ST2859 serogroup A meningococcal meningitis outbreak in Nouna Health District, Burkina Faso: a prospective study

A. Sié¹, V. Pflüger², B. Coulibaly¹, J. P. Dangy², A. Kapaun³, T. Junghanss³, G. Pluschke² and J. Leimkugel³

¹Centre de Recherche en Santé de Nouna, Nouna, Burkina Faso
²Molecular Immunology, Swiss Tropical Institute, Basel, Switzerland
³Section of Clinical Tropical Medicine, University Hospital Heidelberg, Heidelberg, Germany

Summary

We analysed cerebrospinal fluid samples from suspected meningitis cases in Nouna Health District, Burkina Faso, during the meningitis seasons of 2004–2006. Serogroup A ST2859 meningococci belonging to the ST5 clonal complex of subgroup III meningococci were the predominant causative agent. ST2859 bacteria were associated with focal outbreaks in the north of the district. While >10% of the population of an outbreak village carried ST2859, the population in the south of the district was predominantly colonised by serogroup Y ST4375 meningococci, which were associated with only sporadic cases of meningitis. Colonisation with the less virulent Y meningococci may interfere with the spread of the ST2859 to the south of the district, but there are concerns that this serogroup A clone may cause a third wave of subgroup III meningococcal disease in the African Meningitis Belt.

Keywords meningococcal meningitis, Neisseria meningitidis serogroup A

Introduction

Epidemic meningococcal meningitis continues to threaten the countries of the meningitis belt of sub-Saharan Africa. For the past 100 years, outbreaks of meningococcal meningitis with incidence rates of up to 1% of the general population have hit this region in periodic waves (Greenwood 1999). In recent years, the periodicity of epidemic cycles has been varying considerably from country to country; their dynamics are still not well understood. Over the past 10 years, Burkina Faso was affected by repeated outbreaks caused by serogroup A and serogroup W135 meningococci (Taha et al. 2002; Nicolas et al. 2005; Traore et al. 2006). Little outbreak activity was recorded in 2005 (http://who.int), but 19 000 cases and >1500 deaths caused by meningococcal meningitis were reported in 2006 (WHO 2007).

While serogroup A meningococci have been responsible for most epidemics in the meningitis belt in the last 100 years, other serogroups such as C (Whittle et al. 1975), W135 (Taha et al. 2000; Fonkoua et al. 2002; Njanpop-Lafourcade et al. 2005) and X (Gagneux et al. 2002; Djibo et al. 2003; Boisier et al. 2007) have also caused outbreaks of considerable size. Clonal waves of colonisation and disease are a characteristic feature of the epidemiology of meningococcal meningitis in the African Meningitis Belt (Leimkugel et al. 2007a). In the case of serogroup A, meningococci genoclouds associated with the sequence types (STs) 5 and 7 have been responsible for the outbreaks in the last two decades (Nicolas et al. 2001). It appears that a ST2859-associated serogroup A genocloud is now spreading through West Africa and increasing the likelihood of a new epidemic sweeping through the belt in coming years (Njanpop-Lafourcade et al. 2005; Traore et al. 2006; WHO 2007). Here, we describe the emergence of this genocloud in a rural district of Burkina Faso. While only a few cases were observed in the south of the district, where serogroup Y meningococci dominated in the pharyngeal flora of the population, an outbreak of serogroup A meningitis in the north of Nouna Health District (NHD) was associated with heavy colonisation with the disease causing ST2859 genocloud.

Methods

Study area

The study was performed in NHD in the Kossi region of Burkina Faso. Its population is 296 007 and it covers 7464.4 km² (ECD Nouna 2005). The district has 25 health centres, each covering on average 11 840 inhabitants, and one district hospital in the city of Nouna (Figure 1). Medical equipment at the health centres is very basic and only a few essential drugs are available. Nurses
are regularly trained in diagnosing bacterial meningitis and in performing lumbar punctures. Most health centres have one room for inpatient care.

Most people live in villages (65 to 6384 population) with an average distance of 9.52 km (range: 0–48 km) to the nearest health facility. Nouna Health Research Centre (CRSN) entertains a demographic surveillance system (DSS) covering 76 847 individuals of the district population, living in roughly 9000 households with a mean household size of 8.0 in the south of the district (Ye et al. 2002).

The meningitis season lies within the dry season from the end of December to late April. The national meningitis programme provides the health facilities with drugs for the treatment of meningitis cases. Treatment is free of charge. According to the national guidelines of the ministry of health, a suspected case is defined as a patient with acute fever of at least 38 °C associated with one of the following signs: neck stiffness, impaired consciousness, petechial or purpuric eruption. For infants, it is defined by fever associated with a bulging fontanel with or without stiff neck. The first-line treatment is chloramphenicol; ampicillin is given in severe cases.

Patient recruitment and cerebrospinal fluid samples

Following the national guidelines for meningitis surveillance, diagnostic lumbar puncture was performed on suspected meningitis patients presenting to one of the 25 Health Centres of the NHD. Patients were enrolled into the study if their cerebrospinal fluid (CSF) could be transported to the laboratory of Nouna District Hospital within 6 h. CSF samples were analysed by latex agglutination test (Pastorex; Bio-Rad, Reinach, Switzerland), direct Gram staining and cultivation of bacteria on Blood and Chocolate Agar (WHO 1999). Results were confirmed by multiplex polymerase chain reaction (PCR) later (Parent et al. 2005). During the pilot phase of the project, CSF samples were collected only during the peak of the meningitis season (between February and April 2004 and January and April 2005). In 2006, samples were collected continuously from January 1st until July 31st.

Demographic information of the patients, as well as date of recruitment, clinical outcome and date of discharge, was collected using a standard questionnaire applied by a trained field worker.

Colonisation surveys

In April 2006, Neisseria colonisation surveys were carried out in different parts of the district. For our longitudinal meningococcal colonisation study with a sample size of 300 participants, we selected 37 compounds by proportional cluster sampling from the Nouna DSS population. For this, the DSS area (Figure 1) was divided into three rural zones and the city of Nouna. Proportional to the size of the village and the total population of the zone, two villages were selected from each of the three areas. Households were selected from the six villages and the city of Nouna, with a probability proportional to the size of the household.

Motivated by a focal disease outbreak, a separate study was conducted in the village with the highest meningitis disease burden in 2006, which is located close to Ira outside the DSS area (Figure 1). In this village, meningococcal carriage was analysed in 10 compounds where meningitis cases were registered and in four compounds with no suspected meningitis patients.
In both studies, throat swabs were taken from all consenting members of the selected compounds present at the time of the visit, yielding 316 samples in the DSS compounds and 180 samples in the epidemic village. The swabs were immediately plated onto Thayer-Martin agar. The agar plates were transported to the lab and incubated at 37 °C in candle jars for 24 h. Neisseria meningitidis and Neisseria lactamica were identified and isolated using classical microbiological methods (Gagneux et al. 2000).

Characterisation of bacterial isolates
Meningococci were serogrouped with serogroup-specific antisera (Difco, Basel, Switzerland) according to the manufacturer’s instructions and serological typing was confirmed by PCR (Taha 2000; Bennett et al. 2004). Multi-Locus Sequence Typing (MLST) was performed as described (http://pubmlst.org/neisseria/) on 10 each of serogroup A carrier, serogroup A disease, and 10 serogroup Y isolates, which were randomly selected.

Results
Focal nature of a Neisseria meningitidis serogroup A disease outbreak in 2006
Between January and July 2006, 410 suspected meningitis cases were notified to NHD. CSF samples from 212 patients were analysed. The epidemic threshold of 10 cases per 100 000 was crossed in week 13 and the outbreak peaked in week 16 (Figure 2). Neighbouring districts reached the epidemic threshold in week 4 in Solenzo, in week 12 in Déou (Djibasso and Bomborokuy; Figure 1). The highest incidence rate (775/100 000) was reported from Ira Health Centre, where 199 cases were reported among the 26 333 inhabitants living in 20 villages and 12 scattered settlements. With 138 cases and six deaths, most patients came from one particular village with a population of 1354, resulting in an incidence rate of 2800/100 000. Another 42 deaths occurring in the village in the beginning of the outbreak period are probably also attributable to the meningitis epidemic.

Laboratory diagnosis confirmed 81 cases of meningococcal meningitis in the NHD in 2006. Of these 72 were positive for serogroup A, four for serogroup Y and one for serogroup W135. MLST performed with nine randomly selected serogroup A case isolates revealed ST2859. For five culture-negative samples, PCR analysis reconfirmed meningococcal meningitis, but failed to determine the serogroup of the causative meningococci. In addition, 15 cases of Streptococcus pneumoniae meningitis and 10 cases of Haemophilus influenzae type b meningitis were recorded.

Mortality among patients with confirmed meningococcal meningitis was 7.4% (6/81), (all attributed to serogroup A) vs. 46.7% (7/15) for pneumococcal meningitis. None of the 10 laboratory-confirmed H. influenzae cases was reported dead, in contrast to 12.3%
(13/106) of all patients with a negative laboratory test of the CSF.

The vast majority of all reconfirmed cases from the north of the district were caused by serogroup A meningococci. Most cases caused by *N. meningitidis* serogroups Y and W135, *S. pneumoniae* or *H. influenzae* were reported from the centre or the south of the district (Table 1, Figure 1). As expected, all *H. influenzae* cases were children under 5 years of age (Figure 3) and the median age (4 years) of these patients was younger than that of pneumococcal (5 years) and meningococcal meningitis patients (7 years). Most of the pneumococcal (85.7% [12/14]; age not known for one patient) and meningococcal meningitis patients (68.3% [56/82]) were children younger than 10 years. Only three cases were older than 30 years (Figure 3).

**ST2859 outbreak strain was already present in the NHD during 2004–2005**

Between February and April 2004, 37 CSF samples were collected from the suspected meningitis patients in the NHD and analysed. Most samples came from the hospital in Nouna (*n* = 11) and the health centre in Wérébére (*n* = 11). Seven cases of serogroup A meningococcal meningitis were reconfirmed by laboratory analysis, in addition to one Hib and three pneumococcal meningitis cases. The only growth positive serogroup A meningococcal case isolate was ST2859. All laboratory reconfirmed meningococcal meningitis patients had reported to Wérébére Health Centre, which covers a population of 6247. With 20 suspected cases (incidence rate = 320/100 000 in this area) and eight deaths reported to district authorities, an epidemic was declared in this area and >4000 inhabitants between 2 and 30 years were vaccinated with anti-meningococcal serogroup A/C polysaccharide vaccine in February 2004.

Between January and April 2005, 55 CSF samples were collected and analysed. In this period, the epidemic threshold was not reached in NHD and thus no outbreak declared. Laboratory analysis confirmed five cases of meningococcal meningitis, three serogroup A, one

### Table 1  Distribution of meningitis cases among the health centres in the Nouna Health District during the 2006 meningitis season

<table>
<thead>
<tr>
<th>Health centre from north to south</th>
<th>Registered cases</th>
<th>Collected CSF</th>
<th>Neisseria meningitidis</th>
<th>Streptococcus pneumoniae</th>
<th>Haemophilus influenzae</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>A</strong></td>
<td><strong>W135</strong></td>
<td><strong>Y</strong></td>
<td><strong>n.s.</strong></td>
</tr>
<tr>
<td>Berma</td>
<td>199</td>
<td>46</td>
<td>25</td>
<td>4</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Ira</td>
<td>55</td>
<td>17</td>
<td>11</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Barani</td>
<td>33</td>
<td>7</td>
<td>4</td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Djibasso</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Werebere</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Kinkeuy</td>
<td>29</td>
<td>28</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Bomborokuy</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yévédougou</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Borekuy</td>
<td>10</td>
<td>7</td>
<td>1</td>
<td></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Doumbala</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Konkuy-Koro</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dembo</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sono</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Koro</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nian</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CMA Nouna</td>
<td>14</td>
<td>36</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>HC Nouna</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Darra</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Toni</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bourasso</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lekuy</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bagala</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Goni</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Dokuy</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Gassingo</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>410</td>
<td>212</td>
<td>72</td>
<td>1</td>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>

Comparison of cases registered at the district health authorities, vs. collected CSF samples and laboratory results.
serogroup W135 and one serogroup Y case. All the three serogroup A isolates were ST2859. Furthermore, four Hib and 11 S. pneumoniae cases were recorded.

**Local differences in Neisseria colonisation within the health district**

As part of a longitudinal carriage study, a Neisseria meningitidis carriage survey was conducted in April 2006 in the DSS area in the south of NHD. It revealed a N. meningitidis colonisation rate of 11.4% (36/316). All 36 pharyngeal isolates were serogroup Y and all 10 randomly selected Y isolates analysed by MLST were ST4375. No colonisation with serogroup A meningococci was observed in this part of the district and colonisation with non-groupable meningococci was very low (2/316 = 0.6%).

This surprising result prompted us to conduct an additional carriage survey on May 6/7th in the outbreak village close to Ira Health Centre in the north of the district. A colonisation rate of 13.3% (24/180) was observed and here most (22/24 = 92%) of the pharyngeal isolates were serogroup A. Only one isolate was serogroup Y and one was non-groupable; the N. lactamica colonisation rate was 7.8% (14/180). All 10 randomly selected serogroup A carriage strains analysed by MLST were ST2859. Serogroup A meningococcal colonisation rates in compounds with at least one suspected meningitis case and in compounds without patients were comparable (12.2% and 10.5%, respectively). The age distribution of serogroup A carriers was much broader than that of the cases (Figure 4).
Discussion

This prospective study of meningococcal colonisation and disease documents the emergence of serogroup A ST2859 meningococci in the NHD, Burkina Faso. They belong to the ST5 clonal complex of subgroup III serogroup A meningococci, which have been causing subsequent waves of epidemics in the African Meningitis Belt for 20 years. Beginning in Mecca in 1987, the first genocloud, associated with ST5, has caused a series of epidemics in the late 1980s and early 1990s with approximately 150 000 cases (Teyssou & Muroz-Le Rouzic 2007). In the mid 1990s, the ST5 clone was replaced by ST7, differing from ST5 in only one allele (adk3 vs. adk1). Population genomic analyses are required to understand the genetic basis for the emergence of new clones with epidemic potential (Leimkugel et al. 2007b). However, our preliminary comparative proteome analyses are indicating that ST5, ST7 and ST2859 serogroup A meningococci differ substantially from each other, speaking against a very recent separation of these lineages. Currently it is unclear whether the ST2859 clone is of comparable virulence as its ST5 and ST7 counterparts with the same potential to cause a new epidemic wave throughout the African Meningitis Belt and beyond. While no major outbreak was recorded in the NHD in 2007 (Sie et al., unpublished observation), 34 other districts of Burkina Faso crossed the epidemic threshold in 2007 and the Ministry of Health of Burkina Faso reported 22 255 suspected cases between the January 1st and April 8th 2007 (MoH, personal communication; http://www.who.int/csr/don/2007_04_17/en/index.html). CSF specimens from all affected areas have tested positive for N. meningitidis serogroup A by latex test and/or culture. Sudan was hit by a major outbreak in 2007 with 9646 suspected cases of meningococcal diseases reported by the Ministry of Health of Southern Sudan by March 11th 2007 (http://www.who.int/csr/don/2007_3_15a/en/index.html). CSF specimens collected from the eight affected states have tested positive for N. meningitidis serogroup A. Currently it is unclear whether the ST2859 clone is the major causative agent of these outbreaks.

Multilocus sequence typing analysis of the four bacterial isolates out of all 10 confirmed meningococcal cases from 2004 and 2005 demonstrates that serogroup A ST2859 meningococci have been circulating in the NHD at least since 2004 but only causing focal outbreaks. This supports the view that apart from climatic factors characteristic for the meningitis season in the African Meningitis Belt additional triggering factors, such as copathogens, are required to initiate a major meningococcal disease outbreak. In our study, the local carriage rate of A meningococci at the time of the outbreak in the most affected village was high (13.3%) and the age spectrum among carriers was broad. In contrast, a study conducted over 5 months in Bobo-Dioulasso, Burkina Faso in 2003 revealed a substantial number of serogroup A ST2859 meningococcal meningitis cases, but no carriage (Mueller et al. 2007). In our long-term meningococcal carriage and disease study in Ghana (Leimkugel et al. 2007a), we have observed strong correlations between the carried population of virulent meningococci and the disease isolates. Only at the beginning of a serogroup A ST7 colonisation and disease wave did we find patient isolates from CSF that were initially unrepresented among the carriage isolates from the population sample comprising 300 individuals of the total district population of about 140 000 (Leimkugel et al. 2007a). Dependent on disease incidence rates and the proportion of individuals included, colonisation surveys can thus be less sensitive than studies of disease isolates in detecting the emergence of new hyper-virulent clones in a study area. Since no carriage surveys were performed during our pilot studies in 2004 and 2005, the geographical pattern and extent of colonisation with ST2859 meningococci in the NHD prior to 2006 is unknown.

Case fatality rates of 6.3% among patients with meningococcal meningitis and of 46.2% among patients with pneumococcal meningitis are similar to those observed in other studies in the African Meningitis Belt (Peltola 2001). The case fatality rate of 12.4% (13/105) within suspected meningitis patients with no reconfirmed bacterial aetiology raises concern about a yet unidentified aetiology associated with high mortality. Interestingly, our colonisation studies show that in the south of NHD, where only sporadic meningitis cases were seen, the population was primarily colonised by serogroup Y ST4375 meningococci belonging to the ST23 clonal complex in 2006. Here, an overall colonisation rate of >10% was associated with only sporadic reconfirmed cases of serogroup Y meningitis, reflecting the low virulence of this clone. So far only three serogroup Y ST4375 isolates have been notified to the MLST database,
one carrier strain isolated in Burkina Faso in 2004 and two disease strains isolated in 2006 in Burkina Faso and in Niger. Serogroup Y meningococci of the ST23 clonal complex have been associated with invasive disease particularly in Asia, Canada and the United States (Claus et al. 2005; Harrison et al. 2006; Tsang et al. 2007).

While in the U.S. serogroup Y is currently responsible for a third of the meningococcal disease burden (Bilukha & Rosenstein 2005), serogroup Y meningococci appear to be isolated in Europe more frequently from healthy carriers than from patients with invasive diseases (Yazdankhah et al. 2004). The planned introduction of a serogroup A polysaccharide–protein conjugate vaccine in the countries of the African Meningitis Belt has raised concern about potential serogroup replacement. Outbreaks caused by serogroups such as X and W135, traditionally regarded as relatively apathogenic, have been recorded in recent years (Djibo et al. 2003; Gagneux et al. 2002; Traore et al. 2006; Boisier et al. 2007). This may either indicate a change of the epidemiological situation in sub-Saharan Africa towards a more complex picture (Greenwood 2007) and/or reflect improvements made in meningococcal surveillance during the last two decades. For now, the significance of less pathogenic serogroups in the epidemiology of meningococcal disease in the African Meningitis Belt is not yet well understood. Our results show the simultaneous spread of two meningococcal serogroups in the NHD that would have remained undetected with only case-oriented surveillance. It is not yet clear how and to what extent the spread of different serogroups might influence each other. Due to the long distance between the DSS area and Ira and the orientation of the population around Ira towards local markets in the north, direct contact between the people in the north and south of the district is not very intense. Furthermore, colonisation with serogroup Y meningococci may interfere with colonisation and invasive disease caused by hyper-virulent lineages, such as the serogroup A meningococci belonging to the ST5 clonal complex.

Acknowledgements

This work was supported by the Volkswagen Foundation and the German Research Foundation (DFG) through the Collaborative Research Grant (SFB) 544 ‘Control of Tropical Infectious Diseases’. This publication made use of the Neisseria Multi-Locus Sequence Typing website (http://pubmlst.org/neisseria/) developed by Keith Jolley and Man-Suen Chan (Jolley et al. 2004, BMC Bioinformatics, 5:86) and sited at the University of Oxford. The development of this site has been funded by the Wellcome Trust and European Union.

References


Corresponding Author Thomas Junghanss, Section of Clinical Tropical Medicine, University Hospital, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany. Tel.: +49 6221 5634904; Fax: +49 6221 565204; E-mail: thomas.junghanss@urz.uni-heidelberg.de