Contacts of Leprosy Patients: Occurrence and Prevention of the Disease

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Contacts of Leprosy Patients: Occurrence and Prevention of the Disease

Over het vóórkomen en het voorkómen van lepra onder contactpersonen van leprapatiënten

Proefschrift

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Introduction

Introduction

Leprosy in general

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an organism that, after having gained access to the human body, lives and multiplies intracellularly, especially in the Schwann cells of the peripheral nerves and in macrophages.

In the nerves, inflammatory reactions can cause function loss of these nerves, resulting in sensation loss, paresis or paralysis of the muscles, and diminished sweat production. This again may lead eventually to deformities and handicaps that are so much feared.

The infected macrophages form granulomata in the skin and infiltrate the bony and cartilaginous structures of the nose, resulting in ulceration and destruction, leading to typical facial deformities. Other organs like bones, spleen, lymph nodes, kidneys and eyes can also be involved.

Most human beings can muster sufficient resistance against *M. leprae* and infection with this organism will then be aborted unnoticed. In some individuals, however, the bacilli provoke initially remarkably little or even no response of the immune system in respect to building up cellular immunity, the kind of immunity that is needed to combat the disease. The level of the cellular immunity that the body can build up determines the type of the disease according to the Ridley-Jopling classification.[1] When there is enough immunity to limit the spread of bacilli through the body, but not enough to eliminate them fully, a localised disease called tuberculoid leprosy will develop. When there is no cellular immunity at all, bacilli will spread through the body unconstrained, which will result in a form of the disease called lepromatous leprosy. In between these two poles there is a form of disease called borderline leprosy, which is subdivided into three groups: borderline tuberculoid, midborderline and borderline lepromatous. For treatment purposes the people suffering from leprosy are divided into paucibacillary (PB, meaning "with few bacilli") and multibacillary (MB, meaning "with many bacilli") patients. It will be clear that tuberculoid leprosy patients are PB and lepromatous patients MB. The borderline group is divided based on the number of skin lesions present in the individual patient and on the detectability of bacilli in the so-called skin smear, a diagnostic procedure by which fluid from the skin is collected, stained, and examined for the presence of leprosy bacilli.

Although *M. leprae* multiplies slowly (the generation time is uniquely long: 12-13 days), over the years enormous numbers of bacilli can be built up: it has been estimated that in some lepromatous patients a load of seven billion organisms per gram of tissue can be reached. Over time, instabilities in the immune system can cause acute inflammation around invaded cells with sudden nerve function loss or worsening thereof. These episodes are known as "leprosy reactions". Three types of these reactions are distinguished: type I or reversal reactions, type II or erythema nodosum leprosum (ENL) reactions and the Lucio phenomenon, which is rare.

The present treatment of choice to eliminate the infection from the body is multidrug therapy (MDT), for MB patients a combination of three drugs, rifampicin, clofazimine and dapsone during 12 months and for PB patients a combination of two drugs, rifampicin and dapsone

during 6 months.[2] Of these drugs rifampicin is bactericidal, clofazimine is only slowly bactericidal and dapsone is bacteriostatic.

Besides treatment with agents to kill or inhibit the bacilli, several other treatments have been developed to minimise the disabling effects of the disease. For leprosy reactions corticosteroids like prednison are used. Clofazimine has, besides its antibacillary effect, also an anti-inflammatory action which is used in the treatment of leprosy reactions. Thalidomide has a benificial effect on type II leprosy reactions, but its use is limited to male patients and female patients over their reproductive age, because of its teratogenic effect.

Non-medical methods to treat deformities, disabilities and handicaps due to leprosy include surgical procedures like tendon transfers to compensate for paralysis of certain muscles, physiotherapy, occupational therapy and vocational training.

It is common practice to record the "disability grade" of every patient. Grade 0 means that there is no sensation loss in hand or foot nor visible deformity, grade 1 means that there is sensation loss in hand or foot detectable, but no visible damage or disability, and grade 2 includes those patients with visible damage or disability.[2] The percentage of new patients who already are grade 2 disabled is regarded as a measure of the quality of a leprosy control programme.[3]

History of leprosy

The earliest written records of leprosy come from India and are dated around 600 BC. From there it may have spread to the east, to China, where the first record in writing of leprosy is from around 190 BC, and Japan.[4] It is thought that the soldiers of Alexander the Great transported leprosy in western direction, including Greece, where the disease was first described around 300 BC.[5] Skeletons from the second century BC, found in Egypt, showed the first clear signs of leprosy in that part of the world.[6]

During the first millennium leprosy spread northwestwards in Europe, possibly assisted by returning crusaders. The disease undoubtedly had reached Britain by 950 AD.[7] For reasons yet unknown, the leprosy incidence declined in Western Europe after the fourteenth century while the disease reached its peak in Norway only around 1850. In post-Columbian times Portuguese and Spanish soldiers presumably introduced leprosy in the Americas.[7]

Leprosy situation worldwide

According to the World Health Organisation (WHO), worldwide approximately 410,000 new cases of leprosy have been detected in 2004. The peak in detection was in 1998 when 804,000 new leprosy patients were diagnosed. This suggests a dramatic fall in the leprosy incidence (incidence = number of newly diseased persons over a certain period) worldwide, but case detection does not depend on the real incidence alone, but also on case finding efforts. Before 2000 many countries stepped up their leprosy case finding activities in order to reach the leprosy elimination goal, defined as a prevalence (prevalence = number of patients at a given point in time) of less than 1 per 10,000 population, by the year 2000. Therefore, many

new cases of leprosy were found during the years before 2000. Meima et al. analysed the data on case detection of leprosy and concluded that there was no general decline demonstrable until 2004. [8] They also pointed out that prevalence is an irrelevant indicator for monitoring epidemiological changes in leprosy, as it also depends on the duration of treatment. By reducing the average treatment duration by 50%, the prevalence will also be reduced by 50%, without any changes in new case detection or transmission.

Nowadays there are still 6 countries where the prevalence of leprosy is higher than 1 per 10,000. These countries are Brazil, D.R. Congo, Madagascar, Mozambique, Nepal and Tanzania and these account for 24% of the total global leprosy burden.[9]

Leprosy situation in Bangladesh

According to the WHO, in Bangladesh a total of 9844 new leprosy cases were diagnosed in 2002, which means a case detection rate of 0.76 per 10,000. The distribution over the country is not equal, with highest prevalences in the northwest and in the southeast of the country, and in the capital of Dhaka (figure 1). [10]

Nation-wide Bangladesh has reached the WHO elimination goal by the end of 1998, but there are several areas where the prevalence is still above 1 per 10,000. The following districts and metropolitan areas had not reached the elimination goal by the end of 2002:[11]

Metropolitan areas:

- Dhaka (prevalence 4.0)
- Chittagong (2.6)

Districts:

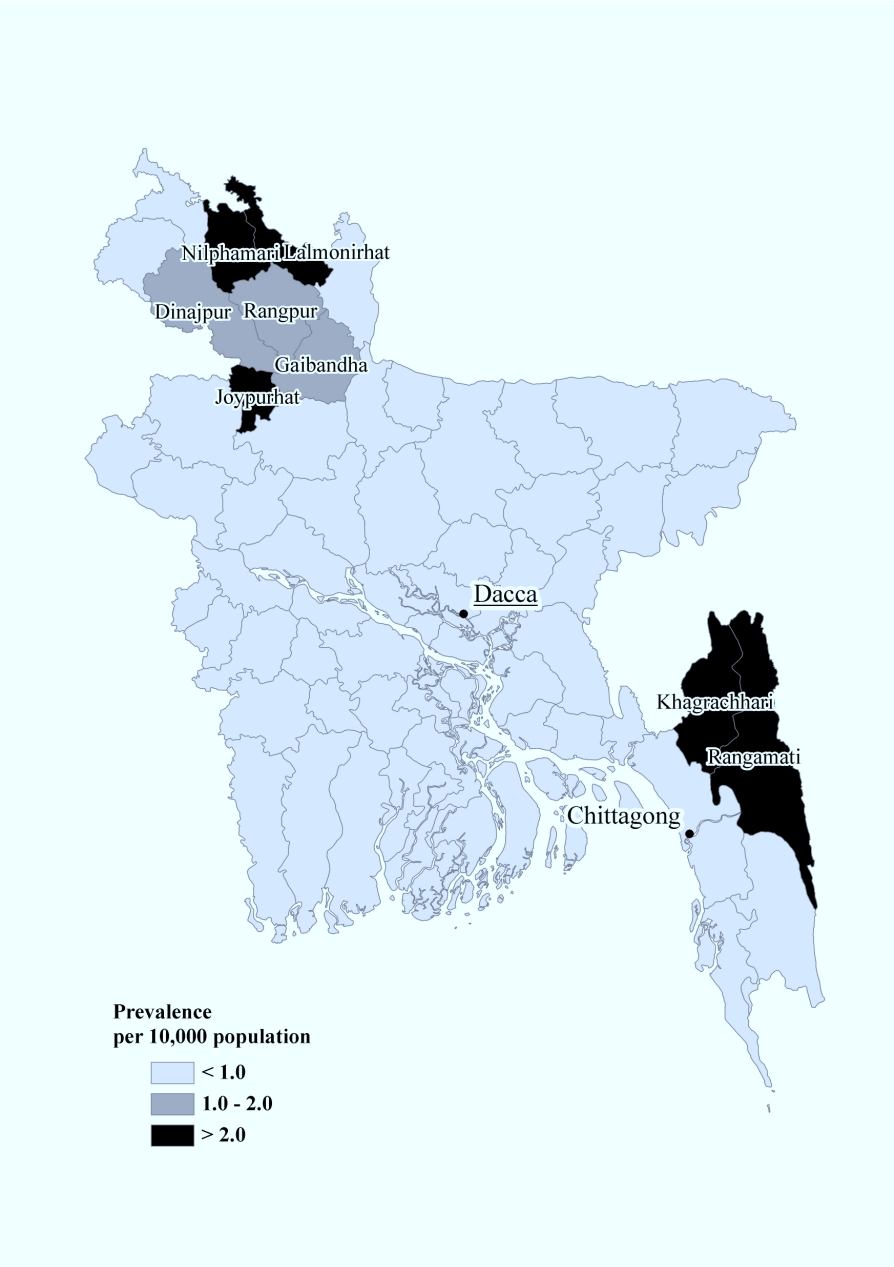
- Chittagong division: Khagrachari (3.0), Rangamati (2.3)
- Rajshahi division: Dinajpur (1.2), Gaibanda (1.4), Joypurhat (2.2), Laimonirhat (2.0), Nilphamari (3.0), and Rangpur (1.3)

Leprosy in Nilphamari and Rangpur

The districts of Nilphamari and Rangpur are located in the northwest of Bangladesh. The Danish Bangladesh Leprosy Mission (DBLM) started its leprosy control programme in Nilphamari in 1977. In 1986 part of Rangpur district was also included and in 1997 the whole of Rangpur district was covered. The peak in new case detection rate was in the mid-nineties of the previous century: in 1994 this was 6.34 per 10,000 population. The registered prevalence dropped from 6.78 per 10,000 in 1994 to 1.39 per 10,000 in 2004 (source: DBLM Annual Report 2004). During this period, the MB rate remained about the same: around 20%. The grade 1 and 2 disability rate dropped from 17.4 to 10.8%

Figure 1. Leprosy prevalence rate in Bangladesh, January 2003

(Source: http://www.searo.who.int/en/Section10/Section20/Section72_886.htm)



Leprosy and contacts of patients with leprosy, prevention of the disease

For many centuries the suspicion among laymen was that contact with a person afflicted by leprosy increased the risk for contracting the disease oneself. More than 60 years ago the first scientific evidence for an increased risk among close contacts was published.[12] In this study only household contacts were actually examined and in the contact studies that followed, different definitions of (close) contact have been used. In this thesis I shall regard all household contacts, neighbours and neigbours of neighbours as well as social contacts who spend 4 h/day on at least 5 days a week as (close) contacts. All others will be regarded as non-contacts, although, of course, occasional contact with a patient with leprosy is likely in this group.

Having concluded that close contacts are more at risk, it has to be kept in mind that most new cases in populations where leprosy is relatively highly endemic are people without known close contact with a patient with leprosy.[13]

The first method used to prevent leprosy was legislation that forced people suffering from a recognisable form of leprosy into isolation and segregation. It is uncertain whether this was an effective way to slow down the transmission of *M. leprae* in the society (see for the situation in Norway [14]).

Another method to prevent disease is vaccination. In leprosy vaccination trials with *M. leprae* alone or in combination with other mycobacteria have been conducted, but to date the best investigated and most promising one is BCG, a vaccine consisting of the mycobacterium of Calmette and Guerin and developed to prevent tuberculosis. Trials with this vaccine have indicated that it gives partial protection against the development of leprosy, especially when administered repeatedly.[15,16] The protective effect varies between 20 and 80%.[17]

The third intervention investigated to prevent leprosy is chemoprophylaxis. After the discovery that dapsone was an effective anti-leprosy drug and patients were treated successfully, trials with this drug to prevent leprosy among those with an increased risk, and also as blanket treatment for whole populations, have been conducted. [18-20] For details and a meta-analysis of these trials see the article by Smith and Smith[21]. They indicated that the protective effect of dapsone was better in trials in which is was given as blanket treatment (around 91%), but also when given to contacts alone it was around 60%. Despite the positive conclusions of these trials, chemoprophylaxis with dapsone never became an accepted preventive measure, partly, doubtlessly, because it had to be given over a longer period of time, which increased the risks for non-compliance and development of drug resistance. Another reason for the weaning interest in chemoprophylaxis was the development of a far more potent treatment of leprosy, multidrug therapy (MDT), a combination of drugs that rendered patients non-infectious after a few doses. It was hoped that early detection and treatment of all new cases with this drug regime would slow down and eventually stop the transmission of *M. leprae*. So far, there is no definite proof that this hope was justified, as new case detection rates of leprosy did fall, but not necessarily because of the treatment.[8] This called for other strategies of leprosy control and the interest in chemoprophylaxis increased again. In recent years a trial with a double dose of rifampicin as chemoprophylactic agent, was conducted in Indonesia by Bakker et al.[22] This study included the population of five small and isolated islands in the Flores Sea where leprosy was highly endemic. The population

of one island served as control group while on one other island only close contacts were given prophylaxis. On the three remaining islands in principle the whole population was treated. The study concluded that only in case rifampicin was given to the total population it was effective in preventing leprosy. If only given to contacts (household, neighbours and neighbours of neighbours) no significant effect could be demonstrated after 3 years of follow-up. The design of the study, an unblinded community intervention trial in which it was not certain whether the communities were in fact completely comparable and with small numbers of people in the different treatment groups, made a confirmation of the findings by another study, with a more robust design and larger numbers of people included, desirable. For this reason the COLEP study (see below) was started.

The main leprosy control method used so far is early case detection and subsequent treatment of all known patients with MDT. Although in general passive case detection has been regarded as the most appropriate method for early case-finding, active case finding methods have been developed and implemented to find hidden leprosy cases. Active mass surveys are not cost-effective and are therefore not routinely applied in leprosy control programmes, therefore many programmes have restricted active case finding to household contacts of newly detected leprosy patients, since this group has an increased risk of disease.[23] Although the majority of the incident cases originate from the pool of people in the population without known household contact,[24] a recent study showed that contact with a leprosy patient is nevertheless the major determinant in incident leprosy, whereby the type of contact is not limited to household relationships, but also includes neighbour and social relationships.[25] Whether this is mainly the result of closer physical contact to the index case, similar genetic and immunological background, environmental factors, or a combination of all, is not yet resolved.[23]

Mathematical modelling suggests that it may not be easy to achieve rapid declines in leprosy transmission solely through intensified case finding in combination with MDT treatment.[26,27] The fact that MDT control has so far failed to convincingly accelerate declines in leprosy incidence necessitates re-thinking of how to control leprosy.[8,28]

Apart from undetected or hidden leprosy patients, other major sources of ongoing transmission are likely to be those who are infected subclinically with *M. leprae*. There is increasing evidence from nasal PCR studies that sub-clinical transmission may exist and that those infected may go through a transient period of nasal excretion.[29] This indicates, as was previously already shown by sero-epidemiological studies, that leprosy is a highly infective disease and transmitted relatively easily in endemic areas.[30,31]

It is likely that high-risk groups (for development of disease and as source of transmission) should be included as target population for the measures to be taken in order to maximise the impact of control measures. In the absence of a fully effective vaccine, one rational intervention strategy would be prophylactic treatment of high risk groups, since it has the potential to greatly reduce the force of infection in the community, as was already expressed by the expert panels during the International Leprosy Congress in China and at other meetings since.[32,33]

Research questions

This thesis describes the methodology and the first results of a large single-centre, double blind, cluster-randomised and placebo-controlled trial to study the effectiveness of a single dose of rifampicin administered to close contacts of recently diagnosed leprosy patients. This trial, called the Prospective (Sero-)epidemiological Study on Contact Transmission and Chemoprophylaxis in Leprosy (COLEP), was conducted in northwest Bangladesh. The research questions for this thesis were:

1. What is the new case detection rate of leprosy in the disricts of Nilphamari and Rangpur in northwest Bangladesh?

2. What is the risk of close contacts of leprosy patients to develop leprosy and what are the contributions of physical and genetic distance?

3. What is the effectiveness of chemoprophylaxis by means of a single dose of rifampicin in preventing leprosy in close contacts?

The COLEP trial had three more research objectives, but these will be dealt with elsewhere. These are: i) to determine the predictive value of anti-PGL-I antibody detection for the future development of leprosy, ii) to determine the usefulness of monitoring the anti-PGL-I antibody levels as a way to measure the effectiveness of chemoprophylactic interventions (Chapter 6 of this thesis is related to this objective), and iii) to determine the cost-effectiveness of chemoprophylaxis-based intervention among close contacts of leprosy patients.

This thesis undertakes to answer the research questions mentioned above by means of various studies related to COLEP. After the general introduction, ***Chapter 2*** provides a literature review on risk factors for the development of leprosy among contacts. ***Chapter 3*** describes the methodology of the trial in detail, as well as some recruitment findings. In ***Chapter 4*** the results of the analyses of the intake data are described, showing that the risk of developing clinical leprosy among contacts is related to both physical and genetic distance, age, and type of leprosy of the index patient (research question 2). In ***Chapter 5*** we estimate the new case detection of leprosy among the general population of northwest Bangladesh and compare these data with the data from the contact population of the COLEP trial (research question 1). ***Chapter 6*** describes the serological findings from the blood samples collected from all patients and contacts included in the trial. ***Chapter 7*** gives the results of the analyses of the data after two years follow-up (research question 3), and in ***Chapter 8*** the main findings are discussed and answers to the research questions of the COLEP trial, as mentioned above, are sought and recommendations given. Finally, ***Chapter 9*** provides a short summary in English and Dutch.

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Risk factors for the development of clinical leprosy among contacts, and their relevance for targeted interventions

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Abstract

Existing knowledge on risk factors for the development of clinical leprosy among contacts of known leprosy patients is reviewed with the aim to identify factors associated with leprosy among contacts that have potential for developing effective targeted interventions in leprosy control. Different definitions of 'contact' have been used and most studies on this subject were among so-called household members. Yet several studies indicate that contacts found in other places than the household are also at risk of developing leprosy. The type of leprosy and the bacterial index are the main patient-related factors involved in transmission, but also contacts of PB patients have a higher risk of contracting leprosy as compared to the general population. The most important contact-related factors are the closeness and intensity of the contact and inherited susceptibility, while the role of age and sex of the contacts is not clear. The role of socio-economic factors is also vague. The significance of immunological and molecular markers in relation to risk of transmitting or developing leprosy is not yet fully understood, but there is an indication that contacts who are sero-positive for anti-PGL-I antibodies are at increased risk of developing clinical leprosy. The presence of a BCG scar is likely to be related to a lower risk. Analogies with tuberculosis suggest that the 'stone-in-the-pond' approach to control may be applicable to leprosy too. Sputum smear negative tuberculosis patients are known to spread the bacteria to others. This analogy strengthens the suggestion that the contacts of paucibacillary leprosy cases should also be included in contact tracing and examination. It is concluded that targeted interventions should be aimed at close contacts of both MB and PB patients inside and outside the household, particularly when genetically related.

Introduction

Contacts of leprosy patients are known to have an increased risk of contracting leprosy themselves. This is not surprising, given the fact that leprosy is an infectious disease caused by Mycobacterium leprae, which is spread from person to person mainly through nasal discharges.[1 - 3] Contact tracing has therefore been a regular activity in many leprosy control programmes with the primary aim of (early) case detection and subsequent treatment. However, in infectious disease control in general, contact tracing and examination may have other objectives as well. Finding new cases of sub-clinical infections among contacts offers the possibility to give passive immunization (e.g. hepatitis B) or, in case of non-viral infectious diseases, prophylactic doses of antibiotics (e.g. meningococcal meningitis). These measures can reduce the risk that infected individuals develop a clinical form of the disease with associated complications and prevent further spread of the disease.

In leprosy control it was hoped that providing multidrug therapy (MDT) to all newly detected leprosy cases would not only lead to healing of the patients, but also to prevention of further spread of Mycobacterium leprae. Unfortunately, there is no convincing evidence for decreased transmission of M. leprae, as the new case detection rate in general has not decreased.[4] Additional interventions need to be considered, preferably focusing on high risk groups for contracting infection with M. leprae and developing clinical leprosy. Prophylactic treatment of contacts is an example of such a possible intervention.[5] In the absence of a method to determine sub-clinical infection with M. leprae reliably, other risk factors for the development of leprosy among contacts need to be identified.

In this paper, we review the literature on data describing the risk of developing leprosy among contacts of leprosy patients and on characteristics of contacts that could be relevant in defining subgroups with different risk levels. Contact definitions will be discussed, followed by a review of potential risk factors. In addition, immunological and/or molecular markers which could be relevant to the development of clinical disease are described briefly. Finally, analogies with tuberculosis are explored as far as these could be relevant for the control of leprosy. The objective of this review is to identify factors associated with leprosy among contacts that have potential for developing effective interventions in leprosy control.

Literature search

First a general literature search using PubMed was carried out using the keywords leprosy, transmission, contact, airborne diseases, tuberculosis and infection transmission either as separate entries or in combination. Then a systematic search using PubMed was carried out for the time period 1940 to 2003, which yielded 253 articles on risk factors and markers in contacts. The Cochrane Library was searched using the keyword strings 'leprosy and contact' and 'leprosy and transmission'. All abstracts that appeared through these searches were scanned on contents, and relevant articles were retrieved. From the references in these retrieved articles, other relevant articles were identified and included into the review.

Definitions of leprosy contacts

One of the first investigations, published in 1942, describing that contacts of leprosy patients had a higher risk of developing leprosy compared to the general population, was that of Doull et al.[6] in the Philippines. Like Doull's, most later studies on contacts in leprosy were on 'household contacts'. The meaning of 'household' is generally regarded to be understood and no further specification is provided. In a number of studies, however, it is more precisely defined as 'those people living in the same house as the index case',[7] or 'a group of people sleeping under the same roof and/or partaking food from the same kitchen as the index case'.[8 - 11] Jesudasan et al.[10] divide household members into two categories: (i) those belonging to the nuclear family (parents, children or siblings) and (ii) others. In some studies in Africa another definition was used: 'a group of people considering the same person as (family) head'.[12,13] Fine et al.[13] divide household contacts into (i) dwelling contacts and (ii) other household contacts, whereby a person was considered a dwelling contact when he or she actually slept in the same dwelling. Amezcua et al.[14] divide household contacts into three categories: (i) those living in the same house, but sleeping in a different room, (ii) those sleeping in the same room, and (iii) those sharing the same bed. Ranade et al.[15] make a division into 'close' household contacts [wife, (grand)parent and (grand)child] and 'not so close' household contacts (all other). The expression 'bedroom contact' has also been used in contrast with other 'house contacts'.[16] Other studies in leprosy use 'close contacts'[17] or 'family members'[18 - 20], without further defining these terms.

As a matter of fact, the definition and the meaning of the word household are culturally determined. Moreover, within cultures there are likely to be groups where the intimacy of contact within a household differs from the other groups (e.g. rich and poor classes).

White et al.[21] distinguish three groups of contacts: (i) house contacts, actually living in the same house, (ii) compound contacts, living on the same compound but in a different house, and (iii) visiting contacts, living outside the compound. A division into six groups of contacts was made by Van Beers et al.:[22] (i) household, (ii) neighbour 1 (living directly adjacent to the patient), (iii) neighbour 2 (living next to neighbour 1), (iv) other relative, (v) daily social, and (vi) daily business.

Literature on other infectious diseases frequently describes contacts in rather vague terms such as 'close' and 'casual'. Freudenstein et al.,[23] for instance, note that the national (UK) guidelines in the management for tuberculosis do not offer a clear definition of close contact. The Division of Tuberculosis Elimination of the Centers for Disease Control and Prevention (CDC) in the United States has developed practical guidelines for contact investigations which still involve subjectivity: a person with a prolonged, frequent or intense contact with a person with TB while that person was infectious is considered to be a close contact.[24] In one study among contacts of tuberculosis patients, close contacts were defined as 'those who slept in the same room, lived in the same house or spent several hours per day with the index case'. The remainder were considered 'casual' contacts.[25] In a social network study on AIDS,

Klovdahl et al.[26] define 'close personal contacts' as those people who were sharing meals or the same house or clothes and other personal possessions together or were having sexual contact or using drugs together. From these studies it is clear that 'closeness' is also associated with the mode of transmission: close contacts may thus be found in places other than the household of the patient.

It can be concluded that, in describing leprosy contacts, various definitions of contact have been used, based on operational and socio-demographic factors. Definitions of close contacts have primarily been confined to household contacts and have generally neglected close contacts found in other places than the household.

Risk factors and relative risks for developing leprosy in contacts

Studies on risk factors in leprosy have been carried out in the general population and among contacts of leprosy patients. Here we focus on risk factors in contacts. The annex lists details of the most important articles describing field studies on contacts. The articles concerning genetics are dealt with in the text, but are not included in the annex. The following (potential) risk factors have been identified.

Type of leprosy

Over the years the definitions and names of the types of leprosy have been subject to change, which should be taken into account when comparing the results. Doull et al.[6] showed that household contacts of all types of leprosy patients had a relative risk of 6, as compared to the general population, to develop clinical disease. Contacts of 'cutaneous' [grosso modo comparable to lepromatous or multibacillary (MB)] patients had an 8-fold increase in risk, whereas for contacts of 'neural' [comparable to tuberculoid or paucibacillary (PB)] patients the risk was four times higher. This study was carried out in the Philippines and included 27,353 person-years with household contact and 307,663 person-years without such contact. Later studies have confirmed the general conclusions that contacts of MB leprosy patients run a higher risk.[9,10,15,22,27 - 30] Fine et al.[13] conducted a study in Malawi including 8741 contacts living in 1656 households among a population of 80,451 people, and found that dwelling contacts of MB patients had a greater risk of contracting the disease than other household contacts, while such a difference was not seen for contacts of PB patients. By dividing PB cases into those whose bacteriological index (BI) was zero and those whose BI was one (presently by definition MB patients), they found evidence that contact associated risk is positively related to the BI.

Intensity of contact and physical distance to a leprosy patient

Sundar Rao et al.[28] (India, the study included 40,625 contacts) found a higher risk for household contacts of leprosy patients as compared to the general population (whereby the contacts of MB patients run a higher risk than those of PB cases). The same was reported by Van Beers et al.[22] from Indonesia, where they did a retrospective and non case-controlled study, in which they also found that 28% of the 101 new leprosy cases they evaluated, could be classified as household contacts. If they included neighbour contacts as well, 63% could be

classified as contacts. Another 15% could be connected to another leprosy patient if social contacts were included. These figures indicate that at least 78% of new leprosy patients could be connected in place and time to a previously diagnosed leprosy patient. The relative risk of household members was 9.4; of neighbour 1 (those living in the house next to a patient) 4.0; and of neighbour 2 (living in a house next to neighbour 1) 1.6. The relative risks for contacts of MB patients were higher than for contacts of PB cases. In the Philippines Cunanan et al.[31] found an even higher relative risk in household contacts: 26 (95% confidence interval 8 - 84) as compared to non-household contacts. This study included 2087 household contacts and 4750 'community' contacts.

In India, Jesudasan et al.[10] and Vijayakumaran et al.[29] studied 9162 and 1661 contacts, respectively, and reported that close household contacts (parents, siblings and children) have a higher risk of contracting leprosy than other household contacts. Several authors also found that the attack rates in households with more than one leprosy patient were twice that of families with only one case.[9,21,27,29] As already mentioned, Fine et al.[13] state that being a dwelling contact of an MB patient is associated with a greater risk of contracting leprosy than being another household contact. It has been argued that family size was relevant, reasoning that the greater the crowding, the more intimate the contact, and it was also suggested that 'bedroom contact' bears a greater risk than other 'house contacts'.[16] Both suggestions could not be confirmed by Newell.[32]

Hausfeld tried to measure exposure in leprosy and described an anthropological method which was used in New Guinea.[33] This method took into consideration that contacts are not limited to households and that the social structure of the community will reflect the transmission and distribution of the disease. A scoring system was developed in order to differentiate between various levels of intensity of contacts. When this method was applied to their study population, it was shown that the incidence of new cases increased rapidly with the closeness of the known level of contact with a lepromatous case.

In summary, it has been established that being a contact of a leprosy patient is a risk factor for contracting leprosy, the extent of the risk being dependent on the closeness of contact. Household contacts (those living in the same house and sharing the same facilities) appear to have the highest risk, but an increased risk for leprosy is not limited to household contacts alone.

Genetic factors

The risk of developing clinical leprosy is thought to be partly determined by hereditary factors.[34 - 39] Most contact studies in leprosy refer to household contacts. As household contacts often share a common genetic background, differences in risk as compared to the general population, could at least in part be attributed to one or more genetic factors. White et al.[21] showed in Uganda where they followed 20,990 children over a period of 8 years, that apparent clustering among closest relatives could well be explained by the more intimate household contact alone and they concluded that if a genetic component of susceptibility existed, it would have a minor influence. They note, however, that in their study group the number of children with contact with a lepromatous patient was too small to draw conclusions on this subgroup. In a review on genetics in leprosy, Beiguelman concluded that

'consanguineous relatives of lepromatous cases are proner to the same form of leprosy than nonconsanguineous relatives (spouses)'.[34] A study in Papua New Guinea among the members of 269 leprosy kindreds showed that leprosy was family related in a population in which the family was not the basic social unit,[35] but Ranade et al.,[15] in India, in another retrospective contact study among 6284 contacts of 1184 leprosy patients, could not find a statistically significant difference in risk of developing leprosy between closely related contacts and those not closely related. A study on twins in India showed that the concordance (the probability that the other of the twins develops disease if one is affected) is more than twice as high in monozygotic twins as in dizygotic.[40] More recent studies concluded that both HLA (DR2) and non-HLA (SLC11A1, formerly NRAMP1 and TNFa) genes contribute to a genetic susceptibility to either leprosy per se or a type of leprosy.[41 - 43] Fitness et al.[37] reviewed this topic in 2002 and concluded that several genes may be involved in susceptibility to leprosy per se or to a type of leprosy, but because many of the associations have only been found in small series of patients or in a single population, these findings would need confirmation in larger studies.

A year later, Mira et al.[39] published the results of a study in Vietnam among 86 families affected by leprosy. They found that a locus on chromosome 6q25 appears to control part of the susceptibility to leprosy per se with a maximum likelihood binomial lod score of 4.31, $P = 0.000005$. This study also confirmed the results of an Indian study showing that a locus on chromosome 10p13 is linked to paucibacillary leprosy, with a maximum lod score of 4.09, $P < 0.00002$.[38]

In conclusion it can be stated that there is accumulating evidence that the risk of developing leprosy is partly genetically determined, although this is as yet not fully quantified. This genetic predisposition could, at least to some extent, explain the observed increased risk to develop leprosy among family contacts of leprosy patients. The contribution of genetic predisposition to the development of leprosy still remains to be disentangled from the effect of relatives living together closely.

Age and sex

Age is found to be a potential risk factor for contacts to develop leprosy. Several authors found that, among the household contacts of MB patients, the risk for children less than 14 years of age was substantially higher than that for adults.[9,10,13,27 - 29] Three of these studies mention a peak rate between the age of 5 and 9 years, but are all referring to the same study population.[9,10,28] Doull et al.[44] reported in 1945 from the Philippines that there was a relation between risk of developing clinical leprosy and the age of initial exposure, the risk decreasing with age of exposure. Noordeen on the other hand states that in high endemic areas like South India (the study of Vijayakumaran was also conducted in South India[29]) the age-specific incidence shows a bimodal distribution with a peak at age 10 - 14, followed by a depression that is again followed by a rise and a plateau over the ages 30 - 60, which is higher than the first peak.[45] He bases this on figures of the WHO. A possible explanation for these seemingly contradictory findings could be the difference in definitions of the age groups as in several studies all people older than 14 are lumped together as adults, while this group is further subdivided in the WHO data.

Considering gender, there have been conflicting findings. Vijayakumaran et al.[29] found no gender difference, which is consistent with the study of Rao et al.,[9] but is in contrast with a study in Malawi where it was found that the risk was significantly greater for males than for females.[13] In an early Indian study, Ali et al.[27] also noted that the attack rate in female contacts was lower than in the male contact group. This was observed by Doull et al. [44] and by Ranade et al.[15] as well.

Several explanations have been proposed for differences in gender related incidence. One might be the differences in diagnostic activities among the two sexes (ascertainment bias). It could also be that men are more exposed to infection as they clothe differently and have more contact with other people. A biological difference cannot be ruled out.[45]

In summary, age and sex have both been shown to be potential risk factors with higher risks seen in young children and older adults as well as in males.

Socio-economic factors

Socio-economic factors could also be of some importance in determining the risk of developing leprosy. Pönnighaus et al.[12] showed a strong inverse relation between the number of completed years of schooling and the leprosy risk, and that good housing conditions were associated with a decreased risk. In contrast, Ali did not find a relation between risk of contracting leprosy and socio-economic factors such as sanitation, housing conditions, economic status, literacy and nutrition.[46] These two studies were carried out in totally different communities and the results are therefore difficult to compare. Moreover, these studies were population studies, not focused on contacts. If socio-economic factors influence the risk of developing leprosy in general, it does not necessarily mean that adverse socioeconomic conditions, once a patient has been identified, increase the risk for the contacts. In airborne diseases in general, however, indoor air quality is a factor that influences the risk of transmission,[47] so it may be assumed that this may also be the case in leprosy.

Immunological and molecular markers

Serological and immunological tests could be helpful in defining groups of contacts at higher risk of developing leprosy, partly because the results of these tests may be an indication of sub-clinical infection. Several studies have shown that antibody levels can be used as a surrogate marker for the bacterial load in the sense that there is a positive correlation between antibody levels and the bacterial index.[48] For a state of the art overview on serology we refer to a recent article by Oskam et al.[49] They state that subclinical infection is far more common than overt disease as antibodies against M. leprae can be detected in 1.7 - 31% of the endemic population. They conclude that serology cannot be used as a single diagnostic test for leprosy, nor can it be used for population screening or for distinguishing past and present infection. It can be used, however, for classification purposes.

From a prospective field study in French Polynesia among 1201 family contacts over a 10-year period, Chanteau et al.[20] concluded that the presence of anti-PGL-I antibodies has a low predictive value for the early diagnosis of leprosy in family contacts (2% risk for seropositive contacts as compared to 1% for seronegative contacts, $P = 0.2$), although the

preliminary results after 2 years of the trial suggested that there was such a relation.[50] In contrast, Ulrich et al.[51] found in a prospective study in Venezuela among 29,000 household contacts, that anti-PGL-I antibody levels indicate a significantly higher risk of developing leprosy in the next 4 years, $P < 0.001$, but that the test would be of very limited value as a screening test in control programmes because of the low sensitivity and specificity. As this study was carried out in the context of a vaccination trial, the results should be regarded with caution as the vaccination could have altered the immune response. Douglas et al.[52] (Philippines) gave preliminary results after 2 years of follow-up of 321 household contacts and 401 controls, stating that the presence of anti-PGL-I antibodies in contacts of MB patients indicated an increased risk of developing leprosy, and in particular MB leprosy: the attack rate for seropositive contacts was 8.3% while the attack rate for seronegative contacts was 0.4%. They also found that only a minority (18%) of MB cases gave rise to sero-reactivity among their contacts. Cunanan et al.[31] found in a study among 6837 contacts, also in the Philippines, that seropositive contacts had a 24-fold increased risk of developing leprosy (95% CI 12 - 45).

The lepromin test is regarded as a marker for the cellular immunity against M. leprae.[53] This test is unfortunately not a good indicator of active or recent infection as in leprosy the specific cellular immunity can be absent, especially in patients with lepromatous disease and the test can be falsely positive due to cross-reactivity between M. leprae and other mycobacteria.[53] Some studies have been carried out combining lepromin reactivity and measurement of antibodies against M. leprae. Dayal et al.[54] found in India, in a prospective study among 455 initially healthy child contacts of different types of leprosy patients, that those children who were antibody positive and lepromin negative had a significantly higher risk of developing leprosy than the other children ($P < 0.01$).

Trials and case-control studies with Bacillus Calmette et Gue´rin (BCG) vaccine both in the general population and in contacts of leprosy patients have indicated that this vaccination gives partial protection against the development of leprosy, especially when administered repeatedly.[55 - 58] The protective effect of BCG vaccination is remarkably consistent in the general population as well as in contacts and is present in countries in South America, Africa and Asia.[59] Although the magnitude of this protective effect differs considerably between the studies, from 20 - 80%, it is likely that BCG vaccination (indicated by a scar) represents a lower risk.

Pattyn et al. examined the presence of specific M. leprae DNA in nasal swabs of a small group of contacts of leprosy patients on the Comores by means of the polymerase chain reaction (PCR).[8] There was no significant difference between the contacts of PB and MB patients (1.9% and 7.9%, respectively, $P = 0:20$), and it was concluded that the observed infection was community-acquired. De Wit et al.[60] (Philippines) found that 19% of the occupational contacts of leprosy patients ($n = 31$) were PCR positive while in the general population this percentage was 12 ($n = 25$). This difference was not statistically significant. In a study in Indonesia, transient positive PCR-tests were observed in 7.7% of nasal swabs obtained from sero-negative individuals in the general population.[7] A correlation between PCR positivity and serology could not be demonstrated.

It can be concluded that immunological and molecular techniques are as yet incapable of identifying individuals with a sub-clinical infection. There is an indication that contacts who are sero-positive for anti-PGL-I antibodies are at increased risk of developing clinical leprosy.

Analogies with tuberculosis

Tuberculosis and leprosy share several characteristics. Both are (at least in part) airborne mycobacterial diseases with a long incubation period. Cellular immunity is necessary to combat both diseases. Moreover, both diseases are capable to give rise to re-infection and relapse. Some of the knowledge from studies in tuberculosis could be relevant for leprosy as well. To date more is known about transmission patterns in tuberculosis, partly because of the availability of molecular epidemiological methods. DNA typing techniques for leprosy are still under development as heterogeneity loci were only identified recently, but developments in this field are going fast. 61 The possibility to use molecular epidemiology will allow a better understanding of transmission patterns, as witnessed by findings from Matsuoka et al.[62] that infection of household members is not necessarily caused by the patient living in that household.

DNA fingerprinting in tuberculosis research made clear that transmission of this disease outside households to other people than close contacts is far more important than previously believed.[63] In a study in San Francisco, it was found that only 10% of the patients who were linked according to fingerprinting techniques, would also have been identified by conventional contact tracing.[64] Klovdahl et al.[65] suggests that outbreak investigations could be more effective if these were not only person oriented ('case-finding'), but also place oriented ('place-finding'), as other places than private households may be involved in outbreaks.

Marks et al.[66] reported a gradual decrease in tuberculin skin test (TST) positivity from contacts belonging to the household, via leisure contacts, relatives not living in the same household and work contacts to other contacts of pulmonary, acid-fast bacilli (AFB) sputum smear (+) TB patients. Beside the distance to the source, source-related factors were found to be important, like the presence of a cavity and high sputum smear positivity. These results were in general consistent with those found by Del Castillo Otero et al.[25] in their study in Spain. In this study, it was also found that not only smear positive patients may transmit the disease, as 43% of the contacts of patients with negative bacteriological results were also infected. This could partly be explained by the fact that there were other close sources who might have caused TB in the index case and infected other contacts as well. Menzies also stresses that contagiousness is not an all-or-nothing phenomenon, depending on more factors than the sputum status alone.[67]

The knowledge about the transmission of tuberculosis and the role of contacts therein raises a number of important and yet unanswered questions for leprosy. These will be addressed in the discussion section.

Discussion

In the literature on leprosy, many different definitions of 'contact' have been used which are based on operational considerations. It can be concluded that people at risk of contracting leprosy are not confined to the group of direct family members living under the same roof, which is the group of contacts currently examined during contact surveys in many leprosy control programmes. Contact events are likely to be more frequent and intense in this group and a higher risk has been demonstrated, but neighbours and social contacts appear to be important contact groups as well. The available data suggest that the risk of contracting leprosy decreases with increasing physical distance to the patient. This hypothesis needs further substantiation because only few studies looked beyond the level of the household in defining contacts.

In order to make maximum use of other sociological parameters of the study population, a scoring system of levels of contact, as described by Hausfeld,[33] would be ideal. However, computer-supported anthropological research among the population concerned would be needed first, which is very time consuming.

Genetic factors probably play a role as well, but genetic distance is often linked to physical distance and many studies do not differentiate between these two parameters. Nevertheless there is an accumulating body of evidence that a genetic relation to a patient is indeed a risk factor.

Gender and age characteristics of the contact could be important, but this has not been established firmly and the data are contradictory. BCG vaccination is partly effective against leprosy as was shown in many studies. Thus the presence of a BCG scar in a contact is likely to indicate a lower risk.

Risk factors, related to the original leprosy patient, for contact transmission are the type of leprosy and the BI.

Current evidence suggests that serological tests could be useful in defining high-risk contacts. The reviewed literature suggests that a contact who is seropositive for antibodies against M. leprae has an (up to more than 20-fold) higher risk of developing leprosy.[31,51,52] Even though the majority of seropositive contacts do not develop clinical leprosy and the majority of new cases develop out of the seronegative group, within a group of contacts of one leprosy patient, those contacts that are seropositive have an increased risk, in particular to develop MB leprosy.[52] Presently, there are no published data relating the serological status of the patient to the risk that this patient spreads the disease. The development of a simple and relatively cheap field test for the detection of anti-PGL-I antibodies makes use of serology in field programmes feasible.[68] Other bio-molecular markers and tests could be useful in defining high risk groups among leprosy contacts as well, but these tests require either follow up visits (reactivity to lepromin) or more sophisticated laboratory facilities than those generally available in leprosy endemic areas.

The available data on leprosy justify the opinion that the stone-in-the-pond model as used in tuberculosis control could be a useful model in leprosy as well. This model is based on a concentric circle approach that assumes that the prevalence of infected individuals is highest near to the source of the infection and gradually decreases as the distance to the source

increases.[69] The results of tuberculosis research strengthen the hypothesis that an effective intervention aimed at prevention of leprosy among contacts of known patients should include other contacts than household contacts and that actively looking for infected individuals elsewhere (e.g. neighbouring houses and the working place) could be effective.

From both leprosy and tuberculosis investigations, it has become clear that the bacterial load of a patient, as measured by a skin smear or a sputum smear, respectively, is an important risk factor for transmission to contacts. However, results of tuberculosis research stress the fact that the sputum status is certainly not the only risk factor. Not only contacts of MB patients but also contacts of PB patients have a higher risk of contracting leprosy than the general population. In analogy to tuberculosis, this suggests that PB patients must not be neglected as a possible source of infection, and that contact examination should also be conducted in case a patient is classified as PB.

Beyond contact tracing and examination to diagnose and treat leprosy in an early phase, other possible interventions for contacts are chemoprophylaxis and (repeated) BCG vaccination. From this review we conclude that targeted interventions should be aimed at close contacts both inside and outside the household, particularly when genetically related. Contacts of PB patients should also be included in such interventions.

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Annex: Publications on risk factors for developing leprosy among contacts

Reference	Design	Contact definitions	Study population	No. index cases or households	Time of follow-up	Risk factors considered	Outcome indicators
Doull et al. 1945 [44] (Philippines)	Retrospective contact study	A person who had lived under the same roof as a leprous person for at least one month	1,520 contacts out of a population of 16,557	Unknown	19,553 PYR	-Age of contact at exposure -Sex of contact	-Annual incidence per 1000 PYR
➢ For those exposed before the age of 5 the male/female incidence ratio in all age groups is > 1. The younger the age group the more males predominated (male/female ratio 4.7 / 1 for the age group of 5-10, and 1.4 / 1 for the age group of 20 years and over). ➢ The age of initial exposure is inversely related to the risk of developing lepromatous leprosy.							
Ali et al. 1966 [27] (India)	Prospective contact study	Family members living with the patient	14,776 contacts	4,383 cases (in 3,666 families, of which 3,104 single patient)	2 years	-Age of contact -Sex of contact and index -Type of leprosy and BI of index -Number of co-prevalent cases	-Attack rate (AR) per 100 per year
➢ Contacts of lepromatous patients suffer the highest attack rate (AR) ➢ The AR in two-source families is almost double that in single-source families. ➢ The AR decreases with age of the contact ➢ The AR among females is lower than among males.							
Rao et al. 1975 [9] (India)	Prospective contact study	Those partaking food from the same kitchen and sleeping under the same roof	22,652 contacts	5,088 families (4,422 single patient families)	77,159.5 PYR	-Age of contact at exposure -Sex of contact -Type of leprosy of index -Number of co-prevalent cases	-Secondary attack rate (SAR) per 1,000 PYR
➢ The secondary attack rate (SAR) among females and males did not differ significantly. ➢ SAR by age at exposure was highest among the age group of 5-9 ($p<0.01$) ➢ SAR almost doubled when there were multiple cases in the family ➢ SAR among contacts of lepromatous patients was 9.5 whereas the SAR among contacts of non-lepromatous cases was 5.8							

Annex-continued

Jesudasan et al. 1984 [10] (India)	Retrospective contact study	Refinement of Rao 1975: same definition of household but divided into two groups: 1. member of nuclear family (parent, sibling, child) 2. others	9,162 contacts with 228 incident cases	1,564 "primary cases"	60,423 PYR	-Age of contact -BI of "primary case" -Number of co-prevalent cases -Closeness of relation	-Incidence rate per 1,000 PYR -Relative risk compared to general population

- Household contacts of non-lepromatous patients had a lower incidence rate as compared to contacts of LL and BL patients ($p<0.05$)
- The relative risk of household contacts of non-lepromatous patients was 2.2 and of BL and LL cases 3.1 as compared to the general population.
- The age specific incidence rate among household contacts reached a peak in the age group of 5-9 years.
- The presence of co-prevalent cases (patients in the household diagnosed as having leprosy at the first survey) increased the incidence rate in that particular household significantly.
- Close household contacts (contacts closely related to the primary case as members of a nuclear family such as parent, child and sibling) have a relative risk of 2 compared to other household contacts.

Sundar Rao et al. 1989 [28] (India)	Retrospective contact study	Same as Rao 1975 and Jesudasan 1984	40.625 contacts	8,642 "primary cases"	176,183 PYR	-Age of contact -Sex of contact -Type of leprosy of index	-Incidence rate (IR) per 1000 PYR -Relative risk compared to general population

- The peak incidence rate (IR) is in the age group 5-9 years.
- The IR among contacts of adult primary cases was significantly higher than the IR among the contacts of child cases ($p<0.01$).
- Household contacts of MB cases have a relative risk (RR, as compared to the general population) of 3-6 whereas the contacts of PB cases have a RR of 2-4.

Annex-continued

Ranade et al. 1995 [15] (India)	Retrospective contact study	Household ("group of people living under the same roof and partaking food from the same kitchen") Two categories: 1. close relation: (grand)parent, spouse, (grand)child 2. others	6,284 contacts	1,184 "primary cases"	74,174 PYR	-Sex of contact -Closeness of relation -Length of contact period -Type of leprosy of index -Treatment compliance of index	- Attack rate (AR) - Relative risk

- The attack rate (AR) for male contacts significantly higher than for female contacts
- No significant difference in AR between close and not close contacts
- No indication that duration of contact influences the AR
- Positive correlation between BI of index and AR among contacts
- Regularity of treatment taken by index had no significant effect on the AR among contacts

Fine et al. 1997 [13] (Malawi)	Prospective contact study	Household = group of people recognizing one person as their head, with two categories: 1. dwelling contacts: sleeping in the same dwelling 2. other household contacts	8,741 contacts living in 1,656 households among a population of 80,451 people	1,887 cases	423,630 PYR (total population regarded as "at risk")	Level of contact by: -Age of contact person -Sex of contact person -Type of leprosy of index -BCG scar of contact	-Incidence rate ratio

- Relative risk (RR) of household and dwelling contacts of MB patients is 5-8 as compared to the general population. RR of contacts of PB patients is 2.
- Dwelling contact with MB cases is associated with a higher risk than just household contact. This association is not seen for contact with PB cases.
- The risk of disease was inversely related to the age of the contact (p=0.08 (MB)-0.14 (PB)).
- Male contacts had a higher risk than female contacts (p=0.05 (MB)-0.72 (PB)).
- RR of individuals without a BCG scar compared to individuals with a scar was 4.8 for MB contacts (95% confidence interval (CI) 2.1-10.9) and 1.8 for PB contacts (CI 1.2-2.6).

Annex-continued

Vijayakumaran et al. 1998 [29] (India)	Prospective contact study	Household contacts divided into two groups: 1. Contacts before MDT of index (original cohort) 2. Contacts after index started on MDT (additional cohort)	1,661 (1094 original cohort, 567 additional cohort)	337 cases	8,403 PYR	-Age of contact -Sex of contact -BI of index -Presence of co-prevalent cases -Index on MDT	-Incidence rate per 1000 PYR -Relative risk

- The original cohort had a higher risk than the additional cohort (relative risk = 2.85, p=0.001)
- Children (up to age 14) had a higher risk than adults (p=0.001)
- No gender difference was seen
- Contacts of patients with a bacterial index (BI) of >2 had a relative risk of 3.01 as compared to contacts of patients with a BI <2 (p<0.001)
- The presence of a co-prevalent case in the household increased the incidence among the original cohort (from 7.5 to 13.4 per 1000 person years of observation (PYR)) (p<0.001).
- The incidence rate (IR) among the general population was 0.9 per 1000 PYR, the IR among the original cohort was 9.1 and the IR among the additional cohort 4.2.

Cunanan et al. 1998 [31] (Philippines)	Prospective population survey	1. Household contacts ("presence of index case at home") 2. Community contact ("other contact")	2,087 (household) 4,750 (community)	37 new cases	6 years	-Type of contact -Seropositivity (Elisa)	-Relative risk

- The relative risk (RR) of household contacts developing leprosy was 26 times greater than of community contacts (95% confidence interval 8-84)
- The RR of Elisa(+) contacts was 24 compared to Elisa(-) contacts (95% CI 12-45)

Annex-continued

De Matos et al. 1999 [30] (Brazil)	Prospective cohort study	Household contacts	758 contacts	Unknown	4 years	-Positivity of Mitsuda skin test of contact -Prior BCG vaccination of contact -Type of leprosy of index	-Incidence rate per PYR -Odds ratio for risk of developing leprosy
➢ The risk was related to the multibacillary form of leprosy in the index case: odds ratio (OR)= 2.547; CI 95%=1.249-5.192. ➢ Prior BCG vaccination was related to a lower risk: OR=0.3802; CI 95%=0.2151-0.06672. ➢ A negative Mitsuda skin test was associated with an increased risk: OR=3.093; CI 95%=1.735-5.514							
Van Beers et al. 1999 [22] (Indonesia)	Retrospective study	Six categories: 1. Household 2. Neighbour 1 3. Neighbour 2 4. Relative 5. Social (daily) 6. Business (daily)	2283 (total population)	101 cases	25 years	-Type of contact -Type of leprosy	-Rate ratio -Relative risk
➢ The rate ratio (defined as the incidence in contact households divided by the incidence in non-contact households) of household contacts was 9.4 (CI 95%=5.7-15.7), of first neighbours 4.0 (CI 95%=2.4-6.9) and second neighbours 1.6 (CI 95%=0.8-3.0) ➢ The relative risk of household contacts of MB patients is 13.7, and of PB patients 5.2, as compared to members of households without any leprosy patient.							

PYR = Person years at risk
BI = Bacteriological index
BCG = Bacille bilié de Calmette et Guérin

3

A study on transmission and a trial of chemoprophylaxis in contacts of leprosy patients: design, methodology and recruitment findings of COLEP

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Abstract

In this article, we describe the design, methodology and recruitment findings of the COLEP study. The objectives of this study were to determine the effectiveness of chemoprophylaxis with a single dose of rifampicin in the prevention of leprosy among close contacts of leprosy patients, and to find characteristics of contact groups most at risk to develop clinical leprosy. These characteristics should be usable by routine leprosy control programmes. COLEP consists of a cluster randomized, double-blind and placebo-controlled trial, a cohort study to determine risk factors characterizing the sub-groups most at risk within the total contact group of a patient, and a cohort study using a reference group from the general population to determine the prevalence and incidence of leprosy in the total population of the study area. The follow-up period will be 4 years. A coding system was developed describing the physical and genetic distance of the contact person to the patient. This study in Bangladesh includes 1037 newly diagnosed and previously untreated leprosy patients and their 21,867 contacts. The prevalence of leprosy among contacts was 7.3 per 1000. A total of 21,708 contacts without signs and symptoms of clinical leprosy are included in a trial of chemoprophylaxis with single dose rifampicin, and randomized at contact group level in treatment and placebo arms. The results of this large field trial will become available in the years to come.

Introduction

The introduction of the relatively short multiple drug treatment regimens (MDT) for leprosy, an infection caused by Mycobacterium leprae, has resulted in a sharp decrease in the number of registered leprosy patients in the world.[1,2] Encouraged by this success, the World Health Organisation (WHO) adopted the goal of elimination of leprosy as a public health problem by the year 2000, defined as a worldwide prevalence of below 1/10,000. This was to be attained by early detection and treatment of patients. This goal was later extended to 2005.[3] However, the world-wide case detection rate is not decreasing, with the detection rate in 1998 being the highest since 1985.[4]

The apparent failure of MDT-based control to achieve declines in leprosy incidence calls for new strategies of leprosy control. High-risk groups for development of disease that may serve as sources of transmission should be included as a target population in new methods to be developed.

This prospective (sero-)epidemiological study on contact transmission and chemoprophylaxis in leprosy (COLEP) is designed to investigate the relative ontributions of several risk factors and the possibilities of chemoprophylaxis to prevent leprosy in close contacts of leprosy patients. The hypothesis behind the study is that close contacts of leprosy patients may already have been infected by M. leprae by the time the patient is recognized and treatment started. By simultaneously treating these subclinical infections among contacts, it is hoped that development of clinical disease and further transmission in this group can be prevented.

The COLEP study has the following objectives:

- To determine the effectiveness of chemoprophylaxis by means of a single dose of rifampicin in preventing leprosy in close contacts.
- To determine the relative risk (as compared to the general population) of contacts of leprosy patients to develop leprosy and to study the contribution of spatial and genetic distance to the index case as risk factors.
- To determine the predictive value of anti-PGL-I antibody detection for the future development of leprosy.
- To determine the usefulness of monitoring the anti-PGL-I antibody levels as a way to measure the effectiveness of chemoprophylactic interventions.
- To determine the cost-effectiveness of chemoprophylaxis-based intervention among close contacts of leprosy patients.
- To determine the incidence and prevalence rates of leprosy in the study area.

In this paper, we describe the design, methodology and basic recruitment findings of COLEP.

Materials and Methods

Study design

The COLEP study is divided into three sub-studies, each one supplementing the others (see also Tables 1 and 2):

1. A single-centre, cluster randomized, double-blind, placebo-controlled trial. There are two treatment groups, one receiving a single standardized dose of rifampicin, the other a placebo. The development of clinical leprosy within these groups will be assessed at 2-year intervals for a period of 4 years by independent (blinded) assessors.

2. These two treatment groups will also be used in a prospective cohort study investigating the predictive value of the presence of anti-PGL-I antibodies for the development of leprosy, and to determine to which extent physical and genetic (kinship) distance to the primary case are risk factors for the development of leprosy.

3. A population-based reference group is formed to determine the prevalence and incidence of leprosy in the general population and to calculate the relative risks of different categories of contacts of leprosy patients to develop the disease.

The Bangladesh Medical Committee granted ethical clearance.

Table 1. Summary of the COLEP trial and expected number of new patients

Reference group (n = 20,000)	Trial group (n = 20,000)	
IR = 0.25-1.0/1000 per year	IR = 1.0-2.0/1000 per year	
	Rifampicin	Placebo
	10,000	10,000
4 years: 20-80 new cases	20-40 new cases*	40-80 new cases

* The expected efficacy of intervention with rifampicin is 50%. It is therefore expected that the incidence rate in the group receiving rifampicin will decrease and that the number of new cases in this group will be lower as compared to the placebo group.

Power calculations

Power calculation for the first sub-study (trial group)

In this power calculation, heterogeneity in the chance of close contacts to develop clinical symptoms of leprosy was taken into account, but no major effect on the numbers needed was found. Given an incidence rate (IR) of 2 per 1000 per year, with a 50% reduction through

intervention (α = 0.05 two-sided, power = 0.80), a total of about 12,000 contacts will be necessary. With an IR of 1 per 1000 per year, this number increases to just over 20,000. This means that about 10,000 contacts in each treatment group is sufficient to detect reliably an expected efficacy of intervention of 50%, even taking into account an expected 10 - 20% loss to follow-up of contacts.

Table 2. Time frame of COLEP trial

	Trial group		
Year/activity	Rifampicin	Placebo	Reference group
2002-2003			
intake	physical examination serology (n = 10,000)	physical examination serology (n = 10,000)	physical examination (n = 20,000) serology (n = 2200)
early analysis			
2004-2005			
1st follow up	physical examination serology	physical examination serology	physical examination serology
mid-term analysis			
2006-2007			
2nd follow up	physical examination serology new cases (estimated): 20-40 *	physical examination serology new cases (estimated): 40-80	physical examination serology new cases (estimated): 20-80
2007-2008			
final analysis			

* The expected efficacy of intervention with rifampicin is 50%. It is therefore expected that the incidence rate in the group receiving rifampicin will decrease and that the number of new cases in this group will be lower as compared to the placebo group.

Power calculation for the second sub-study (serology)

It is assumed that 10% of contacts are seropositive. According to calculations based on a rate of 9:1 for unexposed (seronegative) versus exposed (seropositive), a leprosy incidence of 1 per 1000 per year in the seronegative group (α = 0.05 two-sided; power = 0.80), 10,000 contacts will be sufficient to demonstrate a relative risk of 3 for the development of leprosy in the seropositive group compared with the seronegative group.

Regarding the usefulness of detection of PGL-I antibodies to monitor the effectiveness of chemoprophylactic interventions in preventing leprosy, the power calculations are as follows: based on a initial seropositivity of 10% in contacts at the time of the prophylactic treatment (α = 0.05 two-sided; power = 0.80), a decrease of 3% in seropositivity in the intervention group as compared to the placebo group (e.g. 7% versus 10%) after a period of 4 years can be determined in a random sample of 2000 contacts in each group.

Power calculation for the third sub-study (reference group)

Based on an expected IR over a 4-year period of 1.25 – 5.0 per 1000 population, a representative sample size with 95% confidence level of 20,000 is sufficient when subjects are spread evenly in the population. However, as leprosy is a clustered disease, the reference group will be divided into 20 clusters of 1000 people from the general population in the study area, thus slightly reducing the level of confidence. The clusters will be sampled from the 13 subdistricts of the two districts involved, on the basis of size of the respective populations in the subdistricts. At least one cluster is allocated to each subdistrict, in order to ensure an even geographical distribution. The distribution within the subdistricts is determined at random from lists of villages and unions (larger unit than villages).[5]

Study population

The study will be performed among the population of the districts of Nilphamari and Rangpur in north-west Bangladesh, within the well-developed vertical leprosy control programme of the Danish Bangladesh Leprosy Mission (DBLM). For practical reasons, the upazilla (subdistrict) of Pirgacha has been excluded, as the leprosy control programme there is already integrated into the government health programme. The population of these two districts totals around 4,000,000 (according to the 1991 census), and the number of new leprosy patients among this population during the period 1995 - 2000 was approximately 1800 per year. The population is mainly rural, but within the two districts there are four main towns: Rangpur, Saidpur, Kaunia and Nilphamari. In Saidpur and Rangpur there are sizeable Bihari populations, which are ethnically and socially different from the local Bangladeshi population.

Consent procedure

All eligible subjects (patients, contacts and reference group) will be informed verbally about the study and invited to participate. Written consent is required in the case of blood sampling or taking of prophylaxis. Consent is requested from each adult. For children, consent from a guardian is needed.

Study subjects

Patients

Around 1000 consecutive leprosy patients will be enrolled.

The diagnosis of leprosy is generally carried out according to the DBLM guidelines, which follow those of the national leprosy control programme.[6] However, in the DBLM field programme, a single leprosy lesion with satellites is regarded as single-lesion PB, which is not according to the national guidelines. In order not to interfere with the normal routine programme, this policy has not been changed. All leprosy cases included in the study are confirmed by a medical officer, and this confirmation is written on the patient card.

Exclusion criteria for patients are as follows:

- Any patient who refuses examination of contacts.
- Any patient who suffers from the pure neural form of leprosy.
- Any patient who resides only temporarily in the study area.
- Any new patient found during contact examination of the index case.
- Any new patient living less than six houses (or less than 100 m) away from a patient already included in the study.
- First and second degree relatives of a patient already included in the study.

Contacts

For the 1000 consecutive new leprosy patients, contact groups will be formed consisting of around 20 persons for each patient. Thus the total number of contacts will be around 20,000. All close contacts of the 1000 consecutive new leprosy patients who are recruited for the study will be considered for inclusion. A contact group consists of around 20 individuals. The following categories of contacts have been distinguished:

- Those living in the same house (household members).
- Those living in a house on the same compound, sharing the same kitchen. . Direct neighbours (first neighbours).
- Close business or social contacts, including relatives. In order to be included into this category, one has to be in contact with the patient at least 5 days a week and during at least 4 h/day.
- Neighbours of direct neighbours (second neighbours).

A coding system has been developed to distinguish between several levels of contact. Two parameters were considered: physical and genetic distance to the patient. For physical distance, six categories were defined, based on the local housing situation:

- Those living under the same roof and using the same kitchen (KR).
- Those living under a separate roof, but using the same kitchen (K).
- Those living under the same roof, but not using the same kitchen (R).
- Next-door neighbours (N1).
- Neighbours of the neighbours (N2).
- Social contacts (business contacts, colleagues who stay in the same room at least 4 h a day for 5 days a week) (S).

For the genetic distance, seven categories are defined in which C, P, B and O represent genetic relationships. The others are not genetically related to the patient: spouse (M), child (C), parent (P), sibling (B), other relative (O), relative-in-law (CL, PL, BL or L), non-relative (N).

All contacts are coded according to both types of contact; thus a child-in-law, living next door but using the same kitchen would be coded K and CL. General details of all contacts such as age, gender and presence of a Bacillus Calmette -Guérin (BCG) vaccination scar are recorded, as well as any exclusion criteria, when applicable. All data are entered on a contact registration card.

Exclusion criteria for contacts are as follows:

- Any person who refuses informed consent.
- Any woman indicating that she is pregnant.
- Any person currently on TB or leprosy treatment. . Any person below 5 years of age.
- Any person known to suffer from liver disease or jaundice.
- Any person residing temporarily in the area.
- Any person suffering from leprosy at the initial survey (these patients will be referred to the clinic for leprosy treatment.)
- Any person who is a contact of another (COLEP) patient and is already enrolled in the contact group of the other patient.

Reference group

The reference group (sub-study 3) consists of clusters of inhabitants from 20 selected areas. All people living in the area who are present during the survey and willing to participate are

included, until the number of 1000 per cluster has been reached.

Drugs and placebo administration

The capsules containing either 150 mg rifampicin or placebo were manufactured by Aventis Bangladesh in Dhaka. Independent quality control of the drugs has shown that the quality conforms the requirements of the University Hospital Groningen (The Netherlands). The capsules are the same as used for regular rifampicin production, and thus the name rifampicin is printed on them, regardless of whether it actually contains rifampicin or placebo.

The factory packs the capsules in containers of 120 pieces each, being 30 (maximum number of contacts) x 4 (maximum dosage per contact). One coded container containing either rifampicin or placebo is issued for the complete contact group of one patient. The randomization is done in Rotterdam and the coding of the containers is done in Dhaka by members of the research team from The Netherlands. The codes are kept under lock and key at the Department of Public Health in Rotterdam.

As soon as the prophylaxis is administered to all the eligible contacts of one patient, the container is returned for central storage. The number of unused capsules left in the containers is checked at random. Destruction of the remaining capsules is obligatory to ensure that no placebo medicine labelled rifampicin will find its way into the community. This is done centrally in Nilphamari by incineration.

Chemoprophylaxis regime

A single dose of rifampicin or a placebo is given to all included contacts. The rifampicin comes in capsules of 150 mg and the dosage is the same as recommended in the guidelines of the national leprosy control programme of Bangladesh and DBLM (Table 3).[6] According to body weight and age, 2 - 4 capsules are taken by the contact under direct supervision of a DBLM staff member. All the contacts of one patient receive medication from the same container.

Other possible chemoprophylactic regimes as well as the risk of the development of resistance against rifampicin by M. leprae or M. tuberculosis were considered. Expert opinion made clear that an additional positive effect could not be expected from other regimes and that the risk of development of resistance after a single dose of rifampicin is remote.[7]

Table 3. Dosage of rifampicin according to age and body weight

Age / Weight	*Dose of chemoprophylaxis*
Adult > 35 kg	600 mg
Adult < 35 kg	450 mg
Child 10-14 years	450 mg
Child 5-9 years	300 mg

Serology

Finger prick blood samples are collected from all patients and contacts and from one in every nine persons from the reference group. All samples are collected on Schleicher & Schuell blotting paper GB 002, dried and stored at −20^{0}C until transport to the Netherlands. In addition, finger prick blood from all patients is immediately tested in the field using the ML Flow test, a newly developed lateral flow test that is capable of detecting anti-M. leprae IgM antibodies.[8]

At KIT Biomedical Research, an ELISA for the detection of anti-M. leprae IgM antibodies is performed according to established procedures.[9] The antigen used is NT-PBSA, a semi-synthetic analogue containing the M. leprae-specific trisaccharide moiety of phenolic glycolipid-I.

Monitoring intake and follow-up

After a patient is diagnosed, patient details are recorded such as type of leprosy, duration of symptoms, bacillary index (BI) etc. MDT is started according to the national guidelines. Intake of single-lesion PB (SLPB) patients will be stopped when 400 such patients have been included; the same will apply to the group of other PB patients (PB2-5, with two to five skin lesions on physical examination). This will ensure an intake of at least 200 MB patients. After the patient has received his second dose of MDT, the contact survey is performed. In this way, the chance of re-infection of the contacts by the patient is kept low. During the contact survey, a check for signs and symptoms of leprosy is done. If leprosy is diagnosed, this is recorded and the newly found patient is referred to the clinic for appropriate treatment. This particular contact is then excluded from the trial. All other members of the contact group are asked for a blood sample, which is collected on filter paper for ELISA testing. The results of these tests will not be made available, neither to the contact nor to the field worker, until the study is completed. All field data are recorded on paper initially and entered in a computer database afterwards. The prophylaxis is taken under direct supervision. Follow-up examinations will be carried out after 2 and 4 years.

For the reference group, mass surveys are done in the selected areas. All subjects are checked for signs and symptoms of leprosy and one in every nine subjects is asked for a blood sample. Details are recorded and follow-up examinations will be performed after 2 and 4 years (see Table 1).

Outcome measures

The primary outcome measure is the number of new leprosy patients emerging from the contact groups. The proportions between the rifampicin and the placebo group will be compared at 2-year intervals. Analysis will be carried out in order to define special groups at risk. The results of the serological tests will also be compiled and analysed. The number of leprosy patients found in the reference group will be used to calculate the prevalence rate (at intake) and the incidence rate (during follow-up) in the general population, allowing for calculation of relative

risks among the contacts. A cost-effectiveness study will be part of the analysis.

Data handling and analysis

A separate database has been designed, which is a modification of the database already in use at DBLM. Data are entered in the field during clinic visits and contact/reference group surveys, onto specially designed data sheets. These data are sent to Nilphamari, where they are entered into the database. All paper forms are scanned and filed on hard disk and CD. The paper copies of the data will be retained for a minimum of 2 years after the COLEP study has been completed. An electronic copy of the database is sent to the department of Public Health of Erasmus MC in the Netherlands on a monthly basis. Modern back-up facilities are available at Nilphamari as well. Protection of privacy of patients in the database will be according to Erasmus MC standards. Quality checks on all aspects of the data collection and entry are performed regularly, and feedback on the results is given to the field staff, laboratory personnel and the data entry assistant.

The blood samples on filter paper are sent for ELISA testing to KIT in Amsterdam. The results of these tests will be entered into the database at KIT and an electronic copy is sent regularly to the central database in Rotterdam. The analysis of the data will be done in The Netherlands at Erasmus MC in Rotterdam and KIT in Amsterdam using appropriate statistical methods.

Results

Pilot phase

In the preparatory phase of the study, a 2-month pilot phase was included, which was evaluated before the official start of the study. Evaluation was carried out on the aspects of participation, reasons for exclusion and the practical application of the definitions used in the protocol. The main difficulty in defining contacts was the relative code: parents, spouses, children, brothers and sisters were no problem, but the child of a brother-in-law was sometimes coded as CL (child-in-law) and the difference between O (other relative) and OL (other relative-in-law) was not always clear to the field staff. The distance code was generally filled in correctly, but the difference between N(eighbour)1 and N2 was sometimes vague, as the real situation often differed from the standard lay-out provided. In general, however, it was clear that N1 contacts lived closer by than N2 contacts. By the high number of S (social) contacts and by the housing situation described, it was suspected that many of these S contacts were in fact neighbours of N2 contacts, who did not meet the criteria for the S category. This was indeed the case and special attention was given to correct this, although it remained a weak point during the intake phase.

Recruitment findings: patients and contacts

Contacts of 1037 newly diagnosed leprosy patients were included in the study. Distribution of age, sex and classification of these patients are given in Table 4. A total of 28,083 contacts were registered, of whom 6216 were excluded for various reasons (see Table 5). Although the experimental nature of the study was explained clearly, motivation among the contact population to participate in the study was high, resulting in relatively few refusals (1.2%). The remaining 21,867 contacts were examined for leprosy, of which 159 were confirmed to have previously undiagnosed leprosy. These newly found patients commenced MDT and were excluded from the trial. In total, 21,708 contacts were finally included in the trial. With 159 newly diagnosed leprosy patients, the intake survey revealed a prevalence of 7.3 per 1000 among contacts (Table 6). The prevalence was higher among contacts of paucibacillary index patients with two to five patches and multibacillary index patients, compared with single lesion paucibacillary index cases.

Table 4. Distribution of age, sex and classification of newly detected leprosy patients

	Male				Female				Total
Age	SLPB	PB2-5	MB	Total	SLPB	PB2-5	MB	Total	
5-9	3	5	1	9	2	6	2	10	19
10-14	21	22	12	55	20	19	6	45	100
15-19	37	39	23	99	26	16	5	47	146
20-29	60	43	38	141	33	21	12	66	207
30-39	47	40	39	126	32	27	8	67	193
40-49	32	39	66	137	33	19	14	66	203
50 and older	38	26	52	116	16	20	17	53	169
Total	238	214	231	683	162	128	64	354	1037

Discussion

Although in general passive case detection has been regarded as the most appropriate method for early case-finding, in many settings where awareness, motivation, and diagnostic procedures were regarded as inadequate, active case finding methods have been developed and implemented to find hidden leprosy cases. From this, it became clear that leprosy control programmes face the problem of many leprosy cases remaining undetected. Transmission that took place before the case finding will result in many more new cases in future, years after the completion of these campaigns and most likely also years after the elimination goal as defined by WHO has been reached. Mathematical modelling suggests that it may not be easy to achieve rapid declines in leprosy transmission solely through intensified case finding in combination with MDT treatment.[10,11] It will therefore remain important for leprosy control programmes to detect new patients as early as possible and to do so in an effective and sustainable manner.

Table 5. Reasons for exclusion in contact population

	N	*n*	%
Total number of contacts enumerated	28,083		100%
Reason for exclusion:			
Refusal of informed consent		338	1.2%
Under five years of age		2964	10.6%
Absent		2217	7.9%
Temporary resident		131	0.5%
Pregnancy		438	1.6%
Liver disease or jaundice		51	0.2%
Current TB or leprosy treatment		42	0.1%
Contact of other COLEP patient		4	0%
Not recorded		18	0.1%
Suspected leprosy		16	0.1%
Total excluded from contact examination		6216	22.1%
Total included for contact examination	21,867		77.9%
Confirmed new leprosy		159	0.6%
Total excluded from trial		6375	22.7%
Number of contacts included in trial	21,708		77.3%

Table 6. New cases of leprosy detected on examination of close contacts

Type of leprosy of patient (index)	No. of patients	No. of contacts included	No. of contacts with leprosy	Prevalence per 1000 contacts	95% CI
Single skin lesion (one patch) paucibacillary	400	8835	49	5.5	4.1-7.3
Two to five patches paucibacillary	342	7013	62	8.8	6.8-11.3
Multibacillary	295	6019	48	8.0	5.9-10.6
Total	1037	21,876	159	7.3	6.2-8.5

With the integration of leprosy control activities into the general health service, and the limited resources available, less efforts in active case finding in general populations may be expected. As leprosy disease becomes less frequent (and thus less well-known), detection delays may also increase, with possible negative consequences for both disability and

transmission, since many of the undetected patients may serve as a continuous source of infection.

Mass surveys to actively detect new patients are not cost-effective and are therefore not routinely applied in leprosy control programmes. Many programmes have restricted active case finding to household contacts of newly detected leprosy patients, since this group has an increased risk of disease.[12] However, the majority of the incident cases originate from the pool of people in the population without known household contact.[13] A recent study showed that contact with a leprosy patient is nevertheless the major determinant in incident leprosy, whereby the type of contact is not limited to household relationships, but also includes neighbour and social relationships.[14] This concept shows similarities with the 'stone-in-the-pond' principle describing tuberculosis transmission in concentric circles around a patient.[15] In principle, this concept could be translated into a valuable and sustainable tool for leprosy control programmes and elimination campaigns by focussing case detection, prophylactic intervention and health promotion activities not only on household contacts but also on at least neighbours of leprosy cases. Such a strategy especially becomes feasible in circumstances with reduced caseload because high-risk groups become 'manageable' in size (and distinguishable from the general population). Furthermore, this concept concurs with the view that under such circumstances an outbreak approach of leprosy control is needed, whereby contacts of the index case are examined.

Apart from undetected or hidden leprosy patients, other major sources of ongoing transmission are likely to be those who are infected subclinically with M. leprae; especially for persons incubating multibacillary disease this is easily conceivable. There is increasing evidence from nasal PCR studies that sub-clinical transmission may exist and that those infected may go through a transient period of nasal excretion.[16] This indicates, as was previously already shown by sero-epidemiological studies, that leprosy is a highly infective disease and transmitted relatively easily in endemic areas.[17,18] Household contacts, neighbours, and social contacts have a higher chance to contract the disease. Whether this is mainly the result of closer contacts to the index case of the infection, similar genetic and immunological background, environmental factors, or a combination of all, is not yet resolved.[12] By using standardized contact definitions in the COLEP study, relative risks for developing leprosy can be calculated for several categories of contacts.

The fact that MDT control has so far failed to accelerate declines in leprosy incidence necessitates re-thinking of how to control leprosy.[19,20] It is likely that high-risk groups (for development of disease and as source of transmission) should be included as target population for the measures to be taken in order to maximize the impact of control measures. In the absence of a vaccine, one rational intervention strategy would be prophylactic treatment of high risk groups, since it has the potential to greatly reduce the force of infection in the community, as was already expressed by the expert panels during the International Leprosy Congress in China and at other meetings since.[21,22] It is well documented that dapsone has a chemoprophylactic effect.[23,24] Now, with modern powerful short-course antibiotic combination therapies at hand, the next logical step in this area is to study the feasibility (costs versus effect) of chemoprophylaxis with rifampicin as an intervention strategy alternative to an only-patient-based MDT control of leprosy.

After several years of extensive investigation, it has become apparent that serology with PGL-I is useful for detection of contacts at high risk of developing disease. Furthermore, it has been suggested that seroprevalence in school children is an indicator of the leprosy endemicity in an area and could thus be valuable as an indicator of transmission.[25] The simple lateral flow assay for the detection of antibodies to PGL-I, which is now available, promises to be useful under field conditions.[8]

In 2000, the official registered prevalence of leprosy in the study area (Nilphamari and Rangpur districts) was 2.63 per 10,000 population and the new case detection rate 4.33 per 10,000. In Bangladesh as a whole, the official figures in that year were 0.82 per 10,000 and 1.17 per 10,000 respectively.[26] The intake survey for the COLEP study revealed a prevalence of 7.3 per 1000 among contacts. The COLEP study was designed to answer several urgent questions related to contact transmission, risk factors for contracting leprosy, and the possibility of preventing leprosy by means of chemoprophylaxis. Included in COLEP are 1037 newly diagnosed leprosy patients and 21,708 close contacts. These contacts are included in a trial of chemoprophylaxis with single dose rifampicin, and are randomized at contact group level in treatment and placebo arms. The results of this large field trial will become available in the years to come.

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4

Physical distance, genetic relationship, age, and leprosy classification are independent risk factors for leprosy in contacts of patients with leprosy

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Abstract

Background: Close contacts of patients with leprosy have a higher risk of developing leprosy. Several risk factors have been identified, including genetic relationship and physical distance. Their independent contributions to the risk of developing leprosy, however, have never been sufficiently quantified.

Methods: Logistic-regression analysis was performed on intake data from a prospective cohort study of 1037 patients newly diagnosed as having leprosy and their 21,870 contacts.

Results: Higher age showed an increased risk, with a bimodal distribution. Contacts of patients with pauci-bacillary (PB) leprosy with 2-5 lesions (PB2-5) and those with multibacillary (MB) leprosy had a higher risk than did contacts of patients with single-lesion PB leprosy. The core household group had a higher risk than other contacts living under the same roof and next-door neighbors, who again had a higher risk than neighbors of neighbors. A close genetic relationship indicated an increased risk when blood-related children, parents, and siblings were pooled together.

Conclusions: Age of the contact, the disease classification of the index patient, and physical and genetic distance were independently associated with the risk of a contact acquiring leprosy. Contact surveys in leprosy should be not only focused on household contacts but also extended to neighbors and consanguineous relatives, especially when the patient has PB2-5 or MB leprosy.

Introduction

It has been established that contacts of patients with leprosy have a higher risk of developing leprosy than does the general population. Several risk factors besides being a contact per se have been suggested, such as the type of leprosy of the index patient, the age and sex of the contact, and the genetic and physical distance of the contact to the patient. [1-5] Contact tracing is an important intervention in leprosy control, but it is usually limited to immediate contacts, such as persons living in the same household. Beyond contact tracing and examination to diagnose and treat leprosy at an early phase, other possible interventions for contacts are chemoprophylaxis and repeated bacille Calmette-Guérin (BCG) vaccination. From a review of the literature, it was concluded that targeted interventions should be aimed at close contacts both inside and outside the household, particularly when those persons are genetically related to the index patient, and that contacts of patients with paucibacillary (PB) leprosy should also be included.[6] The independent contribution and relative importance of the various risk factors to the risk of developing leprosy, however, have never been studied in detail or sufficiently quantified. This is particularly the case for genetic and physical distance, 2 important factors that have never been disentangled. The Prospective Seroepidemiological Study on Contact Transmission and Chemoprophylaxis in Leprosy (COLEP) was developed to investigate the potential benefits of chemoprophylaxis among contacts of patients newly diagnosed as having leprosy. It was a double-blind, placebo-controlled trial with a follow-up period of 4 years.[7] To define the subgroups most at risk for developing leprosy, the contacts were coded in detail according to both the physical and the genetic distance from the patient. In the present article, we describe the contact population of the COLEP study and analyze the prevalence of leprosy among the contact population and its relationship to different contact and patient characteristics.

Subjects, Materials, and Methods

Study population

The COLEP study was performed in northwest Bangladesh in 2 districts with a total population of over 4 million people. The study population consisted of contacts of 1037 consecutively found new patients with leprosy. The intake of patients with single-lesion PB (SLPB) leprosy was limited to 400. The number of patients with PB leprosy with 2-5 lesions (PB2-5) and with multibacillary (MB) leprosy was 342 and 295, respectively. Intake started in May 2002 and was completed by the end of October 2003. The following contacts were excluded: those who refused to provide informed consent, pregnant women, any person currently receiving treatment for tuberculosis or leprosy, children <5 years old, any person known to have liver disease or jaundice, any person residing temporarily in the area, and any person known to be a contact of another COLEP patient and who had already been enrolled in the contact group of the other patient.

All eligible subjects (patients and contacts) were informed verbally about the study and invited to participate. Written consent was requested from each adult, and, for children, consent from a guardian was provided. The Bangladesh Medical Committee granted ethical

clearance for the study, which followed the ethical guidelines of the Erasmus MC in Rotterdam. Details of the scope and methodology of the COLEP study have been described elsewhere.[7]

Contacts

A coding system distinguished between several levels of contact, including the parameters of physical and genetic distance from the patient. For physical distance, the categories were based on the local situation, where most people live in single-room houses. Those who do not share the same house often share the same kitchen—a separate structure on a common compound. Sometimes people live in attached houses, sharing a roof without sharing the same kitchen and garden. These last contacts were regarded as being more distant than those who share a kitchen but as being closer than other immediate neighbors.

For physical distance, we defined 6 categories:

- those living under the same roof and using the same kitchen (KR),
- those living under a separate roof but using the same kitchen (K),
- those living under the same roof but not using the same kitchen (R),
- next-door neighbors (N1),
- neighbors of next-door neighbors (N2), and
- social contacts (e.g., business contacts, colleagues, or close friends who stay in the same room at least 4 h/day for 5 days a week; (S).

For genetic distance, 7 categories were defined: spouse (M), child (C), parent (P), sibling (B), other relative (O), relative-in-law (CL, PL, BL, or OL), and nonrelative (N). The C, P, B, and O categories represent genetic (blood) relationships, and the others represent no genetic relationship.

All contacts were coded according to both types of contact; thus, a child-in-law living next door but using the same kitchen would be coded as K and CL. General details of all ontacts—such as age, sex, and the presence of a BCG vaccination scar—were recorded, as was the presence of 1 or more of the exclusion criteria. Persons suspected of having leprosy at the time of the initial survey were excluded from the chemoprophylaxis trial.

Statistical analysis

Data analysis was performed by means of logistic regression using SPSS for Windows (release 11.0.1; SPSS). First, univariate logistic regression was done with leprosy as the dependent variable and with genetic distance, physical distance, age, classification of the index patient, BCG vaccination, and sex of the contact as independent variables. The variables showing significant or nearly significant effects ($P < 0.1$) were included in a multivariate logistic-regression model, and stepwise forward and backward procedures were performed. Because the number of parameters of the remaining 5 variables would have been too high in relation to the number of events (giving a risk of overfitting the model), the categories of the variables genetic distance, physical distance, and age were redefined before the regression

analysis. Genetic distance was divided into 2 categories: closely blood-related contacts (C, P, and B) and those not closely related by blood (all others). The physical distance categories R and N1 were combined, as were the categories N2 and S, which thus created 4 categories in this variable. Age was divided into 5 categories. Together with the variable for BCG vaccination, a total of 16 parameters were thus created. The final model consisted of 4 variables with a total of 14 categories.

Collinearity between the variables was tested in the final model by examining the correlation matrix, which showed no absolute values above 0.58 (the value between physical and genetic distance), by running a logistic regression with genetic distance as the dependent variable and the other 3 as independent variables (Nagelkerke $R^2 = 0.487$) and by running a linear regression with leprosy as the dependent variable and the other 4 as independent variables (highest variance inflation factor, 1.520). This indicated that there is collinearity between genetic and physical distance but that it did not create an unacceptable imbalance in the final regression model. Effect modification was tested by adding all possible interaction terms one by one to the model and repeating the analysis each time. No significant effect was found for any of these terms. Because there may be an increased risk if the patient and contact are of the same sex, the sex of the patient and of the contact were also entered in a regression model, together with their interaction term. This interaction term did not show a significant effect. Finally, the goodness-of-fit test (Hosmer and Lemeshow) showed that the final model was fitting the data. By leaving the least significant variable (genetic distance) out of the model, a reduction in the Nagelkerke R^2 value was seen, so this variable was kept in the model. Odds ratios (ORs) were calculated, but, because of the number of events, these were comparable to relative risks.

Results

For the 1037 patients with leprosy, the total number of contacts counted was 28,083 (the average number of contacts per patient was 27). A total of 6213 contacts were excluded because of the several exclusion criteria, mainly because they were <5 years old (2964) or were absent (2217). Table 1 shows the remaining 21,870 contacts according to physical and genetic distance and divided by sex, age, type of leprosy of the patient, and BCG scar. In 21,701 (99%) of 21,870 cases, the duration of contact was >6 months; in 124 it was shorter, and in 45 the duration was not recorded. Among these contacts, 159 new cases of leprosy, all PB, were found; the detection rate of new cases was 7.3 cases/1000 contacts (95% confidence interval [CI], 6.2-8.5). Table 2 shows the number of contacts with leprosy divided by type of leprosy of the index patient. For 4 of the newly discovered cases, no details about physical and genetic distance had been recorded, leaving 155 cases for the analysis.

Table 1. The contact population included in the COLEP study, by physical and genetic distance.

	Sex		Age years							Type of leprosy[1]			BCG		Total
Physical distance[2]	Male	Female	5-9	10-14	15-19	20-29	30-39	40-49	>49	MB	PB2-5	PBSL	Pos	Neg	
KR	857	1135	408	382	216	251	296	240	199	600	637	755	846	1120	1992
K	847	745	155	249	255	335	176	170	252	428	493	671	593	984	1592
R	60	80	18	22	22	27	20	12	19	86	30	24	52	87	140
N1	2714	2987	882	876	588	1010	1005	679	661	1559	1816	2326	2204	3451	5701
R+N1	2774	3067	900	898	610	1037	1025	691	680	1645	1846	2350	2256	3538	5841
N2	3538	3964	1146	1217	861	1273	1194	897	914	2003	2387	3112	2876	4563	7502
S	2420	2519	719	804	512	867	771	619	647	1418	1576	1945	2037	2850	4939
N2+S	5958	6483	1865	2021	1373	2140	1965	1516	1561	3421	3963	5057	4913	7413	12441
Not recorded	1	3			1			3			2	2		1	4
Genetic distance[3]															
C	748	478	345	346	212	184	100	33	6	417	369	440	662	552	1226
P	355	488			1	23	142	258	419	199	298	346	136	694	843
B	962	384	147	254	202	239	244	164	96	395	431	520	538	795	1346
Total closely related	2065	1350	492	600	415	446	486	455	521	1011	1098	1306	1336	2041	3415

Table 1 – continued

	Sex		Age years							Type of leprosy[1]			BCG		Total
	Male	Female	5-9	10-14	15-19	20-29	30-39	40-49	>49	MB	PB2-5	PBSL	Pos	Neg	
O	3820	2463	1480	1483	900	769	619	467	565	1807	1981	2495	2975	3259	6283
M	183	433		1	28	149	189	137	112	195	183	238	151	462	616
CL	74	230	24	18	40	131	68	14	9	112	81	111	91	210	304
PL	63	128		1	3	8	23	37	119	37	52	102	24	166	191
BL	343	919	28	41	82	362	375	230	144	338	367	557	351	900	1262
OL	781	2328	277	298	263	748	673	455	395	712	1054	1343	1072	2000	3109
N	3107	3580	1027	1108	724	1150	1029	822	827	1882	2124	2681	2608	4018	6687
Total not closely related	8371	10081	2836	2950	2040	3317	2976	2162	2171	5083	5842	7527	7272	11015	18452
Not recorded	1	2						3			1	2			3
Total (%)	10437 (47.7)	11433 (52.3)	3328 (15.2)	3550 (16.2)	2455 (11.2)	3763 (17.2)	3462 (15.8)	2620 (12.0)	2692 (12.3)	6094 (27.9)	6941 (31.7)	8835 (40.4)	8608 (39.4)	13056 (59.7)	21870 (100)

Type of leprosy of the index case. MB = multibacillary, PB2-5 = paucibacillary with 2 to 5 lesions, PBSL = paucibacillary with a single lesion.
KR = sharing roof and kitchen ("household"), K = neighbour sharing the kitchen, R = neighbour sharing the roof, N1 = next door neighbour, not sharing kitchen or roof, N2 = neighbour of neighbour, S = social contact
C = child, P = parent, B = brother or sister, O = other (blood) relative, M = spouse, CL = child-in-law or step child, PL = parent-in-law or step parent, BL = brother- or sister-in-law, OL = other relative-in-law, N = non relative

Table 2. New cases of leprosy among close contacts divided by type of leprosy of index case

Type of leprosy of patient (index)	No. of patients	No. of contacts included	No. of contacts with leprosy	No. (95% confidence interval) of cases of leprosy/1000 contacts
Paucibacillary				
1 lesion	400	8835	49	5.5 (4.1-7.3)
2-5 lesions	342	7013	62	8.8 (6.8-11.3)
Multibacillary	295	6019	48	8.0 (5.9-10.6)
Total	1037	21,867	159	7.3 (6.2-8.5)

The results of the univariate logistic-regression analysis for age, physical and genetic distance, sex, and the presence of a BCG scar in the contact are shown in table 3. The effect of the leprosy classification is shown as the unadjusted OR in table 4.

Age showed a bimodal distribution, with an increasing risk (compared with children 5-9 years old) in persons 10-19 years old and again in those ≥30 years old. This was more apparent among female contacts (Figure 1). Regarding physical distance, there appeared to be no difference between the categories S and N2. There was an increasing risk for N1 (OR, 1.89) and for KR (OR, 3.38) contacts. K and R contacts did not show statistically significant differences from the reference group (S), but this could have been due to the relatively low number of contacts in these groups. Genetic relationship also showed an increasing risk with the closeness of the relationship, compared with N contacts. This was particularly the case for the groups with first-degree blood relationships (C: OR, 3.49; P: OR, 2.39; and B: OR, 2.84). M contacts (OR, 3.29) were also at a high risk for leprosy. CL and PL contacts were relatively small groups, which resulted in wide CIs for the ORs. There was an increased risk for leprosy in contacts of both patients with MB leprosy and those with PB2-5 leprosy, compared with patients with SLPB leprosy ($P = 0.067$ and 0.014), but there was no difference in risk between contacts of patients with PB2-5 and MB leprosy. There were no statistically significant differences in risk between male and female contacts ($P = 0.147$). The presence of a BCG scar had a nearly statistically significant effect ($P = 0.071$) and was therefore initially included in the multivariate model. In the multivariate-regression procedure, this variable was, again, not statistically significant ($P > 0.05$); it was therefore excluded from the final model, which consisted of 4 variables: physical distance, genetic distance, age, and classification of the index patient. All 4 remaining variables showed statistically significant effects (table 4, adjusted ORs). The findings of the univariate analysis for physical and genetic distance and for age were basically maintained in the multivariate-regression model, which indicates that proximity to a patient, blood relationship to a patient, and age (except 20-29 years) contribute independently to the risk of leprosy in contacts of patients with leprosy.

Table 3. Univariate odds ratios (OR's) and 95% confidence intervals (CI's) for the presence of leprosy in contacts by age, distance code, genetic relation, sex, and BCG scar.

Characteristic		OR	95% CI	P
Age in years				
5-9		1		
10-14		1.97	0.96-4.04	0.066
15-19		2.98	1.46-6.09	0.003
20-29		1.53	0.73-3.22	0.263
30-39		2.19	1.08-4.46	0.030
40-49		3.02	1.49-6.12	0.002
>49		3.51	1.76-7.00	<0.005
Distance				
KR	(sharing roof and kitchen)	3.38	1.97-5.81	<0.005
K	(sharing kitchen)	1.62	0.81-3.27	0.175
R	(sharing roof)	1.54	0.21-11.47	0.675
N1	(next-door neighbour)	1.89	1.15-3.10	0.012
N2	(neighbour of neighbour)	1.09	0.65-1.83	0.750
S	(social contact)	1		
Genetic relation				
C	(child)	3.49	1.96-6.23	< 0.0005
P	(parent)	2.39	1.13-5.06	0.022
B	(brother or sister)	2.84	1.56-5.16	0.001
O	(other relative)	1.49	0.93-2.39	0.094
M	(spouse)	3.29	1.56-6.96	0.002
CL	(child-in-law or step child)	0.73	0.10-5.39	0.760
PL	(parent-in-law or step parent)	3.54	1.07-11.70	0.038
BL	(brother- or sister-in-law)	1.42	0.65-3.10	0.384
OL	(other relative-in-law)	1.29	0.72-2.32	0.391
N	(non-relative)	1		
Male sex[1]		1.26	0.92-1.72	0.147
No BCG scar[2]		1.40	0.97-2.01	0.071

[1] Female contacts are the reference group.
[2] Contacts with a scar are the reference group

Table 4. Number of new cases per 1000 contacts, odds ratios and adjusted odds ratios for leprosy by classification, physical distance, genetic distance and age.

Variable	New cases/1000 (95% CI)	OR (95% CI)	P	AOR[1] (95% CI)	P	Wald
Classification			0.039		0.040	6.45
MB	7.9 (5.8-10.4	1.42 (0.96-2.12)	0.083	1.46 (0.97-2.19)	0.067	
PB2-5	8.9 (6.9-11.4)	1.62 (1.11-2.35)	0.012	1.62 (1.10-2.38)	0.014	
SLPB	5.5 (4.1-7.3)	1		1		
Physical distance			<0.0005		0.001	16.24
KR	15.6 (10.6-22.0)	3.21 (2.08-4.96)	<0.0005	2.44 (1.44-4.12)	0.001	
K	7.5 (3.9-13.1)	1.54 (0.83-2.87)	0.172	1.05 (0.52-2.13)	0.898	
R + N1	8.7 (6.5-11.5)	1.79 (1.23-2.60)	0.002	1.69 (1.16-2.47)	0.007	
N2 + S	4.9 (3.8-6.3)	1		1		
Genetic distance						
Closely related	13.2 (9.6-17.6)	2.21 (1.56-3.13)	<0.0005	1.65 (1.05-2.57)	0.029	4.75
Not closely related	6.0 (5.0-7.2)	1		1		
Age in years			0.002		0.003	15.67
5-9	3.3 (1.7-5.9)	1		1		
10-14	6.5 (4.1-9.7)	1.97 (0.96-4.04)	0.066	2.02 (0.98-4.15)	0.056	
15-19	9.8 (6.3-14.5)	2.98 (1.46-6.09)	0.003	3.08 (1.49-6.34)	0.002	
20-29	5.0 (3.0-7.9)	1.53 (0.73-3.22)	0.263	1.72 (0.81-3.63)	0.156	
>29	9.3 (7.4-11.6)	2.84 (1.51-5.34)	0.001	2.94 (1.56-5.54)	0.001	

[1] Adjusted odds ratio: Variables in the final model: classification, physical distance, genetic distance and age.

Discussion

Contacts of patients with leprosy have a higher risk of contracting leprosy than does the general population. Several risk factors—both patient and contact related—have been suggested, but their clinical relevance and relative importance have not been well established. The intake data of the COLEP study enabled us to quantify, in a community where leprosy is highly endemic, the effects of age, sex, BCG scar in the contact, leprosy classification of the index patient, and physical and genetic distance. Because these data are cross-sectional by nature, the number of new patients with leprosy found among the contacts was a prevalence figure and not an incidence rate.

Age and sex of the contact

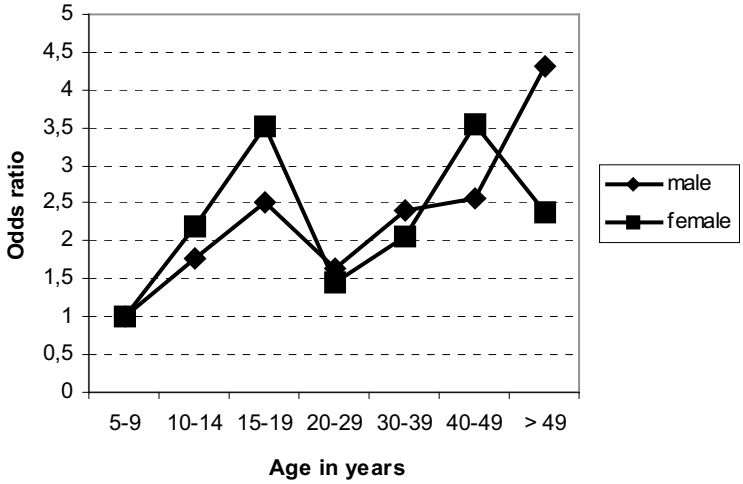
The overall effect of age was highly significant, with older persons being more at risk. Our data showed a bimodal distribution (figure 1) that has been described elsewhere.[2] We observed an increased risk from age 5 to 15 years that peaked between age 15 and 20

years,followed by a decreased risk from age 20 to 29 years. After age 30 years, the risk again increased gradually. This was the case for both male and female contacts. A similar distribution was found for the detection rates of new cases in patients with leprosy who were detected passively in the same area in Bangladesh, but only for females.[8] It was suggested at the time that the observed decrease in the detection rate of new cases in females 20-30 years old could have been due to local social circumstances, with young women being more isolated in the community and, possibly, also shying away from examination, to avoid the stigma of leprosy and its consequences for marriage. The present study of leprosy among contacts of patients with leprosy, however, showed the same trend in both sexes. Immunological effects of pregnancy in young adults would theoretically lead to a higher incidence of leprosy in this age group, so it cannot be an explanation for the observed distribution.[9] In our study, existing pregnancy was one of the exclusion criteria; 438 women were excluded because of this. The leprosy status was recorded for 60% of these women, and none of them had leprosy. In our study, there was a small overrepresentation of males among patients with leprosy, but this was not statistically significant. Because the number of males and females in our contact group was nearly similar, we do not think that the observed small difference can be explained by examination bias. There have been conflicting findings with regard to sex in general as risk factor for leprosy. Two studies in India found no difference between males and females [10,11] but, in Malawi, the risk was significantly greater for males than for females.[3] Other studies also noted that the attack rate in female contacts was lower than that in male contacts.[12-14] It may be concluded from our study that male and female contacts are equally susceptible to contract leprosy and that, for both sexes, persons 20-29 years old has less risk than those 5-19 and ≥ 30 years old.

Type of leprosy of the patient

It has often been observed that contacts of patients with MB leprosy have a higher risk than contacts of patients with PB leprosy, who, again, have a higher risk than non-contacts.[3,5,10-12,14-17] Our data confirm a higher risk for contacts of patients with MB leprosy, but only in comparison to contacts of patients with SLPB leprosy. The contacts of patients with PB2-5 and MB leprosy appeared to have a similar risk. This raises the issue of degree of infectiousness of patients classified as having PB2-5 leprosy. This question cannot be answered in the context of the present (cross-sectional) study in which a common source for both the index patient and the contact with leprosy could not be ruled out. It should be noted that the detection rate of new cases among contacts of patients with SLPB leprosy was also high (5.5 cases/1000 contacts) which justifies contact tracing of all patients regardless of the type of leprosy.

Figure 1. Odds ratios for leprosy in contacts, by age and sex



BCG vaccination

Trials and case-control studies of BCG vaccination in both the general population and contacts of patients with leprosy have shown that it provides protection against leprosy, especially when it is done repeatedly [18-22]. Although the magnitude of this protective effect differs considerably, from 20% to 80%, it is likely that BCG vaccination (as indicated by a scar) indicates a lower risk. It is not always certain, however, that a scar in the shoulder area where BCG vaccination is given is indeed a BCG scar. In our study, it is probably better to speak of a "BCG-like scar." Our data showed a higher risk for persons without a BCG-like scar. Yet the presence of such a scar was statistically correlated with age ($P = 0.01$); younger individuals were far more likely to have received BCG vaccination. Multivariate analysis that included the presence of a BCG scar and age as separate variables showed that the significance of a BCG scar disappeared, whereas age remained a significant factor. This is contrary to what is generally found and could be partly explained by the fact that we used a proxy for BCG vaccination (the BCG-like scar) and, thus, may have underestimated the true effect of BCG vaccination. In addition, BCG vaccination boosts cellular immunity and so could shift the spectrum of leprosy toward the tuberculoid pole. The decreased risk would therefore be mainly for MB leprosy. Because all new cases among the contacts in our study were PB disease, we could not evaluate a possible different risk for MB leprosy. Our findings could well be in line with the suggested underestimation of the protection of BCG vaccination against PB leprosy. [22-24]

Physical distance from the patient

It has been established that there is an inverse relationship between physical distance from a patient with leprosy and the risk to the contact of contracting leprosy.[10,15,25] Our data showed the same trend, but it was not linear. KR contacts—the core household group—had a higher risk than R contacts living in the same house or building and N1 contacts, who, in turn, had a higher risk than N2 and S contacts. There was a similar risk for K contacts, compared with N2 contacts. This might indicate that, for the transmission of leprosy, the category of N2 contacts was more or less homogeneous, irrespective of whether a person shared a kitchen with the patient. Because of the comparable number and age distribution of S and N2 contacts, we doubt whether the field staff strictly followed the guidelines for inclusion in the S category. Many of these contacts appeared to have been neighbors of N2 contacts. This was partly due to the examples of housing schemes that we used for instruction. During the first follow-up period, we will attempt to separate real S contacts from the others. For the present analysis, we regarded them all as having a greater physical distance from the patient than the N2 contacts.

Genetic distance from the patient

Most contact studies of leprosy have referred to household contacts. Because household contacts often share a common genetic background, differences in risk, compared with those of the general population, could be attributed, at least in part, to genetic factors. For half a century, the role of hereditary factors in developing clinical leprosy has been considered.[26] This idea has been supported by twin studies[27], segregation analyses[28], and genome scans.[29,30]

In a review of this topic in 2002, it was concluded that several genes may be involved in susceptibility to leprosy per se or to a type of leprosy, but, because many of the associations have only been found in small series of patients or in a single population, these findings would need confirmation in larger studies.[4] It can be concluded, however, that there is accumulating evidence that the risk of developing leprosy is partly genetically determined. The contribution of genetic predisposition to the development of leprosy still remains to be quantified and disentangled from the effect of relatives living closely together. The results of our analysis strongly support the view that a genetic relationship is indeed a relevant risk factor, independent of physical distance. Univariate analysis showed that closely related contacts of the index patient had a higher risk than the most distant category, N contacts. This was highly significant for C (OR, 3.49), P (OR, 2.39), and B (OR, 2.84) contacts. M contacts are a special category, because they are usually not closely genetically related to the index patient. However, the risk for M contacts is significantly higher (OR, 3.29) than that for N contacts, which can be explained by the close physical distance, because, when it was used as a separate category in a multivariate analysis beside closely related and not closely related contacts, the adjusted OR for M contacts was 1.23 (P = 0.665) (data not shown). In the multivariate analysis, the OR of the closely blood-related group (C, P, and B contacts) taken together was 1.65 (P = 0.029), which demonstrated an independent effect of genetic distance as a risk factor for the development of leprosy. It has to be kept in mind, however,that the physical distance was measured according to dwelling place only. It is possible that close relatives who are neighbors spend more time together than do nonrelated

neighbors.

In conclusion, the intake data of the COLEP study confirmed that the classification of the index patient, the physical distance of the contact from the patient, and the age of the contact are significant risk factors for the presence of leprosy among contacts of patients newly diagnosed as having leprosy. We could not confirm an effect of sex and prior BCG vaccination on this risk. Our data also demonstrated a statistically significant effect of genetic relationship on the risk, independent of physical distance. In practical terms, this means that contact surveys, which are being performed at present mainly among household contacts, should be extended to neighbors and consanguineous relatives, especially when the patient has PB2-5 or MB leprosy.

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5

The prevalence of previously undiagnosed leprosy in the general population and in close contacts of leprosy patients in northwest Bangladesh

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Abstract

Background: The prevalence of previously undiagnosed leprosy (PPUL) and the seroprevalence of anti-PGL-I antibodies against *M. leprae* in the general population were determined to estimate the background level of leprosy transmission in the population. The results were compared with PPUL figures among contacts of leprosy patients in the same area to determine the physical distance between a contact and a patient beyond which the new case detection reaches the level of that of the general population.

Methodology and Principal Findings: Multistage cluster sampling including 20 clusters of 1000 persons each in two districts with over four million population. Physical examination of all individuals included and serological examination from a random sample of 11% of these people. The number of newly found leprosy cases among 17,862 people above 5 years of age from the cluster sample was 27, giving a PPUL rate of 15.1 per 10,000. This was lower than the PPUL rate among the most distant category of a group of contacts of newly detected leprosy patients, namely the neighbours of the neighbours and the social contacts (49 per 10,000). There were marked differences in PPUL between clusters, and no association was found between seroprevalence and the number of newly discovered cases within the cluster. There was no difference between the sexes, but significantly more cases were found in the higher age group.

Conclusions: The overall PPUL rate in the general population is lower than that of the most distant category (neighbours of neighbours) of the contacts of leprosy patients in the same area. Leprosy is higher endemic among the general population of northwest Bangladesh than the figures derived from passive case detection suggest. There are large differences in PPUL rates within the region, which are unrelated to the seroprevalence.

Key words: leprosy, prevalence, contact tracing, seroprevalence

Introduction

For over 60 years it is known that contacts of leprosy patients have a higher risk of developing leprosy than people in the general population.[1] The physical distance is one of the important factors determining this risk[2], and it is likely that, as the distance increases, the relative risk as compared to the general population gradually comes down to one. In many leprosy control programmes contact tracing is an important intervention strategy to find early leprosy cases among close contacts of recently diagnosed leprosy patients, but it is unclear to what level of contact this is justified. In the COLEP study the prevalence of previously undiagnosed leprosy (PPUL) was determined for different levels of contact in a group of people who live in the vicinity of newly detected leprosy patients in northwest Bangladesh.[3] Among the household members, sharing both the roof and the kitchen, the PPUL was 156 per 10,000. The PPUL of the immediate neighbours and those who shared a common roof was 87, while that of the neighbours of the neighbours and social contacts was 49. In order to estimate the background prevalence in the community as a whole, a random sample of the general population was examined.[4] The data available from the intake of this population sample are discussed in this article in more detail. To investigate the association between the PPUL and the prevalence of anti-PGL-I antibodies against *M. leprae* in the community (seroprevalence), blood samples were taken from 11% of this group.

Population and Methods

The study population consisted of the inhabitants of the Rangpur and Nilphamari districts in northwest Bangladesh. The total population is over four million people (estimated population in 2000, based on the 1991 census). The registered new case detection rate of leprosy in this part of the country was 3.21 per 10,000 in 2002 (DBLM Annual Report 2002). This figure is mainly based on passive case detection and active contact (household) surveys.

Out of this population a random sample was taken to estimate the prevalence of previously undiagnosed leprosy. In order to obtain comparable figures with those of the contact group of the COLEP study, the same case-finding strategy – active, door-to-door, screening - was used and performed by the same field staff. As leprosy is a clustered disease, one large sample from a single area may not have given a reliable approximation of the leprosy situation in the two districts, so more samples had to be taken from different areas. A multistage cluster sampling procedure as described in literature was followed.[5]

Sampling procedure

A total of 20 clusters of 1000 people each were randomly sampled from the 13 sub-districts (thana's). One to three clusters were allocated to each sub-district proportionally to the size of its population. A list of unions (in rural areas) and wards (in urban areas) per sub-district was drawn up. A union or ward has an average population of around 23,500. In case the population of a large union was more than three times the size of that of the smallest union, the largest union was split. Then one to three unions (the number of clusters allocated to that sub-district) were selected from the list by means of computerised randomisation.

Per selected union a list of all "sub-unions" (mostly equivalent to villages) was prepared in such a way that the population of the largest village was maximally three times the population of the smallest. These sub-unions have an average population of 5300. Grouping of small villages was sometimes needed, as the accepted minimum size was a population of 1600 (estimation based on census 1991). One sub-union per union was then randomly selected by computer. Three out of the twenty clusters were thus allocated to urban areas, which is a proper reflection of the population figures.

Survey

The surveys of all clusters were performed between November 2002 and February 2003. The population of the village/area was informed in advance about the time the team would perform the survey. During the survey the people were asked about symptoms of leprosy and a body check was performed. Genital areas, and for females also the buttocks and the breasts, were not examined. The survey included all people present whereby female health workers examined the adult females. It started at the northern border of the selected area and stopped when about 1000 people were examined. The criteria used for diagnosis and classification were those of the local leprosy control programme, which follows the WHO guidelines, but those patients with a single lesion with a satellite were recorded as single lesion paucibacillary (SLPB) and not as paucibacillary with 2-5 lesions (PB2-5).[6] All persons suspected of having leprosy were referred to a senior leprosy control officer or a doctor for confirmation. If the disease was confirmed, people were offered regular treatment. All data were entered on registration cards, whereby partly filled cards were used for the next household. From one out of nine persons (every fourth person on a registration card containing nine) a finger prick blood sample was asked and, upon consent, collected on Schleicher & Schuell blotting paper GB 002, dried and stored at –20°C until transport to the Netherlands. If the fourth person on the list refused, the fifth was asked and so on. A total of 2211 samples were tested of whom 81% were taken from the fourth person on the list and 10% from the fifth. The remaining samples were taken from the sixth on the list or further. Because the entry on the cards was continuous, the fourth person was not always the second child as would have been the case if a new registration card were used for every new household.

Serology

An ELISA for the detection of anti-*M.leprae* IgM antibodies was performed according to established procedures.[7] The antigen used was NT-P-BSA, a semi-synthetic analogue containing the *M. leprae*-specific terminal trisaccharide moiety of phenolic glycolipid-I. The specimen was labelled positive when the difference in optical density at 450 nm between NT-P-BSA coated wells and BSA coated wells was 0.200 or higher.

Analysis

Data were analysed by means of descriptive statistics and logistic regression with the Statistical Package for the Social Sciences (SPSS for Windows, release 11.0.1, SPSS Inc., Chicago, Illinois).

Ethical clearance

We obtained ethical clearance from the Ethical Review Committee of the Bangladesh Medical Research Council in Dhaka (ref. no. BMRC/ERC/2001-2004/799). All subjects were informed verbally in their own language (Bangla) about the study and invited to participate. Consent was requested from each adult. For children consent from a parent or guardian was needed. Written consent (in Bangla) was required in the case of blood sampling.

Results

The total number of people enumerated on the referent registration cards was 20,299 of whom 100 were excluded because there were missing data in the records. Of 52 people it was known that they were released from leprosy treatment (RFT) before the survey. As cured leprosy patients presumably can become infected again, these known RFT cases were not excluded. There were 2337 children (1208 male and 1129 female) below the age of five years. As we used the figures in comparison to the figures from the COLEP chemoprophylaxis trial from which under-fives were excluded, the children below the age of five were also excluded from the analysis in this study. This left 17,862 persons for this analysis. Table 1 shows the sex and age distribution by cluster. Among these people, 27 previously undiagnosed cases of leprosy were found. The PPUL is thus 15.1 per 10,000 (95% CI = 9.4-20.8). All newly found cases had PB leprosy (19 SLPB, 8 PB2-5).

None of the children younger than 5 years of age had leprosy, so when they are included, the PPUL comes down to 13.4 per 10,000.

Table 2 shows the PPUL per age group and by sex. As can be seen from this table, our data do not show a difference in risk between the sexes.

When the same age categories are used as in the contact group of the COLEP study (age 5-9, 10-14, 15-19, 20-29, 30-39, 40-49 and 50 and above) age is not a significant risk factor ($p = 0.372$). There is a trend, however, that people of higher age are more at risk. When the subjects are divided into two age groups (under 30 years of age and 30 years and above), age is a significant risk factor. The OR for those 30 years of age or older is 2.55 (95% CI = 1.17 - 5.57, $p = 0.019$) (table 3). Univariate and multivariate logistic regression analysis showed that, in our data, only age is a significant risk factor. Living in an urban area increases the odds for leprosy (table 3), but this is not significant: the adjusted odds ratio (aOR) for city dwellers is 2.04 (95% CI = 0.86 – 4.84, $p = 0.104$) as compared to the population in rural areas.

Table 1. Referent group: sex, age, newly found leprosy patients and seroprevalence by cluster

Cluster	N	M/F ratio[1]	Age				No. of new cases	Newly found cases per 10,000	Registered prevalence[2]	Seropre valence [3]
			Mean	25th percentile	50th percentile	75th percentile				
1	938	0.70	25.9	11	23	36	0	0	4.91	12.7
2	895	0.70	24.2	11	19	35	6	67.0	4.26	9.8
3	871	0.99	25.5	11	21	36	0	0	2.51	6.4
4	866	0.59	25.9	11	23	37	0	0	2.03	3.8
5	897	0.73	29.1	13	25	43	0	0	2.03	4.5
6 (urban)	904	0.53	23.4	11	20	33	2	22.1	3.42	9.6
7	852	0.64	25.3	11	23	36	1	11.7	3.42	5.3
8	892	0.73	26.1	12	21	38	5	56.1	4.21	3.6
9	934	0.85	27.0	13	23	36	0	0	1.71	13.5
10	911	0.58	27.4	12	24	41	3	32.9	3.98	21.2
11	862	0.55	25.1	11	23	35	0	0	1.45	10.7
12 (urban)	862	0.72	26.5	11	23	38	4	46.4	1.61	8.0
13 (urban)	913	0.68	26.4	13	23	38	1	11.0	1.61	8.0
14	903	0.92	28.3	13	27	41	0	0	1.61	8.3
15	848	0.58	30.0	14	26	41	0	0	0.91	9.0
16	950	0.81	28.2	13	26	41	1	10.5	0.91	7.0
17	934	0.63	28.4	13	26	41	1	10.7	0.91	6.3
18	872	0.59	28.8	15	26	40	3	34.4	0.99	5.3
19	865	0.68	27.4	13	25	38	0	0	0.99	4.5
20	893	0.69	26.2	11	23	38	0	0	1.30	19.5
Total	17,862	0.69	26.8	12	23	38	27	15.1	2.31	8.8

[1] M/F ratio = male/female ratio
[2] Registered prevalence (on subdistrict level) per 10,000 population per September 30, 2002, before the survey
[3] Percentage of seropositive samples

Table 4 shows the PPUL in the general population sample, together with the PPUL in the subgroups of contacts of leprosy patients as found during the intake of the COLEP trial.[3] These subgroups were defined by their physical distance to the index patient.

A total of 2211 samples were tested for anti-PGL-I-antibodies, 2016 (91.2%) were negative and 195 (8.8%) positive (Table 1). There is a marked variance between the clusters, both in respect of newly found cases and in respect of seroprevalence. There appears to be no association between these two variables, as the Pearson's correlation coefficient is –0.035 with a p-value of 0.884. The Spearman's rho is –0.098 with a p-value of 0.681. When corrected for age, the Pearson's correlation coefficient is 0.037 (p = 0.877), and the Spearman's rho 0.034 (p = 0.886) respectively.

Table 2. Number of people examined and prevalence of previously undiagnosed leprosy per 10,000 (PPUL) by age and sex

Age	Male			Female			Total
	N	leprosy	PPUL	N	leprosy	PPUL	PPUL
5-9	1542	1	6.5	1597	0	0	3.2
10-14	1277	2	15.7	1378	2	14.5	15.1
15-19	746	1	13.4	1115	1	9.0	10.7
20-29	963	0	0	2091	3	14.4	9.8
30-39	979	4	50.6	1964	2	10.2	20.4
40-49	797	2	25.2	1279	3	23.5	24.1
≥50	973	1	10.3	1159	5	43.2	28.1
Not recorded	1	0	0	1	0	0	0
Total	7278	11	15.1	10,584	16	15.1	15.1

Table 3. Odds ratios and adjusted odd ratios for leprosy

Variable	OR	95% CI	p-value	aOR[1]	95% CI	p-value
Environment						
Urban	1.99	0.84-4.70	0.119	2.04	0.86-4.84	0.104
Rural	1			1		
Age						
5-29 years	1			1		
≥ 30 years	2.55	1.17-5.57	0.019	2.56	1.18-5.65	0.017

[1]aOR = adjusted odds ratio. Variables in final model: environment and age

Table 4. Prevalence of previously undiagnosed leprosy per 10,000 (PPUL) in the subgroups of the contact population and in the general population.

Leprosy contacts and general population	PPUL	95% CI[1]
Sharing kitchen and roof ("household")	156	106-220
Sharing kitchen only	75	39-131
Sharing roof only & next-door neighbour, not sharing roof or kitchen	87	65-115
Neighbour of neighbour & social contact	49	38-63
General population sample	15	9-21

[1] 95% CI = 95% confidence interval for PPUL

Discussion

The PPUL in northwest Bangladesh in the population of 5 years and older, as found by means of a random cluster survey, is 15.1 per 10,000, which is lower than in the most distant subgroup of contacts of leprosy patients. The average seroprevalence is 8.8%. There is no statistical association between the PPUL and seropositivity.

This study, which included about 0.5% of the total population of the area, was based on established multistage cluster sampling techniques. We believe that the results give a reliable picture of the leprosy situation in northwest Bangladesh, in an area where an extensive leprosy control programme has been implemented for more than 10 years. Potential sources for selection and information bias were considered, especially as only those present during the survey were included. Selection bias on cluster level is not likely, but on individual level selection bias is possible as the survey is announced in advance and those afraid of the diagnosis may go into hiding. Males are less likely to be at home during the day and indeed only 42% of those examined are males. In our data, however, the PPUL among males and females is the same. The age distribution in the population examined is similar to the distribution in the contact group, so this will not be a major cause for bias. It is possible that, due to stigma, those with leprosy have a higher chance of being unemployed or rejected at school, so they could be over-represented at the survey, but as all patients found were in the early stage of the disease, this does not seem to be a likely reason for the high number of cases found in our study. We conclude that the possible sources of bias probably have had no effect.

In the past, over-diagnosis has not been a problem in this particular field programme, as was confirmed by an independent evaluator in 2001, but to avoid over-diagnosis in this study, all suspected cases were referred to a senior staff member (medical doctor or leprosy control officer who all had a minimum of 5 years experience in the diagnosis of leprosy at referral centre level) for confirmation.

In the contact group of the COLEP study as a whole, the PPUL rate was 73/10,000, compared to 15.1/10,000 in the population sample.[3,4] With regard to the different categories in the contact group, we conclude that even in the most distant category (the neighbours of the neighbours and social contacts) the PPUL rate (49/10,000) does not come down to the same level as that of the general population. This means that these groups still have an increased risk of developing leprosy and that an extended contact survey including the neighbours of the neighbours of a leprosy patient would still yield more cases per 10,000 than a general population survey would do.

In our study there is no association between the seroprevalence and the number of new leprosy cases found. We therefore could not confirm the findings of Bakker et al. in Indonesia, who described a significant association between these two variables.[8] This might be caused by the fact that the populations of the clusters in northwest Bangladesh are far less isolated than the populations of small islands in Indonesia, but similar studies in different countries have more often lead to conflicting findings in this respect. A study in Sulawesi (Indonesia) found a significant relation between seroprevalence among 2844 schoolchildren and the leprosy burden in the community[9], while a study in Brazil among 7073 schoolchildren could not establish such a correlation.[10]

We found that the PPUL (including children under five) found by active screening was nearly 6 times higher than the registered prevalence (13.4 vs. 2.31). A large difference between the official new case detection (NCD) or prevalence, based on passive case detection, and the NCD or prevalence found by door-to door surveys has been described before. For example, Schreuder et al. found by a rapid village survey in Java, Indonesia, 2½ times the number of known cases[11] and Bakker et al. found during a survey on a few small Indonesian islands 96 cases of leprosy of whom only 11 were previously known.[12] Different sample surveys in India have also revealed sample prevalences 4-5 times the recorded prevalence.[13]

It can be expected that among the newly detected cases in our population sample, a considerable number would have healed without treatment if they had not been discovered, as self-healing has been documented before. Ekambaram et al., for instance, found in South India that the percentage of self-healing among non-lepromatous patients was around 74%.[14] Browne, in Africa, found that 34% of non-treated patients healed spontaneously.[15]

As leprosy is a clustered disease, it is not surprising that there is a marked variance in PPUL among the different clusters. A gradient along geographical lines cannot be discovered. The clusters with a low number of newly found cases are scattered over both districts, as are the clusters with the highest numbers. In the three urban clusters, however, relative high numbers of cases were found. This is in contrast to the findings of Kumar et al. in Agra, India, where the prevalence of leprosy in the urban areas was about 1/3 lower than in the rural areas.[16] Sterne et al. observed a lower incidence of leprosy in the semi-urban district capital of the Karonga District in Malawi[17], while Lapa et al. report that in the State of Pernambuco, Brazil, leprosy is mainly an urban disease.[18] Other factors clearly play a role, and in the data from Agra factors as cleanliness of the houses could partly explain the differences. In our study this kind of data were not recorded, but it is a subject of a study that is linked to the COLEP study (HCC de Jonge et al., submitted for publication).

Our data do not show a difference in risk between males and females. We could also not demonstrate a significant difference between the sexes in the contact group of the COLEP trial, but interestingly, in the original patient population of our study, the male/female ratio was 1.9. These patients were mainly found by passive case detection, while in the population sample and in the contact groups the patients were found by active screening. This could mean that for one or more reasons, women do not present themselves as easily as men, but then one would expect more advanced cases with disabilities among women who were diagnosed by passive case detection than in men. Among the 1037 index patients of the COLEP study this was not the case, on the contrary, only 4% of the female patients were WHO grade 1 or 2 disabled, while 17% of the male patients was recorded with a disability. This is in line with the experiences of Pfaltzgraff in northeast Nigeria, who concluded that leprosy involved men more severely than women.[19] Peters et al., however, in the southeast of the same country, did find significantly more disabilities and a significantly longer period of untreated disease among women.[20]

Another explanation of the differences in male/female ratio between passively found patients and actively found cases could be that among women the disease is more often self-limiting. Browne wrote that, in Africa, he found 2749 self healing leprosy cases among a group of 8098 leprosy patients and that 1630 of them were male and 1119 female.[15] As he does not mention the male/female ratio in the group as a whole it is difficult to interpret his figures in relation to sex differences, but other studies have mentioned a male/female ratio of 2 to 1 in Africa.[20] The ratio found by Browne in the self-healing group was 3 to 2, which could indicate that self-healing among women is indeed slightly more common.

The PPUL shows a bimodal age distribution as was also found by other authors[21] and in the contact group of the COLEP study.[3] There is a trend that higher ages have a higher risk for showing clinical signs of leprosy. When the population of 30 years and older is compared to that younger than 30 years, a significant increased risk can be demonstrated.

In conclusion, our data show that the PPUL in the general population is lower than that in the most distant subgroup of contacts of leprosy patients. It has to be kept in mind, however, that still most new cases in populations where leprosy is relatively highly endemic come out of the non-contact group (EAJ Fischer et al., submitted for publication). Hence full village surveys might be preferable to contact surveys under such circumstances. There are indications that in lower endemic areas the incidence of leprosy among contacts declines faster as the physical distance to the patient increases.[22] If that is indeed the case, screening of contacts further removed from the patient might not be as useful in lower endemic areas.

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6

Association between anti-PGL-I IgM ELISA and clinical and demographic parameters in leprosy

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Abstract

Objective: To determine the risk factors and clinical significance of anti-PGL-I seropositivity.

Design: A large-scale sero-epidemiological study (COLEP) was carried out in northwest Bangladesh. Blood on filter paper from 1025 newly diagnosed patients was collected before treatment was started and tested with an anti-PGL-I ELISA; the relation between patient determinants and seropositivity was calculated using logistic regression .

Results: The median age was 30 years and the male:female ratio 1.9. Overall, 342 patients (33.4%) were seropositive. The following determinants showed a significant correlation with seropositivity ($P < 0.05$) in multivariate analysis: sex, age, disability grade, bacterial index and classification according to the World Health Organization (WHO) system. The number and extent of clinical signs correlated with seropositivity. People with or without a BCG vaccination scar had a similar risk to be seropositive.

Conclusion: Serology is a marker for a higher systemic bacterial load and may identify potential infectious sources among patients with few clinical signs. The size of skin lesions was positively correlated with seropositivity. We did not find different levels of seropositivity among patients with one or two skin lesions, neither did we find different levels among patients with or without satellite lesions.

Introduction

Leprosy is a chronic infectious disease, which is still a major public health problem, mainly in Africa, Asia and Latin America.[1] When left untreated, infection with *Mycobacterium leprae* may eventually lead to severe disabilities. Differences in the cellular immune response of the host determine the clinical features, which form a spectrum and vary from one or a few hypopigmented anaesthetic skin lesions to extensive skin involvement and irreversible damage to the peripheral nerve system.

Accurate diagnosis and classification of leprosy patients is important for treatment purposes as correct treatment may prevent disabilities, relapse and continued transmission. Currently, there are two classification systems in use, which are at least partially complementary:

- The classification according to Ridley and Jopling is based on immunological and histopathological features and makes a distinction between tuberculoid (TT) and lepromatous (LL) leprosy. Between these poles there are three borderline groups (BT, BB and BL) and a separate indeterminate group (I).[2]
- The World Health Organization (WHO) designed a less demanding classification system for treatment purposes. The WHO classification system is based on clinical (and when available bacteriological) features and divides leprosy patients into multibacillary patients (MB, 6 or more skin lesions/satellite lesions and/or a positive bacterial index [BI as determined by microscopy]) and paucibacillary patients (PB, up to 5 skin lesions/ satellite lesions and a negative BI).[3] In some control programmes PB patients with a single lesion (SLPB) are recorded separately.

In the WHO classification system satellite lesions, small (secondary) lesions in the vicinity of a larger (primary) lesion, are counted as separate lesions. The WHO system does not take into account the large variation in the size of lesions. However, there are theoretical arguments for a relation between lesion size and the proliferation of bacteria.[4] Moreover, experience suggests that lesion size may influence the classification decision made by doctors and field workers (unpublished observations). This would decrease the power of any statistical analysis based on classification data.

There are currently two tools available for routine control programmes to aid the correct classification of leprosy patients:

- The BI is a logarithmic scale ranking from zero to six, which defines the bacterial load found by microscopy after acid-fast staining of skin smears or biopsies.[5]
- With the development of rapid tools, serology has become an easily applicable method in the field.[6] The presence of antibodies to the *M. leprae*-specific phenolic glycolipid-I (PGL-I) correlates with the bacterial load of a leprosy patient and its detection can aid the classification of confirmed leprosy patients as MB or PB for treatment purposes.[7]

In this study, we relate the PGL-I-based serology results of 1,025 newly diagnosed, well characterized leprosy patients from Bangladesh to their detailed clinical and demographic characteristics. Factors determining seropositivity are established as well as the clinical

relevance of serology results. This study is part of a prospective (sero-) epidemiological study on contact transmission and chemoprophylaxis in leprosy (COLEP).[8]

Materials and Methods

Patients and samples. This serological study is part of the COLEP study.[8] The patients were from northwest Bangladesh and were detected through passivie case detection. They were diagnosed at Danish Bangladesh Leprosy Mission (DBLM) clinics between May 2002 and October 2003. The districts of Nilphamari and Rangpur in northwest Bangladesh have a total population of approximately 4.3 million with 1,505 new leprosy cases detected by the DBLM staff in 2001 (case detection rate 3.5/10,000 population).[9] Patients were classified based on the WHO classification system.[3] A medical doctor confirmed the diagnosis for every patient and treatment was given according to the WHO/DBLM guidelines. Group sizes were set at a maximum of 400 for SLPB and 400 for PB and a minimum of 200 for MB patients; patients with the pure neural form of leprosy were excluded.[8] Eleven patients with a positive disability grade [14] were reclassified from SLPB into PB. Four patients who were initially classified as SLPB (1) or PB (3) were reclassified as MB based on a positive BI. Ridley & Jopling classification is not performed at DBLM.

A single blood sample was obtained from 1025 of the 1037 patients enrolled in the COLEP study, consisting of 383 SLPB, 348 PB and 294 MB patients.

From each patient demographic and clinical data were collected. Finger prick blood was collected on 0.37 mm blotting paper (GB002 Schleicher and Schuell, 's Hertogenbosch, the Netherlands), air-dried and stored in plastic zip bags with silica gel at –20°C until use.

The study abides by the "International Ethical Guidelines for Biomedical Research Involving Human Subjects" (Council for International Organizations of Medical Sciences, CIOMS, Geneva, 1993). Ethical clearance was obtained from the Ethical Review Committee of the Bangladesh Medical Research Council and written informed consent was obtained from each patient before inclusion in the study.

Coating of ELISA plates. Serology for the detection of IgM antibodies against *M. leprae* was performed using the ELISA technique previously described[10] with natural tri-saccharide linked to bovine serum albumin via a phenolic ring (NT-P-BSA) as a semi-synthetic analogue of PGL-I.[11] Round-bottomed microtiter plates (NUNC 96 U Invitrogen/Life Technologies, Taastrup, Denmark) were coated with 50 µl/well NT-P-BSA (0.01 µg carbohydrate/ml dilution in 0.1 M ammonium hydrogen carbonate buffer, pH 8.0). Wells to control for non-specific binding were coated with 50 µl of a solution containing 0.082 µg/ml BSA of the same batch that was used for the preparation of NT-P-BSA. Plates were air dried for 2 days at room temperature and stored in sealed plastic bags with silica gel in the dark at room temperature until use (within 6 months).

ELISA. On the day before testing a 3.17 mm diameter disc was punched from the blood impregnated filter paper card into a polypropylene tube and incubated overnight at 4°C in 25 µl phosphate buffered saline (pH 7.2) containing 0.1% (v/v) Tween 20 (PBST). The next day 183 µl of PBST+10% (v/v) normal goat serum (Gibco Invitrogen/Life Technologies,

Auckland, New Zealand; PBST+NGS) was added and incubated for one hour. This corresponds to an approximately 1:167 dilution of serum.

Before adding the eluted samples, the pre-coated plates were washed with PBST (two times short and two times 2-5 minutes), followed by a blocking step with 100 µl/well PBS+1% (w/v) BSA (Boehringer, Mannheim, Germany) at 37°C for 1h. Next, 50 µl of the sample dilution was added to each well followed by incubation at 37°C for 1 h. Plates were washed as described above and 50 µl/well conjugate (1:10,000 dilution in PBST+NGS of a peroxidase-conjugated goat IgG fraction to human IgM 5FC µ; Capple/Organon Teknika, Turnhout, Belgium) was added and incubated at 37°C for 1 h. After another washing procedure 50 µl/well TMB substrate solution (0.4% (w/v) 3,3',5,5'-tetramethyl-benzidine + 0.4% (w/v) urea hydrogen peroxide in DMSO [all three from Sigma-Aldrich, Steinheim, Germany], diluted 1:10 in 0.1 M sodium acetate citrate buffer pH 4.0) was added to initiate a colouring reaction. The reaction was stopped by adding 50 µl/well 0.5 N H_2SO_4 when a standard serum reached a net optical density at 450 nm (OD) of 0.600. The status seropositive was given if the net OD was above 0.199. When the OD of the standard serum was either too low (OD < 0.55) or too high (OD > 0.75) the samples were retested. The ELISA performance was monitored using this standard plus a positive and negative control serum sample on each plate.

Bacterial Index. The BI was determined by microscopy on Ziehl-Neelsen stained slit skin smears[12] taken from the earlobe, forehead and a skin lesion; the highest BI was recorded.

Clinical signs. The clinical signs of the patient were recorded as number of skin lesions (hypopigmented and/or anaesthetic skin patches), number of nerves involved (nerves: facial, ulnar, radial cutaneous, median, lateral popliteal and posterior tibial; involved: enlarged, tender or painful) and as number of body areas affected (with a skin lesion and/or nerve involvement) according to the system described by Van Brakel *et al.*,[13] dividing the body into seven body parts, namely head, torso, back and the four extremities. Satellite lesions are recorded separately from the determinant "number of skin lesions". The size of the largest skin lesion was estimated as being small (<10 cm diameter), medium (10 to 15 cm diameter) or large (>15 cm diameter). The clinical data set was based on body charts drawn by the DBLM-staff.

Data analysis. Patient and serological data were stored in Microsoft Access and Excel, respectively. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS for Windows, version 11.0.1, SPSS Inc., Chicago, Illinois; 2001). Logistic regression was used to identify independent determinants influencing the odds ratio for seropositivity. Determinants associated with seropositivity in univariate analysis ($P < 0.10$) were selected for multivariate analysis. In multivariate analysis, we tested for statistically significant ($P < 0.05$) interactions between determinants in the final model and for confounding.

Results

Patient characteristics.

Blood samples were collected from 1,025 newly diagnosed leprosy patients. The median age was 30 years (range 5 to 84) and the male:female ratio was 1.9. The distribution of the patients' characteristics is presented in Table 1. The distribution of sex, age, BCG vaccination rate and disabilities differed among the three WHO classification groups.

Table 1. **Patients' characteristics in relation to WHO classification**

	MB		PB		SLPB		Total		
Determinants	no.	%	no.	%	no.	%	no.	%	P -value[a]
total	294	28.7	348	34.0	383	37.4	1025	100	
Sex									
Male	230	78.2	221	63.5	222	58.0	673	65.7	
Female	64	21.8	127	36.5	161	42.0	352	34.3	< 0.0001
male:female ratio	3.59		1.74		1.38		1.91		
Age (years)[b]									
5-14	28	9.5	63	18.2	51	13.3	142	13.9	
15-29	78	26.5	118	34.1	150	39.2	346	33.8	
30-44	98	33.3	99	28.6	108	28.2	305	29.8	
45-59	65	22.1	49	14.2	53	13.8	167	16.3	
60 and above	25	8.5	17	4.9	21	5.5	63	6.2	< 0.0001
median age	39		29		28		30		
BCG vaccination[c]									
Yes	58	19.9	100	29.2	113	29.5	271	26.6	
No	233	80.1	243	70.8	270	70.5	746	73.4	0.009
Disability grade(14)									
0	199	67.7	309	89.1	383	100	891	86.9	
1	60	20.4	20	5.7	0	0	80	7.8	
2	35	11.9	19	5.2	0	0	54	5.3	< 0.0001

[a] P-value calculated with Pearson Chi-square.
[b] For two patients no age information was available.
[c] For eight patients no information on the BCG vaccination status was available.

The MB group contained more male and older patients in comparison with the PB and SLPB groups ($P < 0.0001$). The MB group contained fewer BCG vaccinated patients ($P = 0.009$) and more patients with disabilities ($P < 0.0001$). The median age of the BCG vaccinated patients was lower, 25 years (inter quartile range [IQR], 15 to 35) compared with non-vaccinated patients, who had a median age of 32 years (IQR, 20 to 45, $P < 0.0001$). After age and sex adjustment there is an odds ratio of 1.54 for non-vaccinated patients to be classified as MB (exact binomial 95% confidence interval [95% CI], 1.09-2.18).

Factors determining seropositivity.

Out of the 1,025 patients, 342 (33.4%; 95% CI, 30.5 to 36.3) were given the status seropositive based on ELISA testing (Table 2).

WHO classification and BI

A strong relation with seropositivity was shown for the determinants WHO classification and BI in both univariate and multivariate analyses (Table 2). In the multivariate analysis, the odds ratio (OR) for seropositivity was adjusted for differences in sex, age, BCG vaccination and disability distribution. Compared to SLPB patients, MB patients were more likely to be seropositive (adjusted odds ratio [aOR] MB, 11.8; 95% CI, 7.83 to 17.8) while PB patients had no difference in risk for seropositivity (aOR PB, 1.27; 95% CI, 0.86 to 1.86). Seropositivity increased with the BI: the aOR for patients with a BI 1 or 2 was 6.33 (95% CI, 2.42 to 16.5) and the aOR for patients with a BI higher than 2 was 59.0 (95% CI, 25.0 to 139) compared with BI negative patients.

Sex and age

In univariate analysis, the determinants sex and age were not significantly related with seropositivity. However, after adjustment for the other determinants in the multivariate analyses (model contains determinants: WHO classification, sex, age, BCG vaccination and disability grade) they appeared to be significantly related. Females were more likely to be seropositive than males (aOR, 1.46; 95% CI, 1.05 to 2.04) and the positivity prevalence decreased significantly with age (Table 2).

BCG vaccination

In univariate and multivariate analyses with the determinant BCG vaccination, no difference in seropositivity was found between BCG non-vaccinated patients and vaccinated patients (aOR, 1.20; 95% CI, 0.84 to 1.72). In addition, no correlation was found between BCG vaccination and BI positivity (OR BCG non-vaccinated patients, 1.24; 95% CI, 0.80 to 1.93).

Disability grade

In univariate analysis, the determinant disability grade had a significant relation with seropositivity; in the multivariate analysis this relation was reduced. Patients with a disability grade 1 and 2 were more likely to be seropositive compared to disability grade 0 patients, but only disability grade 1 showed a significant difference with disability grade 0 patients (aOR, 1.79; 95% CI, 1.01 to 3.16). Grouping the disability grade 1 and 2 together resulted in a significant aOR of 1.73 (95% CI, 1.09 to 2.76).

Clinical signs

Detailed clinical data from 996 patients were available for analysis (Table 3).

Satellite lesions

Comparison between patients with satellite lesions and patients without satellite lesions showed no differences in sex, age or BI distribution and no correlation with serology was found in multivariate analysis (model contains: skin lesion (size), skin lesion (number), nerve, body area, sex and age). The correlation of satellite lesions in the univariate analysis was

altered from significant to non-significant after adjustment with the determinant skin lesion (number) or with the determinant body area.

Table 2. Logistic regression analysis to determine risk factors for seropositivity among leprosy patients

		%	Unadjusted		Adjusted	
Determinants	no.	seropositive	OR[a]	95% CI[a]	aOR[b]	95% CI
WHO classification						
SLPB	383	16.7	1		1	
PB	348	21.3	1.35	0.93-1.95	1.27	0.86-1.86
MB	294	69.4	11.3	7.84-16.3	11.8	7.83-17.8
			P < 0.0001		P < 0.0001	
Bacterial Index[c]						
0	883	25.1	1		1	
1 - 2	23	69.6	6.81	2.76-16.8	6.33	2.42-16.5
> 2	104	94.2	48.6	21.0-112	59.0	25.0-139
			P < 0.0001		P < 0.0001	
Sex						
Male	673	34.3	1		1	
Female	352	31.5	0.88	0.67-1.16	1.46	1.05-2.04
			P = 0.369		P = 0.027	
Age (years)[d]						
5 - 14	142	31.7	0.90	0.59-1.36	0.92	0.56-1.50
15 - 29	346	34.1	1		1	
30 - 44	305	33.4	0.97	0.70-1.34	0.67	0.45-0.98
45 - 59	167	34.1	1.00	0.68-1.48	0.53	0.33-0.86
60 or above	63	30.2	0.83	0.47-1.49	0.42	0.21-0.86
			P = 0.963		P = 0.024	
BCG vaccination[e]						
Yes	271	28.4	1		1	
No	746	35.0	1.36	1.00-1.84	1.20	0.84-1.72
			P = 0.050		P = 0.310	
Disability grade[14]						
0	891	29.2	1		1	
1	80	63.8	4.27	2.65-6.89	1.79	1.01-3.16
2	54	57.4	3.27	1.87-5.72	1.66	0.85-3.26
			P < 0.0001		P = 0.066	
1 - 2[f]	134	61.2	3.83	2.63-5.58	1.73	1.09-2.76
			P < 0.0001		P = 0.020	

[a] OR = odds ratio, 95% CI = 95% confidence interval.
[b] adjusted OR; Determinants in the final model: WHO classification, sex, age, BCG vaccination and disability. aOR for Bacterial Index was calculated without the WHO classification determinant.
[c] For fifteen patients no BI result was available.
[d] For two patients the age was not recorded.
[e] For eight patients BCG data were not available.
[f] Analyses were performed with a recoded determinant disability grade (grade 1 and 2 were coded as 1 - 2).

Skin lesion size

The size of a skin lesion was found to be a determining factor for seropositivity. The aORs of 'medium' and 'large' skin lesions were 1.45 (95% CI, 0.94 to 2.23) and 2.37 (95% CI, 1.47 to 3.83), respectively, compared with 'small' skin lesions.

Table 3. Logistic regression analysis to determine risk factors for seropositivity among clinical signs of leprosy patients.

		%	Unadjusted		Adjusted	
Clinical signs	no.	seropositive	OR[a]	95% CI[a]	aOR[b]	95% CI
Satellite lesion						
not present	756	29.4	1		1	
present	240	45.0	1.97	1.46-2.65	1.15	0.78-1.70
			P < 0.0001		P = 0.489	
Skin lesion (size)[c]						
Small	623	22.6	1		1	
Medium	174	35.1	1.85	1.28-2.65	1.45	0.94-2.23
Large	191	63.9	6.04	4.26-8.58	2.37	1.47-3.83
			P < 0.0001		P = 0.002	
Skin lesion (number)						
1	477	17.0	1		1	
2	151	17.2	1.02	0.63-1.65	0.90	0.54-1.49
3 - 5	117	34.2	2.54	1.62-3.99	2.54	1.42-4.54
6 - 15	136	61.0	7.66	5.03-11.6	5.15	2.22-11.9
> 15	115	87.0	32.6	18.0-59.0	10.2	3.41-30.6
			P < 0.0001		P = 0.0003	
Nerve						
0	587	24.2	1		1	
1 - 2	274	29.6	1.32	0.95-1.81	1.24	0.85-1.82
> 2	135	79.3	12.0	7.58-18.9	2.01	1.08-3.72
			P < 0.0001		P = 0.078	
Body area[d]						
1 - 2	692	18.6	1		1	
3 - 5	176	50.0	4.36	3.07-6.21	0.78	0.37-1.62
6 - 7	128	88.3	32.9	18.6-58.2	2.91	0.99-8.52
			P < 0.0001		P = 0.004	

[a] OR = odds ratio, 95% CI = 95% confidence interval.
[b] adjusted OR; Determinants in the final model: skin lesion (size), skin lesion (number), nerve, body area, sex and age.
[c] Estimated as small (< 10 cm diameter), medium (10 to 15 cm diameter) and large (> 15 cm diameter).
[d] Seven body areas: head, torso, back and the four extremities (13).

Number of skin lesions

The determinants skin lesion (number), nerve and body area showed a positive correlation with seropositivity, in both univariate and multivariate analyses. The seropositivity rate increased significantly with the number of skin lesions: patients with three to five skin lesions had a significantly increased aOR of 2.54 (95% CI, 1.42 to 4.54), while patients with two

lesions did not have a significantly different aOR (aOR, 0.90; 95% CI, 0.54 to 1.49) compared with patients with one lesion.

Number of nerves and body areas involved

Patients with more than two nerves involved and patients with more than five body areas affected were more likely to be seropositive. Having more than two nerves involved resulted in a significant aOR of 2.01 (95% CI, 1.08 to 3.72) compared with no nerve involvement. When six or seven body areas were affected the aOR for seropositivity was 2.91 (95% CI, 0.99 to 8.52) compared with one and two body areas.

None of the determinants showed any significant interaction. Analyses were repeated with the ELISA cut-off values: 0.149, 0.249 and 0.299 to confirm the conclusions based on the cut-off value 0.199. No significant differences were found (data not shown).

Discussion

Leprosy serology has been studied frequently and many of the factors determining seropositivity are well known, as has been reviewed by Oskam *et al.*[15] However, these studies were often performed on a limited number of patients. Here we describe a study on more than one thousand patients in which serology is compared to a large variety of clinical and demographic data and in which factors determining seropositivity are established.

The ELISA used was based on the detection of specific antibodies in peripheral blood eluted from blood spots on filter paper. Blood on filter paper was chosen for practical reasons: it is cheap, easy to collect in the field and requires no centrifugation or cold chain. However, blood eluted from blood spots is known to give a slightly lower signal in the ELISA compared with serum. [16]

Patient characteristics. The median age (30) and the male:female ratio (1.9) are in agreement with other reports from this area, [17,18] taking into account that, due to the group size criteria described in the Materials and Methods section, our study population included a higher percentage of MB patients (28.7%) than the actual situation (18.4% in 2002 and 21.9% in 2003). [17]

The MB patient group comprised more males, older patients and more patients with a disability grade > 0 than the PB or SLPB groups. These differences between MB and PB patients are reported frequently [18-,20] and are thus in line with expectations.

In previous studies BCG vaccination was held to be protective against leprosy and was highly associated with the development of tuberculoid leprosy instead of lepromatous leprosy, suggesting that BCG vaccination would protect against lepromatous leprosy. [21,22] We see a similar, but less strong effect in our patient population with regard to the development of PB or MB leprosy: MB patients were less frequently BCG vaccinated than PB and SLPB patients (BCG coverage MB approximately 20%; PB and SLPB, 29%). After age and sex adjustment there is an aOR of 1.54 (95% CI, 1.09 to 2.18) for non-vaccinated patients to be classified as MB, compared to PB-SLPB patients. A lower BI and/or seropositivity among BCG vaccinated patients would have been a supplementary argument for this protective role of

BCG and would confirm the hypothesis that BCG vaccination is responsible for a shift in the immune response towards the tuberculoid pole of the spectrum. However, the determinant BCG vaccination did not have any influence on either seropositivity or BI.

Factors determining seropositivity. As expected, a strong correlation was found between serology and the determinants WHO classification and BI.[20,23-25] For the majority of patients who were MB, and particularly those who were skin smear positive, elevated levels of *M. leprae* specific IgM antibodies were found (Table 2).

The prevalence of seropositivity in this study population showed similar age and sex patterns as demonstrated in other studies.[19,25-27] The decline in seropositivity prevalence with increasing age is consistent with the decrease of overall IgM levels with age. It has been suggested that females have higher innate IgM levels than males, which may be the explanation for the higher seropositivity rate found among females.[28] The alteration in significance for the variable sex on seropositivity in multivariate analysis compared to univariate analysis can be explained by the high number of male MB patients (male:female ratio; 3.59, versus 1.91 total population, table 1). The variable WHO classification confounded the correlation between sex and seropositivity in univariate analysis.

A correlation between disability grade and serology (aOR disability 1 and 2, 1.73; 95% CI, 1.09 to 2.76, $P = 0.020$) was found, corresponding with the general trend reviewed by Oskam *et al* [15]: PB patients with a disability generally had higher seropositivity rates than PB patients without a disability.

The seroprevalence among MB patients (69%) was rather low compared with other studies in which it varied between 75 and 100%. [15] Comparison between studies is difficult since the classification criteria have changed over the years and our data collection was done using filter paper blood which gives slightly lower titers than serum. [16] Another possible explanation for the relatively low seropositivity in the MB group may be the short detection delay: the DBLM leprosy control programme has been well established in the area since 1977. DBLM [17] and Richardus *et al.* [29] have reported gradually decreasing percentages of MB cases and disabilities in our study area, which may be caused by a reduction in detection delay due to intensive control efforts. Since the numbers of skin lesions, nerves involved and body areas affected are correlated with both detection delay and seropositivity, a lower number of clinical signs among the MB classified group due to a short detection delay would lead to a lower seropositivity in the MB group. Further study may explore this possibility.

Clinical signs determining seropositivity. For a better understanding of the clinical significance of seropositivity, specific clinical determinants were related to serology results. Based on our results we can make a number of remarks with regard to the WHO classification system as it is currently used:

- There was no serological difference between patients with and without satellite lesions (Table 3). Since there was also no serological difference between patients with one or two skin lesions it can be concluded that there is no serological evidence to distinguish between SLPB and PB with 2 lesions, with and without satellite lesions. This implies that the presence of satellite lesions may be ignored for quantification of skin lesions and that a distinction may be made between PB 1 and

2 lesions on the one hand and PB 3-5 lesions on the other hand, with PB 1 and 2 being equivalent with the current SLPB category.

- The size of a lesion, here subjectively recorded from the largest lesion drawn on the patient information card, may also be a relevant factor for classification. There is a strong indication that the lesion size is a determining factor for seropositivity (Table 3).

The presence of not more than two lesions (regardless of the presence of satellite lesions) and no lesion larger than 10 cm diameter (small sized) may be new criteria for SLPB classification. If a separate SLPB treatment – such as ROM (rifampicin, ofloxacin, minocycline [3])– were used, this insight could have a large impact on the economic aspects of leprosy control. We realize that at the moment this is solely based on serological evidence and more detailed clinical information about response to treatment and risk of impairment will be needed to support our arguments for such an adjustment of WHO classification.

At an individual level seropositive PB patients may have disease that is behaving more like MB disease. It would be interesting to study if seropositive patients would benefit from a longer treatment with regard to relapse and the development of reactions and nerve damage.

The exact number of lesions is less crucial for seropositivity among MB patients, although a difference was seen between patients with up to 15 lesions and patients with more than 15 lesions. The number of nerves and the number of body areas affected seem to be both independent factors for seropositivity.

It may be stated that seropositivity is highly correlated with clinical signs: numbers of skin lesions, nerves involved and body areas affected. All these clinical signs signify the dissemination of the bacterium in the body of the patient, indicating that seropositivity can be used as a marker for a higher systemic bacterial load, and therefore can be used to identify more infectious patients. [30-33]

In conclusion, we have shown that the presence of elevated anti-PGL-I antibody levels is highly correlated with the MB status, BI and the dissemination of clinical signs in a patient. It is clear that serology results reflect the overall systemic bacterial load of a patient. From a serological point of view, it seems reasonable to stop counting satellite lesions as whole lesions, to take skin lesion size into account for clinical decision-making, and consider the possibility to include patients with two skin lesions into the SLPB group. For individual patient management serological testing may give clinicians a better idea about the systemic bacterial load of a patient. The availability of simple serological tests makes this option feasible.

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A single dose of rifampicin is effective in preventing leprosy in contacts of newly diagnosed leprosy patients. Results of a cluster randomised controlled trial

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Abstract

Background: Close contacts of leprosy patients have an increased risk of clinical leprosy, the disease caused by *Mycobacterium leprae*. Uncontrolled and unblinded studies have shown that rifampicin is possibly an effective prophylactic drug against leprosy, but for routine application it is necessary to establish its effectiveness through a double blind, placebo-controlled trial.

Methods: A single-centre, field based, double blind, cluster-randomised and placebo-controlled trial was carried out among 21,711 close contacts of 1037 newly diagnosed leprosy patients. Prophylaxis consisted of a single dose of rifampicin given to close contacts in the second month after the beginning of treatment of the patient. The data were analysed blindly at follow-up 24 months after the intake.

Results: Out of the 21,711 included persons, 19,957 (91.9%) were seen at follow-up. In the placebo group, 66 out of 10,006 developed leprosy. In the rifampicin group this was 29 out of 9951. The overall reduction in incidence by a single dose of rifampicin was thus 56% ($p = 0.0003$). The overall number needed to treat (NNT) to prevent a single case of leprosy among contacts was 271. There were differences between subgroups, both in reduction of incidence and in NTT.

Conclusions: The effectiveness of 56% of a single dose of rifampicin given to contacts of new leprosy patients in preventing the development of clinical leprosy is a promising finding with regard to the potential of this intervention in leprosy control. The effect however, is not consistent in all subgroups of contacts and this has implications for our understanding of the early pathogenesis of leprosy infection.

Introduction

For over 60 years it has been known that close contacts of patients with leprosy have an increased risk of contracting the disease.[1] The risk that a contact of a patient with leprosy develops clinical leprosy is related to the physical and genetic distance to the index patient, to the age of the contact, and to the classification of the disease of the index case.[2]

Since the 1940's of the previous century, the treatment of choice for leprosy became dapsone, which was replaced by multidrug therapy (MDT), a combination of three drugs, dapsone, clofazimine and rifampicin in the early 80's. Before MDT became the standard treatment for leprosy, randomised controlled trials using dapsone or acedapsone were conducted to investigate whether these drugs could prevent leprosy among contacts.[3,4] A meta-analysis of the studies on chemoprophylaxis with prolonged administration of these drugs estimated an overall efficacy of about 60%.[5] The efficacy in household contacts ranged from 34-54%,[3,6] whereas it was 91% in the community intervention trial.[7] The disadvantages of dapsone as a chemoprophylactic agent are the development of drug resistance and the lack of patient compliance, due to the need for administration over a long period of time. Therefore newer drugs were considered and rifampicin was a logical choice because of its strong bactericidal effect against *Mycobacterium leprae*, the micro-organism causing leprosy. It was expected that this drug would have at least a similar prophylactic effect as dapsone, but with fewer doses and shorter duration of administration. An uncontrolled trial with rifampicin was conducted earlier and a protective efficacy of 40-50% was reported.[8,9]

Recently, an unblinded study on five Indonesian islands was conducted with two doses of rifampicin, given with an interval of approximately 3.5 months.[10] In this study three populations were compared, a "blanket group" consisting of the population of three small islands on which prophylaxis was given to all persons, a "contact group", consisting of the population of one island on which prophylaxis was given only to contacts living in the same household or less than 50 meters away, and a "control group" consisting of the population of another island on which no prophylaxis was given. The study showed that in the blanket group, chemoprophylaxis was indeed associated with a 74.6% reduction in leprosy incidence, at least during the first three years after implementation. In the population where only household members and neighbours received prophylaxis, no reduction was observed.

A large-scale, double blind, placebo-controlled trial was started in northwest Bangladesh in 2002, using a single dose of rifampicin as chemoprophylaxis. The Prospective (Sero-) epidemiological Study on Contact Transmission and Chemoprophylaxis in Leprosy (COLEP) is registered under ISRCTN61223447 of Current Controlled Trials. We reported about the methodology of this trial and the analysis of the intake data earlier.[2,11] In this article we describe the results of the analysis after 2 years follow-up. The reasons for this interim analysis (the total follow-up period was planned to be 4 years) were firstly that no effect at 2 years follow-up would be a reason for discontinuation of the study as an effect beginning after two years is unlikely, and secondly that an overwhelming effect would call for earlier recommendations for implementation of the intervention in routine leprosy control. Care was taken that this analysis did not compromise the double-blind design of the study.

Materials and Methods

Participants

The COLEP study was conducted in northwest Bangladesh in the districts of Rangpur and Nilphamari, an area with a total population of over four million people. Two subdistricts where the leprosy control services were provided by other organisations than the Danish Bangladesh Leprosy Mission (DBLM), were excluded. The study population consisted of close contacts of 1037 new leprosy patients who were willing to participate. The diagnosis leprosy was made when at least one of the so-called cardinal signs was present. These cardinal signs are:

One or more skin lesions consistent with leprosy and with definite sensory loss.

Thickened peripheral nerves.

A positive skin smear for acid fast bacilli.

Leprosy can be classified on the basis of clinical manifestations and skin smear results. Patients showing negative smears at all sites and who have not more than 5 skin lesions were grouped as paucibacillary leprosy (PB), while those showing positive smears at any site or who had more than 5 skin lesions were grouped as having multibacillary leprosy (MB). Within the group of PB patients, those who have only one lesion were classified as having single lesion paucibacillary (SLPB) disease. As SLPB disease is the most common form of leprosy in Bangladesh, and because we preferred to include a sufficient number of all categories of patients, in the COLEP trial the number of SLPB patients was limited to 400. Of these, 11 were later reclassified on the basis of the skin smear results taken at intake (but the results became available only after some time), or because of their initial misclassification as judged by the recorded clinical symptoms at intake, so in the end 389 SLPB patients were included. The number of paucibacillary leprosy patients with two to five lesions (PB2-5) and multibacillary (MB) patients was 353 and 295, respectively. The intake of the contacts started in June 2002 and was completed by the end of December 2003. Contacts were categorised according to their physical and genetic distance to the index patient.

For the physical distance six categories were defined based of the local housing situation:

- Those living under the same roof *and* using the same kitchen (KR)
- Those living under a separate roof, but using the same kitchen (K)
- Those living under the same roof, but not using the same kitchen (R)
- Next door neighbours (N1)
- Neighbours of the neighbours (N2)
- Social contacts (business contacts, colleagues who stay in the same room at least 4 hours a day for 5 days a week) (S)

During the intake phase it appeared that only a very small proportion of the social contacts satisfied the criteria mentioned above, and the fast majority of them were in fact neighbours

of the N2 contacts. For this reason these two groups were pooled in the analysis. As there was only a small number of R contacts, these were pooled with N1.

For the genetic distance seven categories were initially defined,[2,11] but for the analysis these categories were combined to form two groups:

- Closely related: parent, child or sibling
- Not closely related: all other

Contacts were excluded when they refused informed consent, when they indicated being pregnant, when on tuberculosis or leprosy treatment during the intake, when found to be suffering from (previously undiagnosed) leprosy at the intake, when younger than 5 years of age, when known to be suffering from liver disease or jaundice or when only residing temporarily in the area. A person could only be included in the contact group of one patient.

Finger prick blood samples for anti-PGL-I antibodies were taken from all index cases during intake and from all contacts during intake and follow-up. All samples were collected on Schleicher & Schuell blotting paper GB 002, dried and stored at –20°C until transport to the Netherlands. An ELISA for the detection of anti-PGL-I IgM antibodies was performed according to established procedures.[12] The antigen used was NT-P-BSA, a semi-synthetic analogue containing the *M. leprae*-specific terminal trisaccharide moiety of phenolic glycolipid-I.

The follow-up started two years after the intake, in June 2004, and was completed in February 2006. The follow-up followed the sequence of the recruitment to achieve a uniform follow-up period of 24 months. During the follow-up visit as many contacts as possible were examined and if not all could be seen, an appointment for a second visit was made. If a contact was still not present then, the person was requested through a relative to report to the clinic for examination. If contacts had moved within reasonable distance, the field staff tried to trace them at their new address. If leprosy was diagnosed, the date of official registration was recorded. In order not to miss any new cases emerging from the contact groups, the main central registry was scanned and all patients found during the two years between intake and follow up were listed per clinic. These lists were sent to the clinics with the request to check if anyone of those patients on the list was in fact a COLEP contact.

Ethical aspects

Ethical approval was granted by the Ethical Review Committee of the Bangladesh Medical Research Council in Dhaka (reference no.: BMRC/ERC/2001-2004/799). All eligible subjects (patients and contacts) were informed verbally about the study in their own language and invited to participate. Written consent was obtained from all participants at recruitment. The consent forms were written in Bengali. Consent was requested from each adult. For children below 18 years of age, consent from a parent or guardian was needed.

Intervention

At intake, that is after the index patient had received the second dose of MDT, all contacts of one patient received treatment from the same numbered container, which contained either capsules with 150 mg rifampicin or identical capsules with placebo. The number on the container was identical to the central registration number of the index case.

According to bodyweight and age, each contact took 2 to 4 capsules under direct supervision of a staff member. The following dosage schedule was used: adults weighing 35 kg and over: 600 mg; adults weighing less than 35 kg and children older than 9 years: 450 mg; and children 5-9 years: 300 mg.

Objectives

The hypotheses underlying this study are:

- Transmission of Mycobacterium leprae from the index patient to contacts takes place prior to the diagnosis and start of the treatment of the disease in the index case.
- A single dose of rifampicin is effective in eradicating small numbers of M. leprae bacteria that are possibly present in the contacts. In this way rifampicin could be effective as a measure to prevent clinical leprosy among close contacts of patients with leprosy.

For the present report the following objective of the COLEP study is relevant:

- To determine the effectiveness of chemoprophylaxis by means of a single dose of rifampicin in preventing leprosy in close contacts.

Subgroups of contacts were formed according to their contact status, age, genetic relation to the index patient, sex, classification of the index patient, presence of a BCG scar and serological status.

Outcomes

The primary outcome was the development of clinical leprosy. The disease of every newly found leprosy patient was confirmed by a leprosy control officer and a medical officer, who also made a digital photograph of the lesions for future reference. The health professionals confirming the diagnosis all had a minimum of 5 years experience in the diagnosis of leprosy at referral centre level.

Sample size

Prior to the trial a power calculation showed that 20,000 contacts, 10,000 in both treatment arms, could detect reliably an expected efficacy of intervention of 50%, even taking into account an expected 10-20% loss to follow-up of contacts. For the power calculation we

assumed an incidence rate of 2 per 1000 per year with an expected 50% reduction through intervention, $\alpha = 0.05$ two-sided, and power = 0.80. The total number of contacts enrolled in the trial was 21,711, divided over 1037 clusters.

Randomisation

Randomisation of the rifampicin/placebo containers was done by computerised methods by the database designer (RF) in Rotterdam, the Netherlands. In this way the randomisation was on contact group (cluster) level. The codes were kept under lock in Rotterdam and could only be accessed by the database designer. The number on the container was identical to the registration number of the index patient.

Blinding

As only the database designer in Rotterdam had access to the treatment codes, the participants, the field and hospital staff, and the primary researchers were blinded. The total follow-up period of the trial is 4 years and in order not to compromise the double blind design because of the mid-term analysis after 2 years follow-up, the file with the data of all the contacts in the trial was given to the database designer. He was asked to merge this file with his file of the treatment codes. The combined file was given to the statistician (GJJMB) who performed the analyses. He was instructed not to give the results of any particular analysis to the primary researchers if the result would compromise the blinding of the study.

Statistical methods

Statistical analyses were done using SAS software, version 9.1. Techniques for the analysis of survey samples were used to account for the clustering on the index patient level in the sample. Bivariate associations were investigated using "proc surveyfreq" and the Rao Scott χ^2 instead of the Pearson χ^2. Also "proc surveylogistic" was used instead of the ordinary logistic regression procedure. Odds ratios were calculated and reported, but because of the low prevalence of the outcome, these are comparable with relative risks. Per subgroup of contacts the number needed to treat (NNT) was calculated. Survival analysis was not performed in order not to compromise the blinding at this point in time. A significance level of 5% was used in all tests.

Results

The numbers of participants in each group are shown in Table 1 and Figure 1. Table 1 shows that randomisation led to groups that are well balanced with regard to the different variables.

Table 1. The contact population (N= 19,957) at first follow-up, divided by treatment allocation (placebo n = 10,006, rifampicin n = 9951)

Variable		Placebo N	%[1]	Rifampicin N	%[1]
Age at intake (years)	5-9	1553	7.8	1605	8.0
	10-14	1656	8.3	1613	8.1
	15-19	1107	5.5	1043	5.2
	20-29	1709	8.6	1680	8.4
	≥ 30	3981	20.0	4010	20.1
Genetic distance	Closely related	1600	8.0	1516	7.6
	Not closely related	8406	42.1	8435	42.3
Sex	Male	4676	23.4	4656	23.3
	Female	5330	26.7	5295	26.6
Classification (of index patient)[2]	MB	2852	14.3	2614	13.1
	PB2-5	3140	15.7	3415	17.1
	SLPB	4014	20.1	3922	19.7
BCG-scar	Absent	5904	29.8	5916	29.9
	Present	4032	20.4	3949	19.9
Physical distance[3]	KR	923	4.6	925	4.6
	K	733	3.7	711	3.6
	R + N1	2782	14.0	2545	12.7
	N2 + S	5568	27.9	5770	28.9
ELISA	Negative	8106	47.0	8137	47.2
	Positive	515	3.0	494	2.8

[1] Percentage of total number of contacts

[2] MB = multibacillary, PB2-5 = paucibacillary with 2 to 5 lesions, SLPB = paucibacillary with a single lesion

[3] KR = sharing kitchen and roof ("household"), K = sharing only kitchen, R = sharing only roof, N1 = next-door neighbour, not sharing roof or kitchen, N2 = neighbour of neighbour, S = social contact

Out of the 21,711 persons included, 19,957 (91.9%) were seen during follow-up. Among these, 95 new leprosy patients were found (incidence rate 47.6 per 10,000). In the placebo group, 66 out of 10,006 developed leprosy (incidence rate 66.0 per 10,000) and in the rifampicin group this was 29 out of 9951 (incidence rate 29.1 per 10,000) (Table 2). The reduction in incidence in the rifampicin group was thus 56% (Rao Scott $\chi^2 = 12.964$ [df = 1], $p = 0.0003$). The overall NNT to prevent one new case of leprosy was 271.

Table 3 shows the results by subgroup. The odds ratios for leprosy in the rifampicin group versus the placebo group are given, with the NNT. Rifampicin appears to be especially effective (OR < 0.5, $p < 0.05$) in contacts not closely related to the index patient, in contacts of patients with paucibacillary disease, in contacts with the largest physical distance from the

index patient, in female and seronegative contacts, in those without a BCG-like scar, and in the age groups 10-14 and 20-29. The OR's and the NNT's in the different age groups show a trend that broadly mirrors the trend in incidence over age in the placebo group, in other words, the higher the incidence, the more effective prophylaxis appears to be.

Table 2. Incident cases of leprosy in the contact population after two years follow-up.

	Leprosy				No	Total	Incidence per
	SLPB	PB2-5	MB	Total	leprosy		10,000
Placebo	28	30	8	66	9940	10,006	66.0
Rifampicin	15	10	4	29	9922	9951	29.1

Overall reduction in rifampicin group = 56%, Rao Scott $\chi^2 = 12.964$ (df = 1), p = 0.0003

Discussion

The results of our trial show that the overall incidence of leprosy among contacts in the first two years after diagnosis of the index patient is reduced by 56% through a single dose of rifampicin.

The COLEP study was designed as a single-centre, prospective, cluster-randomised, double blind and placebo-controlled trial to verify results of earlier studies that did not have all of these methodological qualities. The strength of the COLEP trial is its robust design and the large number of subjects that could be included within a relative short span of time because the incidence of leprosy in the study area is relatively high. We cannot be certain, however, that the results are equally applicable to situations were leprosy is less highly endemic, although there is no reason to assume otherwise. It must also be kept in mind that this is an analysis after two years of follow-up. Longer follow-up will show whether the effect of rifampicin prophylaxis will be sustained over longer periods of time.

The results of our study confirm the results of previous studies regarding the efficacy of rifampicin prophylaxis. However, it appears that this effect is not the same for all subgroups of contacts. Contacts who are not closely related or live further away, and who were, on basis of the intake data, expected to be at a lower risk,[2] benefited more from prophylaxis. This inverse relation between efficacy and expected risk also appears to exist with respect to classification of the disease of the index patient. By contrast, a direct relation in respect to contact age is suggested, higher efficacy being recorded in those groups with higher leprosy incidence.

Female contacts appear to benefit slightly more from prophylaxis than male contacts, but this is not statistically significant as the confidence intervals overlap to a great extend.

Table 3. Effect of rifampicin prophylaxis by variable category

Variable		Placebo		Rifampicin		OR (95% CI)[1]	P-value[2]	NNT[3]
		no leprosy	leprosy	no leprosy	leprosy			
Age (years)	5-9	1548	5	1601	4	0.77 (0.21-2.87)	0.7012	1375
	10-14	1644	12	1611	2	0.17 (0.04-0.76)	*0.0206*	166
	14-19	1101	6	1038	5	0.88 (0.27-2.89)	0.8383	1597
	20-29	1699	10	1679	1	0.10 (0.01-0.79)	*0.0286*	190
	≥ 30	3948	33	3993	17	0.51 (0.28-0.91)	*0.0238*	247
Genetic relation[4]	Close	1583	17	1503	13	0.80 (0.37-1.77)	0.5892	488
	Not close	8357	49	8419	16	0.32 (0.18-0.57)	*<0.0001*	254
Sex	Male	4640	36	4639	17	0.47 (0.26-0.85)	*0.0121*	247
	Female	5300	30	5283	12	0.40 (0.20-0.82)	*0.0121*	297
Classification (of index patient)[5]	MB	2832	20	2604	10	0.54 (0.24-1.25)	0.1513	314
	PB2-5	3118	22	3406	9	0.38 (0.16-0.86)	*0.0212*	229
	SLPB	3990	24	3912	10	0.43 (0.20-0.89)	*0.0240*	292
BCG scar	Absent	5853	51	5894	22	0.43 (0.26-0.71)	*0.0009*	203
	Present	4017	15	3942	7	0.48 (0.18-1.26)	0.1360	513
Physical distance[6]	KR	911	12	919	6	0.50 (0.16-1.50)	0.2128	154
	K	728	5	704	7	1.45 (0.38-5.51)	0.5876	n.a.
	R + N1	2765	17	2537	8	0.51 (0.22-1.19)	0.1213	337
	N2 + S	5536	32	5762	8	0.24 (0.11-0.52)	*0.0184*	229
ELISA[7]	Negative	8054	52	8115	22	0.42 (0.25-0.70)	*0.0009*	269
	Positive	511	4	492	2	0.52 (0.08-3.25)	0.4834	269

[1] OR: Odds ratio for leprosy in rifampicin group versus placebo group
2 P-values < 0.05 are written in italics
3 NNT = Number needed to treat to prevent one case of leprosy
4 Closely related: parent, child or sibling. Not closely related: all others
5 MB = multibacillary, PB2-5 = paucibacillary with 2 to 5 lesions, SLPB = single lesion paucibacillary
6 KR = sharing both roof and kitchen, K = sharing only kitchen, R = sharing only roof, N1 = next-door neighbour, N2 = neighbour of neighbour, S = social contact
7 Negative = optical density < 0.2, positive ≥ 0.2

Although males are generally regarded as being more at risk for leprosy,[13,14] neither our intake data nor the presently discussed figures could confirm a significant higher risk. A reason for the difference in efficacy between males and females, if present, could be that females, who are generally lighter, had a relatively higher dose of rifampicin. This assumption would need further investigation.

Prophylaxis appears somewhat more effective in those contacts who were seronegative at intake. The fact that the protective effect in seropositive contacts is not statistically significant, is mainly due to the small numbers in this group. Studies on the prognostic value of serology have shown contradictory findings,[15-17] but previous research indicated that

contacts who are seropositive for anti-PGL-I are at an increased risk of developing leprosy, especially MB disease.[18]

As can be seen from Table 2, prophylaxis appears to be equally effective in preventing MB as PB disease. It must be realised, however, that MB disease in general has a longer incubation period and therefore a longer follow-up period may be needed to confirm this finding.

Finally, in our data the presence of a BCG scar did not affect the response to prophylaxis as measured by the odds ratios. As BCG is effective in preventing leprosy, the absolute numbers of new leprosy patients among those vaccinated is smaller and therefore the NNT is higher.

The findings of the COLEP trial are consistent with those of Bakker et al. from Indonesia.[10] They found no effect of rifampicin in communities where only household and direct neighbour contacts were given prophylaxis, but they could demonstrate a significant effect in those communities where everybody was given prophylaxis. But even in those communities, rifampicin prophylaxis appeared to be more effective in non-contacts than in household contacts. Studies on dapsone prophylaxis also showed that this was more effective when given as a blanket treatment rather than only to household contacts.[5] A possible explanation of these findings could be that, by the time the prophylaxis is given, the potential bacillary load in physically close contacts, closely related contacts, seropositive contacts and contacts of patients with MB disease is on average already too high to be eliminated by a single (or double, in Indonesia two doses were given) dose of rifampicin. This possibly higher average bacterial load could be caused either by a higher exposure (household contacts, contacts of MB patients) or by a higher vulnerability (genetic make-up, partly reflected in the seropositivity, male sex). If a higher bacterial load is indeed a reason for failure of prophylaxis with a single dose of rifampicin, more extended chemoprophylaxis schedules may be effective in those groups of contacts, but this requires further research.

The data from the two-year follow-up of the COLEP trial show that a single dose of rifampicin given to contacts of new leprosy patients is 56% effective in preventing the development of clinical leprosy in a two-year period. This efficacy is similar to that found in the meta-analysis of dapsone trials, however, is those trials dapsone was given for 1-5 years.[5] This is a promising finding with regard to single dose rifampicin as a cheap and practical preventive intervention for contacts of leprosy patients in leprosy control programmes. The effect, however, is not consistent in all subgroups of contacts, and needs further study before recommendations can be made concerning routine implementation. The results of the 4-year follow-up and subsequent in-depth subgroup analysis may provide more insight into this matter.

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8

General discussion

The occurrence and prevention of leprosy among close contacts of leprosy patients.

This thesis deals with the occurrence and the prevention of leprosy among close contacts of patients with leprosy. Close contacts are one of the high risk groups, as was shown for the first time more than 60 years ago by Doull.[18] Since then more researchers looked at this group and many different definitions of 'contact', based on operational considerations, have been used in literature. From our review of this literature (Chapter 2) we concluded that people at risk of contracting leprosy are not confined to the group of direct family members living under the same roof. The available data suggest that the risk of contracting leprosy decreases with increasing physical distance to the patient, however, only a few studies looked beyond the level of the household in defining contacts.

Genetic factors probably play a role as well, but genetic distance is often linked to physical distance and many studies do not differentiate between these two parameters. Nevertheless there is an accumulating body of evidence that a genetic relation to a patient is indeed a risk factor.

Gender and age characteristics of the contact could be important, but this has not been established firmly and the data are contradictory. BCG vaccination is partly effective against leprosy as was shown in many studies. Thus the presence of a BCG scar in a contact is likely to indicate a lower risk.

Serological tests for anti-PGL-I antibodies could be useful in defining high-risk contacts. The reviewed literature suggested that a contact who is seropositive for antibodies against *M. leprae* has an (up to more than 20-fold) higher risk of developing leprosy. Even though the majority of seropositive contacts do not develop clinical leprosy and the majority of new cases develop out of the seronegative group, within a group of contacts of one leprosy patient, those contacts that are seropositive appear to have an increased risk, in particular to develop MB leprosy.

The available data on leprosy justify the opinion that the stone-in-the-pond model as used in tuberculosis control could be a useful model in leprosy as well. This model is based on a concentric circle approach that assumes that the prevalence of infected individuals is highest near to the source of the infection and gradually decreases as the distance to the source increases.

From both leprosy and tuberculosis investigations, it has become clear that the bacterial load of a patient, as measured by a skin smear or a sputum smear respectively, is an important risk factor for transmission to contacts. However, results of tuberculosis research stress the fact that the sputum status is certainly not the only risk factor. In analogy to tuberculosis, this suggests that, in leprosy control, paucibacillary (PB) patients must not be neglected as a possible source of infection, and that contact examination should also be conducted in case a patient is classified as PB.

From our literature review we concluded that targeted interventions should be aimed at close contacts both inside and outside the household, particularly when genetically related. Contacts of PB patients should also be included in such interventions.

The COLEP study

The COLEP study was designed to investigate the effect of one of the possible intervention strategies, namely the use of chemoprophylaxis (Chapter 3). Included in the study were 1037 newly diagnosed leprosy patients and 21,708 of their close contacts. The contacts were included in a double-blind, placebo-controlled trial of chemoprophylaxis with a single dose of rifampicin. This kind of design is regarded to be superior if number of patients and time allow. Randomisation was done at contact group level. All contacts were categorized according to their physical and genetic distance to the index patient.

Beside the patients and their contacts, the COLEP study also included a so-called 'referent group'. This group consisted of a sample from the general population of the the districts were the COLEP study was conducted. In a random cluster survey, twenty clusters of 1000 people each were examined during house-to-house surveys. This is about 0.5% of the total population. In this way the new case detection of leprosy in the general population could be determined and compared to that of the different categories in the contact group (Chapter 5).

The **intake** data of the COLEP study enabled us to quantify the correlation of the occurrence of leprosy in the contact population with the age, sex and the presence of a BCG scar, with the leprosy classification of the leprosy patient, and physical and genetic distance between the contact and the patient, in a community where leprosy is relatively high-endemic (Chapter 4). These data are transversal by nature, and because the duration of the disease among those found to be afflicted cannot be ascertained, the number of new leprosy patients found among the contacts is a prevalence rather then an incidence measure. The intake data of the referent group provided insight in the new case detection of leprosy among the general population. There is no reason to assume that the average duration of the disease in the contact group is different from the referent group, therefore the data are supposed to be comparable.

The first research question was:

What is the new case detection rate of leprosy in the districts of Nilpamari and Rangpur in northwest Bangladesh?

The new case detection (NCD) rate (or prevalence rate of undetected leprosy) could be calculated after the intake of the referent group was completed and was 15.1 per 10,000 excluding children younger than 5 years, and 13.4 including under-fives, which is relatively high. This figure is not completely comparable to the general prevalence rate as found by passive case detection as it includes early cases that would not have come to the attention of the health services this early or even not at all because it could be self-limiting (see below).

From the presently available data the incidence rate of leprosy in the study area cannot be calculated. Only after the follow-up data from the referent group will be available the incidence rate can be determined.

In our study there was no association between the seroprevalence in the area and the number of new leprosy cases found. We therefore could not confirm the findings of Bakker et al. in Indonesia, who described a significant association between these two variables.[19] This might be caused by the fact that the populations of the clusters in northwest Bangladesh are far less isolated than the populations of small islands in Indonesia.

We found that the NCD rate, including children under five, found by active screening was 5-6 times higher than the registered yearly NCD rate (13.4 vs. 2.4 per 10,000). A large difference between the official NCD or, in other studies, prevalence, based on passive case detection, and the NCD or prevalence found by door-to door surveys has been described before. It can be expected that among the newly detected cases in our population sample, a considerable number would have healed without treatment if they had not been discovered, as self-healing has been documented before. Ekambaram et al., for instance, found in South India that the percentage of self-healing among non-lepromatous patients was around 74%.[20] Browne, in Africa, found that 34% of non-treated patients healed spontaneously.[21] Also, those who would not have healed spontaneously, would probably have been treated in a later phase anyway, although more damage might have occurred by then.

As leprosy is a clustered disease, it is not surprising that there was a marked variance in NCD among the different clusters. A gradient along geographical lines could not be discovered. The clusters with a low number of new cases are scattered over both districts, as are the clusters with the highest numbers.

We could not demonstrate a difference in risk between males and females.

The NCD showed a bimodal age distribution which was also found in the contact group. There is a trend that higher ages have a higher risk for showing clinical signs of leprosy.

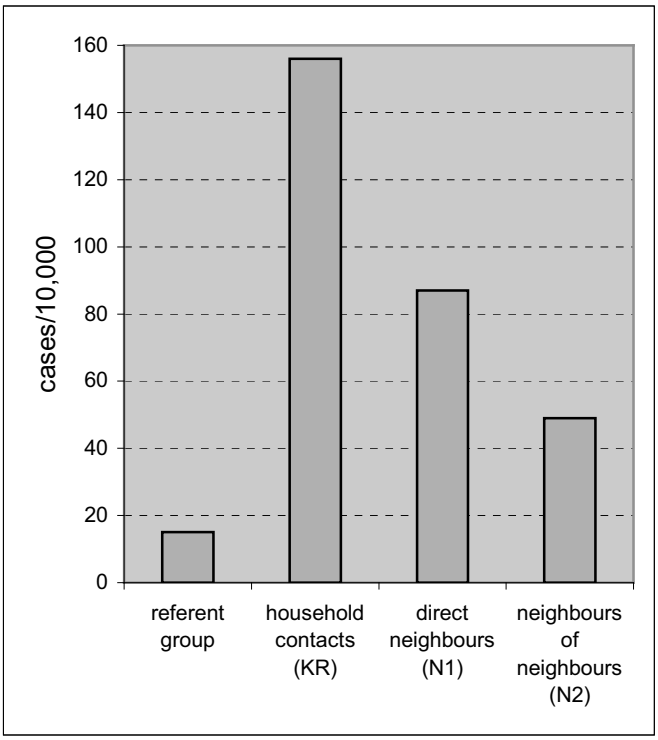
The second research question was:

What is the risk (as compared to the general population) of contacts of leprosy patients to develop leprosy and what are the contributions of physical and genetic distance to the index case as risk factors?

The risk appears to be inversely related to the physical distance. The NCD of leprosy among the most distant group of the contacts (the neighbours of the neighbours and the social contacts) is 3.2 times higher than that in the referent group (49.0 vs. 15.1 when children under five are excluded). This means that a survey among those more distant contacts would still detect more new patients than a general population survey would. In many leprosy control programmes household contacts of new patients are checked for signs and symptoms of leprosy, but our data show that also other, more distant, contacts are at an increased risk (figure 1). This is comparable to the so-called "stone-in-the-pond" principle in tuberculosis: the ripples caused by a stone thrown into the water become wider but also shallower the further they move from the place where the stone hit the water. In the same way the number of potential contacts of a patient becomes larger but the infection risk smaller the further one moves away from the patient.

A close genetic relation with a leprosy patient increases the risk of contracting the disease. According to our data, both factors contribute independently to the risk for contacts.

Figure 1. New case detection rates in different subgroups at intake



Other risk factors considered were:

Age and sex of the contact

The overall effect of age is highly significant, with older ages being more at risk. Our data show a bimodal distribution that has been described before.[22] We observed an increased prevalence from the age of 5 to 15 years, peaking between 15 and 20 years, followed by a decreased prevalence during the ages 20 to 29 years. After 30 years of age the prevalence increased again gradually. This is the case for both the male and female contacts.

The data from our study suggest that male and female contacts are equally susceptible to contract leprosy and that for both sexes the age group 20-29 years has less risk compared to the age groups 5-19 and beyond 30 years.

Type of leprosy of the patient

It has often been observed that contacts of multibacillary (MB) patients have a higher risk than those of PB patients who, again, have a higher risk than non-contacts.[7,23-25] Our data

confirm a higher risk for contacts of MB patients, but only in comparison to contacts of single lesion paucibacillary (SLPB) patients. The contacts of paucibacillary patients with 2-5 lesions (PB2-5) and MB patients appear to have a similar risk. This raises the issue of degree of infectiousness of patients classified as PB2-5. As the data from the intake are transversal, a common source to both the index patient and the contact with leprosy cannot be ruled out. However, if we look at the data in the placebo group after two years of follow-up (so at incidence figures) we see a similar pattern: the incidence among contacts of both MB and PB2-5 is 71/10,000 and among contacts of SLPB 60/10,000 (see table 3 Chapter 7). Thus, according to the data from the COLEP study, the infectiousness of patients with PB2-5 disease might be similar to that of patients with MB disease.

BCG vaccination

The presence of a BCG scar might indicate a reduced risk for leprosy. In our study it is probably better to speak of a BCG-like scar as it is not always certain that a scar in the shoulder area, where BCG vaccination is given, is really a BCG scar. Our data show a higher prevalence (intake) and incidence (follow-up) among those without a BCG-like scar, but corrected for age (and treatment in the follow-up data) this effect is not statistically significant. As BCG boosts the cellular immunity, it could be that in the incident cases (at follow-up) with a BCG scar, the proportion of MB disease is smaller. This appears not to be the case, however, in the PB group, the proportion of PB2-5 cases among those with a scar is lower than among those without, suggesting some shift towards the tuberculoid pole. This effect, however, is statistically not significant.

In conclusion, the intake data from the contact and referent group showed that the NCD rate of leprosy in the general population is lower than that in the most distant subgroup of contacts of leprosy patients. It has to be kept in mind, however, that still most new cases in populations where leprosy in relatively highly endemic areas are people without known close contact with a leprosy patient.[26] Hence full village surveys might be preferable to contact surveys under such circumstances. There are indications that in lower endemic areas the incidence of leprosy among contacts declines faster as the physical distance to the patient increases.[27] If that is indeed the case, screening of contacts further removed from the patient might not be as useful in lower endemic areas.

The third research question was:

What is the effectiveness of chemoprophylaxis by means of a single dose of rifampicin in preventing leprosy in close contacts?

The data available after 2 years follow-up show that the overall efficacy of a single dose of rifampicin is 56% (Chapter 7). The efficacy of a single dose of rifampicine is thus similar to that found in the meta-analysis of dapsone trials, however, in those trials dapsone was given for 1-5 years.[28] The results of our study confirm the results of previous studies regarding the efficacy of rifampicin prophylaxis.

It appears, however, that this effect is not the same for all subgroups of contacts. Contacts who are not closely related or live further away, and who were, on basis of the intake data,

expected to be at a lower risk,[29] benefited more from prophylaxis. With regard to close genetic relationship, this is also reflected in the number needed to treat (NNT), but with regard to close physical distance, the effect on the NNT is outbalanced by the higher risk among household contacts. The inverse relation between efficacy and expected risk also appears to exist with respect to classification of the disease of the index patient: rifampicin prophylaxis was more effective among contacts of PB patients as compared to contacts with patients with MB disease, but the NNT is again the same among the different subgroups of this variable. By contrast, a direct relation in respect to contact age is suggested, higher efficacy being recorded in those groups with higher leprosy incidence, which is also reflected in the NNT.

Female contacts benefited slightly more from prophylaxis than male contacts as judged by the odds ratio's, but the NNT was about similar. Although males are generally regarded as being more at risk for leprosy,[7,24] neither our intake data nor the data from the follow-up could confirm a significant higher risk. A reason for the difference in efficacy between males and females could be that females, who are generally lighter, had a relatively higher dose of rifampicin. This assumption needs further investigation.

Prophylaxis appears somewhat more effective in those contacts who were seronegative at intake. The fact that the protective effect in seropositive contacts is not statistically significant, is mainly due to the small numbers in this group. Studies on the prognostic value of serology have shown contradictory findings,[30-32] but previous research indicated that contacts who are seropositive for anti-PGL-I are at an increased risk of developing leprosy, especially MB disease.[33] In our data, an odds ratio (given the number of events this is comparable with relative risk) of 1.31 (95% CI = 0.57-3.01, p = 0.53) is found for leprosy at follow-up in those contacts who were seropositive at intake. Regarding type of leprosy: of 80 out of the 95 new cases at follow-up, serology results at intake were available. Of these, 6 were seropositive and 4 of these developed MB disease while of the 74 seronegative persons 5 developed MB leprosy. This is highly significant (likelihood ratio p=0.002).

The findings of the COLEP trial are consistent with those of Bakker et al. from Indonesia.[5] They found no effect of rifampicin in communities where only household and direct neighbour contacts were given prophylaxis, but they could demonstrate a significant effect in those communities where everybody was given prophylaxis. But even in those communities, rifampicin prophylaxis appeared to be more effective in non-contacts than in household contacts. Studies on dapsone prophylaxis also showed that this was more effective when given as a blanket treatment rather than only to household contacts.[28] A possible explanation of these findings could be that, by the time the prophylaxis is given, the potential bacillary load in physically close contacts, closely related contacts, seropositive contacts and contacts of patients with MB disease is on average already too high to be eliminated by a single (or double, in Indonesia two doses were given) dose of rifampicin. This possibly higher average bacterial load could be caused either by a higher exposure (household contacts, contacts of MB patients) or by a higher vulnerability (genetic make-up, partly reflected in the seropositivity, male sex). If a higher bacterial load is indeed a reason for failure of prophylaxis with a single dose of rifampicin, more extended chemoprophylaxis schedules may be effective in those groups of contacts, but this requires further research. Another implication of this explanation is that in the higher risk groups more people are in fact undertreated with a single

antibiotic agent, which in theory raises the possibility of selection of rifampicin resistant mutants.

It should be kept in mind that the results presented in this thesis are derived from the follow-up after two years. As clinical leprosy develops slowly, further follow-up studies will be needed to determine whether the effect will be sustained. Three different scenarios are conceivable:

1. Chemoprophylaxis only delayed the development of clinical leprosy due to a marked reduction (but not elimination) of the bacterial load. In this case the number of new cases will increase again in the coming years.
2. Chemoprophylaxis did eliminate the bacilli completely and so the reduction will be permanent, at least until a possible re-infection occurs. Due to the reduction of new cases in the first years, less people might become secondarily infected.
3. A combination of both scenarios: in a part of the contact group *M. leprae* is eliminated by a single dose of rifampicin, while in another part this is not the case.

From the presently available data the combined scenario seems the most likely as the efficacy in higher risk subgroups appears to be lower than in lower risk subgroups, which could be explained by a higher initial bacterial load among the first. If that is the case, regimens for chemoprophylaxis should be different for different subgroups of contacts which makes it far more complicated in field situations and therefore less feasable.

In conclusion, I believe that the present available data do not justify the routine use of a single dose of rifampicin as chemoprophylaxis among contacts of newly diagnosed leprosy patients, as in household contacts and other higher risk subgroups the effect is not (yet) statistically significant, while the possibility of undertreatment appears realistic. Treating different subgroups with different regimens could deal with that problem, but that needs further research first and it might possibly not be acceptable because it could be too complicated. The results of the second follow-up will be important in this respect because it might provide stronger evidence for the efficacy of chemoprophylaxis also in higher risk subgroups.

Conclusions and recommendations

Conclusions

1. The new case detection of leprosy by active house-to house survey is 5-6 times the official registered yearly new case detection in the districts of Nilphamari and Rangpur which is based on passive case-finding.
2. The occurrence of leprosy in contacts of leprosy patients is inversely related to the physical distance between the patient and the contact. Leprosy is still more prevalent among the neighbours of the neighbours of a patient as in the general population.

3. Other variables associated with the occurrence of leprosy among contacts are the age of the contact, the genetic relation between contact and patient, and the type of leprosy of the patient.
4. The serological status of a patient is highly correlated with the type of disease, the bacillary index and the dissemination of the clinical signs.
5. The serological status of a contact is not significantly related to the risk of developing clinical signs of leprosy within a 2 year period. However, if a seropositive contact develops leprosy, it is significantly more often multibacillary disease.
6. The overall efficacy of a single dose of rifampicin after 2 years is 56%. The efficacy is higher in lower risk contacts.

Recommendations

1. Contact surveys in leprosy control programmes should not only include household contacts, but also neighbours and neighbours of neighbours, especially when genetically related.
2. The data presently available do not justify the introduction of chemoprophylaxis in routine leprosy control programmes.
3. Further follow-up of the COLEP cohort will be needed to determine whether the overall effect of prophylaxis will be sustained and to confirm a protective effect in higher risk subgroups.
4. Routine serological examination of contacts of leprosy patients is not justified.

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9

Summary / Samenvatting

Summary

This thesis addresses the risk for developing clinical leprosy for close contacts of leprosy patients and possible prevention thereof by means of chemoprophylaxis.

Chapter 1 is an introduction to the disease in general. It gives a short description of the clinical aspects, the history and the occurrence at present. Possible methods of control are discussed:

Segregation: a method used in the past and of uncertain effectiveness.
Vaccination: the effectiveness of BCG vaccination varies, but seems beyond doubt.
Chemoprophylaxis: trials with both (ace)dapsone and rifampicin showed that this method could be effective. The effectiveness of rifampicin needed further confirmation by a randomised controlled trial.
Early case detection and treatment: the main method used so far. It was hoped that, by treating all known patients and rendering them uninfectious, transmission would slow down. This is not convincingly the case.

At the end of this chapter three research questions are formulated (see chapter 8).

Chapter 2 is a literature review on the risk factors for leprosy among contacts of leprosy patients with the aim to identify factors associated with it. Different definitions of 'contact' have been used and most studies on this subject were among so-called household members. Yet several studies indicate that contacts found in other places than the household are also at risk of developing leprosy. The type of leprosy and the bacterial index are the main patient-related factors involved in transmission, but also contacts of PB patients have a higher risk of contracting leprosy as compared to the general population. The most important contact-related factors are the closeness and intensity of the contact and inherited susceptibility, while the role of age and sex of the contacts is not clear. The role of socio-economic factors is also vague. The significance of immunological and molecular markers in relation to risk of transmitting or developing leprosy is not yet fully understood, but there is an indication that contacts who are sero-positive for anti-PGL-I antibodies are at increased risk of developing clinical leprosy. The presence of a BCG scar is likely to be related to a lower risk. Analogies with tuberculosis suggest that the 'stone-in-the-pond' approach to control may be applicable to leprosy too. Sputum smear negative tuberculosis patients are known to spread the bacteria to others. This analogy strengthens the suggestion that the contacts of paucibacillary leprosy cases should also be included in contact tracing and examination. It is concluded that targeted interventions should be aimed at close contacts of both MB and PB patients inside and outside the household, particularly when genetically related.

Chapter 3. As mentioned above, the effectiveness of rifampicin as a chemoprophylactic agent needed further confirmation and therefore the COLEP trial was designed. This chapter describes the methodology of this trial. COLEP consists of a cluster randomized, double-blind and placebo-controlled trial, a cohort study to determine risk factors characterizing the sub-groups most at risk within the total contact group of a patient, and a cohort study using a reference group from the general population to determine the prevalence and incidence of leprosy in the total population of the study area. The follow-up period was planned to be 4 years. The study included 1037 newly diagnosed and previously untreated leprosy patients and their 21,867 contacts. The new case detection of leprosy among contacts was 7.3 per

1000. A total of 21,708 contacts without signs and symptoms of clinical leprosy were included and randomized at contact group level in treatment and placebo arms.

Chapter 4 describes the results of the logistic regression analyses of the intake data of the contact group. It was concluded that age of the contact, the disease classification of the index patient, and physical and genetic distance were independently associated with the risk of a contact acquiring leprosy. Contact surveys in leprosy should therefore not only be focused on household contacts but also extended to neighbours and consanguineous relatives, especially when the patient has PB2-5 or MB leprosy.

Chapter 5 describes and analyses the intake data of the referent group. The results are compared to those of the contact group. The overall new case detection (NCD) rate of leprosy in the general population appeared to be lower than that of the most distant category (neighbours of neighbours) of the contacts of leprosy patients in the same area. Leprosy is higher endemic among the general population of northwest Bangladesh than the figures derived from passive case detection suggest. There were large differences in NCD rates within the region, which were unrelated to the seroprevalences.

Chapter 6 is not related to one of the main research questions of this thesis, but to one of the other objectives of the COLEP trial, namely the studying of the usefulness of determining and monitoring the anti-PGL-I antibody levels. A significant influence on the probability to be seropositive in multivariate analysis was found for the determinants sex, age, disability grade, bacterial index and classification according to the World Health Organization system. The presence of a BCG vaccination scar did not have a significant association with this risk. Except for satellite lesions, the number and extent of clinical signs correlated with seropositivity. It was concluded that serology appears to be a marker for a higher systemic bacterial load and that it may identify potential infectious sources among patients with few clinical signs.

Chapter 7 describes the results of the COLEP trial after two years of follow-up. Of the included persons, 19,957 (91.9%) were seen at follow-up. In the placebo group, 66 out of 10,006 developed leprosy and in the rifampicin group this was 29 out of 9951. The overall reduction in incidence by a single dose of rifampicin was thus 56% ($p = 0.0003$). The overall number needed to treat (NNT) to prevent a single case of leprosy among contacts was 271. This is a promising finding with regard to the potential of this intervention in leprosy control, however, this effect is not consistent in all subgroups of contacts. Further study is therefore needed before routine implementation can be recomended.

Chapter 8 is a general discussion of the research questions and the answers thereof. The research questions were:

1. What is the new case detection rate of leprosy in the disricts of Nilphamari and Rangpur in northwest Bangladesh?
2. What is the risk of close contacts of leprosy patients to develop leprosy and what are the contributions of physical and genetic distance?
3. What is the effectiveness of chemoprophylaxis by means of a single dose of rifampicin in preventing leprosy in close contacts?

The following answers were given:

1. The new case detection rate was 13.4 per 10,000 population

2. Both physical and genetic distances are inversely related to the risk to develop leprosy. Other factors associated with this risk are age of the contact and type of leprosy of the index patient. The risk for the physically most distant group (the neighbours of the neighbours) is still 3.2 times higher than the risk for the general population.
3. The overall efficacy of a single dose of rifampicin after two years is 56%. This effect is not the same for all subgroups.

It is discussed that the data presently available do not justify the routine use of rifampicin as chemoprophylactic agent in contacts of leprosy patients for two reasons: the first being the fact that in high risk subgroups the effect appears to be smaller than in higher risk subgroups and this effect is not yet statistically significant. Further follow-up of the COLEP cohort will give more observed person-years and therefore statistical significance can be reached. However, thoughts should also be given to the second reason, namely the fact that in the higher risk subgroups the chance of undertreatment is realistic because of a possible higher bacterial load in this group, which could have implications for the selection of rifampicin resistant strains of *M. leprae*.

Chapter 8 is closed by a list of conclusions and recommendations.

The conclusions are summarized as follows:

1. The new case detection of leprosy as found by active house-to house survey is 5-6 times the official registered yearly new case detection.
2. The occurrence of leprosy in contacts of leprosy patients is inversely related to the physical distance between the patient and the contact.
3. Other variables associated with the occurrence of leprosy among contacts are the age of the contact, the genetic relation between contact and patient, and the type of leprosy of the patient.
4. The serological status of a patient is highly correlated with the type of disease, the BI and the dissemination of the clinical signs.
5. The serological status of a contact is not significantly related to the risk of developing clinical signs of leprosy within a 2 year period.
6. The overall efficacy of a single dose of rifampicin after 2 years is 56%.

The recommendations are summarized as follows:

1. Contact surveys in leprosy control programmes should not only include household contacts, but also (neighbours of) neighbours.
2. The data presently available do not justify the introduction of chemoprophylaxis in routine leprosy control programmes.
3. Further follow-up of the COLEP cohort will be needed to determine whether the overall effect of prophylaxis will be sustained.
4. Routine serological examination of contacts of leprosy patients is not justified.

Samenvatting

Dit proefschrift handelt over het risico van contactpersonen van leprapatiënten om ook lepra te ontwikkelen, en over de mogelijkheid om dat te voorkómen door middel van het innemen van een éénmalige dosis rifampicine.

Hoofdstuk 1 is een introductie m.b.t. de ziekte in het algemeen. Kort worden de klinische aspecten, de geschiedenis en het vóórkomen heden ten dage beschreven. Bovendien worden mogelijke methoden ter voorkóming besproken:

Isolatie: een methode die in het verleden veel werd toegepast, maar waarvan het effect onduidelijk is.
Vaccinatie: de effectiviteit van BCG vaccinatie wordt wisselend beoordeeld, maar lijkt boven twijfel verheven.
Het *profylactisch toedienen van medicamenten*: onderzoeken met zowel (ace)dapsone en rifampicine toonden aan dat deze methode effectief zou kunnen zijn. Bevestiging van de effectiviteit van rifampicine door middel van een gerandomiseerd dubbelblind onderzoek was zeer wenselijk.
Vroege opsporing en behandeling: de belangrijkste methode tot nu toe. De hoop was dat, door alle bekende patiënten te behandelen en op die manier niet-infectieus te maken, de transmissie zou verminderen. Dit is tot nu toe niet overtuigend het geval.

Hoofdstuk 2 is een literatuuronderzoek naar de factoren die van invloed zijn op het risico voor contactpersonen om lepra te ontwikkelen. In de literatuur worden verschillende definities voor contactpersonen gebruikt, maar meestal worden huisgenoten bedoeld. Diverse onderzoeken maken echter aannemelijk dat ook contactpersonen buiten het directe huishouden extra risico lopen op lepra. Voor wat betreft patiëntgerelateerde factoren zijn daarbij vooral het type lepra en de zgn. bacteriële index (een maat voor het aantal bacillen dat bij microscopisch onderzoek wordt waargenomen) van belang. Contactpersonen van PB patiënten (patiënten die weinig leprabacillen bij zich dragen) hebben echter ook een groter risico op lepra dan mensen die geen bekend contact hebben met een patiënt. De belangrijkste contactafhankelijke variabelen die van invloed zijn, zijn de fysieke afstand tot de patiënt, de intensiteit van het contact met de patiënt en de erfelijk bepaalde gevoeligheid, terwijl de rol van leeftijd, geslacht en socio-economische factoren niet duidelijk is. De betekenis van immunologische en moleculaire markers voor het risico de ziekte door te geven of te ontwikkelen is niet geheel duidelijk, maar er zijn aanwijzingen dat contactpersonen die seropositief zijn voor anti-PGL-I antilichamen een verhoogd risico lopen om lepra te ontwikkelen. De aanwezigheid van een litteken van een BCG vaccinatie duidt waarschijnlijk op een lager risico. De analogieën met tuberculose suggereren dat de zgn. "stone-in-the-pond" benadering welke bij tuberculosecontrole wordt gebruikt, ook bij lepra toepasbaar zou kunnen zijn. Het is bekend dat sputum positieve tuberculose patiënten ook besmettelijk zijn. De analogie met PB leprapatiënten suggereert dat ook de contacten van deze patiënten opgespoord en onderzocht zouden moeten worden. Geconcludeerd wordt dat interventies m.b.t. voorkóming van lepra onder contactpersonen zowel op contactpersonen van MB patiënten (patiënten die veel bacillen bij zich dragen) als op die van PB patiënten gericht moeten zijn, met name als deze genetisch verwant zijn.

Hoofdstuk 3. Zoals hierboven uiteengezet, was bevestiging van de effectiviteit van rifampicine als chemoprofylacticum nodig en om die reden werd het COLEP onderzoek gestart. Dit hoofdstuk beschrijft de methodologie van dit onderzoek. COLEP bevat een clustergerandomiseerde, dubbelblinde en placebogecontroleerde trial, een cohort onderzoek ter bepaling van de subgroepen binnen de totale groep van contactpersonen, welke het grootste risico lopen om lepra te ontwikkelen, en een onderzoek van een groot cohort mensen uit de algemene populatie in het studiegebied (de referentiegroep), dit om het vóórkomen van lepra onder personen zonder bekend contact met een leprapatiënt vast te stellen. De geplande follow-up duur was 4 jaar. Het onderzoek omvatte 1037 nieuw ontdekte en onbehandelde leprapatiënten en 21.867 contactpersonen. Het aantal nieuwe gevallen van lepra onder deze contactpersonen was 159 (7,3 per 1000). De overige contactpersonen zonder tekenen van lepra werden opgenomen in de trial en gerandomiseerd op contactgroep niveau. De helft van de groepen kreeg éénmalig een dosis rifampicine en de andere helft een placebo.

Hoofdstuk 4 beschrijft de resultaten van de logistische regressie analyses van de intake data van de groep contactpersonen. Geconcludeerd werd dat de leeftijd van de contactpersoon, het type lepra van de patiënt en de fysieke en genetische afstand tussen patiënt en contactpersoon onafhankelijk geassocieerd waren met het risico van lepra onder de contactpersonen. Opsporingsactiviteiten gericht op contactpersonen zouden daarom niet alleen gericht moeten zijn op contacten binnen het huishouden van een patiënt, maar ook op buren en genetisch verwante personen verder van de patiënt verwijderd, met name indien de patiënt PB2-5 of MB lepra heeft.

Hoofdstuk 5 beschrijft en analyseert de intake data van de referentiegroep. De resultaten worden vergeleken met die van de groep contactpersonen. Het blijkt dat het aantal patiënten met lepra onder de referentiegroep lager is dan dat in de fysiek verst verwijderde subgroep van contactpersonen, namelijk de buren van de buren. Lepra komt meer voor onder de algehele bevolking van noordwest Bangladesh dan de officiële data suggereren. Binnen de regio waren er bovendien grote verschillen welke niet gerelateerd waren aan de seroprevalenties in de verschillende gebieden.

Hoofdstuk 6 is niet gerelateerd aan één van de onderzoeksvragen van dit proefschrift, maar aan één van de andere doelstellingen van het COLEP onderzoek, namelijk het bestuderen van de zin van serologisch onderzoek (m.b.t. anti-PGL-I antilichamen). Uit multivariate analyse blijkt dat er een significante relatie is tussen de kans om als patiënt seropostitief te zijn en het geslacht, de "disability grade", de bacteriële index en het type lepra dat vastgesteld is. Het hebben van een BCG litteken was niet significant geassocieerd met seropositiviteit. Het aantal en de uitgebreidheid van de ziekteverschijnselen was gecorreleerd met seropositiviteit, maar niet het aanwezig zijn van zgn. satellietlesies. Geconcludeerd werd dat seropositiviteit een indicator lijkt te zijn voor het aanwezig zijn van een groter aantal leprabacillen in het lichaam en dat het daarom mogelijk gebruikt zou kunnen worden om meer infectieuze patiënten te identificeren, ook als ze weinig klinische verschijnselen vertonen.

Hoofdstuk 7 beschrijft de resultaten van het COLEP onderzoek na twee jaar. Van de geïncludeerde personen werden 19.957 (91,9%) opnieuw gezien en onderzocht. In de placebogroep hadden 66 van de 10.006 personen lepra ontwikkeld en in de rifampicine groep 29 van de 9.951, hetgeen een reductie betekent van 56% ($p = 0,0003$). Over het geheel

genomen moesten 271 personen behandeld worden om te voorkómen dat er zich bij één lepra ontwikkelde. Dit is een hoopgevend resultaat m.b.t. de potentiële mogelijkheden van deze interventie, maar het blijkt dat het effect in de verschillende subgroepen niet gelijk is. Verder onderzoek is daarom nodig voordat routine implementatie kan worden aanbevolen.

Hoofdstuk 8 is een beschouwing van de onderzoeksvragen en de antwoorden die daarop gegeven zijn.

De onderzoeksvragen waren:

1. Hoe groot is het aantal nog niet eerder gediagnosticeerde leprapatiënten in de districten Nilphamari en Rangpur in noordwest Bangladesh?
2. Hoe groot is het risico onder contactpersonen van leprapatiënten om zelf ook lepra te ontwikkelen, en wat is de invloed daarop van de fysieke en genetische afstand?
3. Hoe groot is de effectiviteit van chemoprofylaxe met een éénmalige dosis rifampicine met betrekking tot het voorkómen van lepra onder contactpersonen?

De volgende antwoorden konden gegeven worden:

1. Het aantal niet eerder gediagnosticeerde leprapatiënten onder de algemene bevolking van Nilphamari en Rangpur was 13,4 per 10.000.
2. Zowel fysieke als genetische afstand zijn omgekeerd gerelateerd aan het risico van lepra. Andere factoren die daarop een invloed hebben zijn: leeftijd van de contactpersoon en het type lepra van de patiënt. Het risico voor de verst verwijderde subgroep die werd onderzocht (de buren van de buren) is 3,2 maal zo hoog als het risico onder de algemene bevolking.
3. De effectiviteit van een éénmalige dosis rifampicine na twee jaar is 56%. Dit effect is echter niet gelijk voor alle subgroepen.

Aangegeven wordt dat de huidige gegevens het toepassen van deze interventie om twee redenen nog niet rechtvaardigen. Ten eerste lijkt het effect van rifampicine kleiner in die subgroepen die, vooraf gezien, een hoger risico op lepra hebben en het effect in deze subgroepen is nog niet significant. Verdere follow-up van het COLEP cohort waardoor meer persoonjaren beoordeeld kunnen worden, zal dat laatste kunnen ondervangen. Ten tweede moet rekening gehouden worden met de mogelijkheid dat de subgroepen met een groter *a priori* risico met een éénmalige dosis rifampicine in feite onderbehandeld worden, wat implicaties kan hebben met betrekking tot het selecteren van rifampicine resistente *M. leprae* stammen.

Hoofdstuk 8 besluit met een lijst van conclusies en aanbevelingen.

De conclusies worden als volgt samengevat:

1. Het aantal nieuw gevonden leprapatiënten dat gevonden wordt bij een huis-aan-huis bevolkingsonderzoek in Bangladesh is 5-6 maal groter dan het aantal officieel geregistreerde nieuw gevallen (hetgeen gebaseerd is op passieve methodes).
2. Het vóórkomen van lepra onder contactpersonen van leprapatiënten is omgekeerd gerelateerd aan de fysieke afstand tussen contactpersoon en patiënt.

3. Ander variabelen die geassocieerd zijn met het vóórkomen van lepra onder contactpersonen zijn de leeftijd van de contactpersoon, de genetische relatie tussen de contactpersoon en de patiënt en het type lepra waar de patiënt aan lijdt.
4. De serologische status van de patiënt is sterk gecorreleerd met het type lepra, de bacteriële index en de uitgebreidheid van de klinische verschijnselen.
5. De serologische status van de contactpersoon is niet significant gerelateerd aan het risico om binnen een periode van twee jaar lepra te ontwikkelen.
6. De effectiviteit van een éénmalige dosis rifampicine ter voorkóming van lepra onder contactpersonen is na twee jaar 56%.

De aanbevelingen werden als volgt samengevat:

1. Onderzoek van contactpersonen van leprapatiënten in het kader van lepracontrole programma's zou, behalve de personen in het huishouden van de patiënt ook de (buren van de) buren moeten omvatten.
2. De gegevens die nu beschikbaar zijn rechtvaardigen het gebruik van chemoprofylaxe onder contactpersonen van leprapatiënten binnen de reguliere lepracontrole programma's niet.
3. Verdere follow-up van het COLEP cohort is nodig om vast te stellen of het effect van chemoprofylaxe blijvend dan wel tijdelijk is.
4. Routinematig bepalen van de serologische status van een contactpersoon is niet gerechtvaardigd.

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Curriculum vitae

Fake Johannes (Hans) Moet was born on August 6, 1957 in Almelo, The Netherlands. He obtained his diploma Gymnasium β from the Christelijk Lyceum Almelo in 1975. In 1976 he started his study medicine at the State University of Utrecht, from which he graduated in March 1983. In 1984 and 1985 he worked as a medical officer at the Benedictine Hospital in Nongoma, KwaZulu, South Africa after which he was trained as general practioner at the State University of Utrecht while working in a general practice in Epe. From 1989 until 1996 he worked for The Leprosy Mission in Bhutan and Indonesia. After having returned to The Netherlands, he worked at nursing home Sonnevanck in Harderwijk until 1999. Since then he was employed by UWV, the organisation responsible for the implementation of the legislation on social security in The Netherlands. In October 2001 he also joined the Department of Public Health, Erasmus MC, University Medical Center Rotterdam where he became involved in the COLEP trial, a research project on leprosy, carried out in Bangladesh. He has obtained his Master of Public Health degree in June 2007.