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Diplomarbeit:

Epidemiology of Neisseria meningitidis transmission; a case-crossover study

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Hamburg, den 3. August 2004

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1 Abstract

Background: Meningococcal disease is a serious public health problem with a case fatality rate of about 10 %. Recent acquisition of the bacteria is generally regarded as an important risk factor for developing the invasive disease. A case-crossover study to examine the effect of transient exposures on the acute outcome, which is the acquisition of Neisseria meningitides, was undertaken.

Methods: In the case-crossover design each case serves as its own matched control while case-times are compared to earlier time periods. Data from a longitudinal study was used for a case-crossover analysis. Healthy students aged 14 to 19 were tested for meningococcal carriage and interviewed about potential risk factors. 121 matched pairs of students who were non-carriers in the first survey and became carriers in the second were analysed. Mantel Haenszel Odds Ratios were calculated and a conditional logistic regression analysis was done.

Results: Both bivariate and multivariate analysis showed a significant association between meningococcal carriage and the predicting variables rhinitis, visits to cinema, and travelling abroad. While the adjusted results for rhinitis (OR 0.33; 95% CI 0.13-0.82) and cinema visits (OR 0.17; 95% CI 0.05-0.65) indicate a protective association, travelling abroad (OR 3.50; 95% CI 1.45-8.34) is a risk factor.

Discussion: Transient exposures that trigger the infection with N. meningitidis are generally difficult to study. This case-crossover study allows new insights in this timely process. For the interpretation of the results methodological issues and potential confounding (e.g. seasonal variation) need to be taken into account, especially while comparing the results with those from studies with traditional designs.

2 Introduction

Neisseria meningitidis is the most common cause of bacterial meningitis, a disease that has been examined by many researchers throughout a long time in history. The interest of health professionals probably arises through the sometimes dramatic course of the infection and because it mainly occurs in small children and adolescents.

In 2001 there have been 782 confirmed cases of invasive meningococcal disease (IMD) in Germany. Regarding the high case fatality rate of up to 10 % this is a significant public health problem even in industrialized countries. Vaccination is available for serogroups A, C, Y and W135 but not for serogroup B, which is responsible for almost 70 % of all IMD cases in Germany [RKI 2002]. Therefore it is important to identify potential risk factors for the acquisition of Neisseria meningitidis and to understand the dynamic of the transmission.

Rather than the disease, the normal outcome of meningococcal colonization is asymptomatic nasopharyngeal carriage. Whether one then develops the invasive disease depends on the virulence of the bacterial strain, the hosts immunity and other facilitative conditions [Conyn-van Spaendonck et al. 1999]. The overall carriage rate in Europe is about 10 %, but can be much higher under certain conditions. Prevalence of meningococcal carriage is unusual in infancy and early childhood, increases through the first two decades to peak at 25 % in teenagers and young adults and then declines [Cartwright 1995]. Therefore this age group is well suited to serve as study population in carriage studies.

Cartwright [1995] states that the disease is still mystifying and paradoxical for doctors and epidemiologists and though individual cases can be treated with antibiotics the epidemiology of the disease cannot be influenced. He further says that despite considerable research during the last hundred years *"... we still understand only incompletely the process of acquisition and the ensuing factors which lead in a few cases to the development of invasive disease. There is no clear-cut relationship between meningococcal carriage rates and disease rates."*

In her review of the meningococcal carrier state Claire Broome summarised the situation as follows: *"Since most patients with meningococcal disease have not had contact with a case, and the organism has no known reservoir outside man, asymptomatic carriers have been recognized as the source of transmission. Thus understanding the meningococcal carrier state has been seen as crucial to explaining the epidemiology and pathogenesis of meningococcal disease." [Broome 1986]*

The data base of the present paper is a longitudinal study that was conducted in the year 2000 in North Rhine Westphalia. Prior to the data collection invasive meningococcal strains of the phenotype B:4:P1.4 were observed more frequently. B:4 isolates of Neisseria meningitidis predominate in Belgium and the Netherlands since many years and there was a increase in Germany in the last decade.

Smoking and living in closed or semi-closed communities like military camps, schools and universities as well as visits to bars and other crowded places have been considered as risk factors for meningococcal carriage [Cartwright 1995; Conyn-van Spaendonck et al. 1999; Caugant et al. 1994]. While numerous studies have provided information about persistent exposures that might influence the carrier state, little is known about short-term risk factors.

With the current investigation we focused on those exposures that have an acute character. Our aim was to find out if there are factors that can trigger asymptomatic carriage. The structure of the data base allowed us to use the case-crossover design. So far this design has been used for example in studies on myocardial infarction [Meier 1998; Mittleman 1993] or injury onset [Sorock 2001]. In his study on haemorrhagic fever with renal syndrome Dixon [1997] showed that the case-crossover design is also well suited for research on infectious diseases and that it can help to identify risk factors clearer than the classical case-control design. Meningococcal carriage can be regarded as an acute outcome and predicting factors can be constant over time, as well as short and transient exposures. The case-crossover design has its strength when exposures have a short induction time and the effect is immediate.

In order to determine whether there are acute events in the life of adolescents that can trigger the acquisition of N. meningitidis, we undertook this case-crossover study.

3 Background of Meningococcal Disease and Carriage

The causative agent of meningococcal disease is Neisseria meningitidis. The following chapter gives a brief insight into the characteristics and mechanisms of the bacterial strain, the distribution of the disease and asymptomatic carriage and also gives information about the to date knowledge of potential risk factors for carriage and disease.

3.1 The Pathogen

3.1.1 Microbiology and Pathogenesis

Neisseria meningitidis are gram-negative, aerobic diplococci and isolation is best on chocolate agar. There is no other natural reservoir than humans. The major site of colonisation is the nasopharynx and transmission takes place by aerosol or secretions to others during direct and close contacts [Rosenstein 2001].

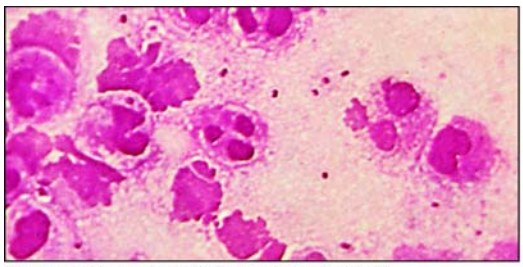

Figure 1: N. meningitidis in cerebrospinal fluid

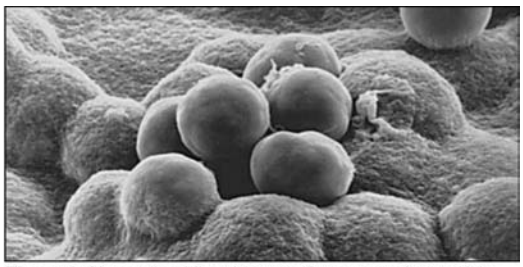

Figure 2: N. meningitidis, electron microscope photograph

Neisseria meningitidis expands a capsule, which protects the bacteria from desiccation during transmission. On the basis of structural differences in the capsular polysaccharides, meningococci can be divided into at least 12 serogroups (A, B, C, 29-E, H, I, K, L, W-135, X, Y, Z). Further classification makes use of variations in the outer-membrane proteins (OMP). Class 2 and 3 OMP (Porin B) define 8 serotypes and there are 14 serosubtypes on the basis of class 1 OMP (Porin A) [Cartwright 1995]. Thus the antigenetic formula results from *serogroup : serotype : serosubtype* like for example C:2a:P1.5.

Only a minority of inhaled infected aerosols actually result in meningococcal disease. In most cases the bacterial strain leads to asymptomatic carriage of Neisseria meningitidis. The virulence of a meningococcal strain is affected by its surface organelles. These surface structures and other secreted products of meningococci help to overcome host defences and are known to be critical in colonisation, invasion and disease pathogenesis [Cartwright 1995].

Virulence factors: The attachment of meningococci to the microvillous surface of mucosal cells is initiated by the binding of *Pili.* These filamentous bacterial lectins are the major adhesions that promote colonisation of the upper respiratory tract. Subsequently the opacity-associated proteins, *Opa* and *Opc*, bind to receptors and strengthen the adhesion. The attached organisms are engulfed by the cells and can then traverse the mucosal epithelium through phagocytic vacuoles. Neisseria meningitidis produces *IgA1 protease,* an outer-membrane protein which can cleave IgA1 – a human secretory immunoglobulin – and thus may play a role in mitigating mucosal immunity. *Porins* are outer-membrane proteins that create pores through which small hydrophilic solutes pass into the periplasmatic space. In addition meningococci can use mechanisms to obtain iron from human lactoferrin, transferrin and haemoglobin to enhance their pathogenic potential [Rosenstein 2001]. Another important characteristic of Neisseria meningitidis is the high level of *blebbing*, the shedding of outer membrane vesicles. Once meningococci gain access to the bloodstream, they release *endotoxin* in the form of membrane *blebs*. These blebs contain *outer-membrane proteins (OMPs)* and *lipopolysacccharides (LPS)* and are able to bind antibodies that would otherwise attach the whole bacteria. LPS then stimulates the cytokine production and thus inflammatory mediators like the *tumour necrosis factor* and *interleukin* are elicited. The inflammation of various organs follows and the typical hemorrhages appear as a sign of the affected blood vessels. The amount of circulating endotoxin correlates with the severity and prognosis of invasive meningococcal disease [Cartwright 1995].

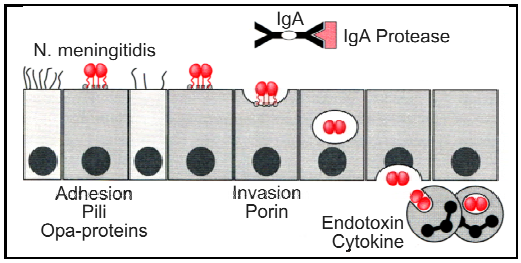

Figure 3: pathogenesis of meningococcal disease

3.1.2 Clinical Manifestations

Meningococcal disease can appear as bacteraemia, septicaemia with or without meningitis and can also take a fulminant and, within hours lethal course. About half of all cases present purulent meningitis. In 25 % of patients septicaemia occurs and between 10 and 15 % of all cases develop a very severe septic shock, the *Waterhouse-Friderichsen syndrome*. 25 % of the cases present mixed forms [RKI 2002].

Invasive meningococcal disease (IMD) has a typically rapid onset. The incubation time is usually between 2 and 4 days but can be up to 10 days. After a short prodromal period with uncharacteristic symptoms that suggest an upper respiratory tract infection, IMD arises with sudden onset of headache, fever, neck stiffness, photophobia, vomiting and vertigo. Further neurological signs like nervousness, somnolence, coma and cranial nerve palsy can occur and imply the involvement of the central nervous system. 75 % of the patients show petechial or purpuric rash or purpura fulminans. The Waterhouse-Friderichsen syndrome, which goes along with massive adrenal hemorrhage and multiorgan failure, is fatal in most cases within a few hours of the first symptoms of IMD appearing. There are several other but less frequent syndromes associated with meningococcal disease like pneumonia, otitis media and conjunctivitis. In infants, meningeal infection can have a slower onset and symptoms may be less characteristic. For instance small children rarely exhibit neck stiffness. A bulging fontanelle can be seen as a sign of the raised intracranial pressure [RKI 2002].

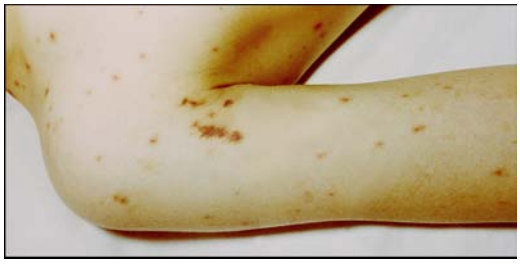

Figure 4: petechial rashes

Meningococci are still highly susceptible to penicillin, which is usually the drug of treatment. In about 10 % of cases an alternative therapy with 3rd generation cephalosporines is necessary. 24 hours after chemoprophylaxis the infectionsness of a patient is stopped. Nevertheless overall mortality from meningococcal meningitis can be up to 10 % even in industrialized countries. Additionally up to 30 % of those who survive IMD suffer from significant neurological sequelae like mental disorders, deafness, palsies and seizures [Köhler 2001].

3.2 Epidemiological Features

3.2.1 Worldwide Distribution of Meningococcal Disease

IMD occurs throughout the world with serogroups A, B and C accounting for the majority of cases. While the serogroups B and C predominate in Europe and America, group A meningococci are responsible for explosive outbreaks in Africa. The two other disease associated serogroups Y and W135 are uncommon causes of meningococcal disease but nevertheless W135 meningococci recently have been responsible for outbreaks in Saudi Arabia and Burkina Faso [WHO].

In industrialized countries the disease is endemic and appears sporadic ally or in small outbreaks with an annual rate of approximately 1 per 100,000 population. But attack rates increase significantly during outbreaks. Serogroup B strains caused outbreaks even in the developed world that reached morbidity rates of 5 to 50 cases per 100,000 population. In the 1990s group B associated outbreaks occurred in Northwestern Europe as well as in North and South America [Rosenstein 2000]. Serogroup C strains of the ET-37 complex were responsible for clusters of meningococcal disease during the 1980s and 1990s in Canada, Europe, the United States and Australasia [WHO]. While an endemic disease pattern predominates in the western world, developing countries in the so called African *meningitis belt* are threatened by much higher rates. The region between Sudan in the east to Senegal in the west suffers from large epidemics that occur every 8 to 12 years. The World Health Organization reported approximately 250,000 cases and 25,000 deaths during an epidemic in 1996 that involved numerous West African countries. The global burden of disease is about 500,000 cases and 50,000 deaths annually [WHO]. The mortality of 10% seems to be similar to that in developed countries but the true mortality in the meningitis belt might be much higher, because patients die before they reach a hospital [Rosenstein 2000].

3.2.2 Invasive Disease in Germany

In 2001 there have been 782 confirmed cases of meningococcal disease in Germany, which corresponds to an incidence rate of 0.9/100,000 population.

The average rate remained relatively constant over the past decade. With more than two thirds, serogroup B strains accounted for the most cases. The detailed serogroup distribution is shown in figure 5.

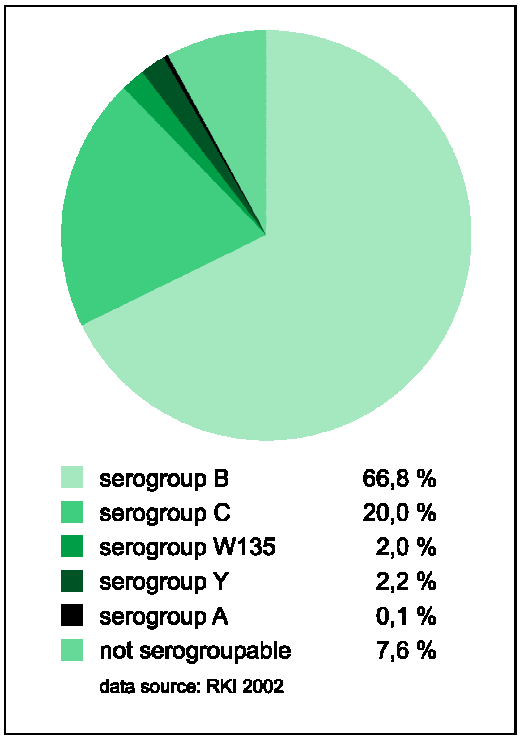

Figure 5: serogroup distribution of meningococcal disease in Germany 2001

Seasonality is indicated by 30 to 40% of all cases occurring in the first quarter of the year. There were 9 regional clusters in 2001 that were responsible for 30 cases, thus the vast majority (more than 90%) appeared as scattered unrelated cases [RKI 2002].

Age-specific incidence rates peak in infants during the first year of life with a rate of 15 cases per 100,000 population. About 40% (289 cases in 2001) affect children under 5 years of age. Another smaller peak of disease frequency can be observed in the age group 15 to 19 years, where the incidence can be three times higher than the average [RKI 2002].

3.3 Risk Factors for the Acquisition of Neisseria Meningitidis

The virulence factors of the bacterial strain that were described earlier, are essential to whether a colonised person develops invasive disease or stays an asymptomatic carrier of Neisseria meningitidis. These surface structures and endotoxins that aid the evasion of the host's immune system are found in the disease associated serogroups. Rather than the disease the normal outcome of meningococcal colonisation is asymptomatic carriage. The following section shows some further conditions that might facilitate meningococcal infection.

Thus the development of IMD depends on the chance that a person is exposed to the bacterial and subsequently is colonised and many factors that are associated with meningococcal disease particularly concern the acquisition of the bacterial strain.

"The process of development of meningococcal disease can be broken down into a sequence of three events - exposure, followed in some cases by acquisition, followed occasionally by the development of invasive disease." [Cartwright 1995]

3.3.1 Age

Though the disease can occur in all age groups, small children bear the highest risk. Age specific disease rates peak in the first year of life at about six months and than decline. The reason lies in immunologic susceptibility. Passively transferred maternal antibodies protect infants against meningococcal infection during the first 3 month of life, subsequently infections rise to the high incidence rates that are recorded for the age group 0 to 4 years. The elevated risk of disease for adolescents may lie in typical behavioural patterns that can be observed in this age group and is discussed later [WHO].

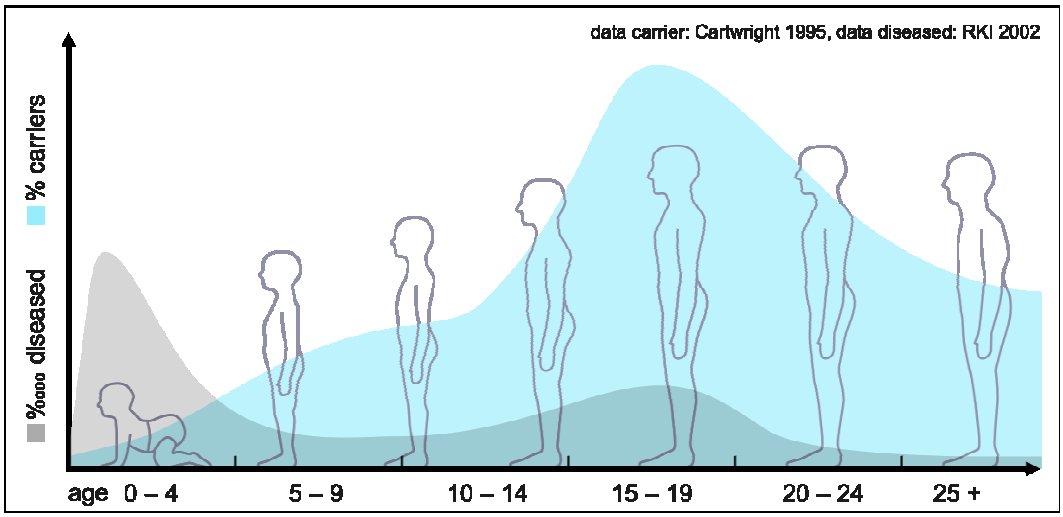

Figure 6: age distribution of meningococcal carriage and disease

The overall rate of asymptomatic carriage has been estimated as high as 10%, but can be much higher under certain conditions. In contrast to the prevalence of the disease meningococcal carriage is unusual in infancy and early childhood, increases through the first two decades to peak at about 25% in teenagers and young adults and then declines [Cartwright 1995].

3.3.2 Season

The reason for the seasonality of meningococcal disease is not yet clear. In temperate climates the disease appears with a characteristic peak during winter and early spring followed by a summer decline. Epidemics in the meningitis belt start typically between January and March, which is the dry season, and come to an end with the onset of the rainy season in May and June. Humidity and weather patterns that might go along with changes in social behaviour and respiratory virus activity are discussed to play a role in contracting the disease at certain times of the year [Cartwright 1995].

One might expect that the seasonality of respiratory tract infections and meningococcal disease itself also results in higher rates of asymptomatic carriage during the winter months. But unlike the invasive disease, no association between meningococcal carriage and season could be observed so far [Ichhpujani 1990].

3.3.3 Contacts with a Case or Carrier

Meningococci are susceptible to drying and thus aerosol spread needs to occur direct and in close contacts. Family members of cases thus are at increased risk of contracting the disease. Secondary cases are also well documented through outbreaks in schools and universities [Fitzpatrick 2000; Bruce 2001]. Immediate chemoprohylaxis and targeted vaccination of potential contact persons is essential to prevent secondary cases.

3.3.4 Immunity

The immunity of the host also plays an important role in meningococcal infection. Bactericidal antibodies, like for example the maternal antibodies in infants, protect against meningococcal disease. Once these antibodies are lost an upsurge in infections can be observed. Antibodies that protect people from developing meningococcal disease can also be acquired through exposure to other bacteria with surface antigens that cross-react with Neisseria meningitidis, such as Neisseria lactamica, a non-pathogenic bacterial which is closely related to the meningococcus. The Stonehouse survey shows high carriage rates of Neisseria lactamica in children aged 0 to 4 years but in this age group carriage of Neisseria meningitidis strains is rare. This is a sign for the immunogenic potential of cross-reacting bacterial strains [Cartwright 1995].

A predisposition to invasive disease arises for those people with underlying immune defects like deficiency of terminal complement components or deficiency of properdin and asplenia. The influence of an infection with the human immunodeficiency virus (HIV) on invasive meningococcal disease epidemiology is not yet clear [Rosenstein 2000]. Nevertheless the high prevalence of HIV in Sub-Saharan Africa and the potential of Neisseria meningitidis to cause large epidemics in the same region is a fact that needs further research.

In a study that investigated which factors preceded the onset of meningococcal disease, symptoms and signs of ill health during the last two weeks were found significantly more frequent in IMD patients [Haneberg 1983]. Viral infections of the upper respiratory tract are discussed as risk factors for the disease due to their

potential to enhance the spread of respiratory droplets by increased coughing. For the colonised person the irritation of the mucosa during respiratory infections might diminish the natural barrier to invasion [Rosenstein 2000].

3.3.5 Smoking

Numerous studies refer to the relationship between smoking and the development of meningococcal disease. In a norwegian study children under 12 years of age that were exposed to passive smoking were found more often among IMD cases than in the control group [Haneberg et al. 1983]. Fischer et al. [1997] additionally found dose-response effects in passive smoke exposure and the disease. Tobacco use has been described as being responsible for about one third of all cases. Active and passive smoking has also been associated with asymptomatic carriage [Cartwright 1995; Conyn-van Spaendonck et al. 1999; Caugant et al. 1994]. Cartwright discusses as reason that smokers associate socially with other smokers and may disseminate oropharyngeal infections more efficiently through increased coughing.

Smoking affects the immunity of a person by influencing the amount of immuno globulines. Salivary IgA, which is essential for the immune response to meningococci, is reduced and IgM concentrations rise due to smoking. The composition of the oral flora might as well be affected by smoking as the gram-negative flora increases at the expense of the inhibitory flora. But nevertheless it is still unclear if there is an interaction between meningococci and the nasopharyngeal flora [Cartwright 1995]. By inhibiting the mucociliary clearance tobacco smoke promotes the adherence of the bacterial and smoke also damages the epithelium of the respiratory tract [Fischer et al. 1997]. Children are known to have more delicate mucous membranes and may therefore be more likely to acquire the pathogen if living in a smoky atmosphere [Cartwright 1995].

3.3.6 Living Conditions

Several studies reveal that certain living conditions affect meningococcal carriage as well as the disease. Living in closed or semi-closed communities and sleeping in dormitories like it usual in army camps, prisons or sometimes in student

residences lead to increased carriage rates [Bruce 2001; Cartwright 1995]. In a dutch study increasing family size has been associated with higher carriage rates [Conyn-van Spaendonck 1999]. Researchers in the USA found black race and low socioeconomic status to be linked with higher rates of IMD [Rosenstein 2000]. Probably because these factors indicate differences in the living environment. Smoking habits are for example known to be more prevalent in persons with low socioeconomic status and bedrooms often have to be shared with siblings. Cartwright summarized the situation as follows: *"High rates of smoking and respiratory infections, poor diet, damp, poor quality housing and overcrowding…could all contribute to increased risk." [Cartwright 1995]*

College students were shown to have an elevated risk of acquiring meningococci during the first week of term. These so called freshmen experience a substantial change in their living environment [Neal 2000].

3.3.7 Behavioural patterns

The high carriage rates among teenagers and young adults lead to the assumption that typical patterns of social behaviour might promote the transmission of the bacterial. Visits to discotheques, hall bars, youth and sport clubs as well as kissing have been considered as risk factors for meningococcal carriage [Conyn-van Spaendonck 1999; Neal 2000; Imrey 1996]. An outbreak among North American university students was found to be associated with recent bar patronage [Imrey 1996] and in another outbreak in Northern Argentina the infected persons were significantly more likely to have attended a particular disco than the control group [Cookson 1998]. Factors that facilitate the transmission of Neisseria meningitis in bar environments might again be exposure to smoking and overcrowding.

Caugant et al. [1994] additionally found that working outside the home and a profession in the transportation or industry bear a greater risk of carriage.

3.4 Vaccination and Public Health Aspects

Serogroups A, B, C, W135 and Y are associated with the disease and vaccination is possible with quadrivalent polysaccharide vaccine against serogroup A, C, W135 and Y. Immunity is usually serogroup-specific. The vaccination commission of the Robert-Koch Institute recommends immunization for people that are at increased risk of meningococcal infection. Individuals with conditions like immune defects, such as complement- and properdin defects or asplenia should undergo vaccination as well as laboratory personnel, Mecca-pilgrims and other people who are travelling into endemic regions [RKI 2000]. The World Health Organisation also recommends emergency vaccination to control outbreaks and immunization of population groups for which a high risk has been documented like people attending army units or boarding schools. With the recently developed conjugate vaccines, protection of children below the age of 18-24 month is also possible, this was a problem in earlier times [WHO].

As seen above, most cases of IMD in Germany are caused by serogroup B strains, for which there is still no effective vaccine. The capsular polysaccharide of serogroup B meningococci has identical structures (α-2,8 linked polysialic acid) than those found in fetal neural tissue. Due to this antigenetic peculiarity no antibodies against the capsule of serogroup B strains can be built and it explains why immunization fails [Köhler].

Secondary cases among close contacts of patients with IMD can be prevented through chemoprophylaxis. But of all patients with meningococcal disease only 1–2% are secondary cases and as there are high rates of asymptomatic carriage in certain population groups, chemo-therapeutic elimination of nasopharyngeal carriage is practically impossible [WHO].

From the public health perspective meningococcal disease gains further significance through the epidemiological fact that young children and adolescents are the most threatened age groups. Though the disease can be treated successfully with antibiotics, IMD still has a high mortalitiy rate, respectively a high rate of sequelae. Therefore it is of considerable importance

to define alternative approaches for the prevention and control of meningococcal disease and carriage.

3.5 Dynamics in the Transmission of N. meningitidis

Asymptomatic carriage of virulent meningococcal strains is usually low. This suggests a high risk of developing the disease once a person is infected with virulent strains. There are a number of studies that reported the carriage rates of causative meningococcal strains during outbreaks. An investigation of a group C outbreak in the Netherlands showed low carriage of the invasive strain in the population that recently experienced the outbreak and the causative strain was not found in the reference population [Conyn-van Spaendonck 1999]. In a study about a school outbreak in Wales 11 of 744 students carried the epidemic group B strain and among the 119 identified contacts of three ill students, two carried the pathogenic strain [Fitzpatrick 2000]. In a norwegian study 8,8 % of the asymptomatic carriers in a randomly sampled population harboured virulent strains that are known to have invasive capacity [Caugant 1994].

In the african meningitis belt carriage rates are low during time periods between epidemics but increase dramatically during an epidemic [Cartwright 1995]. This indicates a high transmissibility of the epidemic strain.

Interpreting these findings is difficult. Due to the low point prevalence of virulent strains in endemic regions and in inter-epidemic times in Africa it might be suggested that the transmissibility and the acquisition rate of the meningococcal strain seems to play an important role in disease frequency.

4 Methods

4.1 Data Background

Data for the case-crossover analysis was taken from a longitudinal study on meningococcal carriage that was conducted between February and October 2000 in North Rhine Westphalia, Germany. The study was a cooperation of the *Institute of Public Health North Rhine Westphalia, the Robert-Koch Institute in Berlin, the University of Heidelberg and 6 local public health offices.* Prior to this investigation there has been little knowledge about the meningococcal carrier state in Germany.

Students from grade 9 to 12 in the participating counties were asked to volunteer. As mentioned earlier this age group bears an increased risk of disease and is as well known for high carriage rates of 25–30 %. Therefore a sample of about 2000 students was thought to give reasonable results. To avoid a potential influence of the social-economic status different school forms were included.

During the 9 month period the same students were tested on meningococcal carriage three times with intervals of at least two month between the measurements. Tonsillo-pharyngeal swabs were taken and analyzed through the laboratory of the National Reference Centre for Meningococci at the University of Heidelberg. Swabs were immediately plated on Martin-Lewis selective agar as well as on blood and chocolate agar. To avoid a reduction of germs no other transport medium was used. Plates were then incubated at 37°C in 5–7% CO2 atmosphere. Subsequently serogroups and serotypes of the meningococcal isolates were determined. With mono- and polyclonal antisera 12 serogroups can be identified. Currently 8 serotypes and 14 serosubtypes can be differentiated. For clonal analysis macrorestriction analysis using pulsed field gel electrophoresis was applied [Glitza 2004].

The same students from whom a swab was taken were surveyed at each of the three dates on factors that might be associated with the acquisition of Neisseria

meningitidis. The students were asked to complete a pre-tested, standardized questionnaire in which they report about their housing conditions, lifestyle and social behaviour, smoking status and health.

4.2 Case-Crossover Design

The case-crossover design was first described by Maclure and is a scientific method that can be regarded as a hybrid between the case-control design and a crossover experiment. The latter is known as experimental study in which every subject receives two or more interventions in a random sequence and the outcome is measured after each intervention. The case-crossover design is a case-control version of the crossover experiment [Rothman 1998].

Maclures approach is similar to the traditional matched-pair case-control design since in both designs each case has a matched control. The key difference is that for the case-crossover design no control persons have to be selected because each case serves as its own matched control. This is possible by comparing the exposure status of the same individual during different times instead of using different subjects at the same time [Maclure 2000].

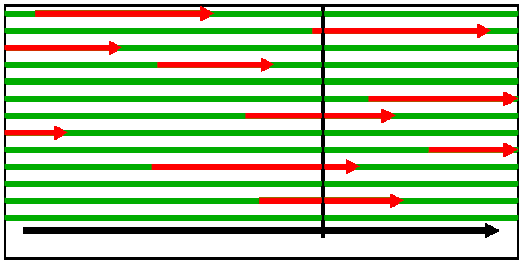

Figure 7: single measure (e.g. case-control study)

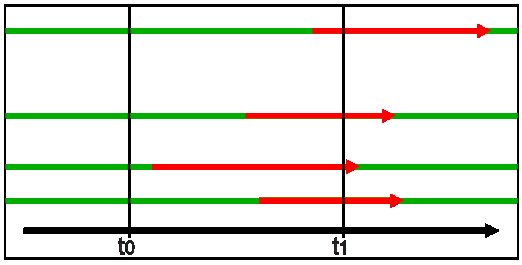

Figure 8: repeated measure (e.g. case crossover study)

Figure 7 and 8 show the different methods to compare cases (red) with controls (green). With the case-crossover design the investigator can assess whether anything unusual that happened just before an event, was able to trigger the outcome. It determines the effect of a brief and transient exposure on the risk of the onset of a relatively rare, acute disease [Maclure 2000]. Requirements of the case-crossover design are therefore:

- *Acute cases* – A time of onset of the disease is needed to define the case window, thus an abrupt outcome applies best [Maclure 2000].

- *Crossover in exposure status* – Risk estimates are derived by comparing the exposure status of an individual in different time windows. Thus there has to be a sufficient number of individuals that crossed from higher to lower exposure level [Maclure 2000].

- *Brief and Transient exposures* – The exposure needs to be short lived. If this is not the case it is difficult to relate an exposure to the outcome due to a potential carryover effect. This means that exposures from the distant past could be the cause of the outcome [Maclure 2000].

- *Time windows* – Time windows that are compared are obtained from the same individual at different times. These person times have to be sampled carefully. The case window is the period preceding the outcome onset in which the person was at risk and the control windows are periods of the same length at a different time. The time windows that are compared may not overlap [Rothman 1998].

The power of a case-crossover study depends on the number of subjects that crossed from low to high exposure level or vice versa. The more discordant pairs are included in the analyses the better is the statistical power. Like in traditional study designs it is also possible to sample additional controls respectively control time windows to increase the efficiency of case-crossover analyses [Maclure 2000].

4.2.1 Design Application

The North Rhine Westphalian study included three measurements of the carrier- and exposure state. The first two surveys which included 1526 students were taken into account for the case-crossover analyses. Subsequently those individuals were selected as cases that were non-carriers in the first (February/March) and then became carrier of N. meningitidis in the second survey (May/June). 121 subjects fulfilled these criteria. Thus 242 person times were sampled and formed 121 matched pairs (see figure 9).

Within these matched pairs the exposure status of the case at the time of meningococcal carriage is compared with the exposure status of earlier, pre-carriage time periods.

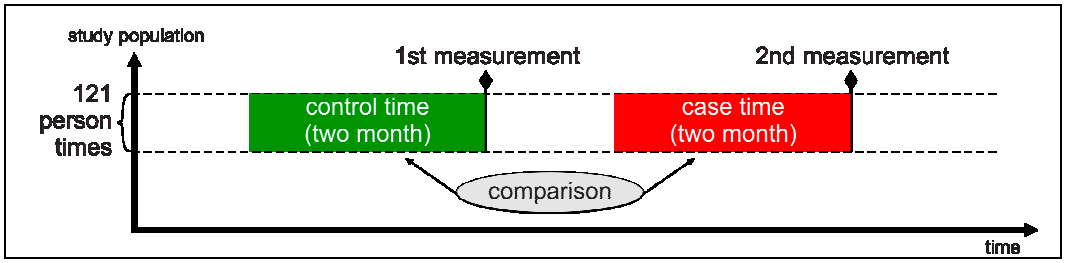

Figure 9: application of the case-crossover design

Meningococcal carriage has an abrupt onset and the predicting factors that were surveyed are both, constant over time as well as short and transient exposures. The questionnaire asked for several factors that are possibly associated with meningococcal carriage. The case-crossover design is putting an emphasis on the proximal causes. For example the socio-economic status of an individual is a chronic effect and therefore can not be investigated with this method but recent infections and antibiotic use are suitable variables.

So far this design has been used several studies on myocardial infarction. Mittleman et al. [1993] investigated whether physical exertion can trigger myocardial infarction and Meier et al. [1998] used the case-crossover design in addition to the classical case-control design to estimate the influence of acute respiratory-tract infections on the risk of developing myocardial infarction. Injury and traffic accidents are also good examples for the usefulness of the case-crossover design. The triggering of injury onset in the work environment was investigated [Sorock 2001] as well as the association between cellular-telephone calls and car collisions [Redelmeier 1997]. But the case-crossover approach has also been used in infectious disease epidemiology. In his study on haemorrhagic fever with renal syndrome Dixon [1997] showed that the case-crossover design is also well suited for research on infectious diseases and that it can help to identify risk factors clearer than the classical case-control design.

4.2.2 Advantages and Limitations of the Study Design

One practical advantage of the case-crossover design is its time and cost effectiveness. Administrative data sources can be used in a lot of settings. The present study was done with secondary data that was originally collected for a longitudinal study. In the car collision study the investigators used police records and a telephone company's billing data [Redelmeier1997]. After linking these data sets, to derive matched pairs, analysis was possible with the case-crossover method. Administrative data often lacks information on chronic factors that might be confounders and hence are important for classical study designs. But the case-crossover design is based on subject matching and therefore automatically controls for confounders that are constant over time [Maclure2000].

Case-crossover analyses might also increase statistical efficiency. Dixon [1997] compared the results of a case-control and case-crossover analysis in his study of risk factors for hemorrhagic fever with renal syndrome. Odds ratios from the crossover approach were higher for risk factors and lower for protective factors.

Maclure started to develop the case-crossover design in the late 1980s and the limitations are still being discovered. As with classical study designs systematic error is possible. Recall bias is a common problem in studies that are based on self-reports of exposures in the past. Differences in the recollection of cases versus controls might be a problem. In the case-crossover design control times are supplied by the cases themselves and the question is therefore whether the timing of transient exposures in the case and control windows can be recollected correctly. This erroneous classification of exposures into a time window is also called differential misclassification [Last 1995]. The problem disappears when objective data sources are used like the telephone company's data in the car collision study.

Like in traditional designs non-differential misclassification can occur in case-crossover studies. This is the case when the probability of erroneous classification is the same in all time windows [Last 1995]. This bias might for example be a problem when the questionnaire is somewhat inaccurate.

The case-crossover design depends on the assumption that the distribution of an exposure is stable over time. But if this is not the case and the exposure status changes over the study period, bias due to trends in time might be introduced. Time can therefore be associated with exposure frequency and the impact of these time trends can be spectacular [Greenland 1996].

To circumvent the above mentioned forms of bias researchers have proposed different approaches. Mittleman et al. [1995] found that the efficiency greatly increased as the number of control time windows that were sampled in each matched set increased. If the data set allows to use the case-crossover design at all, it is often easy to sample more than one time window. It is also possible to compare the case period with the usual frequency of an exposure over a long period of time. In the study of risk factors for the onset of myocardial infarction the usual frequency approach gained the best power.

Suissa [1995] suggested a special type of controlled case-crossover analyses to adjust for time trends. For this so called case-time control design truly non-diseased control persons have to be selected. This control group is then analysed with the same crossover approach than is used in the case-crossover analyses. The comparison of the results of case and control-crossover analyses gives an estimate of the potential influence of time trends.

4.3 Statistical analysis

Case-crossover analysis is similar to the common matched pair analysis. Thus person times are compared while Mantel Haenszel Odds ratios are calculated. For the bivariate analysis EPI-Info Version 6.04d was used (Center for Disease Control and Prevention, Atlanta, USA).

Only those matched pairs that reveal a shift in the exposure status are included in the analysis. These pairs are called discordant pairs. For example the variable rhinitis included 34 discordant pairs. 7 pairs reported a rhinitis during the case window but not in the control window (discordant exposed) and, conversely, 27 pairs had rhinitis during the control time window but not in the case time window (discordant unexposed). The odds ratio is the ratio

of these two and represents the likelihood of the outcome meningococcal carriage. The remaining 87 concordant pairs drop out of the calculation.

In order to describe the relationship between the outcome and a set of risk factors multivariate analysis was done. In matched studies one has to define the variable that specifies the stratum and therefore conditional logistic regression analyses was done using cLOGISTIC Version 1.00Eß3 (Gerard E. Dallal).

5 Results

The meningococcal strain (Phenotype B:4:P1.4) that was associated with the majority of cases of invasive meningococcal disease in North Rhine Westphalia was not isolated from any of the students in both measurements.

Table 1: Bivariate analysis of variables associated with the transmission of N. meningitidis, case-crossover analysis, North Rhine Westphalia 2000 (N = 242; 121 matched pairs)

	No. of discordant pairs	Mantel-Haenszel Odds ratio	95 % Confidence Intervall	p-Value
Travelling abroad	44	**2.67**	1.34 - 5.69	0.004
Rhinitis	34	**0.26**	0.10 - 0.61	0.001
Cinema	25	**0.25**	0.07 - 0.69	0.005
Cold	31	**0.41**	0.17 - 0.92	0.031
Smoking	17	2.40	0.79 - 8.70	0.146
Antibiotics	27	0.69	0.29 - 1.58	0.441
Sport activities	39	0.77	0.39 - 1.52	0.522
Party	18	2.0	0.69 - 6.49	0.239
Pub	26	1.60	0.68 - 3.94	0.327
Discotheque	25	1.27	0.54 - 3.10	0.689

Table 1 presents the results of the bivariate analysis. Students who stated that they had rhinitis during their case period and not during their control period had a reduced risk of becoming a carrier of N. meningitidis (OR 0.26; 95% CI 0.10-0.61). Having had a cold just before the second measurement is also negatively associated with meningococcal carriage (OR 0.41; 95% CI 0.17-0.92). Of all variables that asked for activities like going to pubs, parties or discotheques, none showed a significant association to become a carrier. And while cinema visits in the time before the onset of carriage show a protective association (OR 0.25; 95% CI 0.07-0.69), travelling abroad is a significant risk factor for meningococcal carriage (OR 2.67; 95% CI 1.34-5.69).

The conditional logistic regression analysis shows similar associations as seen in the bivariate results for rhinitis, cinema visits and travelling abroad. Table 2 shows the variables that were included in final model. Rhinitis (OR 0.33; 95% CI 0.13-0.82) and cinema visits (OR 0.17; 95% CI 0.05-0.65) show a protective association, while students were 3.5 times more likely to have travelled abroad

during the case time period than during the control time period (OR 3.50; 95% CI 1.45-8.34). Hence travelling abroad seems to be a risk factor for adolescents to take over carrier state. The Odds Ratio for recent use of antibiotics is 0.39 (95% CI 0.14-1.08). In our study a significant association between onset of meningococcal carriage and smoking status could not been found, neither in the bivariate nor in the multivariate analysis.

Table 2: Multivariate analysis, Conditional logistic regression model, case-crossover analysis, North Rhine Westphalia 2000 (N = 242; 121 matched pairs)

	OR (95% CI)	p-Value
Cinema visits	0.17 (0.05-0.65)	0.0094
Rhinitis	0.33 (0.13-0.82)	0.0171
Travelling abroad	3.48 (1.45-8.34)	0.0052
Antibiotics	0.39 (0.14-1.08)	0.0692

6 Discussion

There are a number of factors that have been linked with meningococcal disease. Active and passive smoking, living in closed or semi-closed communities, seasonal factors and bar or night club visits are known to be potential risk factors for IMD [Fischer 1997; Imrey 1996; Cookson 1998; Neal 2000]. Some studies additionally found asymptomatic carriage to be linked with certain exposures. Conyn-van Spaendonck et al. [1999] reported that increasing family size, visits to discotheques, youth and sports clubs appeared to be a risk factor for carriage while recent antibiotic use was negatively associated with carriage. In a norwegian study Caugant et al. [1994] found age between 15 and 24, male sex and smoking to be risk factors.

A major advantage of the case-crossover design is, that difficulties in the selection of control groups are avoided since each case acts as its own control. As the case-crossover design is based on subject-matching it will control for all possible confounders that do not change within individuals [Rothman 1998]. However there are methodological issues that have to be taken into account for the interpretation of the findings of this study.

The questionnaires collected information on exposures in the past and thus relying on memory related to exposure history. But we were asking for the recent rather than the distant past. In addition carriage of N. meningitidis is asymptomatic and therefore should not produce systematic differences in recall. Thus recall bias is unlikely in the present study.

In contrast to previous studies we found no association between smoking and carriage. This might be the case because smoking is a constant habit. And as described earlier the case-crossover design is not suitable to measure the effect of chronic risk factors.

Antibiotic use can be regarded as a transient exposure and therefore is suitable for this design. But the findings of the present study were statistically not significant and thus do not confirm previous investigations which revealed that

recent antibiotic use can prevent colonization [Conyn-van Spaendonck 1999]. Non-differential misclassification bias could have lead to the insignificant results. The reason may lie in some inaccuracy of the question and in the aspect that students might mix up symptomatic treatment like non-steroidal antiflogistics with actual antibiotic use. Nevertheless the results show p-values close to significance and relative narrow confidence intervals in the bivariate and multivariate analysis. Thus it can be seen as a marker for a protective effect similar to results from other studies.

Rhinitis showed a significant negative association in the bivariate analysis as well as in the conditional logistic regression model. This is interesting since in the literature infections of the upper respiratory tract that cause mucosal irritations are regarded as factors that have the potential to increase the risk of disease [Cartwright 1995; Rosenstein 2001]. One possible explanation for this finding is that the abundant nasal discharge could prevent colonization of the mucosa with N. meningitidis. A study about carriage of N. meningitidis among household contacts of patients with IMD in New Zealand also stated that having a runny nose is negatively associated with carriage [Simmons 2001]. Another possible explanation can be based on immunological alterations during the rhinitis process.

Travelling abroad was positively associated with carriage and seems to be a strong risk factor for taking over the carrier state. The predictor variable travelling is probably an indicator for activities or behaviour while being abroad. Meeting new people (with different strains of N. meningitidis) and being outside of every day life could be aspects that lead to increasing carriage rates. Travellers often have to spend many hours in airports, planes and trains which are air conditioned environments that may cause mucosal irritations due to dry air and hence facilitate meningococcal colonization. In addition travelling frequently goes along with crowded situations which can lead to increased exposure to Neisseria meningitidis.

One special disadvantage of the case-crossover design is the possibility of introducing bias due to time trends in exposure distribution. This means that exposures might not be equally distributed over time. These trends have to be taken into account while interpreting our results of travelling and rhinitis. While

the outcome itself (meningococcal carriage) does not change with season [Ichhpujani 1990], the exposures rhinitis and travelling might do so. The fact that rhinitis is more common in the winter month, where our control time window lies, could have lead to an overestimation of those discordant pairs that contain more exposed controls than exposed cases which would lead to an underestimation of potential effects. An other possible time trend in exposure that could influence our findings could be within the variable travelling abroad. It does not provide detailed information about the journey, for example the duration. Therefore we do not know how valuable the lack of school holidays during the control time window is. However both time windows are earlier than the main holiday and travel season in Germany. To adjust for bias due to time trends a case-time-control analysis was suggested [Suissa 1995] which uses the same crossover approach for cases and controls. But using this study design some of the methodological advantages of a case-crossover design are lost. For example control persons have to be selected. Greenland [1996] considered that this adjustment itself might be confounded. As Mittleman [1995] proposed about control sampling strategies for case-crossover studies, we used a third measurement 4 months after the case time window as additional time window. Due to loss to follow-up the dataset for this additional analysis had only a few discordant pairs which did not allow a useful analysis. Nevertheless, this additional component of the study could help for a better understanding of results in similar case-crossover studies.

Today the epidemiology of N. meningitidis transmission is still not completely understood. This study provides information that gives a more up to date picture of the epidemiological process. The case-crossover design is a useful and cost effective tool to study transient exposures that might trigger the infection with N. meningitides. Knowledge about these factors could provide practical advice for future prevention strategies. When the methodological issues that are discussed above are taken into consideration the case-crossover design allows new insights.

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8 Tables and figures

9 Appendix

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