7 meningococci which had caused an epidemic in Mecca in 1987 and subsequently spread through the meningitis belt (Gagneux et al., 2000).

S. pneumoniae is the commonest cause of meningitis in Accra on the coast of Ghana (Haddock, 1971). *S. pneumoniae* was also found to cause over 50% of bacterial meningitis in Kumasi (a tropical rainforest zone with a long rainy season and a short dry season) and its surroundings with a mortality rate of 36.4% (Mackie et al., 1992). In the above study pneumococcal meningitis was found to be most prevalent during the dry hot season.

1.10 Rationale and research frame work

Following the 1996/97 epidemic of meningitis in Ghana (Tikhomirov et al., 1997; Woods et al., 2000), the Navrongo Health Research Centre (NHRC) [Ministry of Health, Ghana] and the Swiss Tropical Institute (STI), Basel, Switzerland, established a scientific research partnership to address problems relating to epidemic meningococcal disease. The goal of the collaboration is to contribute to the understanding of the epidemiology and pathogenesis of meningococcal meningitis and its control in the meningitis belt.

This collaboration has led to the determination of the causative agents of bacterial meningitis in the KND (Gagneux et al., 2000; Gagneux et al., 2002a; Gagneux et al., 2002b) and the analysis of the genetic population structure and microevolution of the meningococcal strains dominating in the KND. The findings of the molecular epidemiological and clinical research works during the first phase of the collaboration can be found in the PhD thesis of Sebastian Gagneux (Gagneux, 2001) and Abraham Hodgson (Hodgson, 2002).

The NHRC/STI research collaboration made use of a demographic surveillance system (at the NHRC) and geographic location of all the compounds in the KND to give a detailed description of the epidemiological features of the 1996/97 epidemic in the district. The risk factors (Hodgson et al., 2001a), survival and sequelae (Hodgson et al., 2001b) of meningococcal meningitis were also researched into under the above collaboration. Following the above meningococcal meningitis epidemic in the KND and outbreaks in neighbouring Burkina Faso as well as threats of epidemics of serogroup W135 epidemics made it important to continue the long term meningococcal colonisation survey and analysis necessary for the long term understanding of mechanisms underlying epidemics of meningococcal meningitis in the African meningitis belt.

Detailed analysis of CSF samples from suspected meningitis cases from the KND and Bolgatanga regional hospital (Upper East regional hospital) showed that there was an increase in pneumococcal meningitis cases associated with high mortality in the region. There is relatively little information on the burden of pneumococcal meningitis in the African meningitis belt. The answer may contribute to the study of the pathogenesis of pneumococcal meningitis. It is also of practical importance particularly in the development of pneumococcal vaccine and policy change in the management and

prevention of pneumococcal meningitis as well as rehabilitation of survivors of pneumococcal meningitis.

Epidemics of meningococcal meningitis have been shown to have a strong association with environmental conditions (Lapeyssonnie, 1963; Besancenot et al., 1997; Belcher et al., 1977; Greenwood, 1987) though the underlying mechanisms of this association are not well understood (Greenwood et al., 1983). The current recommendation by WHO (Varaine et al., 1997; WHO, 2000) for the declaration of an epidemic is less specific (Kaninda et al., 2000; Lewis et al., 2001) in that before this figure is arrived at many people would have died in the communities since people in developing countries, especially rural areas, mostly seek traditional treatment or self medicate as a result various healthcare seeking behaviours or geographical and financial barriers to healthcare.

There is the need to use an alternative method that can predict an impending epidemic based on prior knowledge of the disease situation in the district from the previous year(s). It is important to consider the use of local environmental factors of the district like humidity, temperature, dust, length of sunshine, wind speed and rainfall (which are recorded by the local weather stations) together with the epidemiological data of the district (recorded at the health facilities) in the prediction of these epidemics.

CHAPTER 2

GOAL AND OBJECTIVES

GOAL AND OBJECTIVES

2.1 Goal

To contribute to the understanding of the epidemiology and pathogenesis of meningococcal and pneumococcal meningitis and assess the burden of pneumococcal meningitis in Northern Ghana.

2.2 Objectives

1. To investigate the dynamics of carriage and disease of *N. meningitidis* in the Kassena Nankana District of Northern Ghana by analysing the persistence of epidemic strains and the acquisition of new clones.

2. To describe the epidemiological features and assess the survival and sequelae of pneumococcal meningitis in Northern Ghana.

3. To describe the influence of climatic factors on the incidence of meningococcal and pneumococcal meningitis in Northern Ghana with the goal to develop a simple early warning system for the prediction of outbreaks.

4. To develop recommendations for the prevention and control of meningococcal and pneumococcal meningitis in Northern Ghana.

CHAPTER 3

METHODS

CHAPTER 3

METHODS

3.1 Study area.

The Kasena Nankana District (KND), one of the most deprived districts in Ghana, has a population of 140000, an area of 1675km^2 and lies within the guinea savannah woodland of northern Ghana between latitude 10^{o}30´ and 11^{o}00` north of the equator and between longitude 1$^o$00`and 1^{o}30` west of the Greenwich meridian. The district lies within the meningitis belt of sub-Saharan Africa with a sub-Sahelian climate of a short rainy season from May to October (average annual rainfall 850-950mm) and a long dry season from November to April, much of which is dusty due to the harmattan winds blowing from the Sahara. The soil type of the KND is mainly sand, clay, gravel and loamy soil. In most places a combination of sandy loam covers a very large acreage. The land cover is generally grassland with thin vegetation during the rainy season and a very dry land with poor vegetation during the dry season.

The general population is rural except for those living in Navrongo, the district capital. People live in compounds with an average population of 10 and a range of 1 to 143. These compounds in most parts of the district are widely dispersed with farmlands around them.

The district has 1 hospital (the War Memorial Hospital) located in Navrongo, the district capital and 4 health sub districts each of which has a health centre. The KND has a state owned meteorological station in Navrongo where daily weather conditions are recorded. The district has a demographic surveillance system in which births, deaths, in and out migrations and other demographic characteristics and residence status are updated every ninety days (Binka et al., 1999).
The district has a weather station where daily climatic conditions are recorded.

3.2 Study design

Detailed description of the methods can be found in the respective chapters. Analysis of the meningococcal colonization and disease in the KND from 1998 to 2005 was carried out to decribe the observed pattern of carriage and disease of meningococci (chapter 4). Analysis of serogroup W135 carriage (following 4 reported cases) was also carried out and a decription of the observed carriage pattern as well as clinical picture are in chapter 5. All cases recorded from 1998 to 2003 were analyzed. The results have been used to describe the epidemiological features of pneumococcal meningitis in chapter 6. A case-control study design, with the facilitation of the NDSS, was used to determine the survival and sequelae of pneumococcal meningitis cases recorded from 1998 to 2004 (chapter 7). Statistical methods include fitting Bayesian autoregressive term order 1 using Markov Chain Monte Carlo simulation in WinBugs version 1.4. Negative binomial regression (in both STATA and WinBugs) was used for the time series analysis of the climate and epidemiological data (chapter 8).

CHAPTER 4

CLONAL WAVES OF COLONIZATION AND DISEASE OF *NEISSERIA MENINGITIDIS* IN THE AFRICAN MENINGITIS BELT. AN EIGHT-YEAR LONGITUDINAL STUDY IN NORTHERN GHANA

CHAPTER 4

Clonal Waves of Colonization and Disease of *Neisseria meningitidis* in the African Meningitis Belt. An Eight-Year Longitudinal Study in Northern Ghana

[1] Julia Leimkugel, [2] Abraham Hodgson, [2] Abudulai Adams Forgor, [1] Valentin Pflüger, [1] Jean-Pierre Dangy, [1] Tom Smith, [3] Mark Achtman, [1*] Sébastien Gagneux and [1] Gerd Pluschke

[1] Swiss Tropical Institute, Basel, Switzerland
[2] Navrongo Health Research Centre, Ministry of Health, Navrongo, Ghana
[3] Max Planck Institute for Infection Biology, Berlin, Germany

*Present address: The Institute for Systems Biology, Seattle, USA

This article has been publish in

Public Library on Science - Medicine 2007; 4 (3): e101

4.1 Abstract

Background The Kassena-Nankana District (KND) of northern Ghana lies in the African meningitis belt where epidemics of meningococcal meningitis have been re-occurring every 8-12 years for the last 100 years. The dynamics of meningococcal colonisation and disease are incompletely understood.

Methodology/Principal Findings Between February 1998 and November 2005, pharyngeal carriage of *N. meningitidis* in the KND was studied by twice yearly colonisation surveys. Meningococcal disease was monitored throughout the 8-year study period, and patient isolates were compared to the colonisation isolates. The overall meningococcal colonisation rate of the study population was 6.1%. Compared to industrialised countries, the colonising meningococcal population was genetically less diverse, less constant in genotype composition over time, and a smaller proportion of the isolates was non-serogroupable. All culture-confirmed patient isolates and the majority of carriage isolates were associated with three sequential waves of colonisation with encapsulated (A ST5, X ST751, A ST7) meningococci. We observed a broad age range in the healthy carriers, resembling that of meningitis patients during large disease epidemics.

Conclusions The observed lack of a temporally stable and genetically diverse resident pharyngeal flora of meningococci might contribute to the susceptibility to meningococcal disease epidemics in the African meningitis belt. Because capsular conjugate vaccines are known to impact meningococcal carriage, effects on herd immunity and potential serogroup replacement should be monitored following the introduction of such vaccines.

4.2 Introduction

The highest burden of meningococcal meningitis occurs in the 'meningitis belt' of sub-Saharan Africa; a region stretching from Senegal to Ethiopia with an estimated population of 300 million (Lapeyssonnie, 1963; Greenwood, 1999). Within individual areas of the meningitis belt, major disease epidemics occur in irregular cycles every 8–12 years, with attack rates ranging from 100 to 1000 per 100,000 population. Epidemics start in the early dry season, stop abruptly at the onset of

the rains, but may break out again in the following dry season. Low humidity and high temperatures may favour the occurrence of meningococcal disease by damaging mucosal surfaces and the immune defence. In any one country, epidemics only last for two to three years (Greenwood, 1999). The periodicity of these epidemics is not well understood, nor is it possible to predict them accurately. The current approach for control of meningococcal disease epidemics is based on early detection of the disease by the epidemic threshold of 10-15 cases per 100,000 inhabitants per week (WHO, 2000) followed by mass immunisations with polysaccharide vaccines (WHO, 1998). However, in settings with limited resources, effective surveillance and timely interventions are difficult to implement. Therefore vaccination campaigns are often delayed (Greenwood, 1999).

N. meningitidis can be classified into thirteen serogroups based on the chemical composition of its polysaccharide capsule (Yazdankhah and Caugant, 2004). Serogroup A accounts for most epidemics in the African meningitis belt, but C and W135 epidemics have also been reported (Greenwood, 1999; 2005). Meningococci that cause epidemics are genetically closely related; specific genotypes plus their epidemiologically associated genetic descendants constitute specific genoclouds (Zhu et al., 2001). The two most recent meningococcal disease pandemics originated in Asia and were caused by serogroup A meningococci belonging to two related genoclouds (Zhu et al., 2001). These two genoclouds have been assigned the sequence types 5 (ST5) and ST7, respectively, based on Multi-Locus Sequence Typing (MLST) (Zhu et al., 2001; Maiden et al., 1998). Serogroup W135 meningococci used to be a rare cause of invasive disease. However, two recent W135 meningitis outbreaks in Mecca were followed by major epidemics in Burkina Faso (Taha et al., 2000; WHO, 2005).

N. meningitidis is a commensal of the human nasopharyngeal mucosa. It is transmitted by aerosol droplets or through contact with respiratory secretions. Because meningococcal transmission is independent of disease, characterisation of the carrier state is crucial for understanding the epidemiology of meningococcal disease. Multiple colonisation studies have been performed in industrialized countries, but little is known about the meningococcal colonisation dynamics in Africa. Here, we report the findings of the first long-term colonisation study carried out in the African meningitis belt. Our results demonstrate a notable absence of a temporally stable and genetically diverse meningococcal flora in the pharynx of healthy individuals, which may result in increased susceptibility for epidemic meningococcal disease.

4.3 Materials and Methods

Study area

The study was conducted in the Kassena-Nankana District (KND) of the Upper-East Region of Ghana. It lies within the guinea Savannah woodland and has two major seasons; a short wet season from June to October and a long dry season for the rest of the year. The district-population is about 140,000, most of them rural, except for the 20,000 inhabitants of Navrongo town. People live in compounds with an average of 10 inhabitants. Between 1997 and 2002, yearly vaccination campaigns with meningococcal serogroup A/C polysaccharide vaccine targeted the whole district population. Between 2003 and 2005, smaller campaigns were carried out. In 2003, 80% of the study participants reported to have been vaccinated within the previous three years. Ethical clearance for this study was obtained from the responsible institutional review boards.

Colonization isolates

Thirty-seven residential compounds were randomly selected from a complete listing of the district population using the Navrongo Demographic Surveillance System (NDSS)(Binka et al., 1999). Throat swabs were taken twice per year from all inhabitants of the 37 compounds who agreed to participate. A total of 16 surveys have been performed since March 1998. One of the compounds was replaced in April 2002 after being deserted by its inhabitants. A throat swab was taken from all consenting compound members present at the time of the visit and directly inoculated on Thayer-Martin agar plates (Gagneux et al., 2000). Two colonies with neisserial morphology were sub-cultured from each positive plate. *N. meningitidis* and *N. lactamica* colonies were identified by standard bacteriological methods as previously described (Gagneux et al., 2000).

Disease isolates

Suspected meningitis patients presenting at the War Memorial Hospital, Navrongo, or one of the four Health Centres of the KND were recruited throughout the study period. A suspected meningitis patient was defined by sudden onset of fever and stiff neck, or fever and stiff neck and altered mental status, in accordance with WHO-guidelines (WHO, 1998). A lumbar puncture was performed before treatment, and the cerebrospinal fluid specimen was analyzed as described previously (Gagneux et al., 2000).

Characterisation of bacterial isolates

Meningococci were serogrouped with serogroup-specific antisera (Difco) according to the manufacturer's instruction. In a subset of isolates, serological typing was confirmed by PCR (Taha, 2000; Bennett et al., 2004). All isolates were analysed by pulsed-field gel electrophoresis (PFGE) after digestion of genomic DNA with *NheI* (Morelli et al., 1997). MLST was performed as described (Maiden et al., 1998).

4.4 Results

Clonal waves of meningococcal colonisation and disease

We monitored the dynamics of pharyngeal carriage of *N. meningitidis* and bacterial meningitis in the KND of northern Ghana from February 1998 to November 2005. Three major waves of clonal colonisation and disease with encapsulated meningococci were observed. A meningitis epidemic in the dry season of 1996/97 (Hodgson A. et al., 2002) was followed by a smaller outbreak with 50 laboratory-confirmed serogroup A meningitis cases in the following dry season. Thirty-six isolates were culture confirmed and identified as subgroup III, ST5 bacteria (Gagneux et al., 2000), that spread throughout the meningitis belt after an epidemic in Mecca in 1987 (Nicolas et al., 2001). Carriage of the serogroup A ST5 meningococci decreased steadily from 2.7% (8/301) in April 1998 to 0.3% (1/308) in November 1999 (Fig. 4.1a). Thereafter, none of the clinical or colonization isolates from the KND belonged to the serogroup A ST5 genocloud. In 2000, no serogroup A meningococci were isolated from either patients or carriers. However, a new wave of serogroup A meningococcal colonisation and disease started in 2001. All serogroup A carrier and disease strains isolated since then belonged to a new genocloud of serogroup A meningococci associated with ST7 that was observed for the first time in Africa in 1995 (Zhu et al., 2001). Although colonisation was still low in April 2001 (i.e. was <0.3%), seven serogroup A ST7 meningitis cases were identified between February and March 2001. In the following three years, serogroup A ST7 colonisation rates of 1.2% to 4.3% were observed. In spite of yearly serogroup A/C polysaccharide mass-immunisations, this low level of colonisation was associated with repeated serogroup A ST7 meningitis outbreaks in the KND (Fig. 4.1a). Seventy laboratory-confirmed cases were identified between January and May 2002, and 56 between January and May 2003, and 114 between

December 2003 and April 2004. Thereafter, the serogroup A ST7 colonisation rate dropped below 1% and only two serogroup A ST7 meningitis cases were recorded in February 2005. Between the two waves of serogroup A colonisation and disease, we documented a wave of colonisation with a serogroup X ST751 genocloud (Fig.4.1b) (Gagneux et al., 2002a; Gagneux et al., 2002b). The extensive spread of this low-virulent serogroup was associated with a total of 15 meningitis cases between 1998 and 2003. Serogroup X carriage and disease peaked in the dry seasons of 1999/2000 and 2000/01 with colonisation rates of 17.3 and 15.1%, respectively. Since November 2003, 23 NG ST192 carriage isolates with closely related PFGE-patterns were collected (Fig 4.1b). With 3.8% (12/313) their colonisation rate peaked in November 2004. NG ST192 strains isolates have been previously reported from the Gambia and Niger.
(*http://pubmlst.org/perl/mlstdbnet/mlstdbnet.pl?page=st-query&file=pub-m_isolates.xml*).

Overall, 311 meningococcal meningitis cases were confirmed by culture and/or Latex agglutination during the study period. We obtained a bacterial isolate in 197/311 (63%) of cases. Latex agglutination confirmed the serogroup A capsule for all 114 CSF samples that were negative in culture. All recovered disease isolates belonged to the three dominating genoclouds of encapsulated meningococci (36 serogroup A ST5, 148 serogroup A ST7 and 15 serogroup X strains). With respect to colonization, meningococcal growth was observed in 6.1% (304/4999) of pharyngeal swab samples. All serogroup A (n=55) and serogroup X (n=161) carriage isolates belonged to the three genoclouds causing the major sequential colonisation waves. In addition, 16 NG isolates shared ST and PFGE-patterns with the serogroup A ST5 (2 isolates), serogroup A ST7 (2 isolates) or serogroup X (12 isolates) isolates, respectively (Fig. 4.1a). These colonisation isolates thus represented unencapsulated variants of the respective genoclouds. There was no evidence for an accumulation of the non-encapsulated variants towards the end of the colonisation waves (Fig. 4.1). In some cases, encapsulated and NG variants of the same genocloud were found simultaneously in the same compound.

Low background of meningococci unrelated to the clonal waves

Only 16.4% (50/304) of the colonisation isolates were unrelated to the dominating serogroup A, X and NG ST192 genoclouds (Fig. 4.1c). Although neighbouring Burkina Faso was hit by repeated W135 ST11 epidemics in the dry seasons of 2002-2004, in the KND, carriers of the epidemic strain

were only found in April 2004 (3/350; 0.9%) and November 2004 (2/313; 0.6%), and not a single W135 meningitis case was recorded between 1998 and 2005 (Forgor et al., 2005). Single carriers of W135 ST11 meningococci were also identified in April (1/300) and in November 1998 (1/299) (Gagneux et al., 2002b), two years prior to a first documented W135 meningitis outbreak in Mecca (Taha et al., 2000). While serogroup Y meningococci (21 isolates) and serogroup Y ST168 related NG strains (7 isolates) were isolated in 10 out of the 16 individual surveys, carriage of serogroup B and serogroup 29E meningococci was anecdotal (Table 4.1). Carriage of serogroup Y meningococci was strongly associated with one particular compound, where during eight of the 16 surveys, 67% (14/21) of the serogroup Y strains were isolated. Altogether, only eight NG isolates had PFGE-patterns and STs unrelated to the dominating serogroup A, X, Y and NG ST192 genoclouds (Table 4.1). While the *N. lactamica* carriage rate remained relatively constant (4.7%– 9.3%) for six years, it declined after April 2004 to 0.3% in April 2005 (Fig. 4.1d). We observed no significant correlation between the A/C meningococcal polysaccharide vaccine immunisation status and meningococcal carriage of all serogroups (RR=1.11; p=0.81), of serogroup A (RR=0.9; p=0.92), or of *N. lactamica* (in the >2year old RR=0.7, p=0.3).

Age distribution of carriers and patients

Colonization with meningococci in the KND exhibited a broad age range (Fig. 4.2a). It peaked in teenagers and young adults (median age 17.9 years; range 5 months to 84 years). In contrast, the carriage rate of *N. lactamica* was highest in the <5 age group (Fig. 4.2b). During the 1996/97 epidemic the age pattern of clinically diagnosed meningitis patients (median age 17.8 years; range 3 months- 80 years) resembled that of meningococcal carriers (Fig. 4.2c), the incidence rates of males (n=628, IR=0.95%) and females (n=713, IR=0.98%) were comparable (RR=0.97, p=0.59). In contrast, during the post-epidemic A meningococcal disease outbreaks between 1998 and 2005, the incidence of meningitis was highest in children <10 years of age and decreased steadily with age (Fig. 4.2c). The median age of A ST5 cases in 1998 and of A ST7 cases in 2001-2005 was comparable (8.0 years; range 4 months- 64 years versus 10.0 years; range 2 months - 75 years, respectively). However, between 2001 and 2005 the incidence rate of males (n=159, IR=0.049) was significantly higher (RR=2.0, p<0.0001) than of females (n=89, IR=0.024). The case fatality rate of A meningococcal meningitis was much higher during the post-epidemic outbreak in 1998 (20%; 10/50) than during the epidemic in 1996/97 (4.7%; 65/1396) or during the outbreaks in 2001-2005 (4.8%; 11/238).

Table 4.1: Carriage rates in % during 16 carriage surveys in the Kassena Nankana District

	Carriage in %															
Survey No.	**1** Apr.'98	**2** Nov. '98	**3** Apr.'99	**4** Nov.'99	**5** Apr.'00	**6** Nov.' 00	**7** Apr.'01	**8** Nov.'01	**9** Apr.'02	**10** Nov.'02	**11** Apr.'03	**12** Nov.' 03	**13** Apr.'04	**14** Nov.'04	**15** Apr.'05	**16** Nov.'05
N. lactamica	9.3	8.7	8.2	9.7	8.4	6.0	8.4	6.5	4.7	5.6	5.4	6.4	3.7	1.9	0.3	0.6
N. meningitidis	4.7	3.0	5.1	4.2	19.8	13.6	17.1	2.0	2.7	2.8	2.9	3.4	8.0	5.8	3.1	0.6
serogroup A	*2.7*	*1.0*	*0.7*	*0.3*					*1.2*	*1.9*	*2.2*	*1.4*	*4.3*	*0.6*	*0.9*	
serogroup X			*3.4*	*1.9*	*17.4*	*11.0*	*15.8*	*1.3*	*0.6*	*0.6*		*1.0*				
serogroup Y	*1.3*	*0.7*	*0.7*	*0.6*	*1.3*				*0.9*	*0.3*	*0.3*	*0.3*	*0.3*			
serogroup W135	*0.3*	*0.3*											*0.9*	*0.6*		
serogroup 29E								*0.3*					*0.3*			
serogroup B											*0.3*		*0.3*			
non groupable	*0.3*	*1.0*	*0.3*	*1.3*	*1.0*	*2.7*	*1.3*	*0.3*				*0.7*	*2.0*	*4.2*	*2.2*	*0.6*
PFGE pattern of NG strains	A	A, X, NT	NT	X, NT	X, Y, NT	X(7), Y	X, Y(3)	X				192, X,	192(5), A(2)	192(12), Y, NT	192(5), NT(2)	NT(2)
Total no. of people swabbed	**300**	**299**	**292**	**308**	**298**	**301**	**310**	**306**	**339**	**319**	**312**	**297**	**350**	**313**	**321**	**334**

Given are percentages of all *N. lactamica* and *N. meningitidis* carriers at each survey. Furthermore, for *N. meningitidis* the carriage rates of the different serogroups are cited. For NG strains the PFGE patterns are given, if more than one NG strain was isolated, the number of carriers are added in brackets;
A, X, Y is the typical pattern of the respective serogroups, NT= Non-typable, PFGE-pattern is not known. 192 is the NG ST192-pattern.

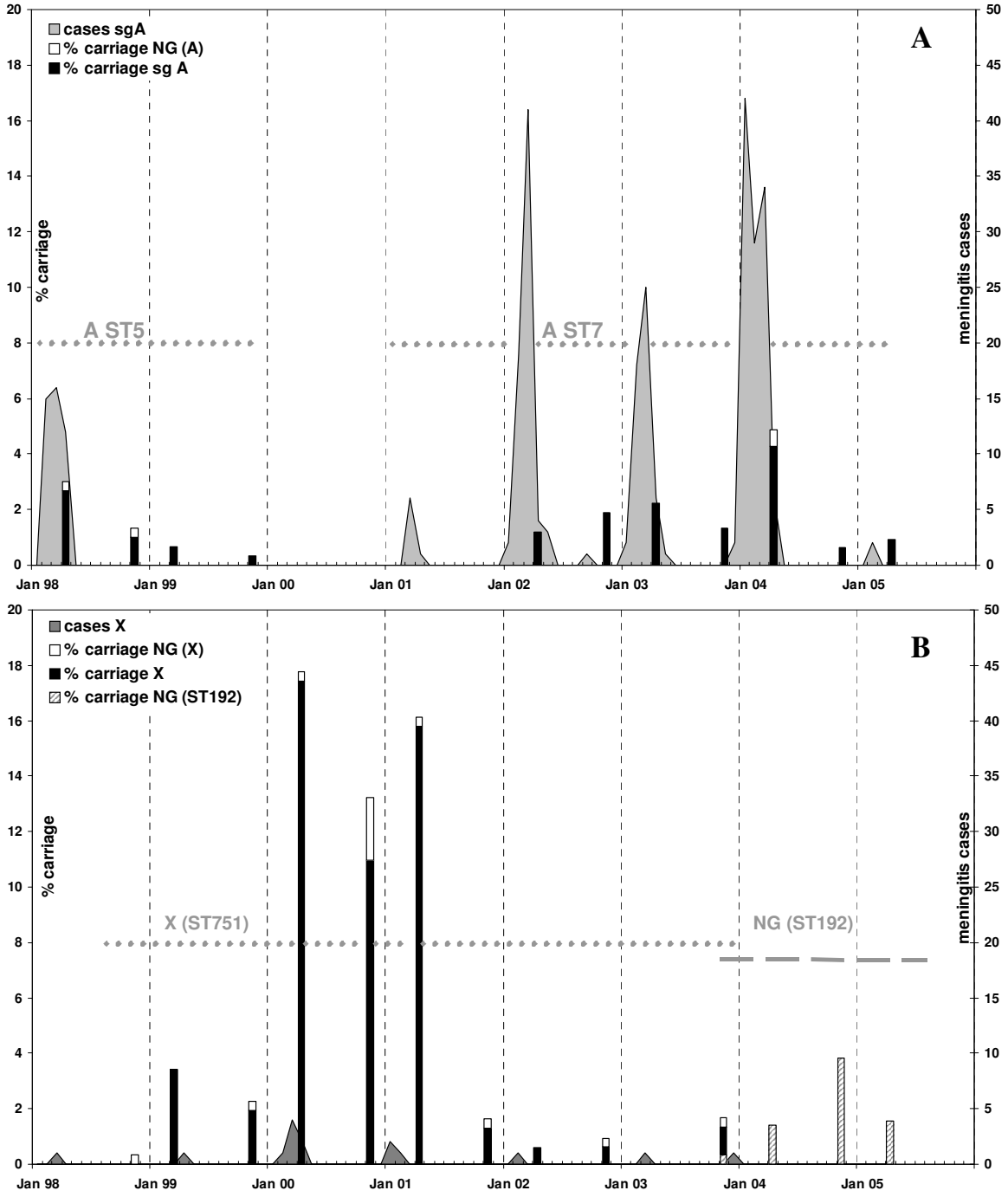

Figure 4.1 A & B. Waves of colonization and disease in the KND from April 1998 until November 2005. Carriage rates recorded during 16 colonization surveys (April and November each year) and monthly numbers of confirmed meningitis cases of *N. meningitidis*

A) genoclouds of serogroup A ST5 and ST7 meningococci

B) genoclouds of serogoup X ST851 and NG ST192 meningococci

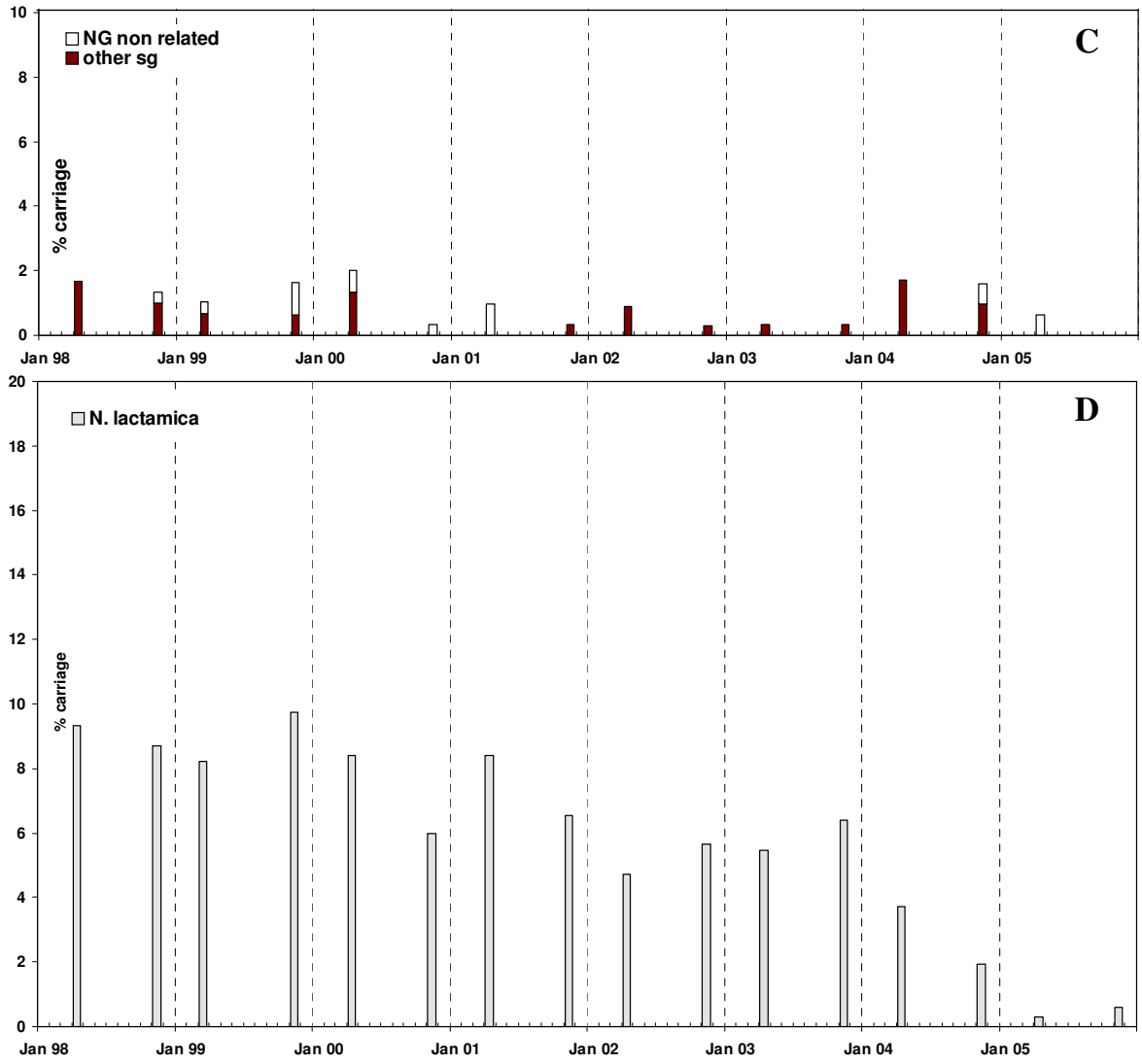

Figure 4.1 C & D. Waves of colonization and disease in the KND from April 1998 until November 2005. **C**) carriage rates of other serogroups and meningococci non related to the A, X, or NG ST192 genoclouds **D**) carriage rates of *N. lactamica*

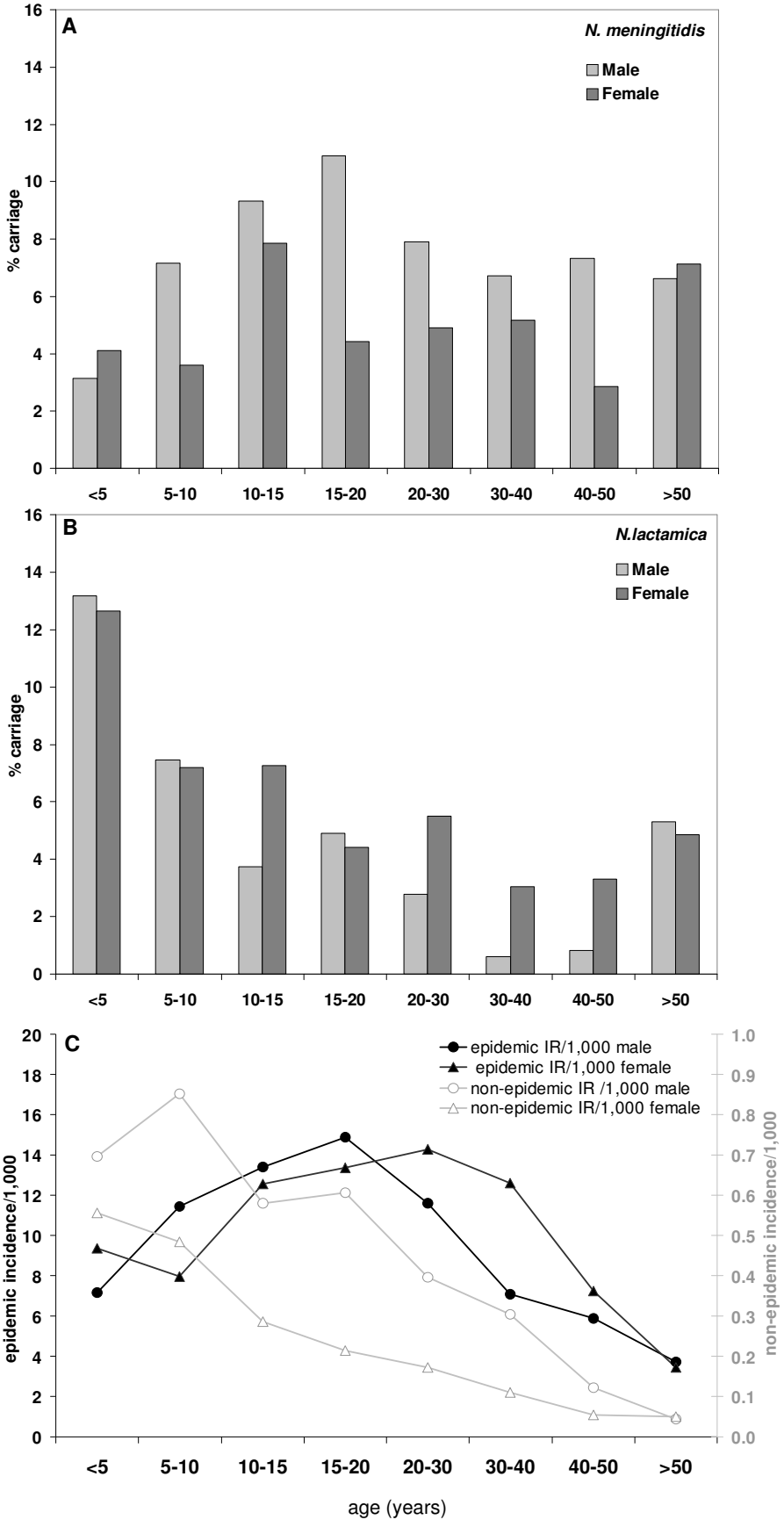

Figure 4.2 Carriage of meningococci and age spectrum of incidence rates of meningococcal meningitis

A) Carriage of meningococci (all serogroups and NG, cumulation of all surveys) in the different age groups of the male (dark grey bars) and female (light grey bars) population.

B) Carriage of *N. lactamica* in the different age groups (cumulation of all surveys) of the male (dark grey bars) and the female population (light grey bars).

C) Age spectrum of incidence rates of meningococcal meningitis in the male (circles) and female (triangle) population of the KND in the epidemic of 1996/97 (light grey) versus the interepidemic period 2001 to 2005. Denominator is the district population 1995-99. On the primary Y-axis the epidemic incidence rates and on the secondary Y-axis the interepidemic incidence rates are indicated

4.5 Discussion

This first longitudinal study of meningococcal colonisation in the meningitis belt of sub-Saharan Africa revealed features which are in many aspects remarkably different from findings of colonisation studies conducted in Europe and North America (Caugant et al., 1988; Maiden, 2004; Jolley et al., 2000; Yazdankhah and Caugant, 2004; Claus et al., 2005). The carried population of meningococci in the KND was i) less genetically diverse, ii) less constant in the genotype composition, iii) it included fewer NG strains and iv) virulent encapsulated strains were dominant. Indeed, the A ST5, A ST7 and X ST751 meningococci responsible for all 197 culture-reconfirmed meningitis cases represented 71% (216/304) of the colonisation isolates.

In industrialised countries, approximately 10% of individuals from the general population are carrying meningococci at any one time (Cartwright et al., 1987). In children younger than 4 years, carriage rates are <3%. They increase to 20–40% in teenagers and young adults (Blackwell et al., 1990; Cartwright et al., 1987; Caugant et al., 1988; Caugant et al., 1994) and decrease again to <10% in older age-groups. In contrast, invasive meningococcal disease is most common in young children and in teenagers. Current endemic rates of meningococcal disease in most industrialized countries range from <1 – 5 cases per 100,000 population. The ratio of cases to asymptomatic carriers is usually smaller than 1:100. In industrialised settings, meningococcal strains collected from patients and carriers differ genetically and serologically (Caugant et al., 1988). Typically, the carried populations of meningococci are highly diverse, with a low representation of the invasive serogroups A, B, C, Y and W135 (Maiden, 2004; Jolley et al., 2000; Yazdankhah and Caugant, 2004; Claus et al., 2005). The diverse spectrum of carried strains is relatively constant over time, and up to 50% are serologically non-groupable (Yazdankhah and Caugant, 2004; Cartwright et al., 1987). Encapsulation is thought to reduce adherence to pharyngeal epithelial cells, and loss of expression of capsular polysaccharide may be an adaptation to long-term carriage (Cartwright, 1995). Furthermore, colonisation with NG strains may be beneficial to the host by eliciting cross-reactive immune responses to non-capsular meningococcal surface antigens (Cartwright, 1995).

The observed lack of a stable and genetically diverse resident pharyngeal flora of meningococci in the KND may explain why incoming new clones may spread so successfully in populations of the African Meningitis Belt. This leads to clonal waves of colonisation typically lasting for about four

years and – in the case of hypervirulent lineages – disease outbreaks or epidemics. We found that the case to carrier ratio was generally much higher for serogroup A than for serogroup X meningococci, reflecting the marked difference in virulence between these two serogroups. Only in the dry season of 2001 at the beginning of the A ST7 colonisation and disease wave did we find patient isolates that were unrepresented during the corresponding colonisation survey. The highest A ST7 colonisation rate (4.3% in April 2004) was associated with the largest meningococcal meningitis outbreak observed during the entire study period. These data give no strong indication for a change in the case to carrier ratio in the course of the serogroup A ST7 outbreak.

However, new contact of the population with genoclouds that have epidemic potential does not always lead to high colonisation rates. For example, we recovered isolates resembling those responsible for the 2002-2004 epidemics in Burkina Faso from a few carriers in KND in 2004, but we did not observe any wave of W135 colonisation. Importantly, fluctuations of the pharyngeal microflora of the population are not confined to the meningococci. For example, the *N. lactamica* colonisation rate also changed in the course of the study. In addition, an outbreak of pneumococcal meningitis occurred during the study period with features (seasonality, clonality and a broad age spectrum) characteristic of meningococcal epidemics (chapter 6). Increasing herd immunity may be responsible for the disappearance of dominating genoclouds. However, changes in herd immunity do, not fully explain the complete disappearance of the A ST5 genocloud two years after the 1996/97 epidemic nor the emergence of the closely related A ST7 genocloud after only a short time interval.

The age distribution of healthy carriers in the KND with peak carriage rates in teenagers and young adults was similar to many European colonisation studies (Caugant et al., 1994; Cartwright et al., 1987; Yazdankhah and Caugant, 2004). The incidence of meningitis during the disease outbreaks in the years 1998-2005 was highest in children <10 years, comparable to endemic disease in industrialised countries. It is thought, that immune responses elicited by colonisation with meningococci and other antigenically cross-reactive microorganisms are responsible for the decreased disease susceptibility in the older age groups. This may imply that natural serum antibody-mediated immunity against invasive disease is developing much more efficiently than secretory IgA-mediated protection against colonisation.

However, during the epidemic in 1996/97, the age-distribution of meningitis patients resembled that of meningococcal colonisation, consistent with reports of most large meningococcal epidemics (Greenwood et al., 1979; Moore, 1992; Lapeyssonnie, 1963). During the epidemic the disease susceptibility of the whole population was increased. The fact that also in children <10 years the epidemic incidence of meningitis was exceeding endemic attack rates dramatically, argues against the '2 hit' hypothesis, associating susceptibility to disease with blocking serum IgA elicited by colonisation of the gut with cross-reactive microorganisms (Griffiss, 1982).

The factors that initiate epidemics in the meningitis belt are incompletely understood. Contact of a population with a hyperinvasive new genocloud that is antigenically distinct enough to escape natural immunity may lead to an epidemic. Loss of natural immunity in exposed individuals over time and new birth cohorts may make a population increasingly susceptible. However, epidemics are not always associated with the appearance of a new clone (Greenwood, 1999). This suggests a role of environmental triggers, such as co-pathogens or social factors. In spite of intense annual A/C polysaccharide vaccination campaigns carried out in the KND since 1998, outbreaks with incidence rates of up to 80 per 100,000 occurred between 2002 and 2004. It is not clear, whether herd immunity elicited by the serogroup A ST5 epidemic, lack of environmental triggers or the vaccination campaigns have prevented a large A ST7 epidemic.

Meningococcal vaccines protect individuals from disease by eliciting bactericidal serum antibodies (Borrow et al., 2001). Recent studies following the introduction of conjugate C vaccines in the United Kingdom have demonstrated that capsule conjugate vaccines also affect carriage and transmission by inducing mucosal immune responses (Maiden and Stuart, 2002; Ennes et al., 1992). Herd immunity may play a key role in the control of meningococcal infection using meningococcal conjugate vaccines (Ramsay et al., 2003). Serogroup replacement and the emergence of escape variants (Maiden and Spratt, 1999) are potential disadvantageous effects associated with developing herd immunity. Therefore, meningococcal carriage studies such as those described here should be performed before and after the introduction of new conjugate vaccines in the African Meningitis Belt, in order to assess protective and potential disadvantageous effects of these interventions.

4.6 ACKNOWLEDGEMENTS

The Stanley Thomas Johnson Foundation, the Meningitis Research Foundation and the Meningitis Vaccine Project funded the study. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing or the report. We acknowledge the use of the meningococcal MLST database, which is located at Imperial College London and is funded by the Wellcome Trust. We are grateful for support and contributions of E. Arnold, F. Binka, S. Droz, I. Ehrhard, B. Genton and M. Tanner. In the Navrongo Health Research Center, we thankfully appreciate the assistance of A. Bugri, S. Abudulai and A. Wahab in the laboratory, all nurses and health workers in the War Memorial Hospital, Navrongo and the Health Centers of the KND, C. Tindana with all fieldworkers and drivers for excellent work in the field, and T. Tei and M. Bugase for logistic support. We acknowledge the use of the NDSS database and we thank all study participants for their trust and contribution.

CHAPTER 5

EMERGENCE OF W135 MENINGOCOCCAL MENINGITIS IN GHANA

CHAPTER 5

Emergence of W135 meningococcal meningitis in Ghana

Abudulai Adams Forgor[1], Julia Leimkugel[2], Abraham Hodgson[1], Akalifa Bugri[1], Jean-Pierre Dangy[2], Sébastien Gagneux[2*], Tom Smith[2] and Gerd Pluschke[2]

[1]Navrongo Health Research Centre, Ministry of Health, Navrongo, Ghana

[2]Swiss Tropical Institute, Basel, Switzerland
*present address: Division of Infectious Diseases and Geographic Medicine, Stanford University Medical Centre, Stanford, USA

This article has been published in
Tropical Medicine and International Health volume 10 no 12 pp 1229–1234 December 2005

5.1 Summary

Neisseria meningitidis serogroup W135, well known for a long time as a cause of isolated cases of meningococcal meningitis, has recently increasingly been associated with disease outbreaks of considerable magnitude. Burkina Faso was hit by W135 epidemics in the dry seasons of 2002-2004, but only four W135 meningitis cases were recorded between February 2003 and March 2004 in adjoining Ghana. This reconfirms previous findings that bottlenecks exist in the spreading of new epidemic *N. meningitidis* clones within the meningitis belt of sub-Saharan Africa. Of the four Ghanaian W135 meningitis patients one died and three survived, of which one had profound neurosensory hearing loss and speech impairment. All four disease isolates were sensitive to penicillin G, chloramphenicol, ciprofloxacin and cefotaxime and had the multi-locus sequence type (ST) 11, which is the major ST of the ET-37 clonal complex. Pulsed-field gel electrophoresis (PFGE) profiles of the Ghanaian disease isolates and recent epidemic isolates from Burkina Faso were largely identical. We conducted meningococcal colonisation surveys in the home communities of three of the patients and in the Kassena Nankana District located at the border to Burkina Faso. W135 carriage rates ranged between 0 and 17.5%. When three consecutive surveys were conducted in the patient community with the highest carrier rate, persistence of W135 colonisation over a period of one year was observed. Differences in PFGE profiles of carrier isolates taken at different times in the same patient community were indicative of rapid microevolution of the W135 bacteria, emphasising the need for innovative fine typing methods to reveal the relationship between W135 isolates.

5.2 Introduction

Epidemic meningococcal disease has occurred in the meningitis belt of sub-Saharan Africa for approximately 100 years (Greenwood, 1999). Historically the epidemics have been primarily caused by *Neisseria meningitidis* serogroup A. Serogroup W135 meningococci identified in 1968 (Evans et al., 1968) and first described in Africa in 1982 (Denis et al., 1982) were initially considered to be a rare cause of invasive disease. However, two W135 meningitis outbreaks coinciding with pilgrimage seasons for Hajj in 2000 and 2001 (Taha et al., 2000; Lingappa et al., 2003) were followed by a first large scale epidemic in Burkina Faso in 2002 (Taha et al., 2002b; Decosas and Koama, 2002). Since

then, each year Burkina Faso has been hit by mixed meningitis epidemics caused by W135 and A meningococci. In Saudi Arabia W135 meningococci were responsible for 13% of all meningococcal disease between 1995 and 1999 and have been present to a notable degree at least since 1990 (Lingappa et al., 2003). From 2002 onwards vaccination with the quadrivalent meningococcal polysaccharide vaccine (A/C/Y/W135) therefore became a visa requirement to participate in the Hajj (Wilder-Smith et al., 2003a). Already before the outbreaks in 2000 the danger of W135 meningitis epidemics in Africa was recognized (Kwara et al., 1998).

The Hajj outbreaks probably led to the expansion of a particular W135 clone within the electrophoretic type-37 (ET-37) complex (Mayer et al., 2002; Popovic et al., 2000). A high acquisition rate of W135 meningococci (15-17%) in pilgrims has been reported (Wilder-Smith et al., 2003b). Throughout the world these carriers have transmitted Hajj-related W135 bacteria after returning home (Aguilera et al., 2002; Hahne et al., 2002; Wilder-Smith et al., 2003b). Related W135 strains also belonging to the ET-37 complex have been circulating worldwide since at least 1970 (Mayer et al., 2002) and currently both the Hajj-related epidemic strain and Hajj-unrelated local W135 strains seem to be responsible for sporadic W135 cases worldwide (Hahne et al., 2002; Taha et al., 2004). Genetic drift of the Hajj-related strain (Hahne et al., 2002) complicates the analysis of the relationship between W135 isolates by standard typing techniques, such as pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) considerably.

In spite of its border with Burkina Faso, no outbreak of W135 meningococcal meningitis has so far occurred in Ghana. Here, we describe properties of four W135 strains isolated between February 2003 and March 2004 from the cerebrospinal fluid (CSF) of Ghanaian meningitis patients and provide evidence for spreading and rapid microevolution of the causative W135 meningococci.

5.3 Materials and Methods

Disease isolates

In the respective hospitals, CSF samples were taken for diagnostic purpose, latex agglutination was performed and the causative agents were isolated by culture using standard microbiological techniques. Bacterial isolates of all four Ghanaian W135 cases were transferred for further analysis to the Navrongo Health Research Centre, where serological grouping was reconfirmed by PCR.

Reference isolates of W135 meningococci were obtained from M. Achtman, Berlin (isolated in Mecca, 2000, strains Z9230 and Z9232), and D. Caugant, Oslo (isolated during the outbreaks in Burkina Faso of 2001 (BF01/01, BF24/01), 2002 (BF06/02, BF67/02) and 2003 (BF01/03)).

Carrier isolates

In three of the affected communities and two control communities throat swabs have been taken and analysed for colonisation with *N. meningitidis* and *N. lactamica*. Community K1 is a small village located in a rural setting directly on the main truck road between the south and the north of Ghana (Fig. 5.1). Nearly the whole population of the village participated in the study. In December 2003 a control community located 2 km away from K1 was included. Communities B1 and B2 are located in Bolgatanga, the Upper East Region's capital. Here throat swabs were taken from the affected and the closest neighbouring compounds (including the majority of the about 30 inhabitants per compound).

After obtaining informed consent, throat swabs were taken and directly plated onto Thayer Martin Agar. The plates were incubated at 37ºC within eight hours after sampling for 24-48 hours. Two colonies with neisserial morphology were sub-cultured from each plate. *N. meningitidis* and *N. lactamica* colonies were identified as previously described (Gagneux et al., 2002b) by standard bacteriological methods. Ethical clearance was obtained from the responsible institutional and national ethical approval committees.

Characterisation of bacterial isolates

Meningococcal isolates were serogrouped with serogroup-specific antisera (Difco). Results were reconfirmed by PCR (Taha, 2000; Bennett et al., 2004; Orvelid et al., 1999). All W135 isolates were analysed by pulsed field gel electrophoresis (PFGE) after digestion with *NheI* as previously described (Morelli et al., 1997). All disease isolates were tested for resistance to penicillin G, chloramphenicol, cefotaxime, and ciprofloxacin with E-test strips (Isenberg Henry D.(ed.), 1998) using the NCCLS breakpoints. Selected strains were analysed by multi-locus sequence typing (MLST). DNA extraction (Vela Coral et al., 2001), PCR (Maiden et al., 1998) and sequencing of PCR products with an ABI Prism 310 Genetic Analysis System were performed according to standard protocols on the MLST homepage (http://pubmlst.org/neisseria/). Allelic profiles were analysed using applications available on the MLST homepage.

5.4 Results

Characterization of W135 disease isolates from Ghana

Between February 2003 and March 2004, four cases of W135 meningococcal meningitis were reported by the regional hospitals in Tamale, Bolgatanga and the Korle Bu Teaching hospital (Accra), respectively. Patients came from the centre, the south or the north of the country (Fig 5.1) and were between 3 and 17 years of age (Table 5.1). One patient died, and of the three survivors one had profound sequelae.

All four disease isolates were sensitive to penicillin G, chloramphenicol, ciprofloxacin and cefotaxime. PFGE profiles of all four Ghanaian disease isolates were compared with disease isolates from the Hajj outbreak in Mecca 2000 and from Burkina Faso between 2001 and 2003. The Burkinian strains isolated in 2001 and 2002 showed identical profiles (shown for strain BF67/02, Fig. 5.2, lane 5, profile C), whereas the 2003 isolate appeared to be very closely related (Fig. 5.2, lane 6, BF01/03, profile D). Profiles of the Ghanaian disease isolates were largely identical (Fig. 5.2, lanes 7-10, profile D) and indistinguishable from that of the 2003 strain from Burkina Faso (Fig. 5.2, lane 6). The reference disease isolates from the Hajj outbreak in 2000 had a distinct, but related PFGE profile (shown for strain N11421, Fig. 5.2, lane 4, profile B; both strains had an identical profile). All four Ghanaian disease isolates had the multi-locus sequence type (ST) 11, which is the major ST of the ET-37 clonal complex.

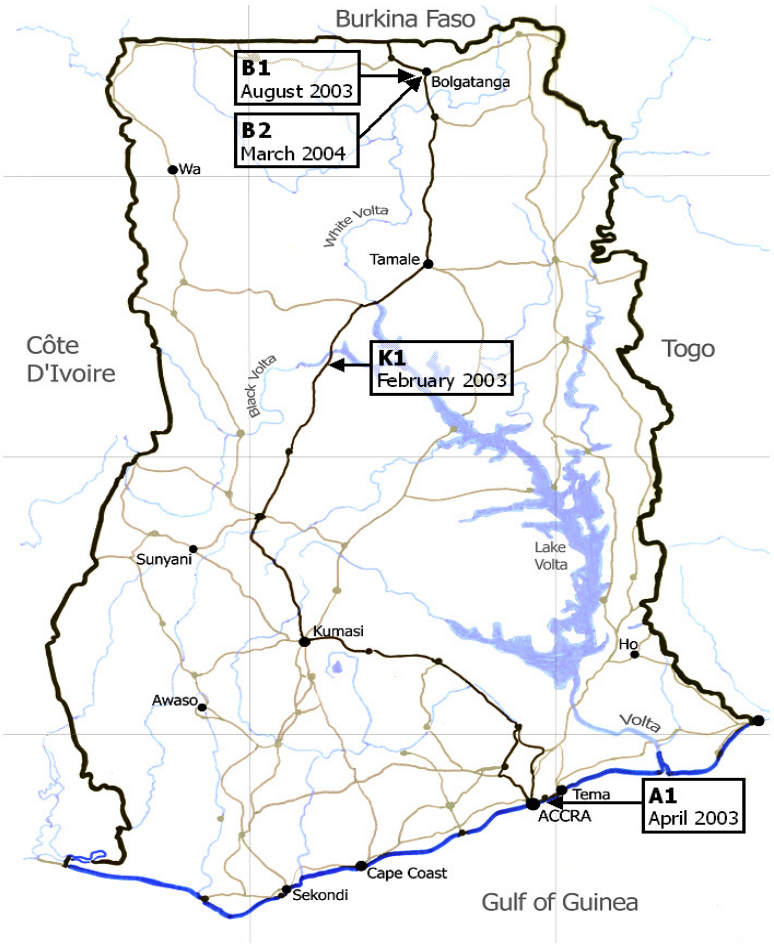

Figure 5.1 Map of Ghana showing the location of home communities of W135 meningitis patients

The sample time points were as follows: Community **K1**: **I**) April 2003, **II**) December 2003 (including control community), **III**) April 2004. Community **B1:** December 2003, Community **B2:** March 2004. The home community of patient A1 could not be identified and has not been sampled.

Table 5.1 W135 cases reported to the Ghanaian disease control authorities in 2003 and 2004

Patient ID	Time of disease onset	Village/city	Region	Age (years)	Sex	Outcome	Sequelae
K1	February 2003	Kpalkpalgbeni	Brong Ahafo	3	male	survived	*multiple
A1	April 2003	Accra	Greater Accra	4	male	died	unknown
B1	August 2003	Bolgatanga	Upper East	17	female	survived	none
B2	March 2004	Bolgatanga	Upper West	3	male	survived	+multiple

*Profound sensorineural hearing loss, speech impairment, transient ataxia, hyperactive left patellar and achillis reflexes

+Arthritis of the knee joints and occasional episodes of brief startling attacks during the first week after discharge but stopped thereafter

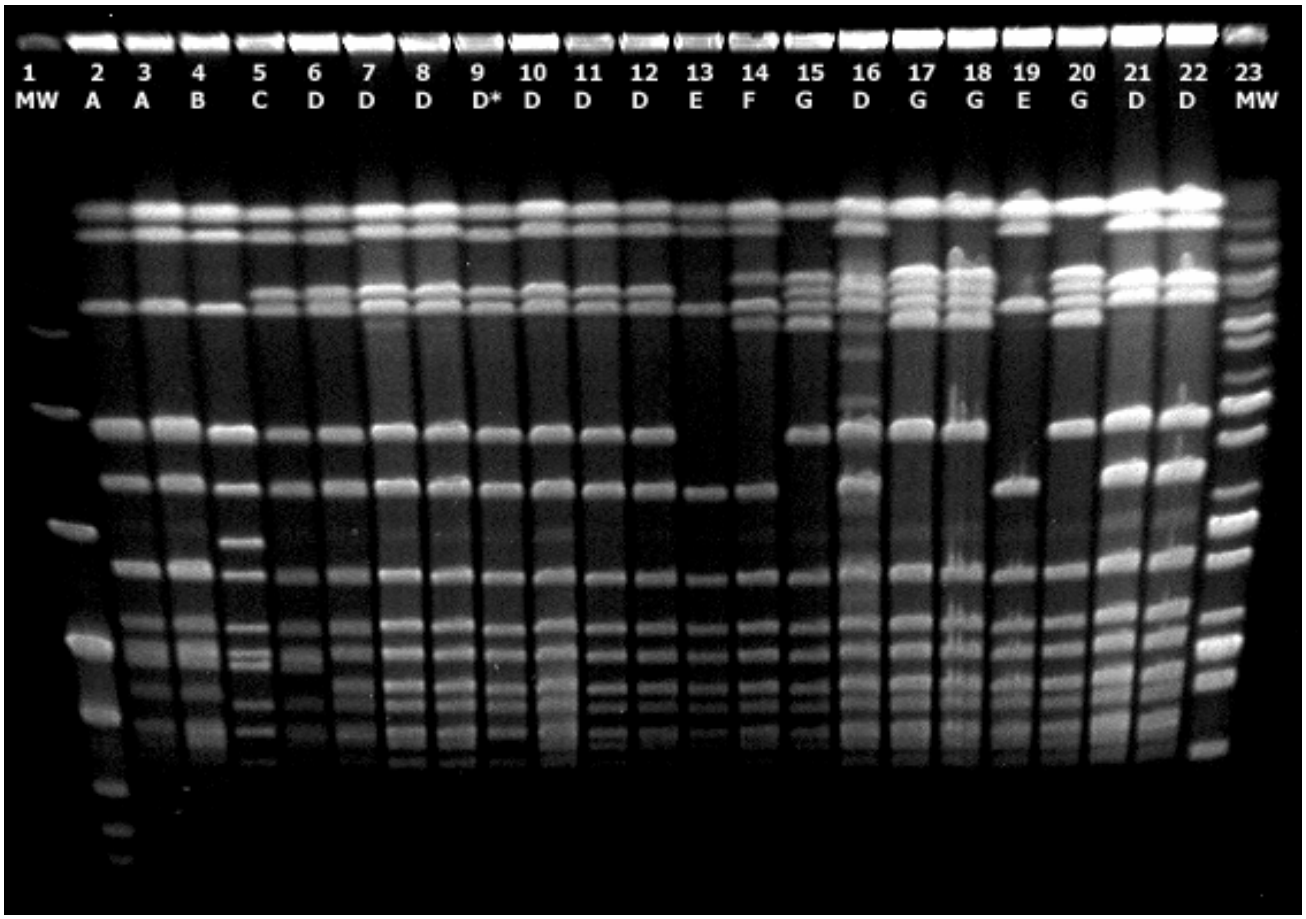

Figure 5.2 PFGE profile of W135 carrier and disease isolates (lane: strain No.; origin) Indicated on the gel are the different band profiles of the W135 strains (A-G). **1:** MW marker, **2, 3:** N1621, N1622, KND 1998, carriage; **4:** N1421 (Z9230), Mecca 2000, reference strains; **5**: N1627 (BF67/02), Burkina Faso 2002, case; **6:** N1628 (BF01/03), Burkina Faso 2003, case; **7:** N1681 Ghana 2003, patient K1; **8:** N1682, Ghana, 2003, patient A1 **9:** N1683, Ghana 2003, patient B1; **10:** N1846, Ghana 2004, patient B2; **11, 12:** N1485, N1487, community K1, April 03, carriage; **13, 14, 15:** N1633, N1640, N1636, community K1, Dec 03, carriage; **16, 17:** N1848, N1857, community B2, March 04, carriage; **18, 19, 20:** N1951, N1953, N1959, community K1, April 03, carriage; **21, 22:** N1888, N1903, KND, April 04, carriage; **23:** MW marker

Table 5.2: Carriage of different serogroups of *N. meningitidis* and of *N. lactamica* in home communities of three W135 meningococcal meningitis patients and in a neighbouring control community.

Time of survey	Community (patient ID)	Volunteers swabbed (n)	Colonization rate % (n)						
			N. lactamica	*N. meningitidis*	W135	A	X	Y	NG
April 2003	Home (K1)	103	7.8 (8)	24.3 (25)	17.5 (18)	2 (2)	0	1 (1)	3 (3)
December 2003	Home (K1)	100	3 (3)	15 (15)	13 (13)	0	0	1(1)	1(1)
April 2004	Home (K1)	96	5.2 (5)	8.3 (8)	3.1 (3)	1 (1)	0	1 (1)	3 (3)
December 2003	Control (K1)	100	1 (1)	9 (9)	0	2 (2)	2 (2)	2 (2)	3 (3)
December 2003	Home (B1)	110	8.2 (9)	3.6 (4)	0	0	0	1 (1)	3 (3)
April 2004	Home (B2)	100	4 (4)	7 (7)	2 (2)	0	2 (2)	0	2 (2)

* Non-groupable (NG) strains were negative both in serological tests and in serogroup A and W135 specific PCR analysis

Table 5.3: Age distribution of colonization with *Neisseria lactamica* and W135 and non-W135 *Neisseria meningitidis* in the patient home community K1 (cumulated data from all three surveys)

	Frequency of colonization						
Age group (years)	<1	1–4	5–9	10–14	15–19	20–39	>40
Neisseria meningitidis serogroup W135	1/6 (16.7%)	5/78 (6.4%)	7/44 (15.9%)	7/37 (18.9%)	6/24 (25.0%)	8/93 (8.1%)	0/17 (0%)
Non-W135 *N. meningitidis*	0/6 (0%)	1/78 (1.3%)	0/44 (0%)	4/37 (10.8%)	3/24 (12.5%)	5/93 (5.3%)	1/17 (5.9%)
Neisseria lactamica	2/6 (33.3%)	9/78 (11.5%)	1/44 (2.3%)	1/37 (2.7%)	0/24 (0%)	0/93 (0%)	1/17 (5.9%)

W135 colonization in patient communities and clonal diversity of bacteria

Three consecutive *N. meningitidis* colonisation surveys were performed in community K1, the first six weeks after the emergence of the case, in February 2003. In April 2003, 17.5% of 103 inhabitants (about 90% of the total population of the village) were colonized with W135 meningococci. Thereafter, the W135 colonisation rate declined to 13% in December 2003 and to 3 % in April 2004. In addition, a few carriers of other meningococci were found. *N. lactamica* colonisation rates were between 3 and 8%. *N. meningitidis* A, X and Y, but no W135 carriers were found in a neighbouring control community included in the December 2003 survey (Table 5.2).

Cummulated data from all three surveys conducted in community K1 were used to analyse the age distribution of colonisation with W135 meningococci in comparison to other serogroups found and to *N. lactamica* (Table 5.3). Logistic regression, including random effects to allow for repeated assessment of the same individuals, indicated that the ratio of carriage prevalence of *N. meningitidis* to that of *N. lactamica* increased with age (Chi-square=7.6, 1 degree of freedom, p=0.006), however there was no significant age trend in the ratio of W135 to other *N. meningitidis* (Chi-square 0.8, 1 d.f. p=0.4).

All isolates from the 18 W135 carriers in community K1 in April 2003, revealed identical PFGE profiles (shown for strains N1485 and N1487, Fig. 5.2, lanes 11 and 12, profile D), indistinguishable from those of the Ghanaian disease isolates (Fig. 5.2 lanes 7-10). However, some genetic diversification became apparent in the December 2003 colonisation survey. While the isolates of nine (of thirteen) W135- carriers revealed the original band profile (data not shown), the isolates from the other four exhibited three new variant profiles (Fig. 5.2, lanes 13-15, profile E, F, G). Two of the three variant PFGE profiles, but not the original profile, were found again in the last colonization survey in April 2004 (Fig. 5.2, lanes 18-20, profile E and G).

In community B1 and B2 only one colonization survey was performed, three months and three weeks, respectively, after the emergence of the case. While no W135 meningococci were found in community B1 in community B2 W135 isolates of two carriers were obtained with the same PFGE profile as the Ghanaian disease isolates (Fig.5.2, lane 16, profile D, Table 5.2). In addition, from

one of them a variant strain was isolated with a PFGE profile (Fig. 5.2, lane 17, profile F) identical to a variant profile found in colonisation isolates from community K1.

W135 colonization in a long-term colonization survey in northern Ghana

Within the framework of a longitudinal N. meningitidis colonization and disease study in the Kassena Nankana District (KND) of northern Ghana (Gagneux et al., 2000; Gagneux et al., 2002b), no W135 meningococcal meningitis case was recorded between 1998 and 2004. During these seven years of twice yearly colonization surveys only single carriers of W135 meningococci have been identified in 1998 (1/300 in April and 1/299 in November 1998) (Gagneux et al., 2000). However, in April 2004 a W135 colonization rate of 0.9% (3/350) was found with isolates showing the same PFGE profile (Fig. 5.2, lanes 21 and 22, profile D) as the Ghanaian disease isolates (Fig. 5.2, lanes 7-10). Profiles of the two 1998 carrier isolates (Fig. 5.2, lanes 2 and 3, profile A), were identical among each other but distinct from all other profiles observed in this study.

5.5 Discussion

In spite of the consecutive W135 epidemics in Burkina Faso in 2002 - 2004, no major outbreak of W135 disease has been observed so far in Ghana, demonstrating that bottlenecks exist for the spreading of epidemic strains within the meningitis belt, as already described for serogroup A meningococci (Achtman, 1995). The four isolated Ghanaian cases described in this paper have probably only been reported because of intensive national surveillance and awareness. W135 strains belonging to the ET-37 complex have been present in Ghana before the Mecca outbreak (Gagneux et al., 2000) and sporadic W135 cases may have easily remained undetected before the year 2000.

PFGE analysis demonstrate that the four Ghanaian W135 meningitis isolates were closely related to recent disease isolates from Burkina Faso, indicating, that these meningitis cases were caused by epidemic-related strains and not by local strains of the ET-37 complex. At least in the north of Ghana colonisation with the Burkina Faso epidemic-related strain is detectable. While visitors from Burkina Faso are frequently met in the border communities B1 and B2, it is not possible to guess the origin of the disease causing W135 strain of patient A1 living close to Accra. In the case of community K1, located in the middle of Ghana, contact with nomads may have been the source of

the W135 bacteria, as a part of a neighbouring community frequently moves to Burkina Faso and back.

W135 carriage rates of healthy contacts in the three home communities of W135 meningitis patients were very different. W135 carriers were found in the home communities (K1 and B2) of the index cases aged 3 years but not in B1, the home community of the 17-year-old patient. Age of the patients may play a role, as suggested by findings of a study carried out during an serogroup C outbreak in Brazil, where contact carriage rates were highest in households, where the index case was an infant (Cartwright, 1995). Carriage rates of outbreak strains tend to be higher in closed or partially-closed communities than in an open communities (Cartwright, 1995). The rural community K1 has the features of a semi-closed community, where inhabitants lived very closely together and shared all living activities, while the urban communities B1 and B2 were much more open and loose. This may explain, why the highest (18%) carriage rate was observed in community K1. The age distribution of W135 colonisation, was not unusual, as the pattern observed in community K1 was characteristic for meningococci in general (Cartwright, 1995).

Changes of the PFGE profile of colonisation isolates with time demonstrate that microevolution of W135 may be rapid. *N. meningitidis* is a naturally transformable species and there is evidence that microevolution is driven more frequently by recombination than by mutation. The observed genetic drift can make it very difficult to distinguish between epidemic-related and local W135 strains belonging to the same ET-37 complex and to prove epidemic spread of a particular clone. While available techniques are suitable to analyse the global population structure of other meningococcal serogroups (Lingappa et al., 2003), new approaches are required for studying the molecular epidemiology of *N. meningitidis* W135.

An affordable vaccine against W135 meningococci (e.g., a trivalent groups A, C, and W135 polysaccharide vaccine) is now available and has been successfully used to contain outbreaks of W135 meningitis in Burkina Faso (Ahmad, 2004). As Burkina Faso epidemic-related W135 meningococci now seem to spread into Ghana, intense surveillance efforts at national and regional levels for timely detection of a potential W135 epidemic is an important issue in future years.

5.6 Acknowledgements

This study was supported by grant GAT.0779-01476-GRT of the Meningitis Vaccine Project. We would like to acknowledge the National Disease control unit of the Ghana Health Service and the National Public Health Reference Laboratory for releasing the CSF samples of the index case and of the case from Accra. Furthermore we are grateful to the laboratory personnel of the Bolgatanga Regional hospital for making available the other two CSF samples. We thank the CSM team of the NHRC for assistance during the study and the communities, health authorities (Upper East Regional Health Directorate, Bolgatanga Municipal Health Directorate, Kintampo District Health Directorate, Kintampo Health Research Centre, Kintampo and Navrongo Health Research Centre, Navrongo) as well as all study participants for the cooperation accorded us. Finally we would like to acknowledge D. Caugant and M. Achtman for helpful comments on the manuscript and the supply with W135 Reference isolates.

CHAPTER 6

AN OUTBREAK OF SEROTYPE 1 *STREPTOCOCCUS PNEUMONIAE* MENINGITIS IN NORTHERN GHANA WITH FEATURES CHARACTERISTIC OF EPIDEMIC MENINGOCOCCAL MENINGITIS

CHAPTER 6

An outbreak of serotype 1 *Streptococcus pneumoniae* meningitis in northern Ghana with features characteristic of epidemic meningococcal meningitis

Julia Leimkugel[1], Abudulai Adams Forgor[2], Sébastien Gagneux[1*], Valentin Pflüger[1], Christian Flierl[1], Elizabeth Awine[2], Martin Naegeli[1], Jean-Pierre Dangy[1], Tom Smith[1], Abraham Hodgson[2] and Gerd Pluschke[1]

[1] Swiss Tropical Institute, Basel, Switzerland
[2] Navrongo Health Research Centre, Ministry of Health, Navrongo, Ghana
*present address: Division of Infectious Diseases and Geographic Medicine, Stanford University Medical Centre, Stanford, USA

This article has been published in
Journal of Infectious Diseases (2005), 195: 192-199

6.1 Abstract

Background The Kassena-Nankana District (KND) of northern Ghana lies in the African meningitis belt where epidemics of bacterial meningitis have been re-occurring every 8-12 years. These epidemics are generally caused by *Neisseria meningitidis*, an organism considered uniquely capable of causing meningitis epidemics.

Methods We recruited all suspected meningitis cases in the KND between 1998 and 2003. Cerebrospinal fluid samples were collected and analysed by standard microbiological techniques. Bacterial isolates were subjected to serotyping, multi-locus sequence typing (MLST) and antibiotic resistance testing.

Results A continual increase in the incidence of pneumococcal meningitis was observed from 2000 to 2003. This outbreak exhibited strong seasonality, a broad host age spectrum, and clonal dominance, all of which are characteristic of meningococcal meningitis epidemics in the African meningitis belt. The case fatality rate for pneumococcal meningitis was 44.4%, the majority of pneumococcal isolates were antibiotic sensitive and expressed the serotype 1 capsule. MLST revealed that these isolates belonged to a clonal complex dominated by sequence type (ST) 217 and its two single-locus variants ST303 and ST612.

Conclusions The ST217 clonal complex of *S. pneumoniae* represents a hypervirulent lineage with a high propensity to cause meningitis. In addition, our results suggest that this lineage might have epidemic potential. Serotype 1 is not included in the currently licensed paediatric heptavalent pneumococcal vaccine. Mass vaccination targeting hypervirulent serotypes with a less complex conjugate vaccine should therefore be considered.

6.2 Introduction

Neisseria meningitidis, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib) are the most common causes of acute bacterial meningitis (Hart and Cuevas, 2003). Meningitis caused by *N. meningitidis* has been considered unique with respect to its epidemic occurrence. A region of sub-Saharan Africa extending from Ethiopia to Senegal, designated the 'meningitis belt', has been particularly vulnerable to meningococcal disease epidemics. In addition to sporadic disease, which

occurs mainly during the annual dry season, epidemics have occurred in the meningitis belt every 8-12 years over the past 100 years (Greenwood, 1999; Achtman, 1995).

Information about the epidemiology of pneumococcal meningitis in the African meningitis belt is fragmentary, but some studies have found *S. pneumoniae* to be the most important causative agent of bacterial meningitis in certain areas (Mar et al., 1979). The incidence in these areas is 10-20 cases per 100,000 and year, which is about ten times higher than in Western Europe and the United States (Greenwood, 1987; Hausdorff et al., 2000b). Cases of *S. pneumoniae* meningitis occur throughout the year, and most studies report the youngest (<2) and the oldest (> 60) age groups to be at greatest risk (Mar et al., 1979; Greenwood, 1987). For unknown reasons, the case fatality rate for pneumococcal meningitis (about 50%) is five to ten times higher than for meningococcal meningitis.

Although there are about 90 pneumococcal serotypes known, only a limited number account for most of the invasive infections. The serotype distribution varies with time, location and age group (Hausdorff et al., 2000b). Clonal dominance and global spread has been described for a small number of highly successful, (often multi-) drug resistant pneumococcal clones (Klugman, 2002). Serotype 1 is one of the most common serotypes causing invasive disease worldwide, particularly in Africa (Greenwood et al., 1980; Hausdorff et al., 2000b; Brueggemann and Spratt, 2003). It has a high attack rate but is rarely isolated from healthy carriers or mild occult bacteraemia. Outbreaks of invasive serotype 1 pneumococcal disease have occurred in several communities (Dagan et al., 2000; Gratten et al., 1993; Hausdorff et al., 2000b; Mar et al., 1979; Porat et al., 2001; Henriques et al., 2001; Tugwell et al., 1976).

The present study was conducted between 1998 and 2003 in the Kassena-Nankana District (KND) in northern Ghana. Following a large meningococcal meningitis epidemic in the dry season of 1997, all suspected meningitis patients were recruited prospectively. Cerebrospinal fluid (CSF) samples were taken and analysed by standard microbiological techniques. Between 2000 and 2003, a continuous increase in incidence of pneumococcal meningitis was observed. We demonstrate that the epidemiological and bacteriological features of this outbreak closely resemble the ones usually associated with meningococcal disease epidemics. The implications of these observations for the control of bacterial meningitis in the African meningitis belt are discussed.

6.3 Methods

Study area

The KND has a population of 140,000 and lies within the Guinea Savannah woodland area of northern Ghana. Two major seasons exist, a short wet season from May to October and a long dry season for the rest of the year. The general population is rural except for those living in the town of Navrongo, which has a population of 20,000. People live in compounds with an average of 10 inhabitants.

Patients

CSF samples were collected from January 1998 to December 2003 from suspected meningitis patients reporting to the War Memorial Hospital, Navrongo, or to one of four Health Centres in the KND. In line with the standard diagnostic procedures in Ghana, samples were analysed at the laboratory of the War Memorial Hospital for confirmation of the clinical diagnosis. Additional samples were obtained from the Regional Hospital of the Upper East Region in Bolgatanga, and from health facilities in the Bongo and Builsa Districts. In 1998 and 1999, only samples collected during the dry season were analysed. Thereafter, samples obtained from the few suspected meningitis cases presenting during the wet season were also included. Ethical clearance for the study was obtained from the responsible institutional review boards and the Ghanaian Ministry of Health. Clinical and demographic information was recorded from all patients. Personal data were linked with the database of the Navrongo Demographic Surveillance System (NDSS). The denominators used for calculation of incidence rates represent the average annual district population between 1995 and 1999 (Nyarko et al., 2002).

Analysis of CSF

CSF samples were analysed by direct Gram staining. Boiled CSF-supernatants were tested serologically for capsular polysaccharide antigens of *N. meningitidis* (serogroups A, B, C and W135), *S. pneumoniae* and Hib (Slidex meningite-Kit, Bio Merieux, Pasteurex-Kit, BIO RAD #61718). CSF specimens were inoculated on blood-, chocolate-, and Thayer Martin Agar and incubated in candle jars for 24 hours at 37˚C. *S. pneumoniae* colonies were identified based on colony morphology, Gram staining behaviour and resistance to Optochin (Taxo P discs, BD

#231046). All pneumococcal isolates were serotyped with the Quellung Reaction using antisera from the Statens Serum Institute, Copenhagen.

Antibiotic resistance testing

All isolates from the KND were tested for resistance to penicillin G, chloramphenicol (the two antibiotics commonly used for standard therapy of bacterial meningitis in Ghana), cefotaxime, and ciprofloxacin using E-test strips (Isenberg Henry D.(ed.), 1998). Breakpoints of the NCCLS protocol have been applied. For ciprofloxacin 4µg/ml has been taken as breakpoint for resistance (Brueggemann et al., 2002). The ATCC 49619 strain was included as control.

Multi-Locus Sequence Typing (MLST)

Bacteria were grown overnight in Todd Hewitt medium. DNA extraction (Vela Coral et al., 2001), MLST (Enright and Spratt, 1998) and direct sequencing of PCR products with an ABI Prism 310 Genetic Analysis System was performed according to standard protocols. Allelic profiles were analysed using applications available on the MLST homepage *(http://spneumoniae.mlst.net).* For the analysis of the relationships between closely related isolates the eBurst software (*http://eburst.mlst.net/)* was used with the most stringent group definition (6/7 alleles identical). All allelic profiles obtained were compared to the complete listing of STs available in the database.

6.4 Results

Meningitis cases

Between 1998 and 2003, a total of 140 meningococcal, 117 pneumococcal and 14 Hib meningitis cases were confirmed by culture and/or Latex agglutination assay in the KND. The number of pneumococcal cases remained low during the first two years of the study, but increased continuously during the following years (Figure 6.1). Two subsequent outbreaks of serogroup A meningococci were reported during the study period. After the large meningococcal meningitis epidemic in Ghana 1997, 50 confirmed serogroup A cases occurred in 1998 (Gagneux et al., 2000). After two years of absence, from 2001 onwards serogroup A meningococcal cases re-emerged causing yearly outbreaks until 2004 (Chapter 4). The number of Hib meningitis cases remained low throughout the study period and included mainly children below 7 years of age (Figure 6.1).

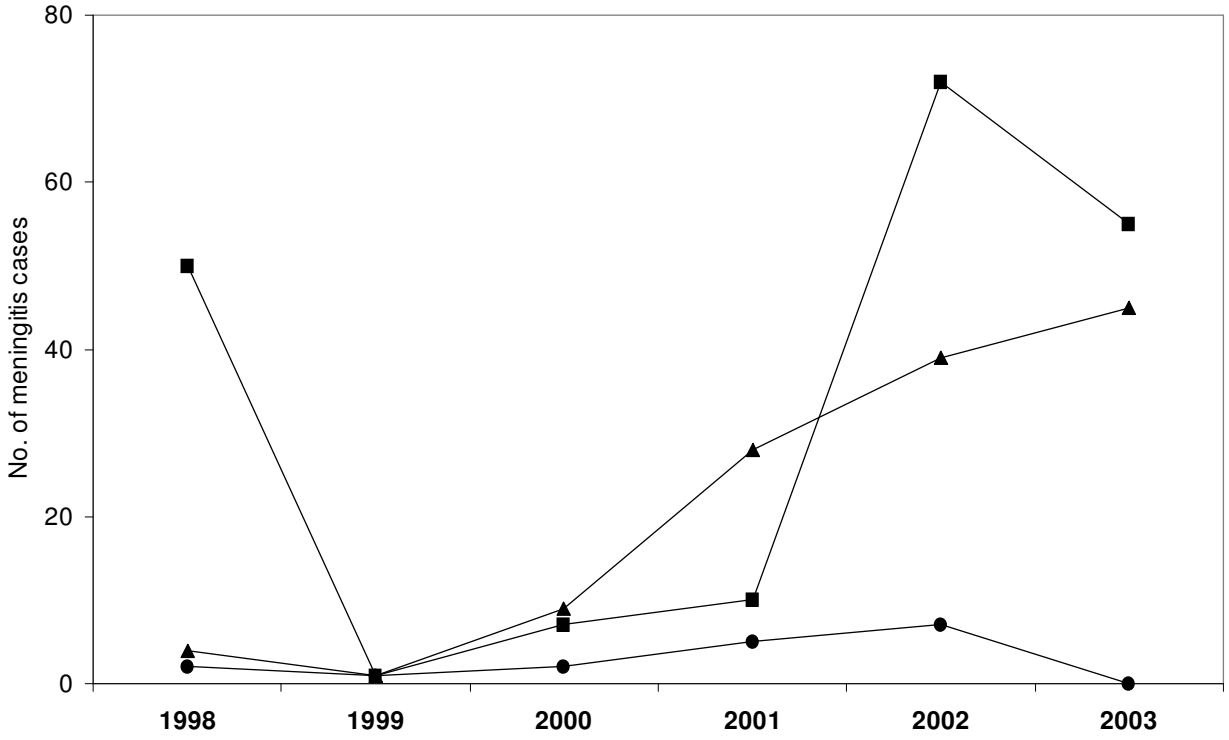

Figure 6.1 Number of laboratory-confirmed (cultivation and/or latex agglutination) meningitis cases in the Kassena-Nankana District of northern Ghana between 1998 and 2003.

■ N. meningitidis, ▲ S. pneumoniae, ● H. influenzae type b

The vast majority of meningococcal and of pneumococcal meningitis cases occurred during the dry season (Figure 6.2). The pneumococcal meningitis cases peaked one to two months earlier than the meningococcal cases. During the rest of the year only sporadic meningitis cases, mostly caused by *S. pneumoniae*, were observed.

The populations of both meningococcal and pneumococcal meningitis patients exhibited a broad age range (Figure 6.3). Infants less then one year had the highest incidence for both pneumococcal and meningococcal meningitis (43 cases/100,000 per year). For pneumococcal meningitis, the incidence in all other age groups was 15 - 26/100,000. For meningococcal meningitis the incidence was comparable for children of all age groups, and decreased steadily for the older age groups. As a result, the incidence of pneumococcal meningitis in the >60 year age group was significantly higher than for meningococcal meningitis (2.6/100'000 versus 23.4/100'000). The overall case fatality rate was 44.4% (51/117) and 4.3% (6/140) for pneumococcal and meningococcal meningitis, respectively.

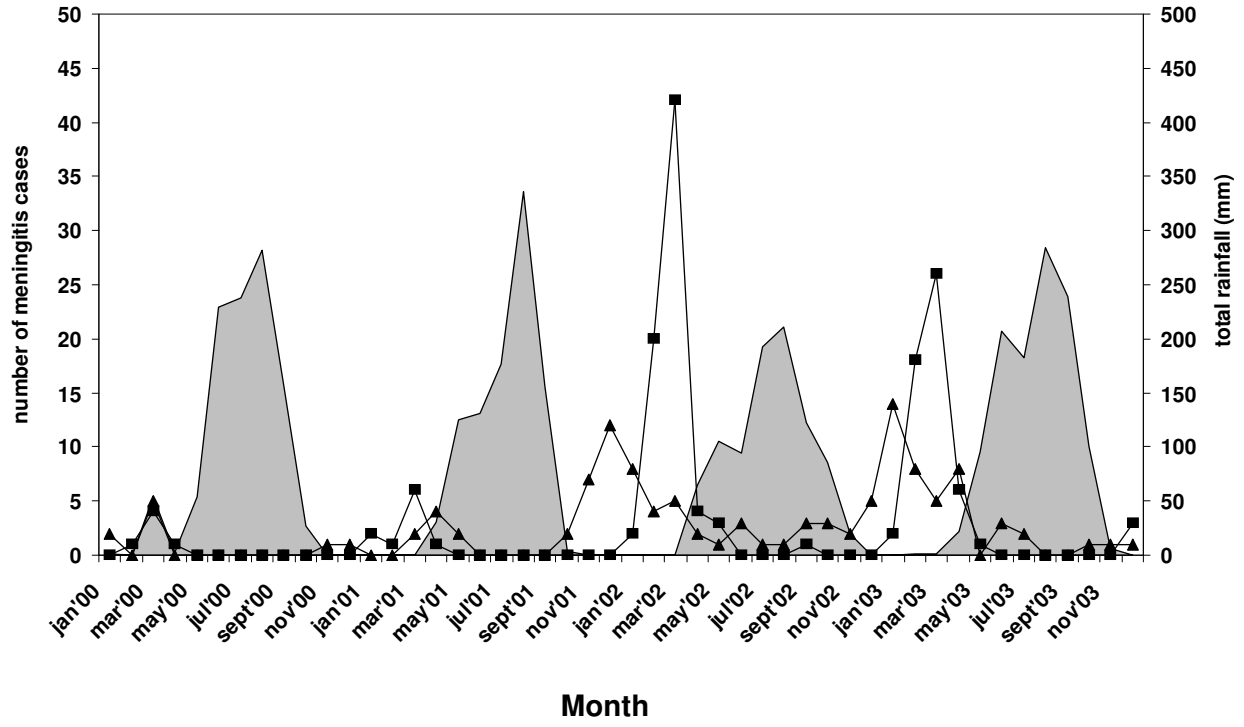

Figure 6.2 Seasonal patterns of rainfall and number of pneumococcal and meningococcal meningitis in the KND.

Laboratory-confirmed meningococcal (■) and pneumococcal (▲) meningitis cases, total monthly rainfall (data from the Meteorological Station of the KND).

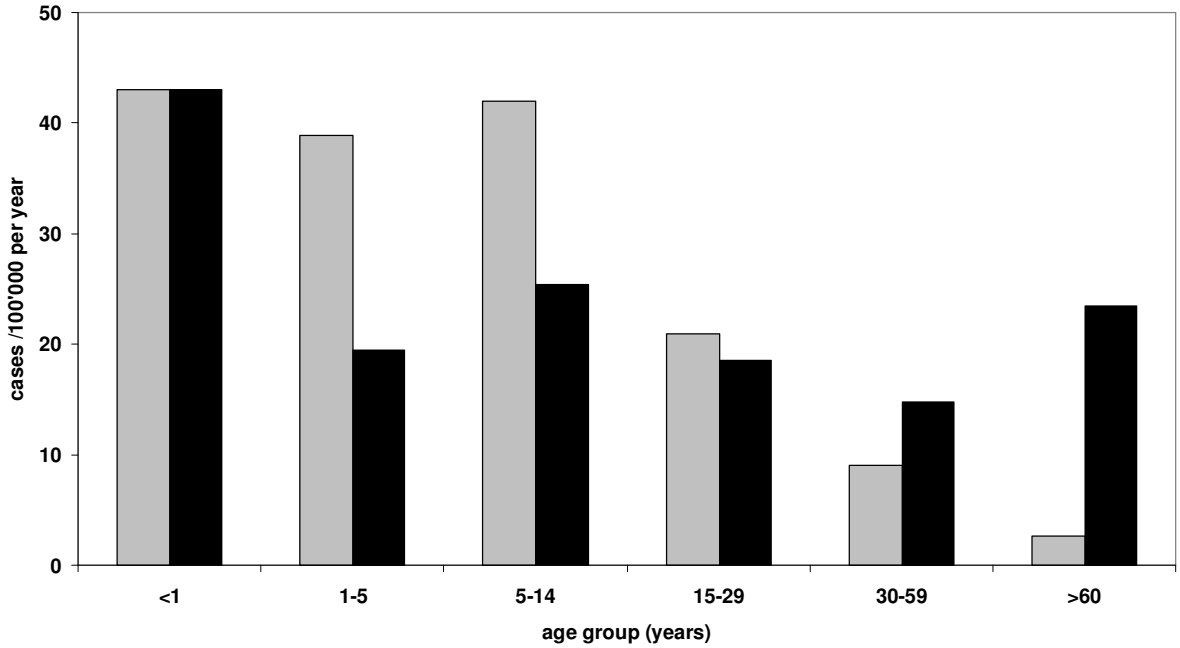

Figure 6.3 Incidence (laboratory confirmed cases by latex agglutination or culture) of meningococcal (grey bars) and pneumococcal (black bars) meningitis in the KND.

The geographic location of the homes of 74 pneumococcal and 102 meningococcal meningitis patients was mapped using the NDSS, but neither pneumococcal nor meningococcal cases were geographically clustered (data not shown). Furthermore no significant family clustering was observed.

Characterization of pneumococcal isolates

Between 1998 and 2003, 76 pneumococcal disease isolates were obtained from meningitis patients in the KND. Fifty-eight of these (76.3%) belonged to serotype 1, which represented the dominating serotype throughout the study (Table 6.1). The 18 non-serotype 1 isolates from the KND belonged to nine other serotypes. Only a third (2/6) of the paediatric disease isolates (<5 year old) were serotype 1, the remaining belonged to serotype 3 and 14. In contrast, in older children (5-14 years), young adults (15-29 years) and grown-ups (30-59 years) the serotype 1 ratio was >80 % (24/29, 11/12 and 11/14, respectively). In patients >60 years the percentage of serotype 1 isolates was 56 % (5/9).

Drug sensitivity testing showed that all but two of the 58 serotype 1 strains from the KND were completely susceptible to penicillin G, cefotaxime, chloramphenicol and ciprofloxacin. Minimal inhibitory concentrations (MIC) determined for the two strains (both isolated in 2002) showing antibiotic resistances were: strain P1036**:** penicillin G 0.5 µg/ml (intermediate), cefotaxim 2µg/ml (resistant), chloramphenicol: 5 µg/ml (intermediate); strain P1037: penicillin G 0.5 µg/ml (intermediate), cefotaxim 1 µg/ml (intermediate), chloramphenicol 8 µg/ml (resistant).

All isolates from the KND and 15 isolates from neighbouring districts were analysed by MLST. The results showed that all serotype 1 isolates were clonally related (Table 6.2). Ten distinct STs were identified; but all shared at least six of seven alleles with one other ST. ST217 and its two single locus variants ST612 and ST303 dominated. In addition, single locus variants of the three dominating STs were sporadically found. All isolates obtained in 1998 and 2000 had ST217. ST303 isolates dominated from 2001 onwards (6/15 in 2001, 9/18 in 2002 and 14/20 in 2003).

An eBurst analysis was done including the STs of the Ghanaian stains and all strains available in the MLST database (Figure 6.4). Three of the 10 STs found in the Ghanaian isolates (ST217, ST303 and

ST612) have been previously described in altogether 34 serotype 1 lineage B isolates (Brueggemann and Spratt, 2003). 16 of these isolates came from Africa, the others from Israel, Europe or the United States. In addition, Brueggemann et al. (Brueggemann and Spratt, 2003) defined three lineage B associated STs (ST613, ST614 and ST 618) represented by four African and one European isolate. The eBurst diagram (Fig.6.5) demonstrates, that all Ghanaian serotype 1 strains found in this study and all the lineage B isolates described by Brueggemann et al. are part of a single clonal complex in which all isolates share 100% genetic identity at six or seven MLST housekeeping loci with at least one other member of the group.

Table 6.1: Age distribution of Serotype 1 and Non-serotype 1 isolates from the KND from 2000 to 2003

Age group (years)	<1	1-4	5-14	15-29	30-59	>60	n.s.*	Total	*age range*	*median*
No. of isolates serotyped	2	4	29	12	14	9	6	**76**	*$^{4}/_{12}$ to 85 y*	*14y*
Serotype 1 isolates	0	2	24	11	11	5	5	**58**	*$1^{9}/_{12}$ to 72 y*	*15y*
Non-serotype 1 isolates	2	2	5	1	3	4	1	**18**	*$^{4}/_{12}$ to 85 y*	*13y*
Serotypes of non-serotype 1 strains	14^{+}	3^{+}	3^{+}, 7F, 8, 12F	8	6A, 8, 10F	8, 12F,14, 38	2			

*Age not specified $^{+}$ two isolates

Table 6.2: Serotype distribution and STs of *S. pneumoniae* strains isolated in northern Ghana between 1998 and 2003.

Serotype	ST	No. of isolates	Year of isolation	Allelic Profile							Origin (District)
				aroE	gdh	gki	recP	spi	xpt	ddl	
1	217	15	1998-2003	10	18	4	1	7	19	9	KND (13), Bongo (1), Builsa (1)
	612	8	2001-2003	10	18	4	1	7	19	31	KND (7), Bolgatanga (1)
	303	36	2001-2003	10	5	4	1	7	19	9	KND (29), Bolgatanga (7)
	1322	1	2001	10	5	4	1	7	19	31	KND
	1316	1	2002	2	18	4	1	7	19	9	
	1325	2	2002	10	8	4	1	7	19	9	
	1331	2	2002	13	8	4	1	7	19	9	
	1327	1	2003	10	18	4	1	13	19	31	
	1328	1	2003	10	18	4	1	7	21	31	
	1323	1	2003	10	5	4	1	7	21	9	
2	74	1	1998	2	13	4	1	6	6	14	KND
3	458	7	2001	2	32	9	47	6	21	17	KND (3), Bolgatanga (4)
4	1321	1	2002	8	8	47	18	46	122	31	Bolgatanga
6A	1320	1	2002	7	13	8	6	6	8	8	KND
7F	1326	1	2002	10	16	4	1	6	21	9	KND
8	1317	1	2003	7	5	15	11	83	58	70	KND
	1318	1	2000	7	9	15	11	83	58	70	
	1335	1	2003	7	9	4	60	83	28	70	
	1319	1	2003	7	9	15	11	83	25	70	
10F	909	1	2003	2	42	2	1	6	19	20	KND
12F	989	1	2003	12	5	89	8	6	112	14	KND
	1330	1	2003	12	5	89	8	13	112	14	
14	1324	1	2002	10	5	4	17	7	21	9	KND
	1313	1	2003	2	5	4	12	7	21	14	
	1315	1	2003	2	5	9	1	7	21	9	
	1314	1	2003	2	5	4	1	7	21	14	Builsa
38	1329	1	2003	12	5	4	10	42	49	9	KND

[a] Values in parentheses indicate the no. of isolates found in each district (given only for those isolates found in >1 district).

Of the non-serotype 1 isolates, only the serotype 14 strains exhibited allelic profiles closely related to those of the serotype 1 complex (Table 6.2). One of the serotype 14 strains (ST1324) was a single locus variant of ST1323 (shown in Figure 6.4), two (ST1314 and ST1315) were double locus variants of ST1323 and the remaining isolate (ST1313) shared five alleles with ST1314 and four alleles with ST1323.

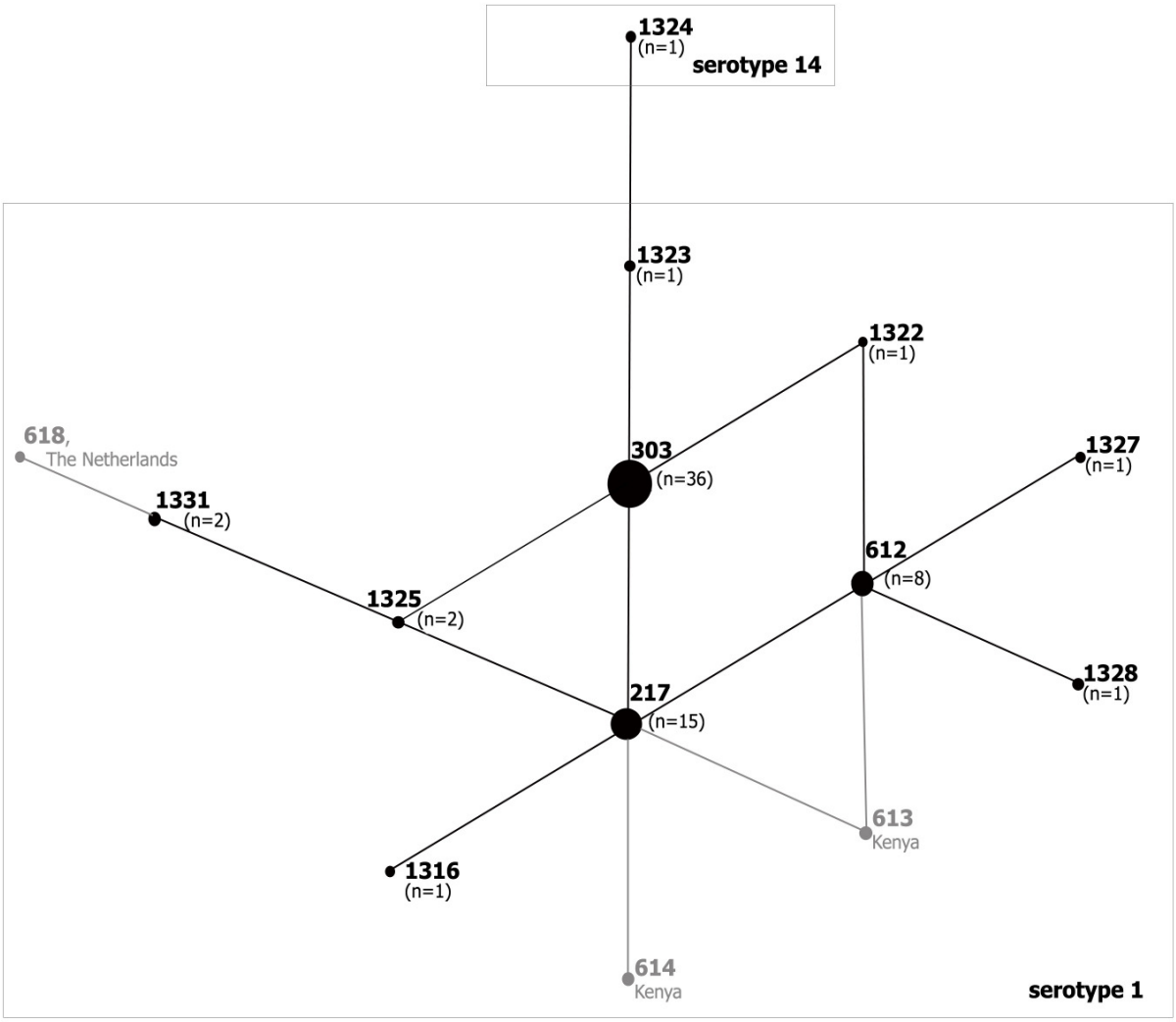

Figure 6.4 e-Burst diagram of the ST217 clonal complex

All Ghanaian serotype 1 and one Ghanaian serotype 14 isolate found in this study and all serotype 1 lineage B isolates described by Brueggemann et al. are included (Brueggemann and Spratt, 2003). Lines connect all single locus variants with each other. ● STs found in northern Ghana (n= number of isolates found in this study); ● serotype 1 lineage B associated STs not found in northern Ghana (Brueggemann and Spratt, 2003) (country of origin of the isolates). The original diagram has been edited for the number of isolates, the origin of non Ghanaian isolates and multiple SLV connections).

6.5 Discussion

N. meningitidis is regarded as uniquely capable of causing bacterial meningitis epidemics. Our observation of a meningitis outbreak caused by *S. pneumoniae* in the KND of northern Ghana is therefore intriguing. The outbreak exhibited epidemiological features characteristic of African meningococcal epidemics (Greenwood, 1999), including strong seasonality, a broad host age range and clonal dominance. The increase in pneumococcal meningitis was accompanied by two successive outbreaks of meningococcal meningitis. In the KND the burden of disease for pneumococcal meningitis has met criteria for the alert status of the WHO definition of epidemic meningococcal outbreaks (threshold of 5 cases per 100,000 per week) and in the neighbouring Bolgatanga District even criteria for the epidemic status with a threshold of 10 cases were fulfilled in March 2001. Cases of both meningococcal and pneumococcal meningitis were concentrated in the dry season, suggesting that similar factors might have triggered both types of outbreak. Such factors may include damaged mucosal defences due to the extreme environmental conditions and/or co-infections of the nasopharynx (Greenwood, 1999). Care was taken to avoid a bias associated with the well-known seasonality of meningococcal meningitis in the study area. Standardized guidelines for lumbar puncture were applied to avoid that lumbar punctures were less likely to be performed during the wet season.

Interestingly, the pneumococcal meningitis cases peaked one to two months earlier than meningococcal meningitis. This may reflect either the very high invasive capacity of the causative clonal complex of serotype 1 pneumococci or indicate that the factors which trigger pneumococcal and meningococcal meningitis are not entirely the same. In this context, differences in climatic conditions during the early dry season (including the Harmattan period with cold nights and extremely dusty air) and the late dry season (intensive heat), may be relevant. The broad age range in both meningococcal and pneumococcal meningitis cases shows that age related differences in the capacity of natural and adaptive immune effector functions are less important for susceptibility to invasive disease than in other epidemiological situations. Lack of spatial clustering suggests that colonization with the serotype 1 pneumococci is not focal.

Clonally related bacteria from a common epidemiological source often show limited genotypic variation (Feil, 2004). Groups of frequent genotypes plus their epidemiologically associated

descendents have been designated *clonal complexes* (Feil, 2004) or *genoclouds* (Zhu et al., 2001) on the basis of a threshold level of MLST allelic identity. The pneumococcal outbreak in the KND was caused by a clonal complex of serotype 1 pneumococci. The three most frequently found STs (ST217 and its two single-locus variants ST303 and ST612) have been described before (Brueggemann and Spratt, 2003), indicating that these genetic variants evolved prior to the outbreak in the KND. However, some of the infrequently isolated locus variants, such as ST1316, ST1322, ST1327 and ST1328 may have emerged locally. It is interesting to note, that ST1331 and ST1325, which were found each twice in the Ghanaian isolates link a ST618 isolate from The Netherlands to the clonal complex.

Serotype 1 pneumococci are a common cause of invasive disease in many parts of the world, but are only rarely found among healthy carriers (Brueggemann and Spratt, 2003; Hausdorff et al., 2000b; Sandgren et al., 2004). Studies comparing the prevalence of *S. pneumoniae* subgroups from invasive disease and from carriage showed that individual serotypes may differ more than a 100 fold in their potential to cause invasive disease (Brueggemann et al., 2003; Sandgren et al., 2004). Individual clonal complexes belonging to the same serotype have different abilities to cause invasive disease (Sandgren et al., 2004), suggesting that complex-specific virulence determinants might be important as well. It is not clear whether the virulence of the three major subgroups of serotype 1 pneumococci with distinct geographic distribution (Brueggemann and Spratt, 2003; Gonzalez et al., 2004) is primarily determined by the capsular serotype and therefore uniform, or whether lineage-specific genetic differences modulate the potential to cause particular types of invasive disease. Our results suggest that the ST217 associated clonal complex might have a particular propensity to cause meningitis. However, further studies are needed in order to verify whether this observation reflects a true bacterial phenotype or merely the influence of host and/or environmental factors.

We do not know whether the ST217 clonal complex has recently been imported into northern Ghana or whether it has been present for a longer time without causing more than sporadic disease. Clonal dissemination of *S. pneumoniae* is usually associated with antibiotic resistance (Klugman, 2002), but we observed no significant resistance in the Ghanaian isolates. Other factors must therefore have led to the increased incidence of pneumococcal meningitis in the KND. Vaccination against *S. pneumoniae* is uncommon in Ghana. However, the massive immunization campaigns with a meningococcal A+C carbohydrate vaccine that have been repeatedly carried out throughout the study period might have played a role. *S. pneumoniae* and *N. meningitidis* both colonize the human nasopharynx, and effective

interventions against one of these bacteria are likely to promote competing micro-organisms. Vaccinations with conjugate vaccines have been shown to reduce nasopharyngeal carriage of the vaccine type bacteria and to lead to replacement by bacteria not included on the vaccine (Bogaert et al., 2004b; Lipsitch, 1999). Even though polysaccharide vaccines, such as the unconjugated *N. meningitidis* A + C vaccine used in the KND, are generally thought to have no effect on the prevalence of nasopharyngeal carriage (Greenwood, 1999), repeated immunization against *N. meningitis* might still modify the bacterial flora of the nasopharynx (Fernandez et al., 2003). Thus, it is conceivable that the increase in pneumococcal meningitis in the KND, as well as the recently observed outbreaks of non-A, non-C meningococcal meningitis (Gagneux et al., 2002b; Djibo et al., 2003; Chonghaile, 2002) may have been promoted by mass vaccination against *N. meningitis*. It will be important to investigate more closely the interactions between these bacteria, especially in the context of vaccination (Bogaert et al., 2004a).

Serotype 1 is not included in the currently licensed paediatric heptavalent pneumococcal vaccine. This vaccine contains polysaccharides from the seven serotypes (4, 6B, 9V, 14, 18C 19F and 23F) that cause over 85% of severe pneumococcal infections in infants and young children in the USA and Canada (Bogaert et al., 2004b; Hausdorff et al., 2000b). The vaccine covers 70% of paediatric disease isolates from Europe, but only 67% and 43% of those from Africa and Asia, respectively (Hausdorff et al., 2000b). In the KND serotypes 3, 7F, 8, 12 and 14 accounted for the non- serotype 1 cases in patients below 15 years of age. The 'pedriatric' serotypes (e.g. 6, 14, 9, 1, 5) (O'Dempsey et al., 1996) were rarely found. Here, the heptavalent conjugate vaccine would have covered 5.7% (2/35) of all cases and 22% of the non-serotype 1 cases in this age group. A nonavalent conjugate vaccine including serotype 1 is currently being developed, but such a complex conjugate vaccine may be too expensive for mass immunization in the African meningitis belt. However, mass vaccination targeting hypervirulent serotypes with a less complex conjugate vaccine should be considered, since increasing trends in pneumococcal meningitis have also been observed in other districts of Ghana (data not shown). Predominance of serotype 1 and a broad age spectrum also seem to be features of the current pneumococcal meningitis situation in Burkina Faso (Robbins et al., 2005; Parent, I et al., 2005). In view of the high case fatality rate of *S. pneumoniae* meningitis, there is also an urgent need for improved treatment options suitable for countries with limited resources.

6.6 Acknowledgements

This work was supported in part by a grant of the Meningitis Research Foundation. We acknowledge the use of the pneumococcal MLST database which is located at Imperial College London and is funded by the Wellcome Trust. We thank Mr Alhassan and his team from the Bolgatanga hospital for access to the data and the provision of samples, the district health authorities of the KND for their support and the health facilities of Bongo and Sandema for their kind collaboration. Furthermore, we would like to acknowledge A. Bugri and A. Wahab for their indispensable contribution in the laboratory in Navrongo, the fieldworkers of NHRC for their efforts, and Prof. Gasser (Basel) for his support and practical advice.

CHAPTER 7

SURVIVAL AND SEQUELAE OF PNEUMOCOCCAL MENINGITIS IN NORTHERN GHANA

CHAPTER 7

Survival and Sequelae of Pneumococcal Meningitis in Northern Ghana

Abudulai Adams Forgor[1], Abraham Hodgson[1], Julia Leimkugel[2], Martin Adjuik[1], Valentin Pflüger[2], Oscar Bangre[1], Jean-Pierre Dangy[2], Gerd Pluschke[2] and Tom Smith[2]

[1]*Navrongo Health Research Centre, Ministry of Health, Navrongo, Ghana*

[2]Swiss Tropical Institute, Basel, Switzerland

This article has been prepared for submission to:
International Journal of Infectious Disease

7.1 Abstract

Background Little information is available about the burden of pneumococcal meningitis (PCM) in sub-Saharan Africa, despite its importance as a leading cause of high mortality and morbidity. We carried out a case control study to assess the survival and sequelae of PCM.

Methods We compared two-year survival of 67 PCM cases hospitalized in Navrongo, Ghana with equal numbers of meningococcal meningitis (MCM) cases and with community controls, all identified in a demographic surveillance system. We also carried out a case-control study of sequelae in 46 traceable survivors of PCM (cases), 46 community controls (CC) and 34 survivors of MCM, matching for age, sex and geographical location using a structured disability questionnaire, and neurological, neuropsychological and audiometric examinations.

Results PCM cases had much higher mortality than either MCM cases or CC (relative hazard compared to MCM=7.0; 95%CI: 2.4-20.3) but this excess was entirely during hospitalization and the first few weeks after discharge. Moderate-profound hearing impairment was found by audiometry in 23.9% of PCM survivors compared with 5.9% of MCM survivors (χ_1^2 =6.2; p=0.01; 95% CI: 1.0, 64.0) and 2.2% of CC (χ_1^2=15.5; p<0.001). 8.7% of PCM survivors had profound speech impairment. More PCM than MCM survivors had psychiatric symptoms (hearing voices: OR=5.0 χ_1^2=5.8; p=0.02; reported self-inflicted injury: χ_1^2=8.3; p=0.004; shutting self up alone: χ_1^2=4.2; p=0.04; panic: OR=4.5; χ_1^2=4.8; p=0.03).

Conclusions Hearing and speech impairment as well as psychiatric disorders, are much more frequent and severe in PCM than in MCM. There is the need for thorough surveillance of PCM in countries at high risk and an accelerated immunization schedule with pneumococcal vaccine containing the appropriate serotypes beginning either maternally or in the perinatal period.

7.2 Introduction

Despite improvements in diagnosis and treatment, morbidity and mortality from PCM remains unacceptably high (Schuchat et al., 1997; Arditi et al., 1998; Fiore et al., 2000; Buckingham et al., 2001; Kellner et al., 2002), with case-fatality rates of about 20% in industrialised countries (Schuchat et al., 1997) and up to 50% (chapter 6; Yaro et al., 2006) in Africa, about 5–10 times higher than for MCM. Bacterial meningitis accounts for approximately 60-90% of acquired hearing impairment in children (Dodge et al., 1984; Richardson et al., 1998; Kulahi et al., 1997).

Most of the studies published on PCM were carried out in developed countries with just a few in developing countries. There is little information on the long-term disability of PCM in the African meningitis belt and none on direct comparism between PCM and MCM. Due to its epidemic nature most studies are related to MCM with a few finding *S. pneumoniae* as the most important causative agent (Mackie et al., 1992; Haddock, 1971; Campbell et al., 2004; Yaro et al., 2006; chapter 6).

From 2000-2005 we observed a continual increase in PCM incidence in northern Ghana, with high mortality and predominance of hypervirulent serotype 1 (unfortunately absent from the currently licensed paediatric heptavalent pneumococcal vaccine (chapter 6). We now report on long-term effects of PCM in the meningitis belt of sub-Saharan Africa based on follow-up of these cases.

7.3 Materials and methods

Study area

The Kassena Nankana District (KND), one of the deprived districts in Ghana, has a population of 140000, an area of 1675km^2 and lies within the guinea savannah woodland of northern Ghana with Burkina Faso as its northern neighbor. The district lies in the sub-Saharan African meningitis belt. The district has 1 hospital (the WMH) located in Navrongo, the district capital and 4 health sub districts each of which has a health centre. The district is endowed with a demographic surveillance system, the Navrongo Demographic Surveillance System (NDSS), in which births, deaths, in and out

migrations and other demographic parameters of the entire district are recorded in a database and updated every 90 days (Binka et al., 1999).

Diagnosis

Between January 1998 and December 2004 cerebrospinal fluid (CSF) samples were collected by lumbar puncture from all suspected meningitis cases presenting at any health facility. Direct Gram staining, and serological testing for capsular polysaccharide antigens of *Neisseria meningitidis* (serogroups A, B, C and W135), *Streptococcus pneumoniae,* and *Haemophilus influenzae* type b (Slidex Meningite Kit, bioMérieux; Pastorex Kit Bio Rad) were carried out at the WMH microbiology laboratory. CSF was also cultured by standard microbiological methods and further aliquots frozen at -80^{o}C and sent to the Swiss Tropical Institute, Basel, Switzerland for confirmation and molecular analysis.

Survival study

Survival was analyzed of all possible laboratory confirmed meningitis cases, including in-patient deaths, from 1998 to 2004 that could be linked to the NDSS database. For each PCM case a CC matched for age (±10%), sex and location of the home on admission date, was selected from the NDSS dataset. Where possible, for each PCM case, a further control, matched by age (±10%), sex and proximity, with a history of MCM prior to the case's admission date was also selected. Where these criteria gave more than one eligible control, the sibling of the case was preferentially included; in the absence of a sibling the control was selected at random. Dates of birth, deaths and migrations of both cases and controls were obtained from the NDSS.

Disability study

The disability study included all survivors of PCM who could be traced. For each survivor two groups of controls matched by age (±10%) and sex were identified in the NDSS database and ordered according to their proximity (by geographical information system) to the case. The first group comprised community members who never had meningitis or meningism up to admission date of the case. The second group of controls comprised survivors of MCM occurring over the same period as the cases. For each case, the community control and MCM control alive at the end of 2005

and living nearest to the home (or in the home) of the case, and matched by age (±10%) and sex, were included in the study.

An appointment was made with each study participant and their relatives after they gave informed consent. Participants were assured of data confidentiality. Participants and their relatives were interviewed by trained field workers blinded to case/control status, using a standard questionnaire previously administered in Kassena-Nankana to survivors of meningococcal meningitis (Hodgson et al., 2001b) and adapted from that of a national disability survey (Ngom et al., 1999). Those ≥6 years old were asked about general conditions of health, exercise of daily living skills (feeding, dressing, cleansing, use of latrines, understanding simple instructions, expression of needs, speaking, hearing, movement in home and community) (table 7.3). Subjects over 6 years were also asked about symptoms of depression, anxiety, addiction and psychosis (table 7.4).

For neuro-psychological status assessment, subjects above 6 years were asked about their orientation in time, place and sense of self. To assess memory, they were asked to recall the composition of the previous day's breakfast, to repeat the names of items mentioned to them, to reverse the order of the names of four animals mentioned to them and to recall these animals after 15 minutes. To assess general knowledge they were asked the names of chiefs of the locality of subjects, the head of state and the biggest town. Those above 10 years old were asked the name of the first head of state of Ghana, to explain a local proverb and to carry out simple arithmetic operations (Berkow, 1992).

In order to evaluate these responses, in the absence of the subjects relatives were also asked about the subject's disabilities, psychiatric history (table 7.5) and changes in general health status. Sensitive issues were explored only after the establishment of a good relationship.

A physician, blinded to the case/control status of subjects, carried out neurological examinations and tested for cranial nerve palsies, motor defects and cerebellar disorders. A portable screening audiometer (Micromate, Denmark) was used for audiometry after otoscopy of cases and controls ≥5years. Those under 5 years were tested by behavioural observational audiometry using thresholds of 500 Hz, 1000 Hz and 2000 Hz. Hearing loss was classified as described by Dodge (Dodge et al., 1984).

Ethical approval

The study was conducted after obtaining informed consent from the chiefs, elders, subjects and parents /guardians of subjects. Ethical clearance for the conduct of this study was also obtained from the Navrongo Health Research Centre Institutional Review Board and the local health authorities.

Data analysis

Data were double entered using visual FoxPro and verified for consistency. Using Stata software version 9.0 (Stata Corp., College Station, TX, USA), Kaplan-Meier estimates of the survival curves for cases and controls were constructed separately for the period up to the end of May 2006. Migration out of the district was treated as a censoring event.

Disability was analyzed using conditional logistic regression, with data stratified as defined in the original matching. Twelve survivors of PCM were dropped in the matched comparison with survivors of MCM.

7.4 Results

From 1998 to 2004 we recorded 145 PCM cases, exhibiting a broad age spectrum with the highest reported incidence and mortality rates in the <1 year age group (figure 7.1). This contrasts with the European pattern where there is an initial decrease with age in PCM incidence and then an increase with age in older age groups (Appelbaum, 1987a) (figures 7.1 and 7.2). Recorded incidence of MCM peaked in the 1-4 year age group declining to a minimum in the 60+ age group (Figure 7.2) resembling the age pattern seen in Europe (van de Beek and de Gans, 2004b).

Tracing

Of the PCM admissions analyzed in the WMH laboratory, 77/145 (53.1%) were discharged alive, and 68(46.9%) died in hospital. Sixty-seven of those discharged alive could be traced in the NDSS database (table 7.1). No patients were admitted more than once for meningitis. Two subjects denied ever having meningitis and were omitted from the analysis, while one survivor could not be interviewed. The low number of identified cases in the NDSS could be due to incorrect addresses or names in the admission records.

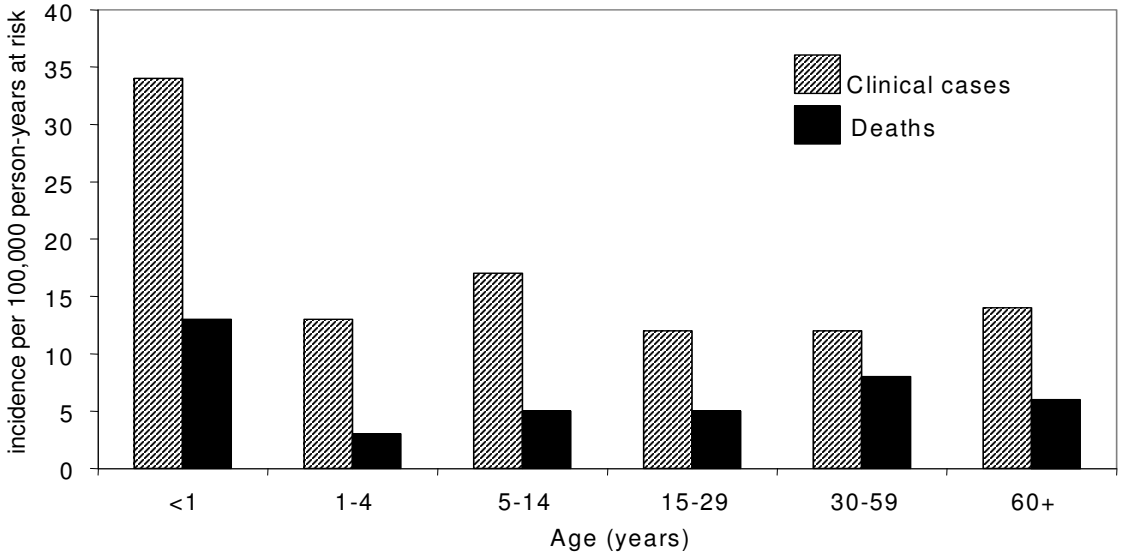

Figure 7.1 Reported incidence and mortality rates of pneumococcal meningitis in the Kassena Nankana District 1998 – 2004

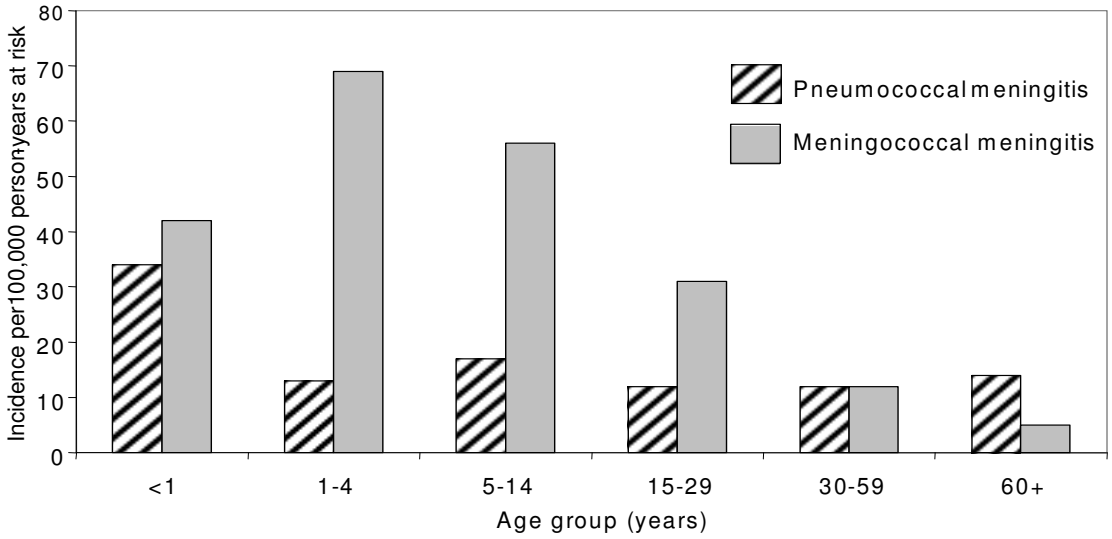

Figure 7.2 Reported incidence rates of meningococcal and pneumococcal meningitis in the Kassena Nankana District 1998 – 2004.

Survival study

21/67 (31.3%) of the PCM cases, 6/67 (9.0%) of the matched CC, and 8/67 (11.9%) of the MCM controls died before the end of the study (May 2006). Most died within the first month of admission (figure 7.3). Nineteen (90.5%) deaths of the PCM group occurred within the first month after admission with only 2 (9.52%) occurring more than one month after admission.

Table 7.1: Results of tracing

	No. of patients(n)	**% of patients**
Found alive (history of pneumococcal meningitis)	46	31.7
Found alive (denied a history of meningitis)	2	1.4
Dead	68	46.9
Absent	22	15.2
Could not be traced	1	0.7
Died after discharge of other causes	6	4.1
Total admissions	**145**	

All 8 deaths in the MCM group occurred within the month of admission and no death occurred more than one month after admission. Deaths in the community controls were spread along the period of study with 2 (33.33%) deaths occurring within the first month after admission of the case. The difference in survival between the three groups over the whole period were highly significant (log-rank test (LR) χ_2^2=17.9, p<0.0001) there was however, no significant difference in survival after the first month of admission (LR χ_2^2=0.18, p=0.67). The relative risk of death (hazard ratio) of PCM compared with MCM was 7.0 (Cox Regression; p<0.0001, 95%CI: 2.4, 20.3).

Disability

Seventy-seven (53.1%) of PCM patients admitted to the hospital were discharged alive. Of these 46(59.7%) were available and participated in the disability study. An equal number of community controls were also included, as were all the MCM cases available (Table 7.2). The mean age of the study participants was 17.6 years (SD 15.1; range 1-73 years) with 13(10.3%) subjects in the 1-4 age group, 60(47.6%) in the 5-14 age group, 32(25.4%) in the 15-29 age group, 16(12.7%) in the 30-59 age group and 5(4.0%) in the 60+ age group.

Levels of disability in the performance of daily skills are shown in table 7. 3. 18 (39.1%) survivors of PCM reported difficulty in hearing normal speech, compared to 11 (32.4%) of survivors of MCM and 9 (19.6%) of community controls (table 7.3).

PCM survivors also had difficulties in expressing their needs and understanding simple instructions more often than the other groups. PCM patients and controls differed in the relatives' perception of changes in the general condition of health in the two years before interview (figure 7.4) (OR=4.5**,**

95%CI: 1.5, 18.3, χ^2 = 0.003 p=0.004), and a higher proportion of PCM survivors were considered to have deteriorated than of MCM survivors (though this difference was not statistically significant) (Table 7.5).

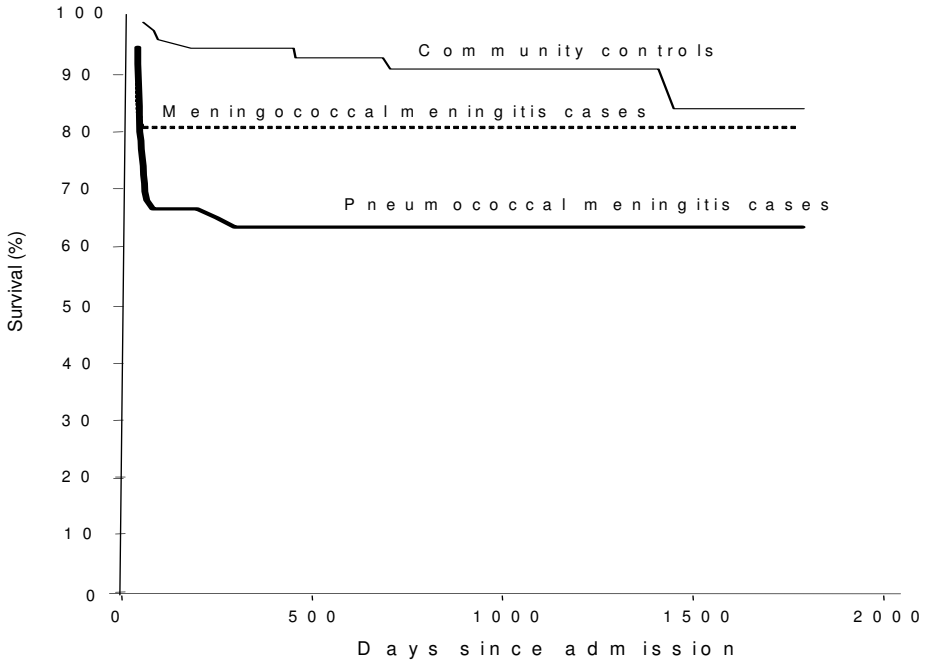

Figure 7.3 Kaplan-Meier survival curves comparing the survival of pneumococcal meningitis cases with meningococcal meningitis cases and community controls in the Kassena Nankana District.

Table 7.2 Distribution of study subjects

	PCM	MCM	CC
Numbers attending	46	34	46
Age (years)	18.6 (SD 15.9; range 2-70)	15.1 (SD 13.0; range 3-60)	18.3 (SD 15.8; range 1-73)
Number female	19(41.3%)	12(35.3%)	19(41.3%)

The main differences in health reported by relatives in the unstructured narrative were that PCM survivors were reported to suffer from difficulties in communication. The neuro-psychological status assessment indicated poor performance in all three groups, with fewer than 70% of each group answering more than 50% of the questions correctly.

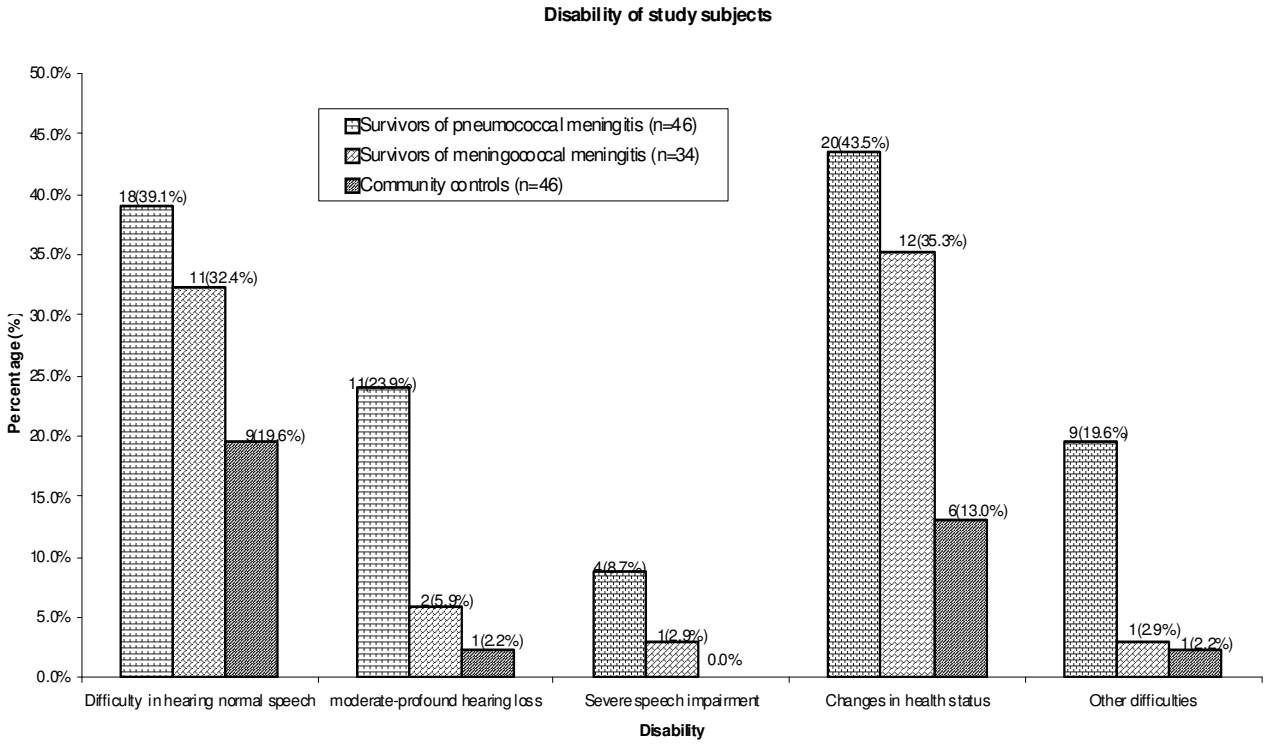

Figure 7.4 Disability of study subjects**.**

The differences between groups in disability recorded by audiometry (tables 7.6 and 7.7 and in figure 7.4) were much greater than those recorded by interview. Overall, moderate-profound hearing impairment was found in 11 (23.9%) of PCM survivors compared with 1 (2.2%) CC (χ_1^2=15.5; p<0.001), and 2 (5.9%) of MCM survivors (OR =8.0; χ_1^2 =6.2; p=0.01; 95% CI: 1.0, 64.0) (Tables 7.6 & 7.7). This compares with only 1.6% of MCM survivors in our previous (much larger) study (Hodgson et al., 2001b). Four PCM survivors had chronic otitis media [left (3) and right (1)] (table 7.8). Two of them had suppuration and one only a tympanic membrane perforation. One CC and 1 survivor of MCM had non-suppurative chronic otitis media, each with tympanic membrane perforations. Swabs of the pus were taken to the laboratory for culture and sensitivity but there was no bacterial growth and these individuals were referred to an Ear Nose & Throat specialist for management.

There was no significant difference in the three groups with regards tests of the other cranial nerves. Nor was there any significant difference in the muscle bulk, tone and reflexes of the survivors of PCM, MCM and CC (figure 7.4).

More PCM than MCM survivors had psychiatric symptoms (hearing voices: OR=5.0 χ_1^2=5.8; p=0.02; reported self-inflicted injury: χ_1^2=8.3; p=0.004; shutting self up alone: χ_1^2=4.2; p=0.04; panic: OR=4.5; χ_1^2 =4.8; p=0.03) (tables 7.4 and 7.5). PCM survivors also have relatively poor social skills (tables 7.4 and 7.5), 17.4% having the tendency to cause self-harm and two behaving strangely.

Other disabilities identified are summarized in table 7.8. Gait ataxia was found in 3 survivors of PCM, 1 community control, and one MCM survivor. All subjects found to have any form of disability were referred to the appropriate specialist(s) for further management and rehabilitation.

7.5 Discussion

Despite the small study size we could show substantial differences in both survival and sequelae between PCM and both MCM and CC groups. The profound excess mortality of about 38% (compared to 6.3% due to MCM reported earlier (Hodgson et al., 2001b)), mostly in the acute phase of PCM is slightly lower than other reports in the African meningitis belt (Bijlmer et al., 1990; Campbell et al., 2004; Goetghebuer et al., 2000) but far higher than the 5-20% reported in industrialised countries (Baird et al., 1976; Kornelisse et al., 1995; Arditi et al., 1998; Fiore et al., 2000; Buckingham et al., 2001; Schuchat et al., 1997).

Possible reasons for the high mortality (hazard ratio approximately 7) are the young age of the PCM cases, hypervirulence of *S. pneumoniae* serotype 1 (chapter 6), late presentation, and initial diagnosis of cerebral malaria (especially in children) leading to delay in starting appropriate antibiotics. Bedside diagnostic test kits for malaria parasites and for bacteria in CSF could prevent the latter.

The lack of significant differences in survival between PCM cases and CC after the acute phase of PCM (χ^2=0.15 p=0.7), confirms that the PCM indeed accounts for the high mortality. PCM

survivors more frequently reported changes in health status, especially hearing impairment, speech impairment and psychosocial changes, than did either CC or MCM (table 7.5 and figure 7.4).

The lack of difference between PCM cases and CC (χ^2=0.15 p=0.7) in the survival rates after the acute phase of PCM, confirms that the high mortality was indeed due to the PCM.

Approximately one out of every four survivors of PCM (24%) has moderate-profound hearing loss, while our earlier study of MCM survivors found a rate of only 1.6% (Hodgson et al., 2001b) comparable to the 2.2% profound hearing loss in the MCM controls in the present study (tables 7.3 & 7.4). This confirms earlier studies that found Sensorineural Hearing Loss (SNHL) to be most often associated with *S. pneumoniae* meningitis (Dodge et al., 1984; Baraff et al., 1993; Daoud et al., 1995; Pikis et al., 1996; Pikis et al., 1996; Richardson et al., 1997; Goetghebuer et al., 2000). Not all hearing loss is detected on admission and hearing evaluation is recommended as part of routine follow-up after bacterial meningitis (Fortnum, 1992; Fortnum and Hull, 1992; Fortnum and Davis, 1993; Woolley et al., 1999).

The high virulence of *S. pneumoniae* serotype 1 (chapter 6) or late presentation are possible explanations for the high incidence of SNHL. SNHL arises at an early stage of pathogenesis (Nadol, Jr., 1978; Kaplan et al., 1986) and early reporting, early diagnosis and prompt appropriate treatment reduce its incidence in survivors (Richardson et al., 1997).

Late identification and lack of rehabilitation of those with impaired hearing could also account for the approximately 9% of PCM survivors with profound speech impairment. Early identification and rehabilitation of hearing loss in children is essential for language acquisition and for educational and social development (Yoshinaga-Itano et al., 1998; Yoshinaga-Itano and Apuzzo, 1998a; Yoshinaga-Itano and Apuzzo, 1998b).

Though our assessments were rudimentary, the incidence of cognitive disability in PCM survivors that we recorded [14(30.4%)] is far higher than in other reports (Grimwood et al., 1995; Grimwood et al., 2000). This could well be a further consequence of hearing and speech impairment coupled with late detection and absence of rehabilitation. The incidence is much higher than in survivors of MCM or CC (OR=6, χ_1^2 =3.96, p=0.0465, 95%CI: 0.7, 49.8).

It is advisable that every child, following bacterial meningitis, should undergo a complete and repeated audiological assessment to detect any lesser impairments and/or unilateral hearing losses since that may damage the development of speech and language. This assessment should begin as soon as possibly because early identification of hearing impairment is needed to ensure that any cochlear implantation is carried out before ossification of the cochlear occurs (Dodds et al., 1997; Marx and Baer, 2001).

The high level of mild hearing impairment in both groups of controls is also of concern since this very likely increases the risk of motor traffic accidents and limits academic performance. The many possible causes of this impairment, including congenital factors, other infections, and drug side-effects, require further investigation. Screening of hearing in newborns before they leave the hospital or maternity home, of infants during postnatal clinics, and of school children (at least annually) is thus necessary even when there is no meningitis epidemic. In the absence of early screening the average age of detection of significant hearing loss is approximately 14 months (Erenberg et al., 1999). Those found to be impaired need early referral to the appropriate specialist for further management.

Consistent with earlier reports (Baraff et al., 1993; Daoud et al., 1995) survivors of PCM were more likely to suffer psychiatric disorders than survivors of MCM and we found indications of psychiatric disorders than were reported in the earlier study of MCM survivors (Hodgson et al., 2001b). This may result from the need to adjust to a sudden drastic change in the health status and the accompanying stigma.

The gait ataxia may have resulted from peripheral vestibular dysfunction or neurological damage from central nervous system involvement of the disease since most survivors with hearing impairment have vestibular areflexia (Rasmussen et al., 1991).

In view of the high morbidity associated with PCM there is the need for a multi diciplinary and multisectorial approach in the management and rehabilitation of survivors of PCM. The identification of long-term sequelae in survivors of pneumococcal meningitis before and after discharge from hospital will enable the institution of programmes for long term follow-up and rehabilitation of survivors. As part of such programs, the survivors and their relatives should receive serious counselling on the condition and changes in the health during and before discharge from the hospital, and the need to make adjustments for their poorer social skills.

At the same time there is the need for thorough surveillance of pneumococcal diseases, with isolation of invasive serotypes by cultures (blood, CSF, ear swabs etc) and agglutination tests. This will be very helpful for future vaccine development and introduction in view of the diversity of pneumococci. Considering the high incidence, mortality and morbidity rates of PCM in the <1 age group and the lack of effect of the 23- valent polysaccharride vaccine on children <2 years and the absence of the hypervirulent serotype1 (found in the district) in the currently licensed heptavalent pneumococcal conjugate vaccine, it is urgent to carry out prenatal maternal vaccination with the pneumococcal polysaccharide vaccine while efforts are being made for conjugate vaccines (with the appropriate serotypes) for perinatal immunization. Protection of the child by transfer of maternal antibodies at birth and by breast-feeding may be possible with antenatal maternal vaccination with pneumococcal vaccine (Deubzer et al., 2004). This approach is currently used successfully in the control of neonatal tetanus and there is good reason for it to prevent neonatal invasive pneumococcal disease.

7.6 Acknowledgements

We thank the study subjects and their relatives for their willing participation, the CSM field workers of the Navrongo Health Research Centre for their efforts and dedication. Akalifa Bugri and Abdul-Wahab Hamid contributed in the microbiology laboratory in Navrongo and Wilson Sama assisted with data analysis. The Navrongo Health Research Centre and both district and subdistrict health authorities of the Kassena Nankana District gave institutional support. This study was carried out in 2006 and financed by the Volkswagen foundation.

Table 7.3 Disability (self reported)

Disability	Cases: Survivors of pneumococcal meningitis n (%)	Controls		Survivors of pneumococcal meningitis Community controls			Survivors of pneumococcal meningitis Survivors of meningococcal meningitis		
		Community controls n (%)	Survivors of meningococcal meningitis n (%)	OR[a] (95%CI)	χ^2	p-value	OR[a] (95%CI)	χ^2	p-value
Difficulty in moving any part of the body	9(19.6)	11(23.9)	8(23.5)	0.7(0.2, 2.3)	0.3	0.6	0.8(0.2, 3.0)	0.1	0.7
Difficulty in seeing	11(23.9)	11(23.9)	7(20.6)	1.0(0.3, 3.5)	0.0	1.0	1.2(0.4, 3.5)	0.1	0.8
Difficulty in hearing normal speech	18(39.1)	9(19.6)	11(32.4)	2.8(1.0, 7.8)	4.4	0.04	1.6(0.6, 4.1)	0.9	0.3
Episodes of fits in the last year	2(4.4)	6(13.0)	2(5.9)	0.3(0.1, 1.7)	2.1	0.15	0.0(0.0, ∞)	2.8	0.1
Inability to move inside the home	2(4.4)	1(2.2)	2(5.9)	2.0(0.2, 22.1)	0.3	0.6	1.0(0.1, 7.1)	0.0	1.0
Difficulty in speaking like a person of same age	9(19.6)	6(13.0)	2(5.9)	1.8(0.5, 6.0)	0.8	0.4	3.5(0.7, 16.8)	2.9	0.09
Inability to move around village	2(4.4)	1(2.2)	2(5.9)	2(0.2, 22.1)	0.3	0.6	1.0(0.1, 7.1)	0.0	1.0
Inability to use latrine unaided	8(17.4)	11(23.9)	8(23.5)	0.5(0.1, 2.0)	1.1	0.3	1.3(0.3,6.0)	0.1	0.7
Loss of feeling in hand or foot	7(15.2)	6(13.0)	5(14.7)	1.2(0.4, 3.9)	0.1	0.8	1.3(0.3, 6.0)	0.1	0.7
Inability to feed unaided	4(8.7)	3(6.5)	1(2.9)	1.3(0.3, 6.0)	0.1	0.7	4.0(0.4, 35.8)	1.9	0.2
Inability to dress unaided	3(6.5)	3(6.5)	2(5.9)	∞			2.0(0.2, 22.1)	0.3	0.6
Inability to keep self clean	6(13.0)	7(15.2)	6(17.7)	0.5(0.0, 5.5)	0.3	0.6	1.0(0.2, 5.0)	0.0	1.0
Inability to express needs	3(6.5)	1(2.2)	0	3.0(0.3, 28.8)	1.1	0.3	∞(0.0, ∞)	4.2	0.04
Inability to understand simple instructions	4(8.7)	1(2.2)	0	4.0(0.4, 35.8)	1.9	0.17	∞(0.0, ∞)	5.6	0.02
Other difficulties	3(6.5)	3(6.5)	2(5.9)	7(0.2, 2.3)	0.3	0.6	0.8(0.2, 3.0)	0.1	0.7

Total number of community controls =46, survivors of pneumococcal meningitis=46; survivors of meningococcal meningitis=34

[a] Odds ratio

∞ Odds ratio could not be determined because of zero denominator

χ^2 Likelihood ratio chi squared (degrees of freedom=2)

Table 7.4 Self-reported psychiatric symptoms

Symptoms	Cases: Survivors of pneumococcal meningitis n(%)	Controls		Survivors of pneumococcal meningitis Community controls			Survivors of pneumococcal meningitis Survivors of meningococcal meningitis		
		Community controls n (%)	Survivors of meningococcal meningitis n(%)	OR[a] (95%CI)	χ^2 (df)	p-value	OR[a] (95%CI)	$\chi^2_{(df)}$	p-value
Aches and pains	2(4.4)	3(6.5)	0(0.0)	0.7(0.1, 4.0)	0.2	0.7	∞	2.8	0.10
Tiredness or having little energy	1(2.2)	1(2.2)	1(2.9)	1(0.1, 16.0)	0	1.0	104(0.1, 16.0)	0	1.0
Difficulty in sleeping	1(2.2)	2(4.4)	0(0.0)	0.5(0, 5.5)	0.5	0.8	∞(0)	3.0	0.4
Tendency to worry a lot	9(19.6)	12(26.1)	4(11.8)	0.7(0.2, 1.9)	0.6	0.4	2.0(0.5, 8.0)	1.0	0.3
Auditory hallucinations	15(32.6)	15(32.6)	5(14.7)	1.0(0.4, 2.4)	0	1.0	5.0(0.4, 1.6)	5.8	0.02
Visual hallucination	13(28.3)	14(30.4)	6(17.7)	1.1(0.5, 2.6)	0.1	0.8	1.4(0.2, 8.3)	0.1	4.0
Episodes of great fear or panic	20(43.5)	18(39.1)	8(23.5)	1.2(0.5, 2.9)	0.2	0.7	4.5(1.0, 20.8)	4.8	0.03
Persecutory delusions	18(39.1)	19(41.3)	11(32.4)	0.9(0.4, 2.2)	0	1.8	2.7(0.7, 10.0)	2.4	0.12
			Addiction						
Drinking alcohol	6(14.0)	6(14.3)	5.0(17.2)	1.0(0.2, 5.0)	0	1	0.3(0.0, 2.2)	1.9	0.17

Total number of community controls =46, survivors of pneumococcal meningitis=46; survivors of meningococcal meningitis=34

[a] Odds ratio ∞ Odds ratio could not be determined because of zero denominator

χ^2 Likelihood ratio chi squared (degrees of freedom=2)

Table 7.5 Psychiatric symptoms reported by relatives

Symptoms	Case: Survivors of pneumococcal meningitis n(%)	Controls		Survivors of pneumococcal meningitis Community controls			Survivors of pneumococcal meningitis Survivors of meningococcal meningitis		
		Community controls n (%)	Survivors of meningococcal meningitis n(%)	OR[a] (95%CI)	χ^2 (df)	p-value	OR[a] (95%CI)	χ^2 (df)	p-value
Changes in health status	20(44.4)	6(13.0)	12(35.6)	5.7(1.7, 19.3)	10.8	0.001	1.6(0.6, 4.1)	0.9	0.3
Other difficulties	9(19.6)	1(2.2)	1(2.9)	9.0(1.1, 71.0)	7.4	0.007	7.0(0.9, 56.9)	5.1	0.02
Depressive and anxiety symptoms									
Shuts himself up alone	9(19.6)	4(8.7)	1(2.9)	2.3(0.7, 7.3)	2.0	0.16	∞	4.2	0.04
Difficulty in sleeping	1(2.2)	2(4.4)	1(2.9)	0.5(0, 5.5)	0.3	0.6	1.0(0.06,16.0)	0	1.0
Tendency to cry	12(26.1)	15(32.6)	7(20.6)	0.7(0.2, 1.9)	0.6	0.4	2.0(0.6, 6.6)	1.4	0.2
Suicidal tendencies	7(15.2)	7(15.2)	5(14.7)	1.0(0.3, 3.1)	0	1.0	1.5(0.3, 9.0)	0.2	0.7
Tend to worry a lot	10(21.7)	12(26.1)	5(14.7)	0.8(0.3, 2.2)	0.3	0.6	1.5(0.4, 5.3)	0.4	0.5
Easily annoyed or irritable	16(34.8)	18(39.1)	12(35.3)	0.8(0.3, 2.0)	0.2	0.7	1.1(0.4, 3.2)	0.1	0.8
Psychotic symptoms									
Auditory hallucinations	13(28.3)	14(30.4)	6(17.7)	0.9 (0.3, 2.4)	0.1	0.8	2.7(0.7, 10.1)	2.4	0.1
Visual hallucination	10(21.7)	10(21.7)	7(20.6)	1.0(0.4, 2.7)	0	1.0	1.7(0.1, 37,7)	0.5	0.8
Persecutory delusions	16(34.8)	18(39.1)	10(29.4)	0.8(0.4, 1.9)	0.2	0.7	1.4(0.5, 8.0)	1.0	0.3
Hurt self	8(17.4)	5(10.9)	1(2.9)	1.6(0.5, 4.9)	0.7	0.4	∞	8.3	0.004
Strange behaviour	2(4.4)	0	0	∞	2.8	0.1	*	0	1.0
Refusal of food	11(23.9)	5(10.9)	6(17.7)	2.2(0.8, 6.3)	2.3	0.1	2.7(0.7, 10.1)	2.4	0.1
Unprovoked fighting	9(19.6)	7(15.2)	4(11.8)	1.7(0.4, 7.0)	0.5	0.5	2.0(0.5, 8.0)	1.0	0.3
Addiction									
Drinking alcohol	6(13.0)	5(10.9)	5(14.7)	1.3(0.3, 6.0)	0.1	0.7	0.3(0.0, 2.2)	1.9	0.2

Total number of community controls =46, survivors of pneumococcal meningitis=46; survivors of meningococcal meningitis=34

[a] Odds ratio

∞ Odds ratio could not be determined because of zero denominator

χ^2 Likelihood ratio chi squared (degrees of freedom=2)

* No matched cases

Table 7.6 Hearing assessment. a. Left ear

Hearing class	Cases: Survivors of pneumococcal meningitis n (%)	Controls		Survivors of pneumococcal meningitis Community controls			Survivors of pneumococcal meningitis Survivors of meningococcal meningitis		
		Community controls n (%)	Survivors of meningococcal meningitis n (%)	OR[a] (95%CI)	χ^2	p-value	OR[a] (95%CI)	χ^2	p-value
500hz									
Normal hearing(<30dB)	29(63.0)	28(60.9)	21(61.8)	reference			reference		
Mild hearing loss(30-55dB)	6(13.0)	17(37.0)	11(32.4)	0.3(0.1, 1.1)			0.1(0.0,0.9)		
Moderate hearing loss(55-70dB)	3(6.5)	1(2.2)	1(2.9)	∞	19.2	<0.01	2.8(0.1, 66.2)	13.4	<0.01
Severe/profound hearing loss(≥70dB)	8(17.4)	0(0.0)	1(2.9)	∞			6.5(0.6, 66.1)		
1000hz									
Normal hearing(<30dB)	31(67.4)	37(80.4)	22(64.7)	reference			reference		
Mild hearing loss(30-55dB)	7(15.2)	8(17.4)	11(32.4)	1.3(0.3, 4.7)		0.01	0.2(0.1, 1.1)		0.02
Moderate hearing loss(55-70dB)	1(2.2)	1(2.2)	0(0.0)	∞	11.2		∞	9.4	
Severe/profound hearing loss(≥70dB)	7(15.2)	0(0.0)	1(2.9)	∞			5.3(0.6, 44.5)		
2000hz									
Normal hearing(<30dB)	32(69.6)	37(80.4)	26(76.5)	reference			reference		
Mild hearing loss(30-55dB)	4(8.7)	9(19.6)	6(17.6)	0.4(0.1, 1.7)		<0.01	∞		
Moderate hearing loss(55-70dB)	3(6.5)	0(0.0)	1(2.9)	∞	15.5		1.0(0.1, 16.0)	10.9	0.01
Severe/profound hearing loss(≥70dB)	7(15.2)	0(0.0)	1(2.9)	∞			6.0(0.7, 49.8)		

Total number of community controls =46, survivors of pneumococcal meningitis=46; survivors of meningococcal meningitis=34

χ^2 Likelihood ratio chi squared (degrees of freedom=3)

∞ Odds ratio could not be determined because of zero denominator.

Table 7.7 Hearing assessment. b. Right ear

Hearing class	Cases: Survivors of pneumococcal meningitis n (%)	Controls		Survivors of pneumococcal meningitis Community controls			Survivors of pneumococcal meningitis Survivors of meningococcal meningitis		
		Community controls n (%)	Survivors of meningococcal meningitis n (%)	OR[a] (95%CI)	χ^2	p-value	OR[a] (95%CI)	χ^2	p-value
500hz									
Normal hearing(<30dB)	29(63.0)	25(54.4)	21(61.8)	reference			reference		
Mild hearing loss(30-55dB)	9(19.6)	19(41.3)	9(26.5)	0.3(0.1, 1.1)			0.1(0.0, 1.0)		
Moderate hearing loss(55-70dB)	2(4.4)	1(2.2)	3(8.8)	2(0.2, 22.1)	8.5	0.04	0.2(0.0, 2.0)	10.5	0.01
Severe/profound hearing loss(≥70dB)	6(13.0)	1(2.2)	1(2.5)	3.4(0.4, 30.9)			5.1(0.6, 43.6)		
Total	46	46	34						
1000hz									
Normal hearing(<30dB)	31(67.4)	35(76.1)	27(79.4)	reference			reference		
Mild hearing loss(30-55dB)	9(19.6)	10(21.7)	4(11.8)	1.0(0.4, 2.9)			1(0.1, 7.1)		
Moderate hearing loss(55-70dB)	1(2.2)	1(2.2)	2(5.9)	1.0(0.1, 16.0)	6.9	0.03	0.8(0.1, 9.7)	3.0	0.4
Severe/profound hearing loss(≥70dB)	5(10.9)	0(0.0)	1(2.9)	∞			4.8(0.5, 42.3)		
Total	46	46	34						
2000hz									
Normal hearing(<30dB)	28(68.3)	34(81.0)	24(80.0)	reference			reference		
Mild hearing loss(30-55dB)	6(14.6)	7(16.7)	3(10.0)	1.4(0.37, 5.5)			1(0.1, 7.1)		
Moderate hearing loss(55-70dB)	3(7.3)	1(2.4)	2(6.7)	3.3(0.3, 32.9)	6.9	0.07	0.7(0.1, 9.1)	2.0	0.6
Severe/profound hearing loss(≥70dB)	4(9.8)	0(0.0)	1(3.3)	∞			3.8(0.4, 35.2)		
Total	41	42	30						

χ^2 Likelihood ratio chi squared (degrees of freedom=3) ∞ Odds ratio could not be determined because of zero denominator

.

Table 7.8 Other identified disabilities

Identifiable disability	Survivors of pneumococcal meningitis (PCM) n (%)	Community controls (CC) n (%)	Survivors of meningococcal menngitis (MCM) n (%)	Differences between PCM and CC			Differences between PCM and MCM		
				OR[a] (95%CI)	χ^2	p-value	OR[a] (95%CI)	χ^2	p-value
Squint	1(2.2)	1(2.2)	2(5.9)	1.0(0.06, 16.0)	0	1.0	0.5(0.05, 5.5)	0.3	0.56
Unstable gait	3(6.5)	1(2.2)	3(8.8)	3.0(0.3, 28.9)	1.1	0.3	0.7(0.1, 4.0)	0.2	0.65
Chronic otitis media	4(8.7)	1(2.2)	2(5.9)	4.0(0.4, 35.8)	1.9	0.17	2.0(0.4, 10.9)	0.7	0.4
Inability to identify smell of alcohol	16(34.7)	21(45.7)	14(41.2)	0.4(0.1, 1.4)	2.0	0.2	0.5(0.2, 1.3)	2.0	0.2
Facial palsy	1(2.2)	2(4.4)	1(2.9)	0.5(0.18, 22.06)	0.3	0.6	1.0(0.0, ∞)	1.4	0.2

Total number of community controls =46, survivors of pneumococcal meningitis=46; survivors of meningococcal meningitis=34

χ^2 Likelihood ratio chi squared (degrees of freedom=3)

∞ Odds ratio could not be determined because of zero denominator.

a Odds ratio

CI Confidence interval

CHAPTER 8

INFLUENCE OF CLIMATIC FACTORS ON THE INCIDENCE OF MENINGOCOCCAL AND PNEUMOCOCCAL MENINGITIS IN NORTHERN GHANA

CHAPTER 8

Influence of Climatic Factors on the Incidence of Meningococcal and Pneumococcal Meningitis in Northern Ghana

Abudulai Adams Forgor[1], Abraham Hodgson[1], Penelope Vounosou[2], Martin Adjuik[1], Julia Leimkugel[2], Elizabeth Awine[1], Gerd Pluschke[2] and Tom Smith[2]

[1] Navrongo Health Research Centre, Ministry of Health, Navrongo, Ghana

[2] Swiss Tropical Institute, Basel, Switzerland

This article has been prepared for submission to:

International Journal of Health Geographics

8.1 Abstract

Background Epidemics of both meningococcal (MCM) and pneumococcal meningitis (PCM) occur in the African meningitis belt. It is not well understood how climate affects the timing of these epidemics and whether both diseases are triggered by the same factors.

Methods Surveillance of MCM and PCM was carried out between January 1998 and December 2004 in the Kassena Nankana District (KND) of northern Ghana by collecting and analyzing CSF samples of all suspected meningitis cases reporting to health facilities in the district. Weekly means of meteorological data were obtained from the local meteorological station. Measurements of relative humidity taken at 06.00 hours (highest humidity of the day) and at 15.00 hours (lowest humidity of the day), maximum and minimum air temperature, number of days of dust haze, length of sunshine in a week, total rainfall in a week and wind speed were provided by the station. We assessed the relationship between climatic variables and reported MCM and PCM cases using negative binomial regression adjusting for temporal correlations using autoregressive term (AR) order 1 model.

Results The results of our models show that concurrent weekly increase in maximum temperature (IRR=1.18; 95%CI: 1.11, 1.24) and concurrent weekly decrease in total rainfall (IRR=0.97; 95%CI: 0.95, 0.99) significantly influenced the risk of MCM. A concurrent weekly decrease in rainfall (IRR=0.98; 95%CI: 0.96, 0.998)] significantly influenced the risk of PCM.

Conclusion Climatic factors that trigger MCM and PCM outbreaks are similar, not always the same and often result in different timing of outbreaks of the two diseases, with PCM outbreaks preceding those of MCM. While the risk of MCM is significantly associated with concurrently weekly increase in maximum temperature and concurrent decrease in rainfall, the risk of PCM is significantly associated with concurrent decrease in rainfall. The duration of preceding absence of rainfall appear to be the best predictor of both PCM and MCM outbreaks.

8.2 Introduction

Bacterial meningitis epidemics occur world-wide, but are particularly devastating in the African Meningitis Belt stretching from Senegal to Ethiopia (Belcher et al., 1977; Greenwood, 1999; Horn, 1908; Waddy, 1957). These epidemics are frequently not recognised until they are well underway. Despite the availability of effective vaccines, control measures are often instituted too late to be very effective. It is currently recommended to trigger a response when the attack rate reaches 15 cases per 100,000 (WHO, 2000; Varaine et al., 1997) but this requires an excellent surveillance system. The need for reporting from the district to regional to national level and to WHO, and the time required to prepare a vaccination programme, introduce further delays.

Most of the epidemics in the African Meningitis Belt are caused by *Neisseria meningitidis.* These epidemics show a very strong seasonality (Lapeyssonnie, 1963; Belcher et al., 1977; Greenwood et al., 1983; Greenwood et al., 1984; Greenwood et al., 1987; Besancenot et al., 1997) and so there is a clear potential for climate-based early warning systems. However, in recent studies in northern Ghana, we have also observed outbreaks of pneumococcal meningitis, indicating that the epidemiology of bacterial meningitis in the Meningitis Belt may be changing (chapter 6).

Outbreaks of meningococcal meningitis start in the dry season when it is dry and dusty and stop during or shortly after the onset of the rains. Though this seasonality is well recorgnised, the underlying mechanism is not well understood (Greenwood et al., 1983). Recent analyses of remote sensed climate data (Molesworth et al., 2003; Thomson et al., 2006) and climate models (Molesworth et al., 2002; Sultan et al., 2005) have provided algorithms for locating epidemic-prone areas, but it remains uncertain how environmental data can best be used to predict the timing of outbreaks. Nor is it clear whether pneumococcal outbreaks result from the same complex of environmental factors.

Between January 1998 and December 2004 in the Kassena Nankana District of northern Ghana (KND) CSF samples were collected from all suspected meningitis cases reporting to local health facilities, and bacteria speciated by latex agglutination tests and bacteriological techniques. Using locally recorded meteorological data we have now analysed separately how the incidence of

laboratory confirmed meningococcal and pneumococcal meningitis depends on recent environmental conditions.

8.3 Methods

Study area.

The Kassena Nankana District (KND), one of the most deprived districts in Ghana, has a population of 140,000, an area of 1675km^2 and lies within the guinea savannah woodland of northern Ghana between latitude 10^{o}30´ and 11^{o}00` N and between longitude 1$^o$00`and 1^{o}30` W. The district lies within the meningitis belt of sub-Saharan Africa with a sub-Sahelian climate of a short rainy season from May to October (average annual rainfall 850-950mm) and a long dry season from November to April during which temperatures increase to daily maxima in March-April of about 40°C. During January-April the atmosphere fills with dust blown from the Sahara by the harmattan winds. The main soil type is a sandy loam.

The district has one hospital (the War Memorial Hospital) located in Navrongo, the district capital and four health sub districts each of which has a health centre. There is a state owned meteorological station in Navrongo where daily weather conditions are recorded.

Epidemiological data

Surveillance of meningococcal and pneumococcal meningitis was carried out between January 1998 and December 2004 in the KND by collecting and analyzing CSF samples of all suspected meningitis cases reporting to any of the above health facilities in the district. The CSF samples are sent together with demographic data of the patients to the War Memorial Hospital microbiology laboratory. Here, the CSF samples are analysed by direct staining with Gram stain, serological testing for *Neisseria meningitidis* (A, B.C and W135) (Nm), *Streptococcus pneumoniae* (SP) and *Hemophilus influenza*e b with slidex meningite kit, biomerieux; Pastorex kit Bio rad. Part of the CSF is cultured by standard microbiological methods at the same laboratory and the rest frozen at -80^{o}C and sent to the Swiss Tropical Institute, Basel, Switzerland for confirmation and further molecular analysis.

A case is said to be confirmed when the CSF of a suspected case has positive antigen detection for *N. meningitidis* or *S. pneumoniae* or positive culture of CSF. The demographic characteristics and

residence status of cases are confirmed using a demographic surveillance system in which the entire resident population is registered.

Meteorological data

Weekly means of meteorological data were obtained from the meteorological station at Navrongo. Measurements of relative humidity (%) taken at 06.00 hours (highest humidity of the day) and at 15.00 hours (lowest humidity of the day), mean maximum and minimum air temperature (^{o}C), number of days of dust haze in the week, length of sunshine (hours) in a week, total rainfall (mm) in a week and wind speed (knots) were provided by the station.

Statistical analysis

Weekly and monthly aggregates of MCM and PCM cases (from the WMH microbiology laboratory dataset) and the corresponding meteorological data (of similar time intervals) were double entered using visual FoxPro. Due to zero-inflation and over dispersion of the data, negative binomial regression was used for data analysis in Stata software version 9.0 (Stata Corp., College Station, TX, USA). The district population as at 21st November 2001 was used in the calculation of the incidence rates.

For each environmental variable, and for both MCM and PCM, negative binomial regression models were used to determine the lag period in the environmental variable that best predicted the incidence of meningitis as determined using the Akaike`s information criterion (AIC). Models were adjusted for age, sex and year.

To identify which of the environmental factors is more important we fixed negative binomial models simultaneously including multiple environmental factors. In order to allow for serial correlation in the responses we included autoregressive term of order 1. Markov Chain Monte Carlo simulation (MCMC) was applied (one chain) to estimate model parameters. After an initial burn-in of 10000 the number of iterations thereafter depended on convergence, which was assessed using ergodic averages of the parameter estimate. After convergence a final sample was collected to obtain medians of the posterior distribution of the parameters. To obtain incidence rate ratios (IRR) per unit change in incidence for each covariate, model estimates were exponentiated. For comparison of model fit, the Deviance Information Criterion (DIC) was used where small values of DIC indicate

superior model fit. The final models include a combination of climatic factors for the short-term prediction of epidemics of MCM and PCM.

8.4 Results

Epidemiological data

During the period under review (1998-2004) 474 cases of bacterial meningitis were confirmed by the laboratory, of which 145 were SP and 329 Nm. Of the total number of cases 189 were females and 285 males. Of all the meningococcal cases, 127 were females and 202 males while for the SP 62 were females and 83 males. The highest number of cases was recorded in the 5-14 age group with SP being 45 and Nm 147 while the 60+ age group recorded the lowest number of cases with SP being 11, Nm 4. There were 9 SP cases and 11 Nm cases recorded in the age group <1; 13 SP and 70 Nm cases recorded in the 1-4 age group; 27 SP and Nm 72 in the 15-29 age group while the 30-59 age group recorded 32 and 33 for SP and Nm respectively.

The environmental factors we considered all showed strong seasonality, and were highly correlated with each other (figure 8.1-8.3 and 8.5-8.8). The pattern of rainfall is a main determinant of the maximum daily temperature, which is lowest in the wet season, from May to December.

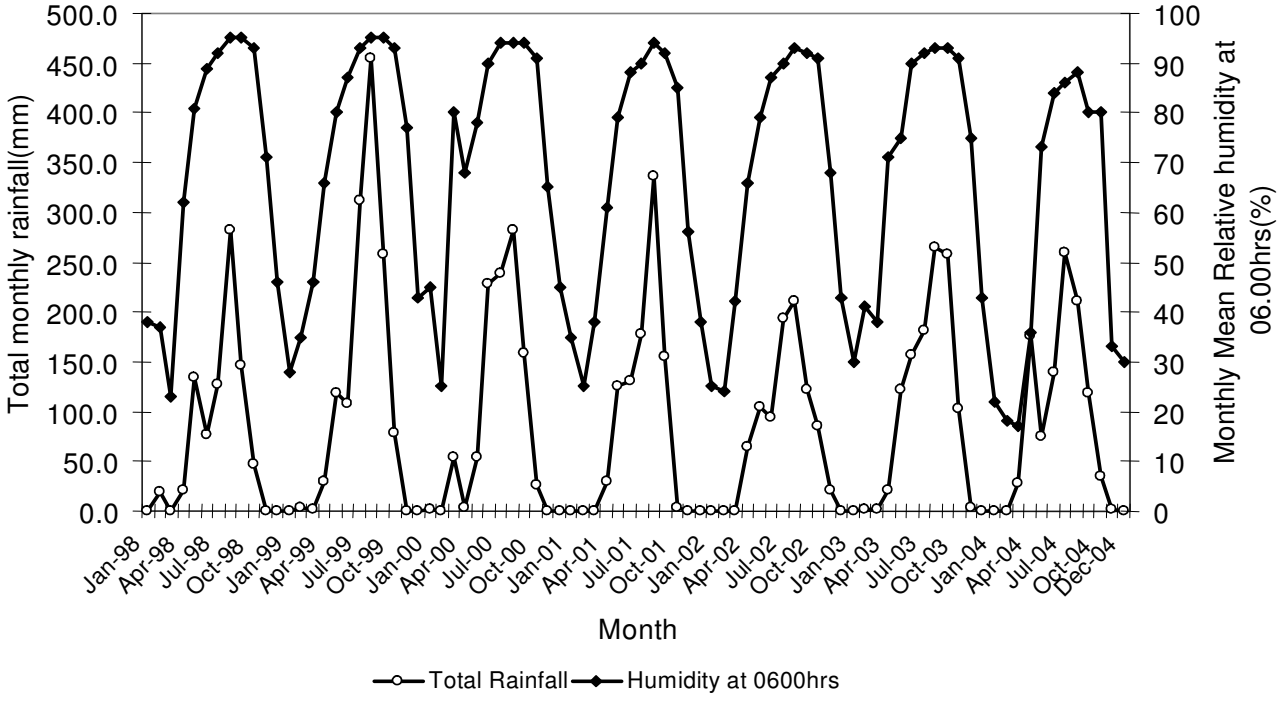

Figure 8.1 Relationship between rainfall and humidity in the KND, 1998 - 2004

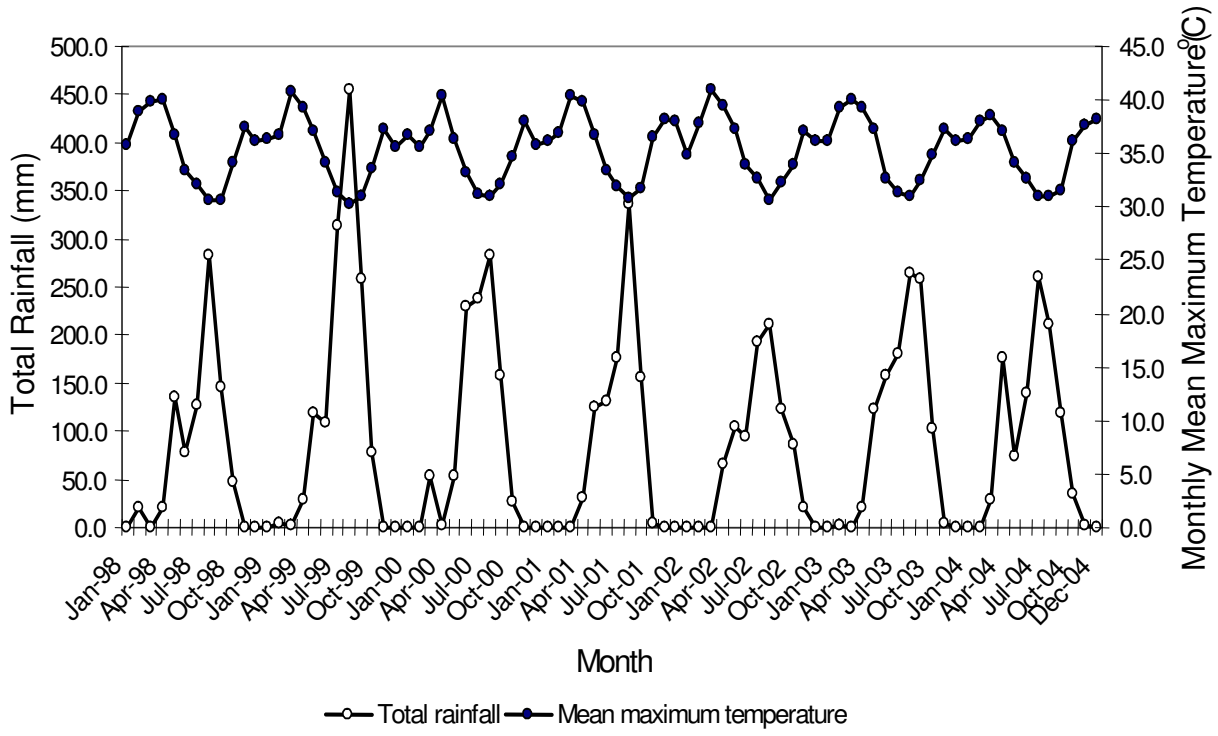

Figure 8.2 Relationship between rainfall and maximum temperature in the KND, 1998 – 2004.

Dust levels peak during the harmattan period from January to April with an inverse relationship between dust and minimum humidity. The peak of humidity corresponds with the minimum of dust, and the minimum humidity corresponding to the middle of the harmattan. Minimum temperatures are relatively low (figure 8.3) at the start of the harmattan, but increase during the period when the night sky is obscured by dust, reaching a maximum at the end of the harmattan.

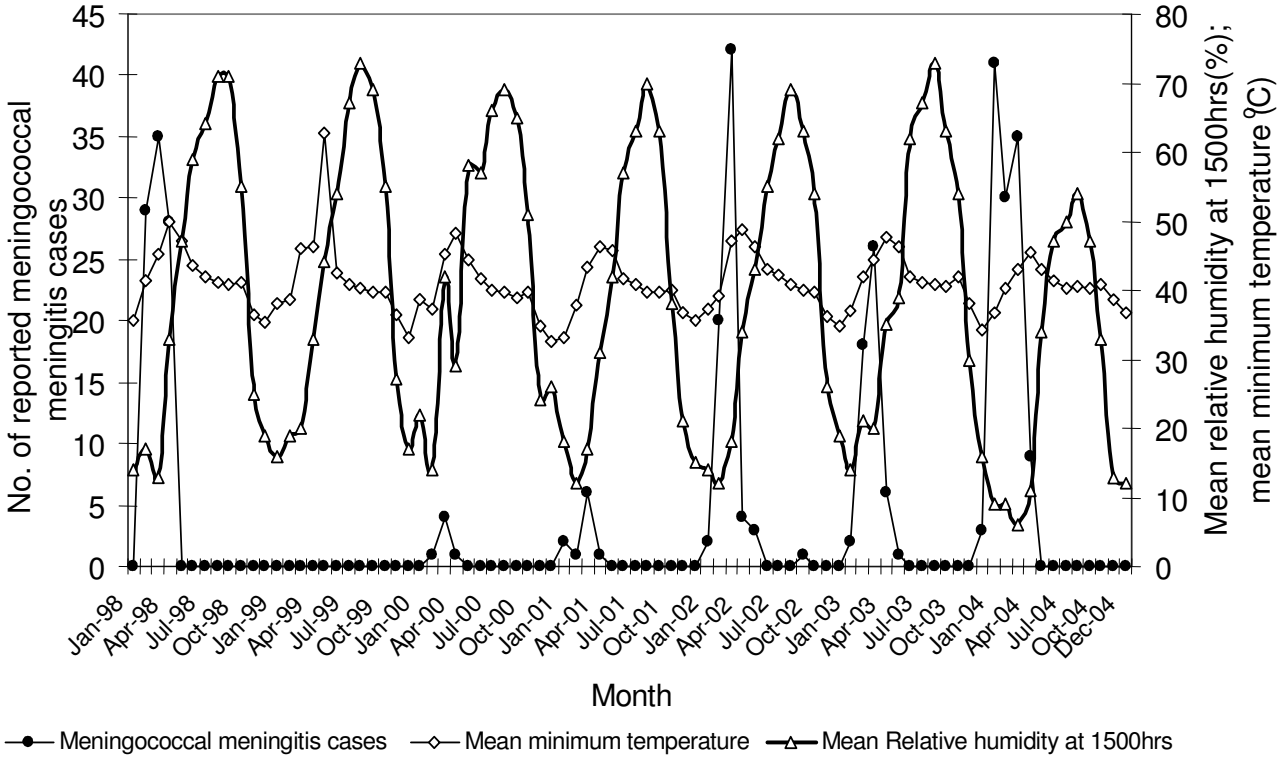

Figure 8.3 Relationship between minimum temperature, relative humidity (recorded at 15.00hrs) and number of reported meningococcal meningitis cases in the KND, 1998 – 2004.

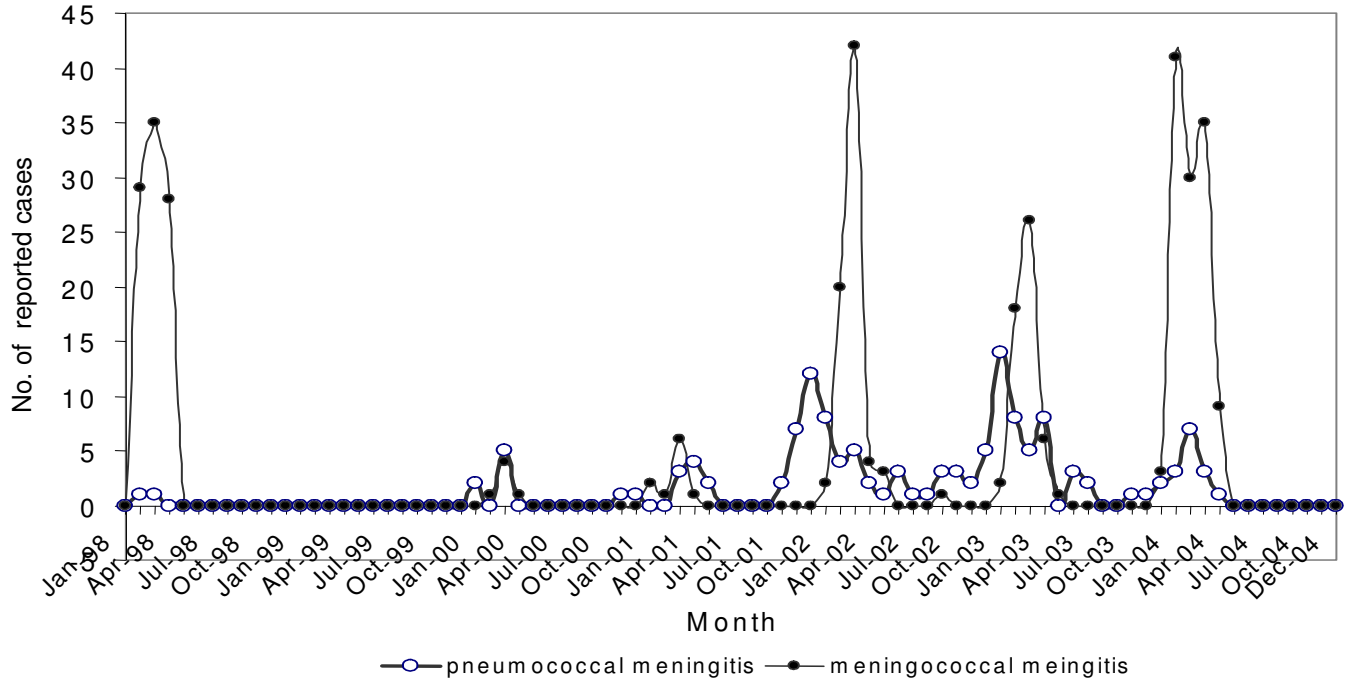

Figure 8.3 Reported pneumococcal and meningococcal meningitis cases in the KND 1998 – 2004.

There was considerable heterogeneity over time in the incidence of both MCM and PCM (Figure 8.4). In some years there were hardly any cases of MCM, while in others there were substantial outbreaks. PCM also showed considerable inter-annual variation in incidence (figure 8.4). We have previously shown that the inter-annual variation in MCM is associated with changes in patterns of colonization (chapter 4), however, the seasonal patterns of the outbreaks are related to the seasonal changes of environmental factors. In general, outbreaks of PCM started earlier than those of MCM, and were biphasic with the first peak preceding meningococcal outbreaks and the second coinciding with the meningococcal outbreak (figure 8.4).

The dust and MCM incidence are strongly correlated but in a typical year the dust rises to a maximum and plateaus for about two months before the MCM outbreak begins, so that the MCM peaks at the same time as the minimum daily temperature (Figure 8.5 and 8.6). The dusty conditions last for 3-4 months, while the MCM outbreaks are rather shorter than this. Because of the lag time between the peak in dustiness and that in MCM incidence, the peak in MCM cases occurs as the dust level starts to go down and the humidity starts to increase. The MCM outbreaks thus often continue after the end of the dusty period and any model for the relationship between dust and MCM must consider the lag period between maximum dust levels and the epidemics. Correspondingly, low humidity is associated with MCM risk, but again with a lag period between the curve of humidity and that of incidence of disease.

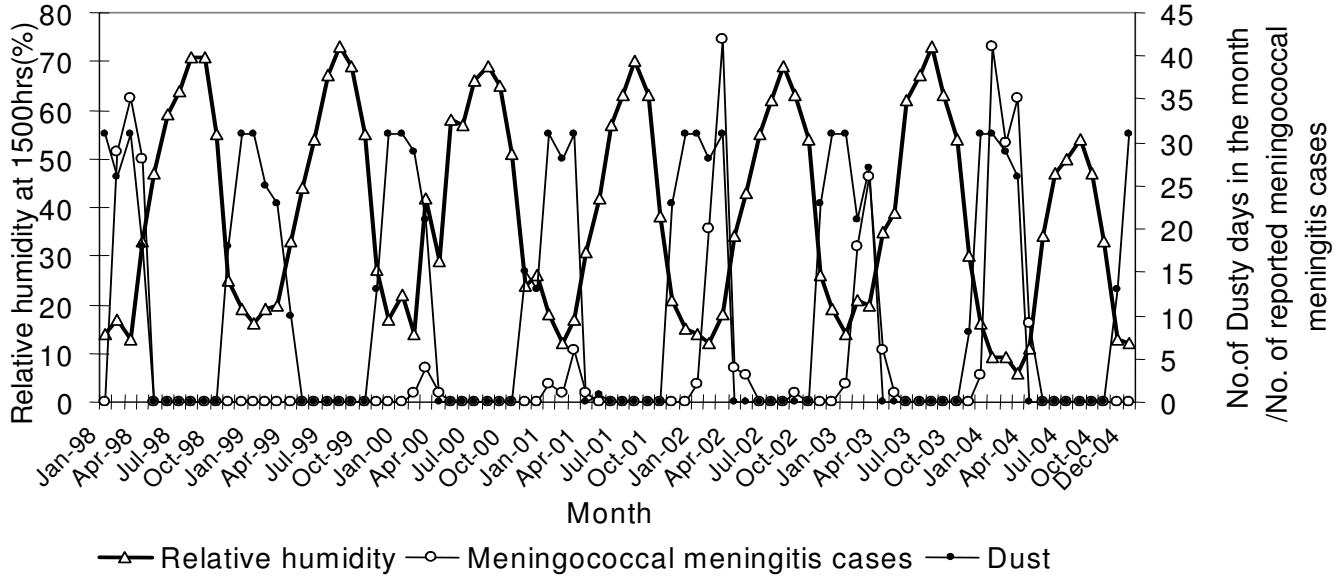

Figure 8.5 Relationship between dust, relatinve humidity (recorded at 15.00hrs) and reported meningococcal meningitis cases in KND, 1998 - 2004

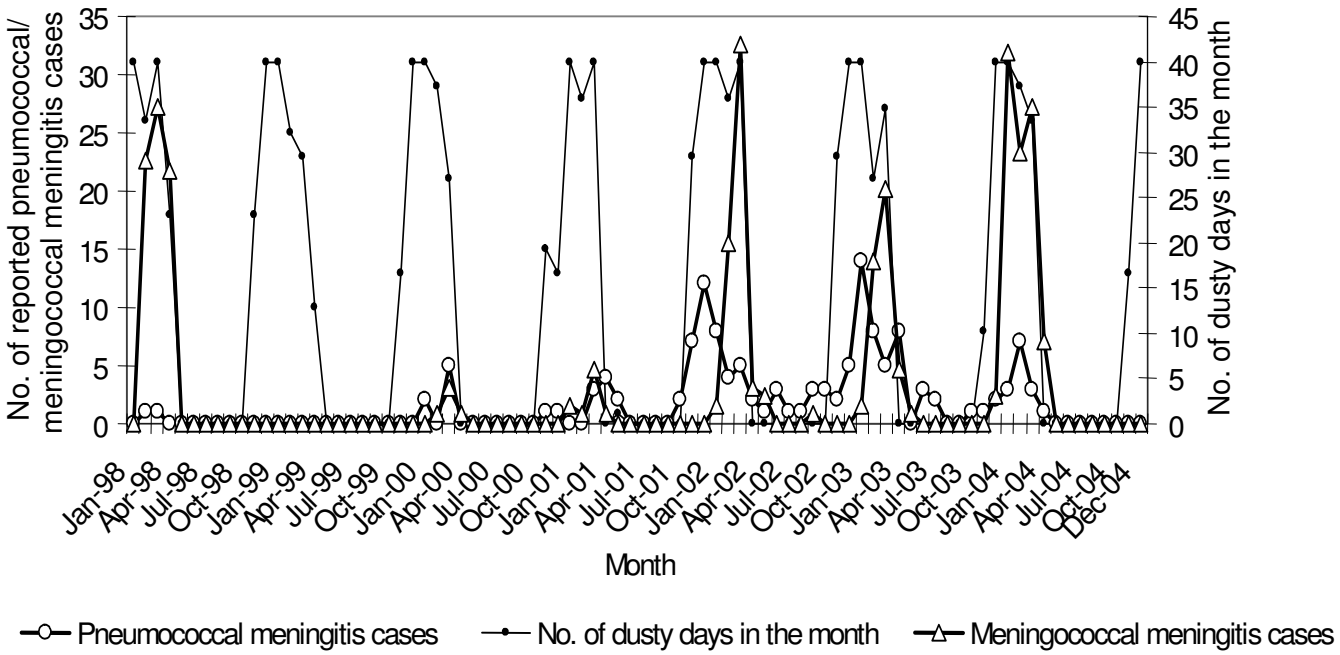

Figure 8.6 Relationship between reported pneumococcal and meningococcal meningitis cases and dust in the KND, 1998 – 2004

Both PCM and MCM have a strong correlation with maximum temperature with peaks of their outbreaks coinciding with the peak of maximum temperature (figure 8.7)

The patterns of lag periods for the two different bacterial infections are very different (Tables 8.1 and 8.2). MCM incidence is more closely related to humidity and sunshine at least 10 weeks previously (we did not explore lags longer than 10 weeks) than to the values taken by these variables closer in time to the incidence. There is no such lag in the relationships between humidity, sunshine, and rainfall and the time of onset of PCM disease. The best fitting lag in the relationship between MCM and dust was 9 weeks, while only a 4-week lag fitted best for PCM (tables 8.1 and 8.2). There appears to be a temperature effect with a long lag for PCM, but concurrent temperatures fit best for MCM.

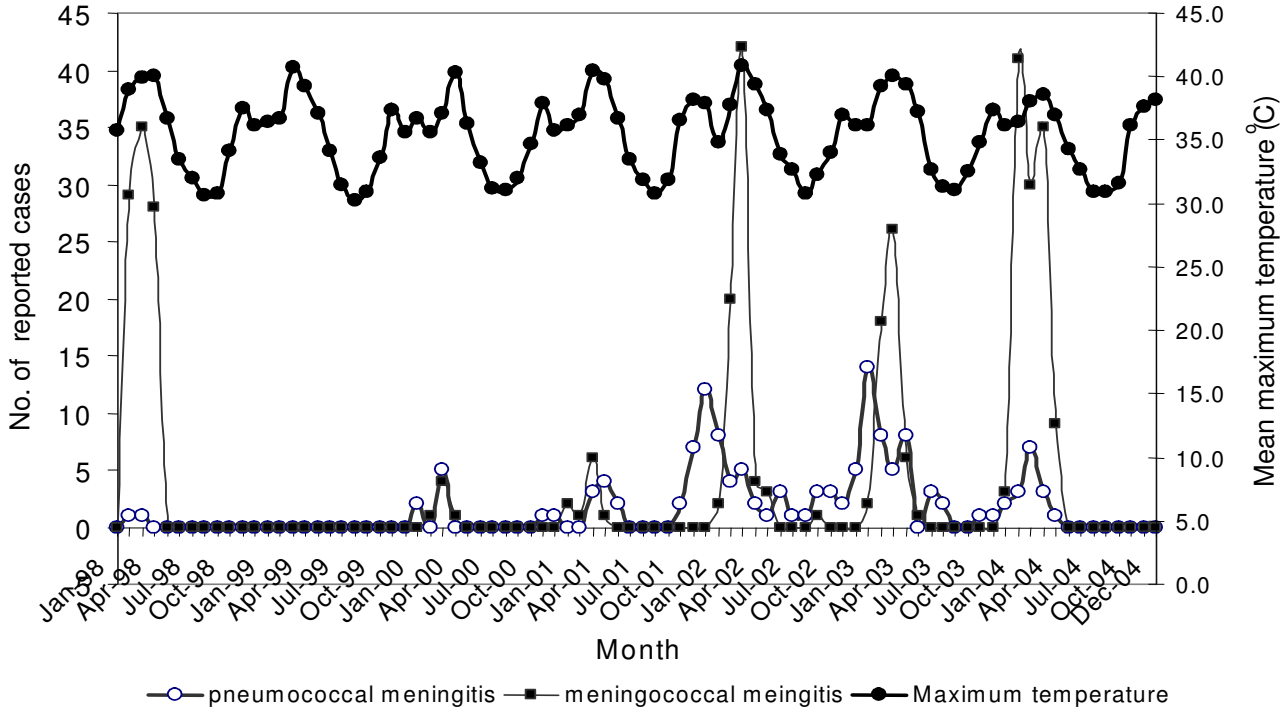

Figure 8.7 Relationship between reported pneumococcal and meningococcal meningitis cases and maximum temperature in the KND, 1998 – 2004.

Since these environmental variables are highly correlated with each other, we fitted further multiple regression models in which the terms corresponding to the best fitting lags were simultaneously included in order to exclude those effects that arise because of confounding (tables 8.1 & 8.2). The incidence of PCM and MCM was influenced by different climatic factors.

The significant risk factors for MCM, after adjusting for other factors appear to be the absence of rainfall and the concurrent weekly maximum temperature (table 1). The significant risk factors for PCM, after adjusting for other factors appear to be the dust levels 4 weeks previously the maximum temperature 9 weeks previously, and the concurrent decrease in maximum weekly humidity (table 2). This is consistent with the onset of outbreaks being early in the harmattan season.

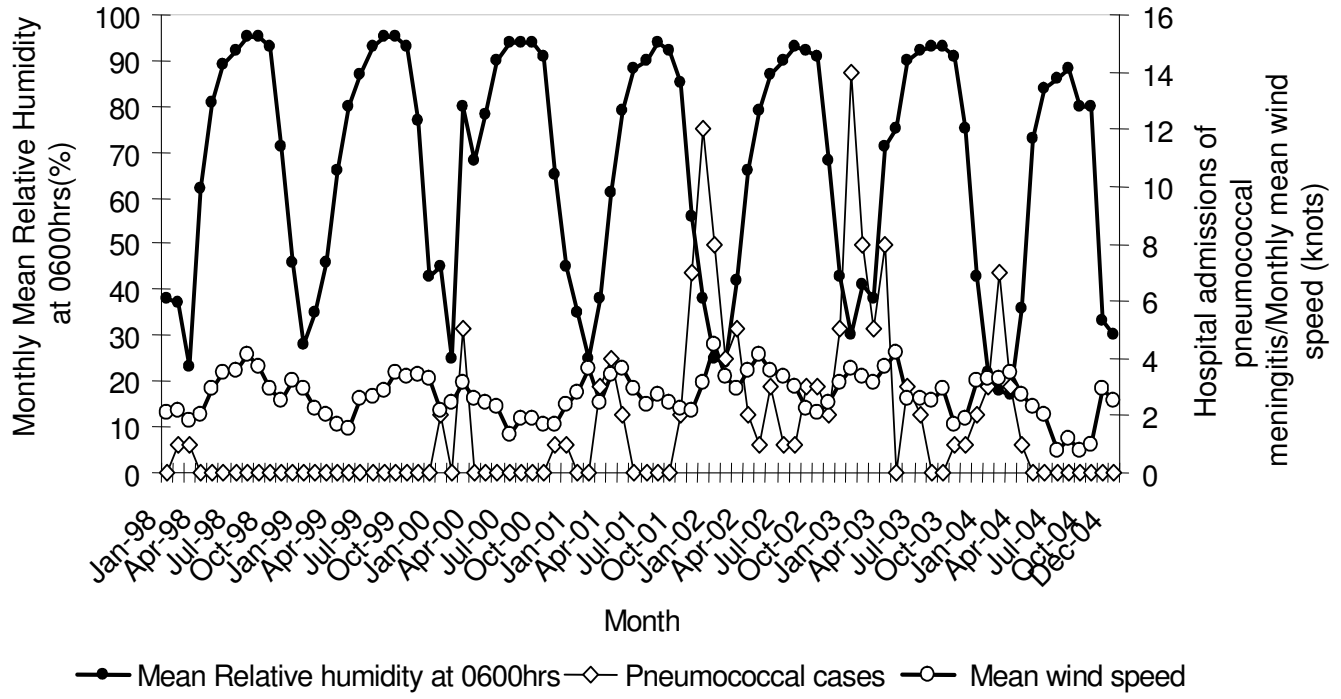

Figure 8.8 Relationship between wind speed, relative humidity and reported pneumococcal meningitis cases in the KND, 1998 – 2004

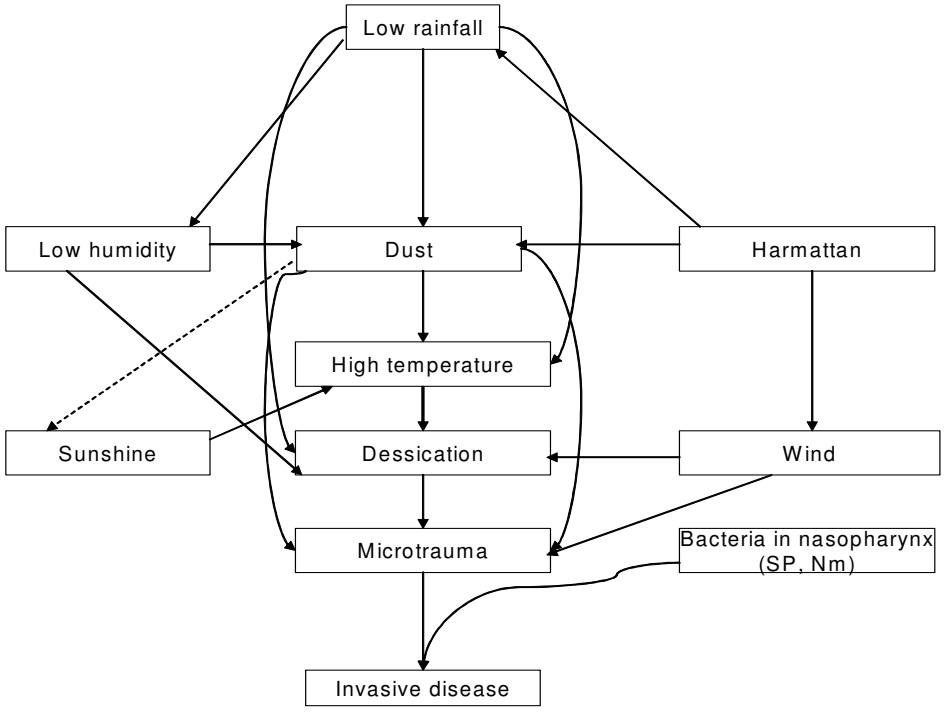

Figure 8.9 Causal web indicating relationships of Environmental factors with pathogenesis of meningitis

Table 8.1 Results of modelled maximum likelihood and Bayesian estimates of the effects of climatic covariates on reported incidence of meningococcal meningitis in the Kassena Nankana District obtained by fitting bivariate and multivariate negative binomial models.

Explanatory variables	Bivariate independent model estimates (95% CI)	Multivariate independent model estimates (95% CI)	Multivariate Bayesian temporal posterior medians (95% BCI)
Climatic variables			
Rainfall (mm)	0.93(0.91, 0.96) ❁	0.98(0.96, 0.995) ❁	0.97♦(0.95, 0.99)
Maximum temperature (^{o}C)	1.61 (1.42, 1.81) ❁	1.12(1.02, 1.22) ❁	1.18♦(1.11, 1.24)
Minimum temperature (^{o}C)	1.24 (1.07, 1.44) ❁	1.24(1.14, 1.33) ❁	
Relative humidity[1] (%) at 06:00	0.94 (0.93, 0.95) ❁	0.96(0.94, 0.98) ❁	
Relative humidity[2] (%) at 15:00	0.91(0.89, 0.93) ❁	0.99(0.95, 1.02)	0.96(0.93, 1.00)
Sunshine (hours)	1.14 (1.00, 1.30)❁	1.0(0.93, 1.08)	1.07(0.95, 1.17)
Dust (days)	1.48 (1.36, 1.62) ❁	1.15(1.04, 1.27) ❁	1.13(0.97, 1.31)
♣Wind speed (knots)	0.93(0.82, 1.07)		
Age group			
0 - <1		1.00	
1 - 4		1.53(0.73, 3.22)	1.68(0.79, 3.04)
5 - 14		1.50(0.74, 3.07)	1.61(0.79, 3.04)
15 - 29	0.63(0.58, 0.69)	0.82(0.39, 1.70)	0.88(0.41, 1.71)
30 - 59		0.35(0.16, 0.76)	0.36(0.16, 0.74)
60+		0.17(0.05, 0.58)	0.16(0.03, 0.44)
Sex			
Female	1.60(1.26, 2.04)	0.58(0.43, 0.77)	0.59(0.46, 0.76)
Over dispersion			16.08(5.02, 40.18)
Temporal correlation			0.97(0.90, 0.998)
Temporal variance			0.44(0.22, 0.79)
DIC			1046.13

The estimates of covariate effects are expressed in terms of incidence rate ratios (IRR)

Bayesian credible intervals (BCI)

❁♦: CI, BCIs do not overlap unity, corresponding to statistical significance.

♣: Not included in temporal model because wind speed effect was not statistically significant up to lag 10 weeks in the bivariate non temporal analysis.

♣: Not included in temporal model because wind speed effect was not statistically significant up to lag 10 weeks in the bivariate non temporal analysis.

Not included in the multivariate analysis to avoid confounding (selection done using AIC)

Table 8.2 Results of modelled maximum likelihood and Bayesian estimates of the effects of climatic covariates on reported incidence of pneumococcal meningitis in the Kassena Nankana District obtained by fitting bivariate and multivariate negative binomial models.

Explanatory variables	Bivariate independent model estimates (95% CI)	Multivariate independent model estimates (95% CI)	Multivariate Bayesian temporal posterior medians (95% BCI)
Climatic variables			
Rainfall (mm)	0.96(0.94; 0.98) ⊛	0.99(0.98, 0.998)⊛	0.98♦ (0.96, 0.997)
Maximum temperature[1] (^oC)	1.21(1.12, 1.30) ⊛	1.10(1.02, 1.18) ⊛	1.09(0.98, 1.19)
♣Minimum temperature (^oC)	0.91(0.83, 1.00)		
Relative humidity[2] (%)	0.97(0.96, 0.98) ⊛	0.98(0.96, 1.00)	0.99 (0.97, 1.01)
Relative humidity[3] (%)	0.96(0.95, 0.97) ⊛	1.0(0.97, 1.03)	1.0(0.96, 1.04)
Sunshine (hours)	1.22(1.06, 1.41) ⊛	1.06(1.0, 1.12) ⊛	1.03(0.89, 1.15)
Dust (days)	1.20(1.12, 1.29) ⊛	0.96(0.87, 1.06)	1.02 (0.91, 1.15)
Wind speed (knots)	1.31(1.14, 1.50) ⊛	1.40(1.13, 1.74) ⊛	0.87(0.64, 1.16)
Age group			
0 - <1		1.00	
1 - 4		0.43(0.17, 1.05)	0.47(0.17, 1.08)
5 - 14		0.54(0.25, 1.17)	0.60(0.27, 1.28)
15 - 29	0.88(0.76, 1.00)	0.36(0.16, 0.82)	0.41(0.17, 0.89)
30 - 59		0.34(0.15, 0.76)	0.39(0.17, 0.86)
60+		0.45(0.18, 1.15)	0.51(0.18, 1.18)
Sex			
Male	1.38 (0.98, 1.96	1.42(0.98, 2.06)	1.44(0.99, 2.04)
Over dispersion			8.09(0.73, 29.6)
Temporal correlation			0.94(0.94, 0.999)
Temporal variance			0.14(0.03, 0.36)
DIC			916.094

The estimates of covariate effects are expressed in terms of incidence rate ratios (IRR)

Bayesian credible intervals (BCI)

⊛♦: CI, BCIs do not overlap unity, corresponding to statistical significance.

♣: Not included in temporal model because minimum temperature effect was not statistically significant up to lag 10 weeks in the bivariate non temporal analysis.

8.5 Discussion

The KND lies in a zone of very high risk for meningococcal meningitis epidemics (Molesworth et al., 2003) with the two factors most clearly associated with bacterial meningitis in our study being high temperatures and airborne dust. Both these risk factors reach extreme levels in the dry season in northern Ghana, but affect the risks of MCM and PCM in different ways. MCM risks are highest at the hottest time of the year, when dust exposure has already been accumulating for several weeks, while PCM risk peaks earlier in the dusty period, and seems to relate to high temperatures several weeks earlier. The early dry season, when most of the PCM cases occur, includes the harmattan period, has very cold nights and very dusty air while the late dry season when the MCM peaks, is marked by intense heat.

We analysed the recorded dates of onset of outbreaks. Colonization prevalence does not show strong seasonality (chapter 4), so the date of onset relates to the processes of pathology rather than those of transmission and this appears to be dependent on other factors that follow after the infection.

The effect of dust could presumably be due to both the quantity and physico-chemical characteristics of the dust particles (Goudie and Middleton, 2001) which cause irritation and microtrauma of the respiratory mucosa thereby making it possible for the bacteria to transverse the nasal mucosa. Dust from the Sahara has been found in the northern Caribbean to contain viable microorganisms (Griffin et al., 2001; Griffin et al., 2003; Kellogg et al., 2004). Considering the poor sanitation and free range rearing of animals there is the possibility of pulverized fecal matter being inhaled together with dust. The interaction between the different bacteria in the nasopharynx could then facilitate the meningococci or pneumococci to traverse the nasal mucosa and to cause invasive disease.

High temperatures increase pharyngeal dryness and irritation (Backman and Haghighat, 1999). The peak of the incidence of meningitis in Nigeria has been found to significantly correlate with highest mean temperature, and inversely correlated with absolute humidity (Greenwood et al., 1984; Greenwood, 1999; Moore, 1992) a finding consistent with ours. Conversely, rainfall leads to high humidity and is hence negatively correlated with both MCM and PCM.

The effect of wind speed is presumably secondary to that of dust, since high winds during the harmattan winds cause dessication of the nasopharyngeal mucosa and also increase the penetration of dust, thus causing mucosal damage that facilitates the entry of the meningococci and pneumococci to cause invasive diseases.

The extreme temperatures appear to dominate the risk factors for MCM in KND. The term in rainfall appears in the model because the epidemics are clearly terminated by the onset of the rains. Periods of very low humidity seems to be important in triggering the PCM outbreaks, and in other settings, humidity appears to play a role independent of that of the other variables as a risk factor for MCM. Anomalies in dust and rainfall have been shown to be important predictors of the location of meningitis epidemics in Africa (Thomson et al., 2006; Lewis et al., 2001) but this analysis did not analyse the seasonality and timing of the epidemics within the dry season. Low humidity causes reduction in the perception of dryness of the nasal mucosa (Norbäch et al., 2000). This prevents the release of vasoactive amines and leukotrienes leading to severe dessication and microtrauma of the nasal mucosa (Burgess and Whitelaw, 1988). Humidity thus, has a direct effect on dessication of the nasal mucosa, leading to damage that could enable pneumococci or meningococci to traverse the nasopharyngeal mucosal membrane resulting into bacterial spread into mucosal tissue, lymphatics and finally potentially into the blood stream. It seems likely that low humidity plays an important role in the pathogenesis of bacterial meningitis also in Europe, where extremes of dust exposure and high temperatures are less frequent but periods with cold and dry air are common in winter. Furthermore, during winter rooms are heated up and this lowers the humidity further. This could explain why bacterial meningitis cases are frequent in winter in Europe than in other seasons.

Increase in the wind speed increases the rate at which the nasal mucosal dries up making it liable to cracking (microtrauma). This may make it possible for potentially virulent pneumococci or meningococci to traverse the nasal mucosa.

The environmental factors that we measured are not the only important risk factors for bacterial meningitis. Socioeconomic and cultural practices were not taken into consideration, nor were effects of health systems, migration or immunization considered. The year to year variations in MCM incidence reflect spreading of distinct meningococcal clones (chapter 4), rather than inter-annual environmental variation. The same may hold true for PCM (chapter 6).

There may be distinct patterns of risk factors within a geographical area. We assumed the climatic variables from the weather station to be representative for the entire district. Land cover and soil types of different areas of the district vary, as do potential other risk factors. An earlier study in Navrongo demonstrated effects of indoor smoke from cooking and heating fires (Hodgson et al., 2001a) on MCM risk. During the dry harmattan season the low minimum temperatures make people cluster around wood fires in their rooms or just outside the homes. This may lead to smoke-induced damage of mucosa and thus allowing meningococci and pneumococci to traverse the nasopharyngeal mucosa. Coinfections, especially viral respiratory infections (RTI) such as caused by Respiratory Syncytial Virus (RSV) or influenza virus may also be important risk factors (Cartwright, 1995; Plotkowski et al., 1986) but we have not analyzed this in the KND. Seasonality of pneumococcal disease in the USA is related to that of RSV (Kim et al., 1996). In the KND the hospital records show an increase in RTI cases during the harmattan season (data not shown).

There are also seasonal variations of behaviour in the KND, with a peak of migration and social activities in the dry season which could facilitate the spread of bacteria, however our longitudinal carriage surveys have found that there is little seasonal variation in carriage of *N. meningitidis* (chapter 4). Since man is the only host of *N. meningitis* there is an ever-present reservoir of carriers enabling the infection to be maintained during inter-epidemic periods leading, during the dry season, to epidemic disease.

A small field study on dust exposure and meningitis incidence, monitoring respirable dust exposure in Navrongo, could further elucidate whether cumulative exposure to dust is responsible for the risks and could also collect data that could be used to calibrate remote sensed data, as well as the local meteorological station readings. Dust exposure levels at the micro level could complete the picture and could be important for respiratory infections other than meningitis also.

Our study involves micro epidemiological analyses, analyzing the time series of individual cases within a single area. This can usefully complement work using remote sensing for predicting space-time patterns of epidemics. Our analyses suggest that a simple algorithm based on environmental factor(s) for short-term prediction of epidemics may be possible. Levels of dust, maximum temperature, humidity and rainfall can be used to predict the timing of epidemics, with epidemics of PCM at the start of the dry season representing a warning of likely MCM later. In contrast to remote-sensing based prediction, which best identifies the places and years most at risk (Molesworth

et al., 2003), local environmental data are likely to be more suitable to predict the timing of the epidemics and hence trigger vaccination campaigns. Currently, vaccination programs are available only for the control of MCM and there is a need for programs to control PCM as well as other pneumococcal disease.

The study demonstrates the importance of integrating environmental data into epidemic forecasting. Intersectoral collaboration (health sector and meteorological services) is needed for the surveillance of meningitis and other diseases with seasonal patterns.

8.6 Acknowledgements

This study was supported by grant GAT.0779-01476-GRT of the Meningitis Vaccine Project and in part by the Meningitis Research Foundation. We thank Mr Joseph Achana and E. S. Boafo of the meteorological services department, Navrongo. Our gratitude goes to Joseph Asampana Akolgo and the CSM field workers of the Navrongo Health Research Centre for their efforts and dedication to the study. We are grateful to the patients who were involved in this study. We also acknowledge Mr Akalifa Bugri and Abdul-Wahab Hamid for their contributions in the microbiology laboratory in Navrongo. We also wish to acknowledge the institutional support provided by the administration of the Navrongo Health Research Centre and the patients who were involved in the study.

CHAPTER 9

DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

CHAPTER 9

DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

The results of the individual studies have been discussed in detail in each of the respective chapters. This section is devoted to the discussion of the main findings as well as their implications and suggestions for further research work.

9.1 Discussion of main findings and recommendations

The results of the 8-year carriage survey demonstrate a notable absence of a temporarily stable and genetically diverse meningococcal flora in the pharynx of healthy individuals. This may result in increased susceptibility for epidemic meningococcal disease in the African meningitis belt. Polysaccharide-protein conjugate vaccines are known to impact meningococcal carriage, effect herd immunity (Palmer, 2002; Maiden and Stuart, 2002; Trotter et al., 2005) and potentiate serogroup replacement. This needs to be monitored using long-term carriage surveys of this type following the introduction of such vaccines. In view of the limited resources in countries of the African meningitis belt the introduction of conjugate vaccines might require targeting the age groups in which carriage is most prevalent for a meaningful herd immunity and cost effectiveness of the vaccine (Trotter et al., 2005) in which case such long term colonization surveys would be very useful.

The observed rapid microevolution of the W135 bacteria in the W135 carriage survey (chapter 5) requires constant surveillance and the need for improved methods of identification of epidemic-prone strains.

The striking seasonality of the peaks of pneumococcal meningitis cases suggests that the mucosal defense mechanism might have been damaged by the extreme weather conditions making it possible for the hypervirulent *S. pneumoniae* serotype 1 to traverse the mucosa. The peaks of the pneumococcal cases, preceding the meningococcal meningitis cases with a lag of about 2 months, coincide with the early period of the dry season when there is some considerable amount of humidity. This may be the reason why the incidence of pneumococcal meningitis is throughout the year while that of meningococcal meningitis is only during the dry season. The incidence of meningococcal meningitis is generally during the period when the humidity is lowest. The peak

onset of the pneumococcal meningitis cases coincides with the early dry season when the harmattan is present with cold nights, strong winds and dusty air. The late dry season is characterized by intense heat.

The significantly high excess mortality in the group of pneumococcal cases (chapter 7) could be due to the presence of the hypervirulent *S. pneumoniae* serotype 1 (Chapter 6) which might not be present in the nasopharynx since it is rarely isolated from the nasopharynges of asymptomatically colonized people (Feikin and Klugman, 2002). The excess mortality could also be due to some genetic factors of patients making them more susceptible to pneumococcal meningitis with this unfavorable outcome (Lin and Albertson, 2004; Cariou et al., 2002). This could also be due to the racial affinity of the *S. pneumoniae* serotype 1 (Gratten et al., 1993; Gratten et al., 1996; Rudolph et al., 2000; Fraser et al., 2001).

Most of the deaths occurred during the first 48 hours of admission, the period when intensive care is most needed. This calls for the establishment of functional and efficient intensive care units in all the hospitals since this can reduce the mortality rate.

This study has shown that hearing impairment is a major sequel of pneumococcal meningitis and that it is much more a problem and more common in survivors of pneumococcal meningitis than survivors of meningococcal meningitis. Speech impairment during the case-control study has been shown to also be a major sequel in survivors of pneumococcal meningitis than meningococcal meningitis. This is disturbing since hearing impairment in infants and young children is of great importance due to the critical time period during which language acquisition and speech development are accomplished (Yoshinaga-Itano et al., 1998; Yoshinaga-Itano and Apuzzo, 1998a). In infants and children bilateral hearing impairment is associated with delays in language development and academic achievement (Davis et al., 1986) even if only mild to moderate hearing loss is involved (Geers et al., 1989).

The early detection of survivors of bacterial meningitis with hearing and speech impairment is very important for their rehabilitation. They should therefore have hearing assessment before discharge from the hospital and also undergo a hearing evaluation as part of their routine follow-up. This will allow for the early detection of any hearing impairment and those with impairment to be provided with simple hearing aids or prepared for cochlear implant where possible. A delay in the detection

will lead to osteoneogenesis and ossification of the affected cochlea making implantation ineffective or not feasible (Dodds et al., 1997). There should be arrangements with teachers of survivors of bacterial meningitis with unilateral hearing impairment so that they can have sitting arrangements in the classroom such that they are not disadvantaged. Those with bilateral impairment should have arrangements for special education at schools for the hearing impaired.

An appropriate management strategy through a multidisciplinary healthcare approach is needed to provide optimal care to survivors of meningitis. There is the need for effective communication between healthcare professionals, parents/guardians or relatives, teachers and patients for the provision of education. It is also necessary to ensure that there is frequent detailed assessment and intervention of ongoing problems in order not to miss important deficits. The patients and their families/guardians would require a great deal of ongoing support which can come from the healthcare team, friends, family members as well as non-governmental/voluntary organizations. There is the need for an understandable approach to be able to detect fine effects like emotional and psychological consequences of meningitis while very obvious effects may be easily observable and treated. Community based rehabilitation of survivors should be encouraged in all communities.

The effects of environmental factors on the incidence of pneumococcal and meningococcal meningitis are similar but not always the same. The weather early in the dry season (cold nights and early mornings) results in the heating of rooms using firewood (or sitting around bound fire in the morning) together with respiratory tract infections seem to acutely damage the integrity of the nasal mucosa making it more advantageous to pneumococci and facilitating the spread of infection. Since outbreaks of pneumococcal meningitis precede those of meningococcal meningitis, the detection of increased numbers of the former should be a warning sign that the latter might occur and measures to curb it should be put in place.

9.2 Suggestions for further research

It is very important to continue the longitudinal carriage surveys and their extension to cover the middle belt (transitional zone between the forest and the savannah woodland), the coastal and the forest area of Ghana. This will contribute to the understanding of the dynamics of carriage of meningococci and pneumococci not only in the meningitis belt but also outside the belt and thus help in the understanding of the pathogenesis of bacterial meningitis as a whole. It will be important

to carry out immunological surveys to assess the antibody levels of the residents. This will complement the findings of the carriage surveys as well as help in modeling future early warning systems. Immunological studies would also give an insight of the extent to which the current polysaccharide A+C vaccine in use confers protection against meningococcal meningitis.

Since individual clonal complexes that belong to the same serotype have different virulence there is the need for further studies into the clonal complex-specific virulence of the *S. pneumoniae* serotype 1 for future vaccine development. It is not known whether this hypervirulent serotype is responsible for pneumonia in the district and also whether the healthy population carries it. Further studies on the carriage of *S. pneumoniae* and causes of pneumonia as well as immunological studies on pneumococcal meningitis and pneumoniae are necessary to address this issue for purposes of management of pneumococcal disease and future vaccine introduction. Since the risk factors for meningococcal meningitis in the district are known there is the need to identify the risk factors of pneumococcal meningitis in view of the fact that the factors that influence the incidence of meningococcal and pneumococcal meningitis are not always the same (chapter 8). There is the need to find out how the various serotypes of pneumococcal meningitis and serogroups of meningococcal meningitis influence the course and outcome of acute bacterial meningitis in the district.

Mucosal immunity is crucial for pneumococcal colonization (Stenfors and Raisanen, 1993) while low serum retinol concentrations are associated with impaired mucosal immunity and alterations in tissue integrity (Sirisinha et al., 1980; Chandra, 1988; Biesalski and Stofft, 1992; Semba et al., 1996). Meningococcal disease in sub-Saharan Africa is characterized by vitamin A deficiency (Semba et al., 1996) while its supplementation delays pneumococcal colonization in neonates (Coles et al., 2002). It will be a good idea to carryout more studies on the impact of vitamin A supplementation (or adjuvant therapy) on the incidence (or outcome) of pneumococcal and meningococcal colonization (or meningitis).

The socio-cultural practices that influence the incidence of pneumococcal and meningococcal meningitis in the district are not known. It is also not known whether socio-cultural practices have the same influence on the incidence of pneumococcal and meningococcal meningitis. The level of stigmatization experienced by survivors of bacterial meningitis is not known. There is therefore, the need for cultural epidemiological studies of bacterial meningitis. The economic burden of bacterial

meningitis in the district needs to be studied. This will help speed up the introduction of conjugate vaccines.

The high level of mild hearing impairment in both cases and controls (chapter 7) calls for the need to carry out a community survey of hearing assessment to find out other causes of hearing impairment in the district.

The high case fatality of pneumococcal meningitis despite the absence of penicillin resistance calls for further investigations for antecedent causes or contributory factors like comorbidity associated with the pneumococcal meningitis. The study of genetic polymorphism in pneumococcal meningitis may provide an insight in the complexity of pneumococcal meningitis. This may not only lead to different treatment and vaccination strategies but also contribute to further decline of mortality and morbidity rates among patients with pneumococcal meningitis.

Carrying out a small field project on dust exposure and meningitis incidence, in particular, monitoring respirable dust exposure in Navrongo, could be used to calibrate remote sensed data, as well as the local meteorological station readings. Dust exposure levels at the micro level could complete the picture and could be important for respiratory infections other than meningitis also.

9.3 Control of pneumococcal meningitis in Africa

Man has evolved to commensally live with *S. pneumoniae* over many thousands of years with probably all humans having nasopharyngeal colonization of it early in life. In most cases this colonization, as explained earlier, does not lead to disease due to the commensal relationship between the bacteria and the host mediated by the human immune system and nonspecific barriers to infection in the respiratory tract of human beings (Johnston, Jr., 1991) all under the influence of the climate. It is assumed, that the disruption of this equilibrium may occur when there is confrontation with a new, possibly more pathogenic, serotype of pneumococcus, other external factors like viral infection or host factors like malnutrition or immune deficiency and sometimes changes in the climate. The control of pneumococcal meningitis and pneumococcal disease in general, is geared towards the maintenance of the equilibrium between the pathogen and man, interruption of transmission of pathogen or boosting the host immunity. These are achieved by the identification and prevention of risk factors, effective treatment of established disease and vaccination.

Prevention of risk factors

Indoor air pollution, malnutrition, overcrowding, smoking, HIV/AIDS (Burman et al., 1985; Janoff et al., 1993; Nuorti et al., 2000a; Kyaw et al., 2003) are preventable risk factors for pneumococcal disease. There is the need to change cultural practices that encourage burning of firewood in rooms (especially where neonates and infants are) with the aim of providing heat during the harmattan season. As a long term, electric heaters and cookers should be encouraged (this has cost implications) while for the immediate and short term well burned charcoal (without wood) can be used for heating rooms and cooking. Cooking with firewood outside homes or in well ventilated large kitchens should be encouraged. The use of charcoal involves felling of trees, which has implication on the depletion of the forest with further widening of the meningitis belt. It is therefore important to agitate for a sustainable charcoal use where a number of trees are nurtured for each tree that will be burnt for charcoal or firewood. Generally, improvements in housing and indoor air quality represent difficult but long term targets. Large standard windows should be encouraged.

There is the need to introduce a sustainable school health programme where all school children are screened for ear, nasal and paranasal infections and those with these infections are treated appropriately. Pneumonia should be identified early and treated appropriately in the health facilities.

Early identification and prophylactic administration of penicillin to sickle cell (John et al., 1984; Hirst and Owusu-Ofori, 2002) disease and asplenic patients can help prevent pneumococcal infections in these at risk groups. Appropriate treatment of patients with basal skull fracture and CSF nasal leakage can prevent pneumococcal meningitis.

Treatment of pneumococcal meningitis

Early diagnosis and administration of appropriate antimicrobial therapy are very essential for optimum outcome of pneumococcal meningitis. Attention to fluid administration and strategies for reducing intracranial inflammation are good adjuncts.

With most pneumococcal disease occurring in the developing world, treatment is generally limited to simple and cheap antibiotics. Penicillin has been the mainstay in the treatment of pneumococcal diseases since its introduction over 50 years ago. However, over the past decade with the detection of penicillin-resistant strains of pneumococcus found in all parts of the world (Appelbaum, 1987b;

Whitney et al., 2000) third generation cephalosporins are now the drugs of choice for the treatment of pneumococcal diseases although there are also reports of resistant strains. Though penicillin is still being used currently in the treatment of pneumonia with oral route (amoxicillin) at the primary health care level and intravenous at the district and higher levels with success there is still high mortality associated with its use (in recommended doses) in the treatment of pneumococcal meningitis (chapters 6 & 7) even when the causative bacteria are sensitive in vitro. This shows that there are other contributory factors involved in the high mortality and morbidity of pneumococcal meningitis.

Chloramphenicol is another drug used in the treatment of pneumococcal meningitis in combination with penicillin. The long-acting oily form of chloramphenicol (given as a single dose intramuscular) appears to be more effective than the aqueous form (given intravenous 6 hourly). These drugs do not affect carriage and therefore do not disrupt transmission.

Due to the penicillin resistance third generation cephaloporins, ceftriaxone/cefotaxime are the antibiotics recommended for the treatment of pneumococcal meningitis (WHO, 2003b). Ciprofloxacin is also effective in the treatment of pneumococcal meningitis. This has effect on carriage but has a lower concentration in the CSF. It is recommended to give it two days to discharge of the patient. Rifampicin, which has a good concentration in the CSF after administration and also acts on carriage, is not recommended for fear of its abuse with subsequent development of resistance an event that has implication in the treatment of tuberculosis and leprosy.

The use of dexamethasone (a steroid) as an adjunct therapy has been shown to be beneficial in pneumococcal meningitis if used in early treatment (McIntyre et al., 1997; de Gans and van de Beek, 2002; van de Beek and de Gans, 2004b).

The administration of dexamethasone may lead to the masking of clinical signs and symptoms. About 1-2% of children with bacterial meningitis on treatment with dexamethasone have been reported to have gastrointestinal bleeding (de Gans and van de Beek, 2002). It has been shown to have neurotoxic effects - aggravation of hippocampus neuronal apoptosis and learning deficits (Nau et al., 2002; Leib et al., 2003). Generally, there can still be severe morbidity even with the rapid sterilization and administration of potent antibiotics because of the inflammatory reaction within the

central nervous system coupled with its effects on cerebral blood flow as well as direct action of bacterial toxins on the nervous system (van der Flier et al., 2003).

Pneumococcal vaccines

Considering the increasing sophistication of life-saving technology, with increasing life expectancy, pneumococcal disease including pneumococcal meningitis, is becoming more common and more expensive to society. The increasing pneumococcal resistance to essential antibiotics and the ease with which resistant strains are assuming global spread underlie the importance of an urgent need for control through vaccination.

There is a 23-valent polysaccharide pneumococcal vaccine, which contains the 23 most common serotypes responsible for 90% of the most serous pneumococcal disease in the developed countries. This vaccine has been shown to have no effect on HIV patients in Uganda (French et al., 2000) and cannot be used to protect them from pneumococcal diseases.

Prenatal immunization of mothers with either the polysaccharide or the conjugate vaccine (such as the 7-valent, 9-valent and 11-valent) will protect neonates and infants from pneumococcal disease (Obaro et al., 2004) before the latter start routine immunization with conjugate vaccines. One problem with the use of the 23-valent pneumococcal vaccine in this way will be the failure of immunosuppressed pregnant mothers to produce antibodies (French et al., 2000).

With a reduction in pneumococcal colonization in children vaccinated with conjugate vaccine family members are less likely to be infected by the pneumococcus. In the same way unvaccinated children are less likely to bring home the infection if majority of their playmates have been vaccinated because of their reduced risk of pneumococcal colonization. The introduction of conjugate vaccines should be preceded and monitored by colonization studies, which would be used to monitor the pharyngeal microfloral ecology or interspecies interference.

Conjugate vaccines are very expensive and not available in developing countries. With the adverse effect on carriage (Huang et al., 2005) causing ecological imbalance in the ecological niche of vaccine serotypes in the nasopharynx and subsequent serotype replacement (Eskola et al., 2001; Poehling et al., 2006) there is the need to monitor carriage when conjugate vaccines are in use. This will allow early detection of serotypes like 11, 15, and 19A which carry antibiotic resistance (Kyaw

et al., 2006; Huang et al., 2005) and 6B, 9V and 23F which have the propensity for global spread (Crook and Spratt, 1998).

There is also the possibility of different bacteria like *Staphylococcus aureus* replacing *S. pneumoniae* since the latter will no longer be there to inhibit growth of the former through the production of hydrogen peroxide by its catalase (Regev-Yochay et al., 2006). Should this happen with Methicillin-resistant *S. aureus* (Regev-Yochay et al., 2005) then the situation will just be like replacing one form of meningitis with another (pneumococcal meningitis with staphylococcal meningitis). The hope for a lasting suppression of pneumococcal disease still looks distant. Nonetheless conjugate vaccines can be of significant public health use in the developing countries especially in the African meningitis belt.

There are two potential problems associated with the pneumococcal conjugate vaccines: the limited protection due to serotype specificity and the high cost of the vaccine. A strategy to overcome these problems is the use of common protein vaccines. These proteins are common to all serotypes of pneumococcus and appear to be immunogenic and protective in animal models. They are less expensive to manufacture than the current polyvalent vaccines (which use the capsular polysaccharide as the immunizing antigen) since they can be produced in large amounts using inexpensive recombinant technology. They are therefore ideal candidate pneumococcal vaccines for use in developing countries with high burden of disease and limited resources.

Common protein vaccines (which are not serotype specific) are being developed from conserved protein epitopes. This type of vaccines might be the ultimate for the elimination of pneumococcal disease including pneumococcal meningitis as a public health problem. The challenge to be faced by common protein vaccines is antigenic polymorphism of the candidates and species replacement in the nasopharynx.

To ensure an effective and sustained control of pneumococcal meningitis in the African meningitis belt, there is the need to put in place a good and effective surveillance system to be able to identify cases and report disease occurrence. It is also important to carry out antibiotic sensitivity test for all cases to be able to identify emergence of resistant strains as early as possible. This requires equipped laboratories and trained laboratory personnel and logistics for the early detection and confirmation

of diagnosis of *S. pneumoniae* not only at the regional levels but also at the district and sub district levels.

9.4 Control of meningococcal meningitis in the African meningitis belt

Currently, the main strategies for the control of meningococcal meningitis epidemics are epidemic preparedness and epidemic response (WHO, 2003a).

Epidemic preparedness involves enhancing surveillance and laboratory capacity for early detection of epidemics and confirmation of diagnosis. It also involves the establishment of national and regional stocks of vaccine and logistics, development and update of national plans for epidemic response. There is the need for country-specific control programme with Standard Operating Procedures based on the inter-country control programme.

Epidemic response involves enhanced epidemiological surveillance, prompt case management with short-course, long acting oily chloramphenicol given intramuscular and mass vaccination with a vaccine containing the appropriate serogroup. Cases should be notified as soon as possible and a line list including zero reporting kept in place. Oily chloramphenicol is produced exclusively for the control of meningococcal meningitis during epidemics in the African meningitis belt. It is contraindicated in pregnancy and children less than one year. Reports of resistant meningococcal strains to chloramphenicol, coupled with the outmoded methods of production and low demand makes its future in the control of meningococcal epidemics bleak, despite its high efficacy.

It has been shown in Niger that a single-dose of ceftriaxone is a good alternative to chloramphenicol in the control of meningococcal epidemics (Nathan et al., 2005). This drug can be used in pregnant women and infants. The problem is the high cost of ceftriaxone and its misuse during inter epidemic periods since it is a broad-spectrum antibiotic. This could deplete stocks meant for epidemics and during epidemics there would be shortage of the drug. Inadequate and intensive use can also lead to the emergence of ceftriaxone resistance. Despite these concerns, ceftriaxone is been recommended for treatment during meningococcal epidemics (WHO, 2003a).

For mass immunization WHO proposes the use of epidemic thresholds for early detection of epidemics as well as improved control methods (WHO, 2000). This conditionality is only achievable

when there is an efficient surveillance system in place. This is lacking in many areas of the meningitis belt making epidemics often far ahead of logistical support including vaccines.

A typical epidemic starts in the dry season and abates with the onset of the rains. However, the lack of an early warning system in the prediction of meningococcal epidemics makes vaccination almost always start shortly before the onset of the rains, which abate meningococcal epidemics even without the vaccine. Vaccination during epidemics arrests only about half of the cases (Woods et al., 2000) before the onset of the rains.

Since these meningococcal meningitis epidemics have strong relationship with climatic conditions, it would be worthwhile for local public health practitioners to use local epidemiological and meteorological data to model a simple algorithm (with support from models of remote sensing) for the prediction of these epidemics in their localities. Surveillance should continue (even when the epidemic abates) during inter epidemic periods.

Currently available meningococcal meningitis vaccines for epidemic control in the meningitis belt are polysaccharide vaccines A or A+C or A+C+W135 depending on the serogroup causing the epidemic. These polysaccharide vaccines have no effect on carriage and do not induce immune memory and are not effective in children under two years (Reingold et al., 1985; MacLennan et al., 1999; Zhang et al., 2000; Maiden and Stuart, 2002; Jódar et al., 2002). This is quite disturbing since this is a group with very high incidence and mortality rates of meningococcal meningitis.

The recent epidemics in Burkina Faso due to serogroup W135 have raised concern about the use of the monovalent or bivalent vaccine (Taha et al., 2002a; Decosas and Koama, 2002; Traore et al., 2006; Mueller et al., 2006).

Polysaccharide-protein conjugate vaccines are immunogenic in infants and induce immunological memory, confer herd immunity and reduce carriage of the vaccine type serogroup (Zhang et al., 2000; Maiden and Stuart, 2002; Trotter et al., 2004). The polysaccharide-protein conjugate vaccines could be of prophylactic use through the Expanded Programme on Immunization with catch up campaigns to maintain immunity high enough to be able to stop transmission in the community.

There is concern about serogroup replacement with the use of conjugate vaccines. Carriage studies are necessary for the evaluation of the impact of conjugate vaccines on carriage and nasopharyngeal micro flora in general. A phase II trial of a heptavalent conjugate vaccine was successfully carried

out in 2005 in Ghana. This vaccine contains diphtheria, purtusis, tetanus, hepatitis B, Hib, meningococcal serogroups A and C antigens and was given in 3 doses according to the Ghanaian Expanded Programme on Immunization. It could be good for use in immunization programmes of endemic regions like the meningitis belt.

9.5 Control of meningococcal and pneumococcal meningitis in Northern Ghana

Genarally, the principles for the control of meningococcal and pneumococcal meningitis in Ghana are not different from those of other countries in the meningitis belt. The measures for the prevention of the risk factors in Africa are the same. Northern Ghana, which lies within the meningitis belt with a population of about 3.3million (Ghana Statistical Service, 2000), is made up of three regions (Northern, Upper East and Upper West) and 34 districts. It has 22 hospitals, 3 regional hospitals (where bacterial culture and sensitivity tests can be done) and a Public Health Reference Laboratory at the northern regional capital, Tamale.

The control of meningococcal and pneumococcal meningitis as a public health problem in Northern Ghana requires that the Public Health Division of the Ministry of Health draws up a broad policy framework (adapted from the inter-country programme on meningitis) within which all the health adminstrative levels (regional, metropolitan, municipal, district and subdistrict) have to operate. This policy needs to look at epidemic preparedness and response with emphasis on surveillance, case management, laboratory support and diagnosis, immunizations and maintenance of cold chain and rehabilitaion of survivors of meningococcal and pneumococcal meningitis.

As a reportable disease in Ghana, the national Disease Surveillance Unit of the Public Health Division has to proactively ensure that all surveillance returns from the districts are in on time so as to ensure their timely onward submission to WHO. The unit should have good collaboration with the International Coordinating Group for epidemic meningococcal disease and ensure that syringes, needles, incineration boxes, drugs (oily chloramphenicol), vaccines, rapid diagnostic kits (agglutination test kits) and laboratory reagents are always in stock and updated in case there is an impending epidemic. The Disease Control and Surveillance Unit should ensure that the Public Health Reference Laboratory at Tamale is fully equipped to be able to conduct detailed bacteriological and molecular tests. Districts and regions in northern Ghana should be alerted by the Surveillance Unit of outbreaks in neighbouring districts or countries. This Unit should develop a

National Standard Operating Procedures for the implementation of bacterial meningitis surveillance (NSOPIBMS) and a national plan of action as well as train regional trainers on the NSOPIBMS who would inturn train the district trainers. The Unit should support and supervise the other levels to enable them carry out their respective roles.

The Ministry of Health should establish an epidemic preparedness and response committee (as described by Hodgson, 2002) with prototype branches at all levels – regions, metropolitan/Municipals/ districts and subdistrict and strengthen them. This committee at the national level should be made up of the Director of Public Health of the Ministry of Health/Ghana Health Service, the head of disease Surveillance Unit, Head of Disease Control Unit, the Chief Medical Officer of the Ministry of Health, the Director of Health Research Unit of the Ministry of Health, the Public Relations Officer of the Ministry of Health, Head of the EPI, the Director of the National Disaster Management Organization (NADMO), a data manager, the Head of the National Public Health Reference laboratory, a representative from the security services, a representative from Ghana Red Cross.

The Regional Director of Health Services and members of the Regional Health Management Team (RHMT), the head of the Public Health Reference Laboratory (in Tamale), the Medical Director/Superintendent of the regional Hospital, the head of the regional hospital laboratory, the Regional Coordinating Director, the Regional Director of NADMO, a representative each of the private health practitioners, chemical sellers association, Ghana Red Cross, Regional Security Committee, Regional House of Chiefs and medical Research Institute or Centre should make up the epidemic preparedness and response committees at the regional level.

At the district level, the epidemic preparedness and response committee should comprise the Distrct Director of Health Services and members of the Distrct Health Management Team (DHMT), the Medical Superintendent of the district hospital, the District Coordinating Director, a representative from the health and social subcommittee of the district assembly, the District Director of NADMO, a representative from the District Security Committee, a representative of the private health practitioners, a representative from the chemical sellers association, a representative from the Private

Transport Union in the district, a representative of the Ghana Red Cross, a representative of the media, a representative of health oriented NGOs working in the distrct and other co-opted members as the committee will deem necessary.

At the subdistrict level, the epidemic preparedness and response committee should be made up of members of the subdistrict management health team, the local assemblyman, a representative of community based rehabilitation organisation, a representative of community health volunteers, an elder from the community and representatives of NGOs engaged in health activities. These committees would have to meet regulary (especially during the epidemic season) to review records of cases and prepare for any impending or respond to any epidemic.

The DHMTs with support from the RHMTs and the Disease Control/Surveillance Unit should organise and train all categories of health personnel in the district on the NSOPIBMS. There should be an additional and special training of surveillance officers, laboratory staff and data managers. Medical assistants in all the three regions should be taught how and when to perform lumbar puncture while the hospitals are equiped to do latex agglutination test. The DHMTs and district hospitals in their annual budgetting should make provision for meningitis control as part of their epidemic preparedness.

Arrangements should be made for the transportation of CSF samples to the district hospitals from the Health Centres in subdistricts and from the district hospitals to the regional hospitals within the region. Disease Control/Surveillance Officers can transport the CSF samples using motorbikes and where possible the medical assistants should dispatch the CSF samples anytime their vehicle or motorbike is going to the hospital or the DHMT. The regional disease control officer should be responsible for transporting CSF samples from the regional hospital to the Public Health Reference Laboratory, Tamale for culture and sensitivity. All CSF samples should have culture and antibiotic sensitivity tests done at this laboratory.

There should be a reliable communication system through which results can be communicated as soon as possible to the officer who referred the sample to enable the timely submission of weekly surveillance reports. This should provide the causal organism and assist the prescriber in the case management and any possible mass vaccination if necessary. The Public Health Reference Laboratory should store some of the CSF samples for molecular analysis later. There should be

regular monitoring and evaluation of the laboratories as well as the NSOPIBMS system at all the various levels . This could be done at refresher workshops organised on NSOPIBMS or regular visits to the hospitals, RHMTs, DHMTs and subdistrict. This will enable weaknesses or difficulties to be detected and assistance offered where necessary. These visits should not be limited to epidemic periods but also during the interepidemic periods.

The DHMTs should also collaborate with the meteorological services department from which enviromental data can be obtained. Simple analysis using epidemiological and environmental data (past and current data) should be carried out at the various DHMTs so that district based early warning systems (EWS) can be developed and be incoporated in the surveillance system.

Districts and subdistricts should receive training on the calculation, interpretation and use of thresholds (for meningococcal meningitis) based on WHO guidelines (WHO, 2000) and simple models of an early warning system based on environmental factors of the district (subdistrict) and epidemiological data on meningococcal and pneumococcal meningitis. Surveillance has to be intensified and enhanced throughout the year with subdistricts submitting timely, weekly reports including zero reporting to the DHMTs which will inturn summarize these into district reports and submit to the RHMT from where the regional reports would be submitted to the national level. There should be some epidemiological analysis at each level with dissemination of results to the lower level. With information from remote sensors (Molesworth et al., 2003) on the district at risk in a particular year combined with the local early warning model and enhanced sustained surveillance it may be possible to detect epidemics far in advance and be able to put them under control.

When the alert threshold is reached (for meningococcal meningitis) there is the need to inform the higher authorities, investigate and confirm the causal organism, treat cases appropriately, strengthen surveillance while preparations are made for mass immunization when the epidemic threshold is reached (which can be forecasted through climate-based early warning system). Neighbouring districts should be informed about the alert threshold and there should be an efficient communication link so to ensure that the they are notified of the epidemic threshold. When the epidemic threshold is reached mass immunization together with the issuance of immunization cards, distribution of drugs and logistics to the various Health Centres and hospitals and treatment with oily chloramphenicol according to epidemic protocol should be carried out. The public health authorities should be informed. The Unit Committee members should be involved in the planning mobilization of the

population to participate in vaccination campaigns. The health workers should continue with the health education on the disease, its causes, risks, and prevention.

The meningococcal polysaccharride vaccine A+C can be used in the three northern regions since the 1996/7, 1998 outbreaks (Woods et al., 2000; Gagneux et al., 2000), the 2002 and 2004 outbreaks were caused by this serogoup though it will be better to use the quadrivalent A+C+Y+W135 in view of threats of serogroup W135 epidemics in Burkina Faso (WHO, 2002).

For outbreaks of pneumococcal meningitis the same reporting system and procedures should be used though the treatment has to be with cefriaxone according to the standard treatment guidelines of the Ministry of Health, Ghana (MOH(GNDP), 2004). For vaccination against pneumococcal meningitis it will be advisable to conduct an extended enhanced surveillance on pneumococcal meningitis at sentinel sites in the three northern regions of Ghana. The introduction of any pneumococcal vaccine should contain the apropriate pneumococcal serotypes in the region.

The communities should be involved in the control of meningitis right from the planning of the control programme. This will ensure their cooperation and assistance in organization and ensuring the success of the immunization programme as well as reporting of suspected cases.

In the long term, to make pneumococcal and meningococcal meningitis diseases of less public importance in northern Ghana, there is the need to introduce polysaccharide-protein conjugate vaccines (like the heptavalent conjugate vaccine tested in the KND in 2005 which contained seven antigens including *N. meningitidis* serogroups A and C) into the EPI schedule (as well as maternal immunization) which should be preceded by carriage serveys and enhanced surveillance (including pharmacovigilance) at sentinel sites in the three northern regions. The carriage surveys should be continued after the introduction of the vaccines to monitor the dynamics of carriage by non-vaccine serotypes or serogroups and other pharyngeal microflora. Better still, common protein vaccines should be introduced (with concomitant carriage surveys) in the EPI programme when these vaccines become available in future.

Since the year 2000 pentavalent conjugate vaccine containing the Hib antigen was introduced into the EPI programme in Ghana. Hib meningitis is now relatively less a public health problem

compared to pneumococcal and meningococcal meningitis. There is the need to however, still keep surveillance on Hib.

9.6 Conclusions

The clonal waves of nasopharyngeal colonization and disease in the KND observed during the longitudinal study represent natural variations in the predominance of meningococcal serogroups (serotypes) that take place over time independent of vaccination. Potential serogroup replacement should therefore be monitored through meningococcal carriage studies such as those described here before and after the introduction of polysaccharide-protein conjugate vaccines in the African Meningitis Belt since these vaccines impact on carriage.

The observed rapid natural microevolution of W135 meningococci during the W135 colonization survey calls for new approaches for studying the molecular epidemiology of *N. meningitidis* W135 since the available techniques are not suitable for the analysis of the population structure to distinguish between endogenous and epidemic strains.

The *S. pneumoniae* ST217 clonal complex represents a hypervirulent lineage with a high propensity to behave epidemiologically like *N. meningitidis*. There is, therefore, the need for a sustained enhanced surveillance at all levels of healthcare delivery together with longitudinal pneumococcal carriage surveys to monitor the serotype distribution of *S. pneumoniae* in the African meningitis belt. This will ensure that vaccines covering the appropriate hypervirulent serotypes in the meningitis belt are introduced for mass immunization.

The high mortality and morbidity associated with pneumococcal meningitis compared to meningococcal meningitis calls for more political will and sustained commitment with allocation of more resources to curb the unacceptable situation.

Hearing and speech impairment are a much more common problem in pneumococcal meningitis than in meningococcal meningitis. In view of the high burden of pneumococcal meningitis in early infancy coupled with the global growing threat of multi-drug resistance, there is the need for an

accelerated immunization schedule beginning in the perinatal period or maternal immunization with pneumococcal and meningococcal vaccines containing the appropriate serotypes/serogroups.

Environmental factors that influence the incidence of meningococcal and pneumococcal meningitis are similar, not always the same and often result in different timing of outbreaks of the two diseases. The duration of preceding absence of rainfall appear to be the best predictor of both pneumococcal and meningococcal meningitis outbreaks. While concurrent reduction in rainfall significantly predict outbreaks of pneumococcal meningitis, meningococcal meningitis outbreaks are best predicted by concurrent increase in weekly mean maximum temperature and concurrent reduction in rainfall in the Kassena Nankana District. There is the need for prototype district level climate-based early warning systems (micro-epidemiological models) for the prediction of epidemics of meningococcal and pneumococcal meningitis in countries of the African Meningitis Belt.

The introduction of conjugate or common protein vaccines in future in the EPI with enhanced surveillance, carriage surveys and community participation has the potential to substantially reduce pneumococcal and meningococcal meningitis as a public health problem.

Reference

Reference List

Achtman M, 1995. Epidemic spread and antigenic variability of *Neisseria meningitidis*. Trends Microbiol. 3: 186-192.

Adegbola RA, Hill PC, Secka O, Ikumapayi UN, Lahai G, Greenwood BM, Corrah T, 2006. Serotype and antimicrobial susceptibility patterns of isolates of *Streptococcus pneumoniae* causing invasive disease in The Gambia 1996-2003. Trop. Med. Int. Health 11: 1128-1135.

Aguilera JF, Perrocheau A, Meffre C, Hahne S, 2002. Outbreak of serogroup W135 meningococcal disease after the Hajj pilgrimage, Europe, 2000. Emerg. Infect. Dis. 8: 761-767.

Ahmad K, 2004. Vaccination halts meningitis outbreak in Burkina Faso. Lancet 363: 1290.

al-Gahtani YM, el Bushra HE, al-Qarawi SM, al-Zubaidi AA, Fontaine RE, 1995. Epidemiological investigation of an outbreak of meningococcal meningitis in Makkah (Mecca), Saudi Arabia, 1992. Epidemiol. Infect 115: 399-409.

Appelbaum PC, 1987a. World-wide development of antibiotic resistance in pneumococci 1. Eur. J. Clin. Microbiol. 6: 367-377.

Arditi M, Mason EO, Jr., Bradley JS, Tan TQ, Barson WJ, Schutze GE, Wald ER, Givner LB, Kim KS, Yogev R, Kaplan SL, 1998. Three-year multicenter surveillance of pneumococcal meningitis in children: clinical characteristics, and outcome related to penicillin susceptibility and dexamethasone use. Pediatrics 102: 1087-1097.

Aronin SI, Quagliarello VJ, 2001. New perspectives on pneumococcal meningitis. Hosp. Pract. (Minneap.) 36: 43-50, 51.

Artenstein MS, Brandt BL, 1975. Immunologic hyporesponsiveness in man to group C meningococcal polysaccharide. J Immunol. 115: 5-7.

Attia J, Hatala R, Cook DJ, Wong JG, 1999. The rational clinical examination. Does this adult patient have acute meningitis? JAMA 282: 175-181.

Backman H, Haghighat F, 1999. Indoor-air quality and ocular discomfort. J Am Optom. Assoc. 70: 309-316.

Baird DR, Whittle HC, Greenwood BM, 1976. Mortality from pneumococcal meningitis. Lancet 2: 1344-1346.

Baraff LJ, Lee SI, Schriger DL, 1993. Outcomes of bacterial meningitis in children: a meta-analysis. Pediatr. Infect. Dis. J. 12: 389-394.

Belcher DW, Sherriff AC, Nimo KP, Chew GL, Richardson WD, Voros A, Feldman HA, Richardson WD, Feldman HA, 1977. Meningococcal meningitis in northern Ghana: epidemiology and control measures. Am J Trop. Med Hyg. 26: 748-755.

Bennett DE, Mulhall RM, Cafferkey MT, 2004. PCR-based assay for detection of *Neisseria meningitidis* capsular serogroups 29E, X, and Z. J. Clin. Microbiol. 42: 1764-1765.

Berkow R&FAJ, 1992. The Merck Manual of Diagnosis and Therapy. Merck Research Laboratories, Rahway. N. J., USA.

Besancenot J, Boko M, Oke PC, 1997. Weather conditions and cerebrospinal meningitis in Benin (Gulf of Guinea, West Africa). Eur. J. Epidemiol. 13: 807-815.

Biesalski HK, Stofft E, 1992. Biochemical, morphological, and functional aspects of systemic and local vitamin A deficiency in the respiratory tract. Ann N. Y. Acad. Sci. 669: 325-331.

Bijlmer HA, van Alphen L, Greenwood BM, Brown J, Schneider G, Hughes A, Menon A, Zanen HC, Valkenburg HA, 1990. The epidemiology of *Haemophilus influenzae* meningitis in children under five years of age in The Gambia, West Africa. J Infect Dis 161: 1210-1215.

Binka F, Ngom P, Philips JF, Adazu KF, Macleod B, 1999. Assessing population dynamics in a rural African society; Findings from the Navrongo Demographic Surveillance System. Journal of Biosocial Science 31: 375-391 31: 375-391.

Blackwell CC, Weir DM, James VS, Todd WT, Banatvala N, Chaudhuri AK, Gray HG, Thomson EJ, Fallon RJ, 1990. Secretor status, smoking and carriage of *Neisseria meningitidis*. Epidemiol. Infect. 104: 203-209.

Blakebrough IS, Gilles HM, 1980. The effect of rifampicin on meningococcal carriage in family contacts in northern Nigeria. J Infect 2: 137-143.

Bogaert D, De Groot R, Hermans PW, 2004a. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. Lancet Infect. Dis. 4: 144-154.

Bogaert D, Hermans PW, Adrian PV, Rumke HC, De Groot R, 2004b. Pneumococcal vaccines: an update on current strategies. Vaccine 22: 2209-2220.

Borrow R, Fox AJ, Cartwright K, Begg NT, Jones DM, 1999. Salivary antibodies following parenteral immunization of infants with a meningococcal serogroup A and C conjugated vaccine. Epidemiol. Infect 123: 201-208.

Borrow R, Goldblatt D, Andrews N, Richmond P, Southern J, Miller E, 2001. Influence of prior meningococcal C polysaccharide vaccination on the response and generation of memory after meningococcal C conjugate vaccination in young children. J. Infect. Dis. 184: 377-380.

Brandileone MC, de Andrade AL, Di Fabio JL, Guerra ML, Austrian R, 2003. Appropriateness of a pneumococcal conjugate vaccine in Brazil: potential impact of age and clinical diagnosis, with emphasis on meningitis. J. Infect. Dis. 187: 1206-1212.

Briles DE, Tart RC, Wu HY, Ralph BA, Russell MW, McDaniel LS, 1996. Systemic and mucosal protective immunity to pneumococcal surface protein A. Ann N. Y. Acad. Sci. 797: 118-126.

Broome CV, Rugh MA, Yada AA, Giat L, Giat H, Zeltner JM, Sanborn WR, Fraser DW, 1983. Epidemic group C meningococcal meningitis in Upper Volta, 1979. Bull. World Health Organ 61: 325-330.

Brueggemann AB, Coffman SL, Rhomberg P, Huynh H, Almer L, Nilius A, Flamm R, Doern GV, 2002. Fluoroquinolone resistance in *Streptococcus pneumoniae* in United States since 1994-1995. Antimicrob. Agents Chemother. 46: 680-688.

Brueggemann AB, Griffiths DT, Meats E, Peto T, Crook DW, Spratt BG, 2003. Clonal relationships between invasive and carriage *Streptococcus pneumoniae* and serotype- and clone-specific differences in invasive disease potential. J. Infect. Dis. 187: 1424-1432.

Brueggemann AB, Spratt BG, 2003. Geographic distribution and clonal diversity of *Streptococcus pneumoniae* serotype 1 isolates. J. Clin. Microbiol. 41: 4966-4970.

Buckingham SC, McCullers JA, Lujan-Zilbermann J, Knapp KM, Orman KL, English BK, 2001. Pneumococcal meningitis in children: relationship of antibiotic resistance to clinical characteristics and outcomes. Pediatr. Infect. Dis. J. 20: 837-843.

Burgess KR, Whitelaw WA, 1988. Effects of nasal cold receptors on pattern of breathing. J Appl. Physiol 64: 371-376.

Burman LA, Norrby R, Trollfors B, 1985. Invasive pneumococcal infections: incidence, predisposing factors, and prognosis. Rev Infect Dis. 7: 133-142.

Butler JC, 2004 Epidemiology of pneumococcal disease. In: Tuomanen, E., Mitchell, T., Morrison, D., Spratt, B. G. (Eds.), The pneumococcus. ASM Press, Washington, pp. 148-168.

Byington CL, Samore MH, Stoddard GJ, Barlow S, Daly J, Korgenski K, Firth S, Glover D, Jensen J, Mason EO, Shutt CK, Pavia AT, 2005. Temporal trends of invasive disease due to *Streptococcus pneumoniae* among children in the intermountain west: emergence of nonvaccine serogroups. Clin. Infect Dis 41: 21-29.

Campbell JD, Kotloff KL, Sow SO, Tapia M, Keita MM, Keita T, Diallo S, Hormazabal JC, Murray P, Levine MM, 2004. Invasive pneumococcal infections among hospitalized children in Bamako, Mali. Pediatr. Infect. Dis. J. 23: 642-649.

Cariou A, Chiche JD, Charpentier J, Dhainaut JF, Mira JP, 2002. The era of genomics: impact on sepsis clinical trial design. Crit Care Med 30: S341-S348.

Cartwright K, 1995 Meningococcal Carriage and Disease. In: Cartwright, K. ed. (Ed.), Meningococcal Disease. John Wiley & Sons, West Sussex, pp. 115-147.

Cartwright KA, Stuart JM, Jones DM, Noah ND, 1987. The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. Epidemiol. Infect. 99: 591-601.

Caugant DA, Hoiby EA, Magnus P, Scheel O, Hoel T, Bjune G, Wedege E, Eng J, Froholm LO, 1994. Asymptomatic carriage of *Neisseria meningitidis* in a randomly sampled population. J. Clin. Microbiol. 32: 323-330.

Caugant DA, Kristiansen BE, Froholm LO, Bovre K, Selander RK, 1988. Clonal diversity of *Neisseria meningitidis* from a population of asymptomatic carriers. Infect. Immun. 56: 2060-2068.

CCDR, 2001. *Neisseria meningitidis* with Decreased Susceptibility to Penicillin in Ontario, Canada 1997-2000. Canadian Communicable Disease Report Volume 27.

CDC, 2001. Outbreak of Pneumococcal Pneumonia Among Unvaccinated Residents of a Nursing Home — New Jersey, April 2001. MMWR, Morbidity and Mortality Weekly Report 50: 707-710.

Chandra RK, 1988. Increased bacterial binding to respiratory epithelial cells in vitamin A deficiency. BMJ 297: 834-835.

Charpentier E, Tuomanen E, 2000. Mechanisms of antibiotic resistance and tolerance in *Streptococcus pneumoniae*. Microbes. Infect 2: 1855-1864.

Chen FM, Breiman RF, Farley M, Plikaytis B, Deaver K, Cetron MS, 1998. Geocoding and linking data from population-based surveillance and the US Census to evaluate the impact of median household income on the epidemiology of invasive *Streptococcus pneumoniae* infections. Am. J Epidemiol. 148: 1212-1218.

Chonghaile CN, 2002. Meningitis in Africa--tackling W135. Lancet 360: 2054-2055.

Claus H, Maiden MC, Wilson DJ, McCarthy ND, Jolley KA, Urwin R, Hessler F, Frosch M, Vogel U, 2005. Genetic analysis of meningococci carried by children and young adults. J. Infect. Dis. 191: 1263-1271.

Cortese MM, Wolff M, Almeido-Hill J, Reid R, Ketcham J, Santosham M, 1992. High incidence rates of invasive pneumococcal disease in the White Mountain Apache population. Arch. Intern. Med. 152: 2277-2282.

Crook DW, Spratt BG, 1998. Multiple antibiotic resistance in *Streptococcus pneumoniae*. Br. Med Bull. 54: 595-610.

Cuevas LE, Kazembe P, Mughogho GK, Tillotson GS, Hart CA, 1995. Eradication of nasopharyngeal carriage of *Neisseria meningitidis* in children and adults in rural Africa: a comparison of ciprofloxacin and rifampicin. J Infect Dis 171: 728-731.

Dagan R, Gradstein S, Belmaker I, Porat N, Siton Y, Weber G, Janco J, Yagupsky P, 2000. An outbreak of *Streptococcus pneumoniae* serotype 1 in a closed community in southern Israel. Clin. Infect. Dis. 30: 319-321.

Dagan R, Melamed R, Muallem M, Piglansky L, Greenberg D, Abramson O, Mendelman PM, Bohidar N, Yagupsky P, 1996. Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. J Infect Dis 174: 1271-1278.

Daoud AS, Al-Sheyyab M, Batchoun RG, Rawashdeh MO, Nussair MM, Pugh RN, 1995. Bacterial meningitis: still a cause of high mortality and severe neurological morbidity in childhood. J. Trop. Pediatr. 41: 308-310.

Davidson M, Chamblee C, Campbell HG, Bulkow LR, Taylor GE, Lanier AP, Berner J, Spika JS, Williams WW, Middaugh JP, 1993. Pneumococcal vaccination in a remote population of high-risk Alaska Natives. Public Health Rep. 108: 439-446.

Davis J, Elfenbein J, Schum R, Bentler RA., 1986. Effects of mild and moderate hearing impairments on language, educational, and psychosocial behavior of children. J Speech Hear Disord 51.: 53-62.

de Gans J, van de Beek D, 2002. Dexamethasone in adults with bacterial meningitis. N. Engl. J. Med. 347: 1549-1556.

Decosas J, Koama JB, 2002. Chronicle of an outbreak foretold: meningococcal meningitis W135 in Burkina Faso. Lancet Infect. Dis. 2: 763-765.

Denis F, Rey JL, Amadou A, Saliou P, Prince-David M, M'boup S, Cadox M, Mar ID, Etienne J, 1982. Emergence of meningococcal meningitis caused by W 135 subgroup in Africa. Lancet 2: 1335-1336.

Deubzer HE, Obaro SK, Newman VO, Adegbola RA, Greenwood BM, Henderson DC, 2004. Colostrum obtained from women vaccinated with pneumococcal vaccine during pregnancy inhibits epithelial adhesion of *Streptococcus pneumoniae*. J Infect Dis 190: 1758-1761.

Djibo S, Nicolas P, Alonso JM, Djibo A, Couret D, Riou JY, Chippaux JP, 2003. Outbreaks of serogroup X meningococcal meningitis in Niger 1995-2000. Trop. Med. Int. Health 8: 1118-1123.

Dodds A, Tyszkiewicz E, Ramsden R, 1997. Cochlear implantation after bacterial meningitis: the dangers of delay. Arch. Dis. Child 76: 139-140.

Dodge PR, Davis H, Feigin RD, Holmes SJ, Kaplan SL, Jubelirer DP, Stechenberg BW, Hirsh SK, 1984. Prospective evaluation of hearing impairment as a sequela of acute bacterial meningitis. N. Engl. J. Med. 311: 869-874.

Dowell SF, Whitney CG, Wright C, Rose CE, Jr., Schuchat A, 2003. Seasonal patterns of invasive pneumococcal disease. Emerg. Infect. Dis. 9: 573-579.

Durand ML, Calderwood SB, Weber DJ, Miller SI, Southwick FS, Caviness VS, Jr., Swartz MN, 1993. Acute bacterial meningitis in adults. A review of 493 episodes. N. Engl. J Med 328: 21-28.

Ennes HS, McRoberts JA, Hyman PE, Snape WJ, Jr., 1992. Characterization of colonic circular smooth muscle cells in culture. Am. J. Physiol 263: G365-G370.

Enos K. Cerebrospinal meningitis in northern Ghana: the experience of the War Memorial Hospital, Navrongo. 1997. Ministry of Health, Ghana.

Enright MC, Spratt BG, 1998. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology 144 (Pt 11): 3049-3060.

Erenberg A, Lemons J, Sia C, Trunkel D, Ziring P, 1999. Newborn and infant hearing loss: detection and intervention.American Academy of Pediatrics. Task Force on Newborn and Infant Hearing, 1998- 1999. Pediatrics 103: 527-530.

Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, Takala A, Kayhty H, Karma P, Kohberger R, Siber G, Makela PH, 2001. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N. Engl. J. Med. 344: 403-409.

Eskola J, Takala AK, Kela E, Pekkanen E, Kalliokoski R, Leinonen M, 1992. Epidemiology of invasive pneumococcal infections in children in Finland. JAMA 268: 3323-3327.

Evans JR, Artenstein MS, Hunter DH, 1968. Prevalence of meningococcal serogroups and description of three new groups. Am. J. Epidemiol. 87: 643-646.

Fairley CK, Begg N, Borrow R, Fox AJ, Jones DM, Cartwright K, 1996. Conjugate meningococcal serogroup A and C vaccine: reactogenicity and immunogenicity in United Kingdom infants. J Infect Dis 174: 1360-1363.

Feikin DR, Davis M, Nwanyanwu OC, Kazembe PN, Barat LM, Wasas A, Bloland PB, Ziba C, Capper T, Huebner RE, Schwartz B, Klugman KP, Dowell SF, 2003. Antibiotic resistance and serotype distribution of *Streptococcus pneumoniae* colonizing rural Malawian children. Pediatr. Infect. Dis. J. 22: 564-567.

Feikin DR, Klugman KP, 2002. Historical changes in pneumococcal serogroup distribution: implications for the era of pneumococcal conjugate vaccines. Clin. Infect. Dis. 35: 547-555.

Feil EJ, 2004. Small change: keeping pace with microevolution. Nat. Rev. Microbiol. 2: 483-495.

Fernandez S, Arreaza L, Santiago I, Malvar A, Berron S, Vazquez JA, Hervada X, 2003. Impact of meningococcal vaccination with combined serogroups A and C polysaccharide vaccine on carriage of *Neisseria meningitidis* C. J. Med. Microbiol. 52: 75-77.

Fiore AE, Moroney JF, Farley MM, Harrison LH, Patterson JE, Jorgensen JH, Cetron M, Kolczak MS, Breiman RF, Schuchat A, 2000. Clinical outcomes of meningitis caused by *Streptococcus pneumoniae* in the era of antibiotic resistance. Clin. Infect. Dis. 30: 71-77.

Fortnum HM, 1992. Hearing impairment after bacterial meningitis: a review. Arch. Dis. Child 67: 1128-1133.

Fortnum HM, Davis AC, 1993. Epidemiology of bacterial meningitis. Arch. Dis. Child 68: 763-767.

Fortnum HM, Hull D, 1992. Is hearing assessed after bacterial meningitis? Arch. Dis. Child 67: 1111-1112.

Fraser D, Givon-Lavi N, Bilenko N, Dagan R, 2001. A decade (1989-1998) of pediatric invasive pneumococcal disease in 2 populations residing in 1 geographic location: implications for vaccine choice. Clin. Infect Dis. 33: 421-427.

French N, Nakiyingi J, Carpenter LM, Lugada E, Watera C, Moi K, Moore M, Antvelink D, Mulder D, Janoff EN, Whitworth J, Gilks CF, 2000. 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomised and placebo controlled trial. Lancet 355: 2106-2111.

Fry AM, Facklam RR, Whitney CG, Plikaytis BD, Schuchat A, 2003. Multistate evaluation of invasive pneumococcal diseases in adults with human immunodeficiency virus infection: serotype and antimicrobial resistance patterns in the United States. J Infect Dis. 188: 643-652.

Gagneux S, Hodgson A, Ehrhard I, Morelli G, Genton B, Smith T, Tanner M, Binka F, Achtman M, Pluschke G, 2000. Microheterogeneity of serogroup A (subgroup III) *Neisseria meningitidis* during an outbreak in northern Ghana. Trop. Med. Int. Health 5: 280-287.

Gagneux S, Wirth T, Hodgson A, Ehrhard I, Morelli G, Kriz P, Genton B, Smith T, Binka F, Pluschke G, Achtman M, 2002a. Clonal groupings in serogroup X *Neisseria meningitidis*. Emerg. Infect. Dis. 8: 462-466.

Gagneux SP, Hodgson A, Smith TA, Wirth T, Ehrhard I, Morelli G, Genton B, Binka FN, Achtman M, Pluschke G, 2002b. Prospective study of a serogroup X *Neisseria meningitidis* outbreak in northern Ghana. J. Infect. Dis. 185: 618-626.

Gagneux S. Molecular Epidemiology of Meningococcal Disease in Northern Ghana. 2001. University of Basel. Thesis

Galimand M, Gerbaud G, Guibourdenche M, Riou JY, Courvalin P, 1998. High-level chloramphenicol resistance in *Neisseria meningitidis*. N. Engl. J Med 339: 868-874.

Geers A, &, Moog J, 1989. Factors predictive of the development of literacy in profoundly hearing-impaired adolescents. The Volta Review 91: 69-86.

Ghaffar F, Barton T, Lozano J, Muniz LS, Hicks P, Gan V, Ahmad N, McCracken GH, Jr., 2004. Effect of the 7-valent pneumococcal conjugate vaccine on nasopharyngeal colonization by *Streptococcus pneumoniae* in the first 2 years of life. Clin. Infect Dis 39: 930-938.

Ghana Statistical Service, 2000. 2000 Population and Housing census. Population and Housing census.

Givon-Lavi N, Fraser D, Porat N, Dagan R, 2002. Spread of *Streptococcus pneumoniae* and antibiotic-resistant *S. pneumoniae* from day-care center attendees to their younger siblings. J. Infect. Dis. 186: 1608-1614.

Goetghebuer T, West TE, Wermenbol V, Cadbury AL, Milligan P, Lloyd-Evans N, Adegbola RA, Mulholland EK, Greenwood BM, Weber MW, 2000. Outcome of meningitis caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* type b in children in The Gambia. Trop. Med. Int. Health 5: 207-213.

Gonzalez BE, Hulten KG, Kaplan SL, Mason EO, Jr., 2004. Clonality of *Streptococcus pneumoniae* serotype 1 isolates from pediatric patients in the United States. J. Clin. Microbiol. 42: 2810-2812.

Gotschlich EC, Goldschneider I, Artenstein MS, 1969. Human immunity to the meningococcus. V. The effect of immunization with meningococcal group C polysaccharide on the carrier state. J. Exp. Med. 129: 1385-1395.

Goudie A, Middleton N, 2001. Sahara dust storms: nature and consequences. Earth-Science Reviews 56: 179-204.

Granoff DM, Gupta RK, Belshe RB, Anderson EL, 1998. Induction of immunologic refractoriness in adults by meningococcal C polysaccharide vaccination. J. Infect. Dis. 178: 870-874.

Gratten M, Morey F, Dixon J, Manning K, Torzillo P, Matters R, Erlich J, Hanna J, Asche V, Riley I, 1993. An outbreak of serotype 1 *Streptococcus pneumoniae* infection in central Australia. Med. J. Aust. 158: 340-342.

Gratten M, Torzillo P, Morey F, Dixon J, Erlich J, Hagger J, Henrichsen J, 1996. Distribution of capsular types and antibiotic susceptibility of invasive *Streptococcus pneumoniae* isolated from aborigines in central Australia. J Clin. Microbiol. 34: 338-341.

Gray BM, Converse GM, III, Dillon HC, Jr., 1980. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. J. Infect. Dis. 142: 923-933.

Gray GC, Callahan JD, Hawksworth AW, Fisher CA, Gaydos JC, 1999. Respiratory diseases among U.S. military personnel: countering emerging threats. Emerg. Infect Dis. 5: 379-385.

Gray LD, Fedorko DP, 1992. Laboratory diagnosis of bacterial meningitis. Clin. Microbiol. Rev 5: 130-145.

Greenwood B, 1999. Manson Lecture. Meningococcal meningitis in Africa. Trans. R. Soc. Trop. Med. Hyg. 93: 341-353.

Greenwood B, 1987 The Epidemiology of Acute Bacterial Meningitis in Tropical Africa. Bacterial Meningitis. Academic Press Inc. (London) Ltd., pp. 61-91.

Greenwood B, 2006. Editorial: 100 years of epidemic meningitis in West Africa - has anything changed?
1. Trop. Med Int. Health 11: 773-780.

Greenwood BM, Blakebrough IS, Bradley AK, Wali S, Whittle HC, 1984. Meningococcal disease and season in sub-Saharan Africa. Lancet 1: 1339-1342.

Greenwood BM, Bradley AK, Cleland PG, Haggie MH, Hassan-King M, Lewis LS, Macfarlane JT, Taqi A, Whittle HC, Bradley-Moore AM, Ansari Q, 1979. An epidemic of meningococcal infection at Zaria, Northern Nigeria. 1. General epidemiological features. Trans. R. Soc. Trop. Med. Hyg. 73: 557-562.

Greenwood BM, Greenwood AM, Bradley AK, Williams K, Hassan-King M, Shenton FC, Wall RA, Hayes RJ, 1987. Factors influencing susceptibility to meningococcal disease during an epidemic in The Gambia, West Africa. J. Infect. 14: 167-184.

Greenwood BM, Hassan-King M, Onyemelukwe G, Macfarlane JT, Tubbs HR, Tugwell PJ, Whittle HC, Denis F, Chiron JP, M'boup S, Triau R, Cadoz M, Mar ID, 1980. Pneumococcal serotypes in West Africa. Lancet 1: 360.

Greenwood B, Whittle H, Blakebrough IS, 1983. Season and meningococcal disease in Northern Nigeria. Medicine Tropicale 43: 35-38.

Griffin D, Garrison V, Herman J, Shinn E, 2001. African desert dust in the Caribbean atmosphere: Microbiology and public health. Aerobiologia 17: 203-213.

Griffin D, Kellogg C, Garrison V, Lisle J, Borden T, Shinn E, 2003. Atmospheric microbiology in the northern Carribbean during African dust events. Aerobiologia 19: 143-157.

Griffiss JM, 1982. Epidemic meningococcal disease: synthesis of a hypothetical immunoepidemiologic model. Rev. Infect. Dis. 4: 159-172.

Grimwood K, Anderson P, Anderson V, Tan L, Nolan T, 2000. Twelve year outcomes following bacterial meningitis: further evidence for persisting effects. Arch. Dis. Child 83: 111-116.

Grimwood K, Anderson VA, Bond L, Catroppa C, Hore RL, Keir EH, Nolan T, Roberton DM, 1995. Adverse outcomes of bacterial meningitis in school-age survivors. Pediatrics 95: 646-656.

Haddock DR, 1971. Forty-seven cases of pyogenic meningitis in adults in Korle Bu Hospital, Accra. Ghana. Med. J. 10: 3-8.

Hahne S, Handford S, Ramsay M, 2002. W135 meningococcal carriage in Hajj pilgrims. Lancet 360: 2089-2090.

Hansman D, 1978. Chloramphenicol-resistant pneumococci in West Africa. Lancet 1: 1102-1103.

Hart CA, Cuevas L, 2003 Bacterial Meningitis. In: Cook G.C., Zumla A. (Eds.), Manson's Tropical Diseases. Elsevier Science Limited, pp. 981-994.

Hausdorff WP, 2002. Invasive pneumococcal disease in children: geographic and temporal variations in incidence and serotype distribution. Eur. J. Pediatr. 161 Suppl 2: S135-S139.

Hausdorff WP, Bryant J, Kloek C, Paradiso PR, Siber GR, 2000a. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part II. Clin. Infect. Dis. 30: 122-140.

Hausdorff WP, Bryant J, Paradiso PR, Siber GR, 2000b. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin. Infect. Dis. 30: 100-121.

Henrichsen J, 1995. Six newly recognized types of *Streptococcus pneumoniae*. J Clin. Microbiol. 33: 2759-2762.

Henriques NB, Kalin M, Ortqvist A, Akerlund T, Liljequist BO, Hedlund J, Svenson SB, Zhou J, Spratt BG, Normark S, Kallenius G, 2001. Dynamics of penicillin-susceptible clones in invasive pneumococcal disease. J. Infect. Dis. 184: 861-869.

Hickey J, 1997. The Clinical Practice of Neurology and Neurosurgical. J.B Lippincott., Philodelphia.

Hill PC, Akisanya A, Sankareh K, Cheung YB, Saaka M, Lahai G, Greenwood BM, Adegbola RA, 2006. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Gambian villagers. Clin. Infect Dis 43: 673-679.

Hirst C, Owusu-Ofori S, 2002. Prophylactic antibiotics for preventing pneumococcal infection in children with sickle cell disease. Cochrane Database of Systematic Reviews.

Hodgson A., Smith T, Gagneux S, Enos K.E., Adjuik M, Pluschke G, Binka F, Genton B. Meningococcal meningitis in northern Ghana: epidemiological features of the 1997 outbreak in the Kassena-Nankana district. Meningococcal Meningitis in Northern Ghana: Epidemiological and Clinical Features, Risk Factors, Survival and Sequelae. 19-39. 2002. University of Basel. 2002.

Hodgson A, Smith T, Gagneux S, Adjuik M, Pluschke G, Mensah NK, Binka F, Genton B, 2001a. Risk factors for meningococcal meningitis in northern Ghana. Trans. R. Soc. Trop. Med. Hyg. 95: 477-480.

Hodgson A, Smith T, Gagneux S, Akumah I, Adjuik M, Pluschke G, Binka F, Genton B, 2001b. Survival and sequelae of meningococcal meningitis in Ghana. Int. J. Epidemiol. 30: 1440-1446.

Hodgson AVO, 2002. Meningococcal Meningitis in Northern Ghana: Epidemiology and Clinical Features, Risk Factors, Survival and Sequelae. University of Basel, PhD thesis, Basel.

Hoge CW, Reichler MR, Dominguez EA, Bremer JC, Mastro TD, Hendricks KA, Musher DM, Elliott JA, Facklam RR, Breiman RF, 1994. An epidemic of pneumococcal disease in an overcrowded, inadequately ventilated jail. N. Engl. J Med. 331: 643-648.

Horn AE, 1908. Report on an investigation of cerebrospinal fever in the northern territories of the Gold Coast in 1908. Journal of Tropical Medicine and Hygiene 11: 358-364.

Hortal M, Camou T, Palacio R, Dibarboure H, Garcia A, 2000. Ten-year review of invasive pneumococcal diseases in children and adults from Uruguay: clinical spectrum, serotypes, and antimicrobial resistance. Int. J Infect Dis. 4: 91-95.

Huang SS, Platt R, Rifas-Shiman SL, Pelton SI, Goldmann D, Finkelstein JA, 2005. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. Pediatrics 116: e408-e413.

Isenberg Henry D.(ed.), 1998. Essential Procedures for Clinical Microbiology. American Society for Microbiology .

Janoff EN, O'Brien J, Thompson P, Ehret J, Meiklejohn G, Duvall G, Douglas JM, Jr., 1993. *Streptococcus pneumoniae* colonization, bacteremia, and immune response among persons with human immunodeficiency virus infection. J Infect Dis 167: 49-56.

Jódar L, Feavers IM, Salisbury D, Granoff DM, 2002. Development of vaccines against meningococcal disease. Lancet 359: 1499-1508.

John AB, Ramlal A, Jackson H, Maude GH, Sharma AW, Serjeant GR, 1984. Prevention of pneumococcal infection in children with homozygous sickle cell disease. Br. Med J (Clin. Res. Ed) 288: 1567-1570.

Johnston RB, Jr., 1991. Pathogenesis of pneumococcal pneumonia. Rev Infect Dis 13 Suppl 6: S509-S517.

Jolley KA, Kalmusova J, Feil EJ, Gupta S, Musilek M, Kriz P, Maiden MC, 2000. Carried meningococci in the Czech Republic: a diverse recombining population. J. Clin. Microbiol. 38: 4492-4498.

Kaninda AV, Belanger F, Lewis R, Batchassi E, Aplogan A, Yakoua Y, Paquet C, 2000. Effectiveness of incidence thresholds for detection and control of meningococcal meningitis epidemics in northern Togo. Int J. Epidemiol. 29: 933-940.

Kaplan SL, Mason EO, Jr., Wald ER, Schutze GE, Bradley JS, Tan TQ, Hoffman JA, Givner LB, Yogev R, Barson WJ, 2004. Decrease of invasive pneumococcal infections in children among 8 children's hospitals in the United States after the introduction of the 7-valent pneumococcal conjugate vaccine. Pediatrics 113: 443-449.

Kaplan SL, Smith EO, Wills C, Feigin RD, 1986. Association between preadmission oral antibiotic therapy and cerebrospinal fluid findings and sequelae caused by *Haemophilus influenzae* type b meningitis. Pediatr. Infect. Dis. 5: 626-632.

Kastenbauer S, Pfister HW, 2003. Pneumococcal meningitis in adults: spectrum of complications and prognostic factors in a series of 87 cases. Brain 126: 1015-1025.

Kaye KS, Fraimow HS, Abrutyn E, 2000. Pathogens resistant to antimicrobial agents. Epidemiology, molecular mechanisms, and clinical management. Infect Dis Clin. North Am 14: 293-319.

Kaye KS, Kaye D, 2000. Multidrug-resistant Pathogens: Mechanisms of Resistance and Epidemiology. Curr. Infect Dis Rep. 2: 391-398.

Kellner JD, Scheifele DW, Halperin SA, Lebel MH, Moore D, Le SN, Ford-Jones EL, Law B, Vaudry W, 2002. Outcome of penicillin-nonsusceptible *Streptococcus pneumoniae* meningitis: a nested case-control study. Pediatr. Infect. Dis. J. 21: 903-910.

Kellogg C, Griffin D, Garrison V, Peak K, Royall N, Smith R, Shinn E, 2004. Characterization of aerosolized bacteria and fungi from desert dust events in Mali, West Africa. Aerobiologia 20: 99-110.

Kim PE, Musher DM, Glezen WP, Rodriguez-Barradas MC, Nahm WK, Wright CE, 1996. Association of invasive pneumococcal disease with season, atmospheric conditions, air pollution, and the isolation of respiratory viruses. Clin. Infect Dis 22: 100-106.

Klugman KP, 2002. The successful clone: the vector of dissemination of resistance in *Streptococcus pneumoniae*. J. Antimicrob. Chemother. 50 Suppl S2: 1-5.

Kornelisse RF, Westerbeek CM, Spoor AB, van der Heijde B, Spanjaard L, Neijens HJ, de GR, 1995. Pneumococcal meningitis in children: prognostic indicators and outcome. Clin. Infect. Dis. 21: 1390-1397.

Kulahi I, Ozturk M, Bilen C, 1997. Evaluation of hearing loss with auditory brainstem responses in the early and late period of bacterial meningitis in children. Journal of laryngology and otology 111: 223-227.

Kwara A, Adegbola RA, Corrah PT, Weber M, Achtman M, Morelli G, Caugant DA, Greenwood BM, 1998. Meningitis caused by a serogroup W135 clone of the ET-37 complex of *Neisseria meningitidis* in West Africa. Trop. Med. Int. Health 3: 742-746.

Kyaw MH, Christie P, Clarke SC, Mooney JD, Ahmed S, Jones IG, Campbell H, 2003. Invasive pneumococcal disease in Scotland, 1999-2001: use of record linkage to explore associations between patients and disease in relation to future vaccination policy. Clin. Infect Dis 37: 1283-1291.

Kyaw MH, Lynfield R, Schaffner W, Craig AS, Hadler J, Reingold A, Thomas AR, Harrison LH, Bennett NM, Farley MM, Facklam RR, Jorgensen JH, Besser J, Zell ER, Schuchat A, Whitney CG, 2006. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. N. Engl. J Med 354: 1455-1463.

Lapeyssonnie L, 1963. La méningite cérébro-spinale en Afrique. Bulletin of the World Health Organisation. 28 (Supplement): 3-114.

Leib SL, Heimgartner C, Bifrare YD, Loeffler JM, Taauber MG, 2003. Dexamethasone aggravates hippocampal apoptosis and learning deficiency in pneumococcal meningitis in infant rats. Pediatr. Res. 54: 353-357.

Leino T, Auranen K, Jokinen J, Leinonen M, Tervonen P, Takala AK, 2001. Pneumococcal carriage in children during their first two years: important role of family exposure. Pediatr. Infect. Dis. J. 20: 1022-1027.

Lewis R, Nathan N, Diarra L, Belanger F, Paquet C, 2001. Timely detection of meningococcal meningitis epidemics in Africa. Lancet 358: 287-293.

Lin MT, Albertson TE, 2004. Genomic polymorphisms in sepsis. Crit Care Med 32, 569-579.

Lingappa JR, Al Rabeah AM, Hajjeh R, Mustafa T, Fatani A, Al Bassam T, Badukhan A, Turkistani A, Al Hamdan N, Al Jeffri M, Al Mazrou Y, Perkins BA, Popovic T, Mayer LW, Rosenstein NE, 2003. Serogroup W-135 Meningococcal Disease during the Hajj, 2000. Emerg. Infect. Dis. 9: 665-671.

Lipsitch M, 1999. Bacterial vaccines and serotype replacement: lessons from *Haemophilus influenzae* and prospects for *Streptococcus pneumoniae*. Emerg. Infect. Dis. 5: 336-345.

Lloyd-Evans N, O'Dempsey TJ, Baldeh I, Secka O, Demba E, Todd JE, McArdle TF, Banya WS, Greenwood BM, 1996. Nasopharyngeal carriage of pneumococci in Gambian children and in their families. Pediatr. Infect. Dis. J. 15: 866-871.

Mackie EJ, Shears P, Frimpong E, Mustafa-Kutana SN, 1992. A study of bacterial meningitis in Kumasi, Ghana. Ann. Trop. Paediatr. 12: 143-148.

MacLennan J, Obaro S, Deeks J, Williams D, Pais L, Carlone G, Moxon R, Greenwood B, 1999. Immune response to revaccination with meningococcal A and C polysaccharides in Gambian children following repeated immunisation during early childhood. Vaccine 17: 3086-3093.

Maiden MC, 2004. Dynamics of bacterial carriage and disease: lessons from the meningococcus. Adv. Exp. Med. Biol. 549: 23-29.

Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG, 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S A 95: 3140-3145.

Maiden MC, Spratt BG, 1999. Meningococcal conjugate vaccines: new opportunities and new challenges. Lancet 354: 615-616.

Maiden MC, Stuart JM, 2002. Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. Lancet 359: 1829-1831.

Mao C, Harper M, McIntosh K, Reddington C, Cohen J, Bachur R, Caldwell B, Hsu HW, 1996. Invasive pneumococcal infections in human immunodeficiency virus-infected children. J Infect Dis. 173: 870-876.

Mar ID, Denis F, Cadoz M, 1979. [Epidemiologic features of pneumococcal meningitis in Africa. Clinical and serotypical aspects (author's transl)]. Pathol. Biol. (Paris) 27: 543-548.

Marchiafava N, Celli A, 1884. Spora i micrococchi della meningite cerebrospinale epidemica. Gazetta degli Ospitali 8: 59-60.

Marx RD, Baer ST, 2001. Spontaneous recovery of profound post-meningitic hearing loss. J. Laryngol. Otol. 115: 412-414.

Mayer LW, Reeves MW, Al Hamdan N, Sacchi CT, Taha MK, Ajello GW, Schmink SE, Noble CA, Tondella ML, Whitney AM, Al Mazrou Y, Al Jefri M, Mishkhis A, Sabban S, Caugant DA, Lingappa J, Rosenstein NE, Popovic T, 2002. Outbreak of W135 meningococcal disease in 2000: not emergence of a new W135 strain but clonal expansion within the electophoretic type-37 complex. J. Infect. Dis. 185: 1596-1605.

McEllistrem MC, Adams JM, Patel K, Mendelsohn AB, Kaplan SL, Bradley JS, Schutze GE, Kim KS, Mason EO, Wald ER, 2005. Acute otitis media due to penicillin-nonsusceptible *Streptococcus*

pneumoniae before and after the introduction of the pneumococcal conjugate vaccine. Clin. Infect Dis 40: 1738-1744.

McIntyre PB, Berkey CS, King SM, Schaad UB, Kilpi T, Kanra GY, Perez CM, 1997. Dexamethasone as adjunctive therapy in bacterial meningitis. A meta-analysis of randomized clinical trials since 1988. JAMA 278: 925-931.

MOH(GNDP), 2004 Infectious Diseases and Infestations. In: MOH(GNDP) (Ed.), Standard Treatment Guidelines. Ghana National Drugs Programme (GNDP), Ministry of Health., Accra, pp. 355-381.

Molesworth AM, Cuevas LE, Connor SJ, Morse AP, Thomson MC, 2003. Environmental risk and meningitis epidemics in Africa. Emerg Infect Dis 9: 1287-1293.

Molesworth AM, Thomson MC, Connor SJ, Cresswell MP, Morse AP, Shears P, Hart CA, Cuevas LE, 2002. Where is the meningitis belt? Defining an area at risk of epidemic meningitis in Africa. Trans. R. Soc. Trop. Med. Hyg. 96: 242-249.

Monnier Y, 1980. méningite cérébro-spinale, harmattan et déforstation. Cahiers d`Outre-Mer 33: 103-122.

Montefiore D, Alausa OK, Sobayo E, 1978. Pyogenic meningitis in Ibadan, Nigeria. A 15-month prospective study. Scand. J Infect Dis. 10: 113-117.

Montgomery JM, Lehmann D, Smith T, Michael A, Joseph B, Lupiwa T, Coakley C, Spooner V, Best B, Riley ID, ., 1990. Bacterial colonization of the upper respiratory tract and its association with acute lower respiratory tract infections in Highland children of Papua New Guinea. Rev. Infect. Dis. 12 Suppl 8: S1006-S1016.

Moore PS, 1992. Meningococcal meningitis in sub-Saharan Africa: a model for the epidemic process. Clin. Infect. Dis. 14: 515-525.

Moore PS, Harrison LH, Telzak EE, Ajello GW, Broome CV, 1988. Group A meningococcal carriage in travelers returning from Saudi Arabia. JAMA 260: 2686-2689.

Morelli G, Malorny B, Muller K, Seiler A, Wang JF, del Valle J, Achtman M, 1997. Clonal descent and microevolution of *Neisseria meningitidis* during 30 years of epidemic spread. Mol. Microbiol. 25: 1047-1064.

Mueller JE, Yaro S, Traore Y, Sangare L, Tarnagda Z, Njanpop-Lafourcade BM, Borrow R, Gessner BD, 2006. *Neisseria meningitidis* serogroups A and W-135: carriage and immunity in Burkina Faso, 2003. J. Infect. Dis. 193: 812-820.

Nadol JB, Jr., 1978. Hearing loss as a sequela of meningitis. Laryngoscope 88: 739-755.

Nathan N, Borel T, Djibo A, Evans D, Djibo S, Corty JF, Guillerm M, Alberti KP, Pinoges L, Guerin PJ, Legros D, 2005. Ceftriaxone as effective as long-acting chloramphenicol in short-course treatment of meningococcal meningitis during epidemics: a randomised non-inferiority study. Lancet 366: 308-313.

Nau R, Haase S, Bunkowski S, Bruck W, 2002. Neuronal apoptosis in the dentate gyrus in humans with subarachnoid hemorrhage and cerebral hypoxia. Brain Pathol. 12: 329-336.

Ngom P, Debpuur C, Bawah AA, Kanyomse E, Ayuure J, Nazzar A, Binka F, 1999. Survey on disability in Upper East Region, Ghana. Navrongo Health Research Centre documentation note No. 40 (unpublished).

Nicolas P, Decousset L, Riglet V, Castelli P, Stor R, Blanchet G, 2001. Clonal expansion of sequence type (ST-)5 and emergence of ST-7 in serogroup A meningococci, Africa. Emerg. Infect. Dis. 7: 849-854.

Norbäch D, Wieslander G, Nordström K, Walinder R, Venge P, 2000. The Effect of Air Humidification on Symptoms and Nasal Patency, Tear Film Stability, and Biomarkers in Nasal Lavage: A 6 Weeks` Longitudinal Study. Indoor Built Environ 9: 28-34.

Nuorti JP, Butler JC, Crutcher JM, Guevara R, Welch D, Holder P, Elliott JA, 1998. An outbreak of multidrug-resistant pneumococcal pneumonia and bacteremia among unvaccinated nursing home residents. N. Engl. J Med. 338: 1861-1868.

Nuorti JP, Butler JC, Farley MM, Harrison LH, McGeer A, Kolczak MS, Breiman RF, 2000a. Cigarette smoking and invasive pneumococcal disease. Active Bacterial Core Surveillance Team. N. Engl. J Med. 342: 681-689.

Nuorti JP, Butler JC, Gelling L, Kool JL, Reingold AL, Vugia DJ, 2000b. Epidemiologic relation between HIV and invasive pneumococcal disease in San Francisco County, California. Ann. Intern. Med. 132: 182-190.

Nyarko P, Wontuo P, Nazzar A, Phillips J, Ngom P, Binka F. Comparing Mortality Patterns at INDEPTH Sites. Navrongo DSS, Ghana. INDEPTH network. Population and Health in Developing Countries. [1], 247-256. 2002. Ottawa, ON, International Development Research Centre. 2002. Ref Type: Serial (Book,Monograph)

O'Dempsey TJ, McArdle TF, Lloyd-Evans N, Baldeh I, Lawrence BE, Secka O, Greenwood B, 1996. Pneumococcal disease among children in a rural area of west Africa. Pediatr. Infect. Dis. J. 15: 431-437.

Obaro SK, Deubzer HE, Newman VO, Adegbola RA, Greenwood BM, Henderson DC, 2004. Serotype-specific pneumococcal antibodies in breast milk of Gambian women immunized with a pneumococcal polysaccharide vaccine during pregnancy. Pediatr. Infect. Dis. J. 23: 1023-1029.

Orvelid P, Backman A, Olcen P, 1999. PCR identification of the group A *Neisseria meningitidis* gene in cerebrospinal fluid. Scand. J. Infect. Dis. 31: 481-483.

Palmer GH, 2002. The highest priority: what microbial genomes are telling us about immunity. Vet. Immunol. Immunopathol. 85: 1-8.

Parent DC, I, Traore Y, Gessner BD, Antignac A, Naccro B, Njanpop-Lafourcade BM, Ouedraogo MS, Tiendrebeogo SR, Varon E, Taha MK, 2005. Bacterial meningitis in Burkina Faso: surveillance using field-based polymerase chain reaction testing. Clin. Infect. Dis. 40: 17-25.

Pastor P, Medley F, Murphy TV, 1998. Invasive pneumococcal disease in Dallas County, Texas: results from population-based surveillance in 1995. Clin. Infect. Dis. 26: 590-595.

Peltola H, 1983. Meningococcal disease: still with us. Rev Infect Dis 5: 71-91.

Pikis A, Kavaliotis J, Tsikoulas J, Andrianopoulos P, Venzon D, Manios S, 1996. Long-term sequelae of pneumococcal meningitis in children. Clin. Pediatr. (Phila) 35: 72-78.

Plotkowski M-C, Puchelle E, Beck G JJ, Hannoun C, 1986. Adherance of type 1 *Streptococcus pneumoniae* to tracheal epithelium of mice infected with influenza A/PR8 virus. Am Rev Respir Dis 134: 1040-1044.

Poehling KA, Talbot TR, Griffin MR, Craig AS, Whitney CG, Zell E, Lexau CA, Thomas AR, Harrison LH, Reingold AL, Hadler JL, Farley MM, Anderson BJ, Schaffner W, 2006. Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. JAMA 295: 1668-1674.

Popovic T, Sacchi CT, Reeves MW, Whitney AM, Mayer LW, Noble CA, Ajello GW, Mostashari F, Bendana N, Lingappa J, Hajjeh R, Rosenstein NE, 2000. *Neisseria meningitidis* serogroup W135 isolates associated with the ET-37 complex. Emerg. Infect. Dis. 6: 428-429.

Porat N, Trefler R, Dagan R, 2001. Persistence of two invasive *Streptococcus pneumoniae* clones of serotypes 1 and 5 in comparison to that of multiple clones of serotypes 6B and 23F among children in southern Israel. J. Clin. Microbiol. 39: 1827-1832.

Ramsay ME, Andrews N, Kaczmarski EB, Miller E, 2001. Efficacy of meningococcal serogroup C conjugate vaccine in teenagers and toddlers in England. Lancet 357: 195-196.

Ramsay ME, Andrews NJ, Trotter CL, Kaczmarski EB, Miller E, 2003. Herd immunity from meningococcal serogroup C conjugate vaccination in England: database analysis. BMJ 326: 365-366.

Rasmussen N, Johnsen NJ, Bohr VA, 1991. Otologic sequelae after pneumococcal meningitis: a survey of 164 consecutive cases with a follow-up of 94 survivors. Laryngoscope 101: 876-882.

Raymond J, Le T, I, Moulin F, Commeau A, Gendrel D, Berche P, 2000. Sequential colonization by *Streptococcus pneumoniae* of healthy children living in an orphanage. J Infect Dis 181: 1983-1988.

Regev-Yochay G, Dagan R, Raz M, Carmeli Y, Shainberg B, Derazne E, Rahav G, Rubinstein E, 2004a. Association between carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in Children. JAMA 292: 716-720.

Regev-Yochay G, Raz M, Dagan R, Porat N, Shainberg B, Pinco E, Keller N, Rubinstein E, 2004b. Nasopharyngeal carriage of *Streptococcus pneumoniae* by adults and children in community and family settings. Clin. Infect Dis 38: 632-639.

Regev-Yochay G, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, Etienne J, Blech M, Smollen G, Maayan-Metzger A, Leavitt A, Rahav G, Keller N, 2005. Methicillin-resistant *Staphylococcus aureus* in neonatal intensive care unit. Emerg Infect Dis 11: 453-456.

Regev-Yochay G, Trzcinski K, Thompson CM, Malley R, Lipsitch M, 2006. Interference between *Streptococcus pneumoniae* and *Staphylococcus aureus*: In vitro hydrogen peroxide-mediated killing by *Streptococcus pneumoniae*. J Bacteriol. 188: 4996-5001.

Reingold AL, Broome CV, Hightower AW, Ajello GW, Bolan GA, Adamsbaum C, Jones EE, Phillips C, Tiendrebeogo H, Yada A, 1985. Age-specific differences in duration of clinical protection after vaccination with meningococcal polysaccharide A vaccine. Lancet 2: 114-118.

Richardson MP, Reid A, Tarlow MJ, Rudd PT, 1997. Hearing loss during bacterial meningitis. Arch. Dis. Child 76: 134-138.

Richardson MP, Williamson TJ, Reid A, Tarlow MJ, Rudd PT, 1998. Otoacoustic emissions as a screening test for hearing impairment in children recovering from acute bacterial meningitis. Pediatrics 102: 1364-1368.

Richmond P, Kaczmarski E, Borrow R, Findlow J, Clark S, McCann R, Hill J, Barker M, Miller E, 2000. Meningococcal C polysaccharide vaccine induces immunologic hyporesponsiveness in adults that is overcome by meningococcal C conjugate vaccine. J Infect Dis 181: 761-764.

Robbins JB, Schneerson R, Gotschlich EC, 2005. Surveillance for bacterial meningitis by means of polymerase chain reaction. Clin. Infect. Dis. 40: 26-27.

Rudolph KM, Parkinson AJ, Reasonover AL, Bulkow LR, Parks DJ, Butler JC, 2000. Serotype distribution and antimicrobial resistance patterns of invasive isolates of *Streptococcus pneumoniae*: Alaska, 1991-1998. J Infect Dis. 182: 490-496.

Sandgren A, Sjostrom K, Olsson-Liljequist B, Christensson B, Samuelsson A, Kronvall G, Henriques NB, 2004. Effect of clonal and serotype-specific properties on the invasive capacity of *Streptococcus pneumoniae*. J. Infect. Dis. 189: 785-796.

Savory EC, Cuevas LE, Yassin MA, Hart CA, Molesworth AM, Thomson MC, 2006. Evaluation of the meningitis epidemics risk model in Africa. Epidemiol. Infect.: 1-13.

Schmidt H, Heimann B, Djukic M, Mazurek C, Fels C, Wallesch CW, Nau R, 2006. Neuropsychological sequelae of bacterial and viral meningitis. Brain 129: 333-345.

Schuchat A, Robinson K, Wenger JD, Harrison LH, Farley M, Reingold AL, Lefkowitz L, Perkins BA, 1997. Bacterial meningitis in the United States in 1995. Active Surveillance Team. N. Engl. J. Med. 337: 970-976.

Scott JA, Hall AJ, Dagan R, Dixon JM, Eykyn SJ, Fenoll A, Hortal M, Jette LP, Jorgensen JH, Lamothe F, Latorre C, Macfarlane JT, Shlaes DM, Smart LE, Taunay A, 1996. Serogroup-specific epidemiology of *Streptococcus pneumoniae*: associations with age, sex, and geography in 7,000 episodes of invasive disease. Clin. Infect Dis. 22: 973-981.

Semba RD, Bulterys M, Munyeshuli V, Gatsinzi T, Saah A, Chao A, Dushimimana A, 1996. Vitamin A deficiency and T-cell subpopulations in children with meningococcal disease. J Trop. Pediatr. 42: 287-290.

Shattuck KE, Chonmaitree T, 1992. The changing spectrum of neonatal meningitis over a fifteen-year period. Clin. Pediatr. (Phila) 31: 130-136.

Short W, Tunkel AR, 2000. Changing Epidemiology of Bacterial Meningitis in the United States. Curr Infect Dis Rep 2: 327-331.

Shultz TR, Tapsall JW, White PA, Ryan CS, Lyras D, Rood JI, Binotto E, Richardson CJ, 2003. Chloramphenicol-resistant *Neisseria meningitidis* containing catP isolated in Australia. J Antimicrob. Chemother. 52: 856-859.

Sirisinha S, Darip MD, Moongkarndi P, Ongsakul M, Lamb AJ, 1980. Impaired local immune response in vitamin A-deficient rats 3. Clin. Exp. Immunol. 40: 127-135.

Smith AW, Bradley AK, Wall RA, McPherson B, Secka A, Dunn DT, Greenwood BM, 1988. Sequelae of epidemic meningococcal meningitis in Africa. Trans. R. Soc. Trop. Med. Hyg. 82: 312-320.

Soro BN, Rey JL, Davis CE, Coulibaly A, Diomande I, 1988. [Epidemiological aspects of meningitis in the north of the Ivory Coast] 1. Med. Trop. (Mars.) 48: 145-148.

Spanos A, Harrell FE, Jr., Durack DT, 1989. Differential diagnosis of acute meningitis. An analysis of the predictive value of initial observations. JAMA 262: 2700-2707.

Spratt BG, 1994. Resistance to antibiotics mediated by target alterations. Science 264: 388-393.

Stenfors LE, Raisanen S, 1993. Secretory IgA-, IgG- and C3b-coated bacteria in the nasopharynx of otitis-prone and non-otitis-prone children. Acta Otolaryngol. 113: 191-195.

Stephens DS, 1999. Unclocking the meningococcus: dynamics of carriage and disease 3. Lancet 353: 941-942.

Stephens D&FM, 1991. Pathogenic events during infection. Infectious Diseases 13: 22-23.

Sultan B, Labadi K, Guegan JF, Janicot S, 2005. Climate drives the meningitis epidemics onset in west Africa. PLoS. Med 2: e6.

Swartz MN, 2004. Bacterial meningitis--a view of the past 90 years. N. Engl. J Med. 351: 1826-1828.

Taha MK, 2000. Simultaneous approach for nonculture PCR-based identification and serogroup prediction of *Neisseria meningitidis*. J. Clin. Microbiol. 38: 855-857.

Taha MK, Achtman M, Alonso JM, Greenwood B, Ramsay M, Fox A, Gray S, Kaczmarski E, 2000. Serogroup W135 meningococcal disease in Hajj pilgrims. Lancet 356: 2159.

Taha MK, Deghmane AE, Antignac A, Zarantonelli ML, Larribe M, Alonso JM, 2002a. The duality of virulence and transmissibility in *Neisseria meningitidis*. Trends Microbiol. 10: 376-382.

Taha MK, Giorgini D, Ducos-Galand M, Alonso JM, 2004. Continuing diversification of *Neisseria meningitidis* W135 as a primary cause of meningococcal disease after emergence of the serogroup in 2000. J. Clin. Microbiol. 42: 4158-4163.

Taha MK, Parent DC, I, Schlumberger M, Sanou I, Djibo S, de Chabalier F, Alonso JM, 2002b. *Neisseria meningitidis* serogroups W135 and A were equally prevalent among meningitis cases occurring at the end of the 2001 epidemics in Burkina Faso and Niger. J. Clin. Microbiol. 40: 1083-1084.

Talbot TR, Poehling KA, Hartert TV, Arbogast PG, Halasa NB, Edwards KM, Schaffner W, Craig AS, Griffin MR, 2005. Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. Am. J. Med. 118: 285-291.

Talbot TR, Poehling KA, Hartert TV, Arbogast PG, Halasa NB, Mitchel E, Schaffner W, Craig AS, Edwards KM, Griffin MR, 2004. Reduction in high rates of antibiotic-nonsusceptible invasive pneumococcal disease in Tennessee after introduction of the pneumococcal conjugate vaccine. Clin. Infect Dis 39: 641-648.

Thomson MC, Molesworth AM, Djingarey MH, Yameogo KR, Belanger F, Cuevas LE, 2006. Potential of environmental models to predict meningitis epidemics in Africa. Trop. Med Int. Health 11: 781-788.

Tikhomirov E, Santamaria M, Esteves K, 1997. Meningococcal disease: public health burden and control. World Health Stat. Q. 50: 170-177.

Torzillo PJ, Hanna JN, Morey F, Gratten M, Dixon J, Erlich J, 1995. Invasive pneumococcal disease in central Australia. Med. J. Aust. 162: 182-186.

Traore Y, Njanpop-Lafourcade BM, Adjogble KL, Lourd M, Yaro S, Nacro B, Drabo A, Parent DC, I, Mueller JE, Taha MK, Borrow R, Nicolas P, Alonso JM, Gessner BD, 2006. The rise and fall of epidemic *Neisseria meningitidis* serogroup W135 meningitis in Burkina Faso, 2002-2005. Clin. Infect. Dis. 43: 817-822.

Trotter CL, Andrews NJ, Kaczmarski EB, Miller E, Ramsay ME, 2004. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. Lancet 364: 365-367.

Trotter CL, Gay NJ, Edmunds WJ, 2005. Dynamic models of meningococcal carriage, disease, and the impact of serogroup C conjugate vaccination. Am. J Epidemiol. 162: 89-100.

Tugwell P, Greenwood BM, Warrell DA, 1976. Pneumococcal meningitis: a clinical and laboratory study. Q. J. Med. 45: 583-601.

Tunkel AR, Scheld WM, 1993. Pathogenesis and pathophysiology of bacterial meningitis. Clin. Microbiol. Rev 6: 118-136.

Tunkel AR, Wispelwey B, Scheld WM, 1990. Pathogenesis and pathophysiology of meningitis. Infect Dis Clin. North Am 4: 555-581.

Usen S, Adegbola R, Mulholland K, Jaffar S, Hilton S, Oparaugo A, Omosigho C, Lahai G, Corrah T, Palmer A, Schneider G, Weber M, Greenwood B, 1998. Epidemiology of invasive pneumococcal disease in the Western Region, The Gambia. Pediatr. Infect Dis. J 17: 23-28.

van de Beek D, de Gans J, 2004b. Dexamethasone and pneumococcal meningitis. Ann. Intern. Med. 141: 327.

van de Beek D, de Gans J, 2004a. Meningitis-associated hearing loss: protection by adjunctive antioxidant therapy. Ann. Neurol. 55: 597-598.

van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M, 2004. Clinical features and prognostic factors in adults with bacterial meningitis. N. Engl. J. Med. 351: 1849-1859.

van de Beek D, de Gans J, Tunkel AR, Wijdicks EF, 2006. Community-acquired bacterial meningitis in adults. N. Engl. J. Med. 354: 44-53.

van de Beek D, Schmand B, de Gans J, Weisfelt M, Vaessen H, Dankert J, Vermeulen M, 2002. Cognitive impairment in adults with good recovery after bacterial meningitis. J. Infect. Dis. 186: 1047-1052.

van der Flier M, Geelen SP, Kimpen JL, Hoepelman IM, Tuomanen EI, 2003. Reprogramming the host response in bacterial meningitis: how best to improve outcome? Clin. Microbiol. Rev 16: 415-429.

Van Esso D, Fontanals D, Uriz S, Morera MA, Juncosa T, Latorre C, Duran M, 1987. *Neisseria meningitidis* strains with decreased susceptibility to penicillin. Pediatr. Infect Dis J 6: 438-439.

Varaine F, Caugant DA, Riou JY, Kondé MK, Soga G, Nshimirimana D, Muhirwa G, Ott D, Høiby EA, Fermon F, and Moren A, 1997. Meningitis outbreak and vaccination strategy. Trans. R. Soc. Trop. Med. Hyg. 91: 3-7.

Vela Coral MC, Fonseca N, Castaneda E, Di Fabio JL, Hollingshead SK, Briles DE, 2001. Pneumococcal surface protein A of invasive *Streptococcus pneumoniae* isolates from Colombian children. Emerg. Infect. Dis. 7: 832-836.

Visser VE, Hall RT, 1980. Lumbar puncture in the evaluation of suspected neonatal sepsis. J Pediatr. 96: 1063-1067.

Waddy BB, 1957. African epidemic cerebrospinal meningitis. Journal of Tropical Medicine and Hygiene 60: 179-189.

Weisfelt M, van de BD, Spanjaard L, Reitsma JB, de GJ, 2006. Clinical features, complications, and outcome in adults with pneumococcal meningitis: a prospective case series. Lancet Neurol. 5: 123-129.

Weldon K, 1988 Anatomy and Physiology of the Nervous system. Neuro-oncology for nurses. Whurr, London., pp. 1-28.

Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, Reingold A, Cieslak PR, Pilishvili T, Jackson D, Facklam RR, Jorgensen JH, Schuchat A, 2003. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N. Engl. J Med 348: 1737-1746.

Whitney CG, Farley MM, Hadler J, Harrison LH, Lexau C, Reingold A, Lefkowitz L, Cieslak PR, Cetron M, Zell ER, Jorgensen JH, Schuchat A, 2000. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. N. Engl. J Med 343: 1917-1924.

Whittle H, Evans-Jones G, Onyewotu I et al., 1975. Group C meningococcal meningitis in the northern savannah of Africa. Lancet 1: 1377.

WHO. Control of epidemic meningococcal disease. WHO practical guidelines. http://www.who.int/csr/resources/publications/meningitis/WHO_EMC_BAC_98_3_EN/en/ [2nd], 1-82. 1998.

WHO, 1999. Pneumococcal vaccines. WHO position paper. Wkly. Epidemiol. Rec. 74: 177-183.

WHO, 2000. Detecting meningococcal meningitis epidemics in highly-endemic African countries. Weekly Epidemiological Record 75: 305-312.

WHO, 2002. Meningococcal disease, serogroup W135, Burkina Faso, preliminary report, 2002. Weekly Epidemiological Record 77: 141-156.

WHO, 2003a. Meningococcal meningitis. Fact sheet N°141.

WHO. Update on the AFRO Paediatric Bacterial Meningitis Surveillance Network. [38], 1-6. 2003b.

WHO, Regional Office for Africa . Vaccine Preventable Disease Bulletin. WHO.

WHO, 2005. Enhanced surveillance of epidemic meningococcal meningitis in Africa:a three-year experience. Wkly. Epidemiol. Rec. 80: 313-320.

Wilder-Smith A, Barkham TM, Chew SK, Paton NI, 2003a. Absence of *Neisseria meningitidis* W-135 Electrophoretic Type 37 during the Hajj, 2002. Emerg. Infect. Dis. 9: 734-737.

Wilder-Smith A, Barkham TM, Ravindran S, Earnest A, Paton NI, 2003b. Persistence of W135 *Neisseria meningitidis* carriage in returning Hajj pilgrims: risk for early and late transmission to household contacts. Emerg. Infect. Dis. 9: 123-126.

Wilder-Smith A, Memish Z, 2003. Meningococcal disease and travel. Int. J. Antimicrob. Agents 21: 102-106.

Wiswell TE, Baumgart S, Gannon CM, Spitzer AR, 1995. No lumbar puncture in the evaluation for early neonatal sepsis: will meningitis be missed? Pediatrics 95: 803-806.

Wong WY, Overturf GD, Powars DR, 1992. Infection caused by *Streptococcus pneumoniae* in children with sickle cell disease: epidemiology, immunologic mechanisms, prophylaxis, and vaccination. Clin. Infect Dis. 14: 1124-1136.

Woods C, Armstrong G, Sackey S, Tetteh C, Bugri S, Perkins B, Rosenstein N, 2000. Emergency vaccination against epidemic meningitis in Ghana: implications for the control of meningococcal disease in West Africa. Lancet 355: 30-33.

Woolley A, Kirk K, Neumann A, Jr., McWilliams S, Murray J, Freind D, Wiatrak B, 1999. Risk factors for hearing loss from meningitis in children: the Children's Hospital experience. Arch. Otolaryngol. Head Neck Surg. 125: 509-514.

Yamamoto M, McDaniel LS, Kawabata K, Briles DE, Jackson RJ, McGhee JR, Kiyono H, 1997. Oral immunization with PspA elicits protective humoral immunity against *Streptococcus pneumoniae* infection. Infect Immun. 65: 640-644.

Yaro S, Lourd M, Traore Y, Njanpop-Lafourcade BM, Sawadogo A, Sangare L, Hien A, Ouedraogo MS, Sanou O, Parent DC, I, Koeck JL, Gessner BD, 2006. Epidemiological and molecular characteristics of a highly lethal pneumococcal meningitis epidemic in Burkina Faso. Clin. Infect. Dis. 43: 693-700.

Yazdankhah SP, Caugant DA, 2004. *Neisseria meningitidis*: an overview of the carriage state. J. Med. Microbiol. 53: 821-832.

Yoshinaga-Itano C, Apuzzo ML, 1998a. Identification of hearing loss after age 18 months is not early enough. Am. Ann. Deaf 143: 380-387.

Yoshinaga-Itano C, Apuzzo ML, 1998b. The development of deaf and hard of hearing children identified early through the high-risk registry. Am. Ann. Deaf 143: 416-424.

Yoshinaga-Itano C, Sedey AL, Coulter DK, Mehl AL, 1998. Language of early- and later-identified children with hearing loss. Pediatrics 102: 1161-1171.

Zhang Q, Choo S, Everard J, Jennings R, Finn A, 2000. Mucosal immune responses to meningococcal group C conjugate and group A and C polysaccharide vaccines in adolescents. Infect. Immun. 68: 2692-2697.

Zhu P, van der EA, Falush D, Brieske N, Morelli G, Linz B, Popovic T, Schuurman IG, Adegbola RA, Zurth K, Gagneux S, Platonov AE, Riou JY, Caugant DA, Nicolas P, Achtman M, 2001. Fit genotypes and escape variants of subgroup III *Neisseria meningitidis* during three pandemics of epidemic meningitis. Proc. Natl. Acad. Sci. U. S A 98: 5234-5239.

APPENDIX. Procedure for performing lumbar puncture.

All patients with suspected meningitis reporting to any of the health facilities in the Kasena Nankana District between 1998 and 2004 were recruited into the pneumococcal meningitis severe case study. Subjects had lumbar punctures (LP) done and cerebrospinal fluid samples collected were analyzed by standard microbiological methods.

The only way to confirm bacterial meningitis is by examination of cerebrospinal fluid (CSF) via LP since clinical signs are non specific and unreliable and blood cultures may be negative in 15-55% of cases (Shattuck and Chonmaitree, 1992; Visser and Hall, 1980; Wiswell et al., 1995). LP involves withdrawing cerebrospinal fluid by the insertion of a hollow needle with a stylet into the lumbar subarachnoid space (Hickey, 1997). Approximately 500ml of CSF are produced (through filtration from the choroids plexuses of the brain) and reabsorbed each day (Weldon, 1988), with 120-150ml present at one time.

To perform an LP on a patient with bacterial meningitis the following are needed: material for sterile technique (only gloves and mask are necessary), spinal Needle, 20 and 22-gauge, three-way stopcock, sterile drapes, 1% lidocaine without epinephrine in a 5-cc syringe with a 22 and 25-gauge needles, material for skin sterilization, adhesive dressing and sponges10 X 10 cm.

A detailed discussion with the patient and/or the caregivers about the risks/benefits of the LP procedure is done by the physician and informed consent obtained before the procedure is carried out. The patient is placed in the lateral decubitus position lying on the edge of the bed and facing away from the operator in a knee-chest position with the neck flexed and head on a pillow, so that the entire cranio-spinal axis is parallel to the bed. Sitting position is the second choice. The patient must be calm and cooperative.

The spinal cord typically ends at the L1 level in adults (slightly lower in children). The iliac crests are located and an imaginary line drawn joining them. A second imaginary line along the spinous processes is drawn form the base of scull to the sacrum. The L4 spinous process palpated, and the spot marked with a fingernail.

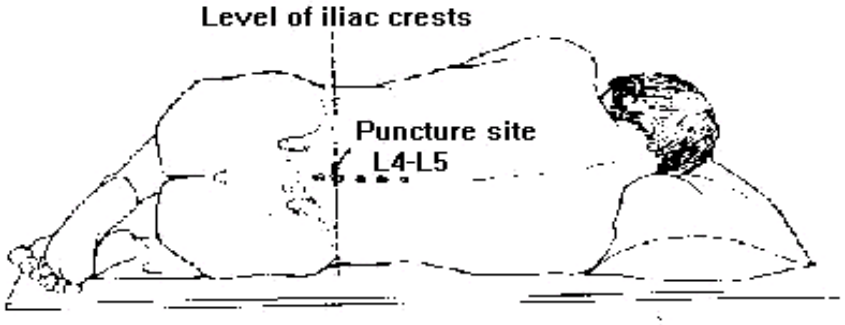

Figure A.1 Position of a patient for lumbar puncture.
(Source: Carlos Eduardo Reis CE (http://www.medstudents.com.br/proced/lumbpunc.htm))

The skin is prepared using chlorhexidine 70% or betadine solution by starting at the puncture site and working outward in concentric circles. Wearing sterile gloves the patient is draped. Aseptic techniques must be used throughout the procedure. To avoid irritative arachnoiditis all traces of iodine with alcohol are removed prior to performing the LP. The skin between the spinous processes (L4-L5) is anaesthetized using the 1% lidocaine in the 5 mL syringe with the 25-gauge needle. The disposable 22-gauge LP needle is inserted at the point of the finger mark in the midline with the needle parallel to the floor and the point directed toward the patient's umbilicus advancing slowly until a "pop" (piercing a membrane of the dura) is heard. The stylet is then withdrawn in every 2- to 3-mm from the needle to check for CSF return. If the needle meets the bone or if blood returns (hitting the venous plexus anterior to the spinal canal), it is withdrawn to the skin and redirected. If CSF return cannot be obtained, one disk space down is tried. To alleviate anxiety of the patient and discomfort the procedure is discontinued after three failed attempts and some else tries at a later time.

When cerebrospinal fluid begins to flow from the needle the first few drops are discarded. Accurate placement of the needle results in a flow of the CSF, which normally is clear and colorless. To avoid trapping a nerve root against the needle and injury, the CSF is never aspirated. 3.5 cc of CSF is allowed to flow into each of the three sterile nunc tubes which are then labeled accordingly and sent to the laboratory as soon as possible for glucose, protein, Gram stain, cell count and differential, culture and sensitivity and the rest frozen at -70 ^{o}C for further molecular analysis. The needle is withdrawn without replacing the stylet. The puncture site is dressed with sterile guaze and the patient made to lie in bed for a few hours.

Contraindications for LP include patients with infections near the puncture site as contamination from an infection could cause meningitis, patients with increased intracranial pressure (as cerebral or cerebellar herniation could occur in these patients), patients that have degenerative vertebral joint disease (it may be difficult to locate and pass a needle through the interspinal space), uncontrolled bleeding diathesis (patients on anticoagulants), lack of patient cooperation.

Complications following LP include, post–spinal tap headache, introduction of bacteria into the CSF leading to aggravation of the meningitis, back or leg pain/paresthesia, accidental puncture of the spinal cord, accidental puncture of the aorta or vena cava, causing serious hemorrhage, herniation of the brain (in a patient with increased pressure, the sudden decrease of pressure through the LP, could cause herniation of the brain - compression of the brain stem), nerve root trauma (eg, previous surgery in the area, scar tissue), cranial, cervical, and lumbar subdural (more common) hematomas (eg, patients on anticoagulation therapy), also possible but very rare are discitis, system/portal venous gas (following a traumatic tap), clinical deterioration in the presence of dural arteriovenous fistula, symptomatic pneumocephalus in a patient with normal pressure hydrocephalus, cranial nerve palsies (4th and 6th).

CURRICULCUM VITAE

Personal data

Name:	Abudulai Adams Forgor
Date of birth:	24 October 1962
Nationality:	Ghanaian
Marital status:	Married

Educational background

1977 – 1982	Damongo Secondary School, Damongo, Ghana (GCE O`Level)
1982 – 1984	Nandom Secondary School, Nandom, Ghana (GCE A`Level)
1985 – 1992	Vitebsk State Medical Institute, Vitebsk, Belarus (MD)
2000 – 2001	School of Public Health, University of Ghana, Legon (MPH)
2005	London School of Hygiene & Tropical Medicine, London.UK (DTM&H of the Royal College of Physicians (London))
2002 – 2006	Swiss Tropical Institute, University of Basel (PhD in Epidemiology)

Professional Experience

1992 – 1993	Housemanship, Komfo Anokye Teaching Hospital, Kumasi, Ghana.
1994 – 1995	Medical Officer, Komfo Anokye Teaching Hospital, Kumasi, Ghana.
1995 – 2002	Senior Medical Officer In-charge, Sampa Government Hospital, Sampa, Ghana.
2002 -	District Director of Health Services, Jaman District, Ghana.
2002 – to date	Senior Medical Officer, War Memorial Hospital, Navrongo, Ghana.
2002 – 2004	Senior Medical Officer, Navrongo Health Research Centre, Navrongo, Ghana.
2002 – to date	Head, Cerebrospinal Meningitis Project, Navrongo Health Research Centre, Navrongo. Ghana.

2004 – to date Head, Heptavalent conjugate vaccine trial Project Navrongo Health Research Centre, Navrongo. Ghana.

Membership of professional bodies

Ghana Medical Association

American College of Epidemiology

International Epidemiological Association

American Society of Tropical Medicine and Hygiene

Royal Society of Tropical Medicine and Hygiene

Canadian Society of Epidemiologist and Biostatisticians

Publications

1.Leimkugel J, **Forgor AA**, Gagneux S, et al. An outbreak of serotype 1 Streptococcus pneumoniae meningitis in northern Ghana with features that are characteristic of Neisseria meningitidis meningitis epidemics. J Infect Dis 2005 Jul 15; 192 (2): 192-9.

2. **Forgor AA**, Leimkugel J, Hodgson A, Bugri A, Dangy JP, Gagneux S, Smith T, Pluschke G (2005). Emergence of W135 meningococcal meningitis in Ghana. Trop Med Int Health 10: 1229-1234.

3. Julia Leimkugel, Abraham Hodgson, **Abudulai Adams Forgor**, Valentin Pflüger, Jean-Pierre Dangy, Tom Smith, Mark Achtman, Sébastien Gagneux and Gerd Pluschke (March 2007). Clonal Waves of Colonization and Disease of *Neisseria meningitidis* in the African Meningitis Belt. An Eight Year Longitudinal Study in Northern Ghana. PLoS Med 4 (3) e101

4. A Hodgson, **AA Forgor**, D Chandramohan, Z Reed, F Binka, D Boutriau, B Greenwood Immunogenicity, reactogenicity and safety of a novel DTPw-HBV/Hib-MenAC conjugate combination vaccine administered to infants in Northern Ghana (Prpared for submition to Int J Infect Dis).

5. Julia Leimkugel, **Abudulai Forgor**, Jean-Pierre Dangy, Valentin Pflüger, Sebastien Gagneux, Abraham Hodgson, Gerd Pluschke. Genetic diversification of *Neisseria*

meningitidis during waves of colonization and disease in the meningitis belt of sub-Saharan Africa (Vaccine (2007), doi:10.1016/j.vaccine.2007.04.035).

6. Julia Leimkugel, Valentin Pflüger, **Abudulai Adams Forgor**, Martin Nägeli, Christian Flierl, Sébastien Gagneux, Gerd Pluschke. Conservation of the Pneumococcal surface protein A (PspA) sequence in a hypervirulent lineage of serotype 1 *Streptococcus pneumoniae*
   (This article will be submitted to Journal of Clinical Microbiology)

7. **Abudulai Adams Forgor**, Abraham Hodgson, Julia Leimkugel, Martin Adjuik, Valentin Pflüger, Oscar Bangre, Jean-Pierre Dangy, Gerd Pluschke and Tom Smith. Survival and Sequelae of Pneumococcal Meningitis in Northern Ghana
   (Prepared for submission to Int J Infect dis).

8. **Abudulai Adams Forgor,** Abraham Hodgson, Penelope Vounosou, Martin Adjuik, Julia Leimkugel, Elizabeth Awine, Gerd Pluschke and Tom Smith. Influence of Climatic Factors on the Incidence of Meningococcal and Pneumococcal meningitis in Northern Ghana.
   (This article will be submitted to International Journal of Health Geographics)