Chapter 7
Past Leprae

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Abstract Although leprosy often results in characteristic morphological alterations to the skeleton, its diagnosis may be difficult in cases of less significant bone changes. Molecular analysis of such cases may help resolve several aspects of the palaeopathology and palaeoepidemiology of leprosy. Several reports have documented the extraction and molecular analysis of *Mycobacterium leprae* DNA from ancient bone samples. Accordingly, a direct palaeomicrobiological approach may be taken to investigate the disease and its sequelae. In addition, the origin and the spread of the disease, as well as the dramatic decline of this infection in post-mediaeval Europe, can now be investigated.

7.1 Introduction

Infectious diseases like tuberculosis and leprosy often result in characteristic morphological alterations to the skeleton, and thus can be identified easily in ancient human remains. However, in cases with less significant bone changes it can be more difficult to come to a clear diagnosis of the underlying disease. Especially in such cases, the analysis of genetic material in ancient tissues may help clarify an unsure morphological analysis. The recent development of modern molecular biological techniques, such as the polymerase chain reaction (PCR) and sequencing techniques, offers a new approach to the identification of pathogenic organisms (Zink et al. 2002; Drancourt and Raoult 2005). Such techniques not only help identify ancient bacterial DNA in human remains, thereby providing direct evidence of the occurrence and frequency of infectious diseases in historic populations, they also yield information about the evolution of microorganisms and the diseases they cause.

In this context, the molecular analysis of cases involving possible infection with *Mycobacterium leprae* is of particular interest, as several aspects of the palaeopathology

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and palaeoepidemiology of leprosy remain controversial (Aufderheide and Rodriguez-Martin 1998). Likewise, both the origin and the obvious spread of this disease, but also its dramatic decline in post-mediaeval Europe are unclear and require elucidation. To date, several reports have documented the extraction of *M. leprae* DNA from ancient bone samples (see below). Accordingly, a direct palaeomicrobiological approach may be taken to investigate this disease and its sequelae.

7.2 Clinical Aspects of Leprosy

Leprosy, or Hansen's disease, is a slowly progressive chronic infectious disease, caused by the bacillus *Mycobacterium leprae*, leading to granulomatous destruction of soft and hard tissues and potentially leading to severe mutilation of the infected individual. The disease was historically a major predator of mankind and – despite its present curability with specific antibiotics – ca. 500,000 individuals worldwide are still infected. Approximately 2–3 million people currently live with mutilations due to leprosy. Nowadays, most cases are concentrated in the tropics of South America, Africa and Asia, although sporadic endemic cases still occur in Europe (e.g. the Baltics, Eastern European countries), North America and the Pacific islands.

Steps in the transmission of the disease are not fully clear. However, it is accepted that the reservoir of the mycobacterium is exclusively human, and that it is most frequently transmitted by aerosolic spread of the bacilli. In most instances, infection seems to occur during childhood, with incubation times ranging from 6 months to several years. Rarely, unusually long incubation periods of up to 40 years have been reported (Gierke et al. 2000), although the infection rate in adults with close contact to infected individuals (e.g. spouses) is as low as 5% even on long-term investigation.

The clinical picture of leprosy is variable and depends on the type of host immune response. The course of the disease can be roughly divided into four stages, which may develop from one another.

Leprosy typically begins as an indeterminate form that can spontaneously heal, remain unchanged for a long time period or proceed to a more severe form. Approximately 95% of contacts with the bacillus will result in spontaneous resolution without development of clinical symptoms. This initial indeterminate form may produce ill-defined skin patches or maculae with slight hypopigmentation. In parallel, such patches may coincide with hypaesthesia of the corresponding skin nerve. If the disease progresses, tuberculoid leprosy may develop provided that the host immune response is still adequately preserved. In this stage, a rapid loss of skin sensation due to severe nerve damage may occur, as well as local paralysis, loss of sweat and sebaceous glands, and hair loss. The skin shows macular lesions with significant hypopigmentation; peripheral nerves are infiltrated and may present as thick subcutaneous bundles. Secondary symptoms include bruising of hypaesthesised skin due to local external damage, and superinfection with poorly healing ulcers.

The lepromatous stage occurs in individuals with a poor immune reaction. Clinically, this is the most severe form and can lead to disfiguring mutilation. The skin lesions may present as maculae, papulae or plaques with hypopigmentation. The regions most affected are ears, the central face, fingers and toes, but the distal extremities, e.g. the extensor surfaces of thighs and forearms, can also be affected. The severe infiltration of skin in the perinasal and periorbital region leads to the "facies leonine" or lion face, which is associated with loss of the eyelashes and lateral eyebrows ("facies leprosa"). Often, the eyes are affected causing blindness. Osseous resorption of the nasal aperture and destruction of the bridge of the nose result in severe mutilation of the face. Affection of the throat may lead to a typical hoarseness. In fact, all other body regions may also be affected leading to a variable clinical picture (see Sects. 7.4 and 7.6 for descriptions of osseous lesions).

A fourth stage, called the borderline stage, also exists, which is somewhat intermediate between the tuberculoid and the lepromatous stage in clinical symptoms.

Considering the wide range of clinical symptoms, especially the early and "milder" stages of the disease, leprosy can easily be confused with various other diseases. This is often important to reconcile in historical terms, since evidence previously interpreted as favouring a diagnosis of "leprosy" must be considered carefully. In contrast, the typical mutilations of the skeleton in the severe forms of leprosy leave such typical traces of the disease that it may be identified in historic remains with a high degree of certainty.

7.3 *Mycobacterium Leprae* – Molecular Features and Potential Typing

The infectious agent of leprosy, *Mycobacterium leprae*, belongs to the acid-fast bacilli group of mycobacteria, but has a number of particular features worthy of note. Like other species of the mycobacteriae group, *M. leprae* has a lipid-rich cell wall, which leads to the unusual staining properties of all acid-fact bacilli and which provides considerable protection to the bacillus. Thus, the conservation of *M. leprae* is much more likely than that of other bacteria in long-stored material, such as bone or mummified soft tissue from past populations.

On the other hand, on the genetic level the *M. leprae* bacillus is a somewhat "degenerated" mycobacterium since its genome has undergone significant downsizing and has accumulated more than 1,130 pseudogenes (Monot et al. 2005). As a consequence, the bacterium requires very particular growth conditions, and has a doubling time of as long as almost 13 days (Shepard and McRae 1965). *M. leprae* cannot be cultivated in in vitro cultures and the only systems available for the in vivo cultivation of the bacterium are the mouse pad model and the nine-banded armadillo *Dasypus novemcinctus* (Kirchheimer and Storrs 1971).

Extensive genetic analysis of the *M. leprae* genome – the entire length of which has recently been sequenced (Cole et al. 2001) – revealed extremely few differences between isolates from different regions of the world. Furthermore, there were no

differences between strains from different sources (collected all over the world) on the level of the complete genome, the copy number of insertion-sequence-like dispersed repetitive sequences, including the mycobacterial interspersed repetitive unit (MIRU), and the variable number of tandem repeats (VNTR). Similarly, genetic fingerprinting and end-sequencing of numerous cosmids from a library of isolates with different origins showed perfect co-circularity between different strains. It was only on the level of single nucleotide polymorphisms (SNPs) that differences were noted (Monot et al. 2005). This latter study described an estimated overall frequency of SNPs in *M. leprae* of approximately one per 28 kb, which is significantly less than that observed in other human pathogens. These data strongly suggest that the *M. leprae* genome is exceptionally well conserved and that the leprosy bacillus is highly clonal (Smith et al. 1993).

The study of the worldwide distribution of SNPs in 175 specimens from 21 countries and all five continents identified only four different patterns, each with a distinct geographical distribution: type 1 occurs predominantly in Asia, the Pacific region and East Africa; type 4 is found in West Africa and the Caribbean region; type 3 resides in Europe, North Africa and the Americas; and, finally, type 2 (the rarest) is seen in Central/East Africa, North India/Nepal and New Caledonia. From this distribution, a general evolutionary scheme for *M. leprae* with two plausible scenarios has been derived. In the first scenario, SNP type 2 preceded type 1, spreading eastward from East Africa or Central Asia to East Asia and the Pacific region, and type 3 was disseminated westward to the Mediterranean and Central Europe before giving rise to type 4, which spread to America by colonialism. Alternatively, type 1 was the progenitor of type 2, followed by type 3 and finally type 4 (Monot et al. 2005).

Despite this recent breakthrough in strain identification patterning, the origin and the time axis of spread remains unclear. Likewise, it is uncertain if the origin of the disease lies in Central Africa or Central Asia; the route of spread is also an open debate. Indeed, these questions may be answerable only by palaeomicrobiological studies of relevant material from well-defined sources. Fortunately, leprosy in its full-blown clinical form leaves very typical traces in hard tissues, thus the analysis of human remains will probably provide adequate answers.

7.4 The Osteopathology of Leprosy

Since the palaeopathological record is restricted mostly to skeletal pathology, the specific and non-specific features of this disease will be outlined here in more detail. As indicated above, the advanced stage of leprosy is distinctive for the ailment, thus a diagnosis can be established with the necessary certainty. However, it is noteworthy that the indeterminate stages of the disease are not at all identifiable by bone pathology.

The typical osteopathology of leprosy was first described by Moller-Christensen (1961) in his superb analysis of the osseous remains from a leper cemetery in

Naestved, Denmark. Moller-Christensen described the typical alterations of the maxilla / nasal aperture and the small bones of the hands and feet (Moller-Christensen 1974). Concomitantly, the long bones of the distal limbs are also affected. However, the osseous pattern of these latter bones show non-specific alterations that are also seen in other chronic infectious diseases, such as tuberculosis or treponematosis, although to slightly differing degrees.

The skeletal involvement in leprosy ranges between 15 and 50% of affected individuals, although methodical examination of the skeletal populations of leprosaria indicates that almost 70% of such burials reveal leprosy-related skeletal alterations (Zimmermann and Kelly 1982; Moller-Christensen 1978). This concurs with modern leprosaria (Steinbock 1976); present day patients with leprosy have skeletal involvement in about 25% of cases (Paterson and Job 1964). Within this population, the most frequently affected body sites are the fingers and toes.

Since *M. leprae* affects nerves and other soft tissues along with direct skeletal affliction, skeletal lesions may be due to direct skeletal involvement, but may also result from secondary destruction due to infection of soft tissues. The latter may be particularly important in the destruction of fingers and toes, where loss of sensation (hypaesthesia) may result in secondary non-specific bacterial inflammation. Accordingly, skeletal involvement can be divided into two types:

1. Specific leprosy-induced skeletal changes include the so-called "rhinomaxillary syndrome" (Andersen and Manchester 1992) leading to the "facies leprosa" (Fig. 7.1). Furthermore, periostitis of long bones with subperiosteal new bone deposition occurs in more than 70% of leper cases (Moller-Christensen 1961). Most frequently, this is seen in tibiae and fibulae although other long bones may also be affected (lepromatous periostitis) (Fig. 7.2).
2. Non-specific inflammation and osseous degeneration occurs due to local trauma and secondary inflammation as a result of sensory loss. This osteitis/osteomyelitis is the same as that in patients without sensory loss and may lead to secondary periostitis, bone resorption and arthritis. These are the most frequent bone lesions found in the small bones of the hands and feet.

Finally, as a secondary effect – such as can result from chronic disuse – osteoporosis of skeletal segments may occur. The absence of periosteal reaction and callus formation in pathological fractures is very typically seen in leprosy (Schinz et al. 1953).

The horribly disfigured facial anatomy, known as "facies leprosa", is engraved in the skull bones as bilateral symmetrical resorption of the maxillary alveolar processes of the incisors with concomitant loss of the nasal aperture and formation of defects of the hard palate. Together, these processes lead to a wide and empty depression where the nose once existed. These bone changes are summarised as "rhinomaxillary syndrome" (Anderson and Manchester 1992). The syndrome – present only in lepromatous leprosy (and those borderline lesions close to lepromatous leprosy) – results from a direct involvement of the affected bones through *M. leprae* infection of the overlying mucosa and skin that spreads to the adjacent bone structures (Aufderheide and Rodriguez-Martin 1998). There is normally only little new bone formation at the periosteal surface, which represents an important differential

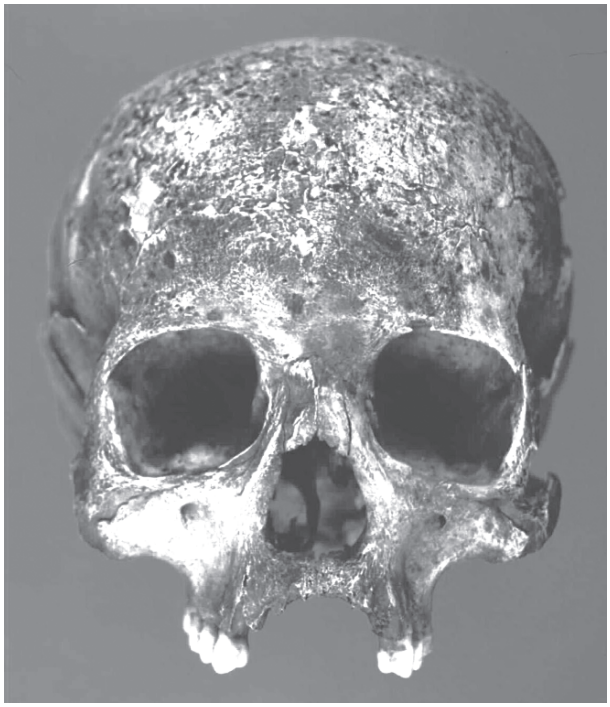

Fig. 7.1 Skull with typical pathological symptoms of 'facies leprosa' of the skull: wide aperture of the nose, extensive resorption of the maxilla and loss of the front teeth

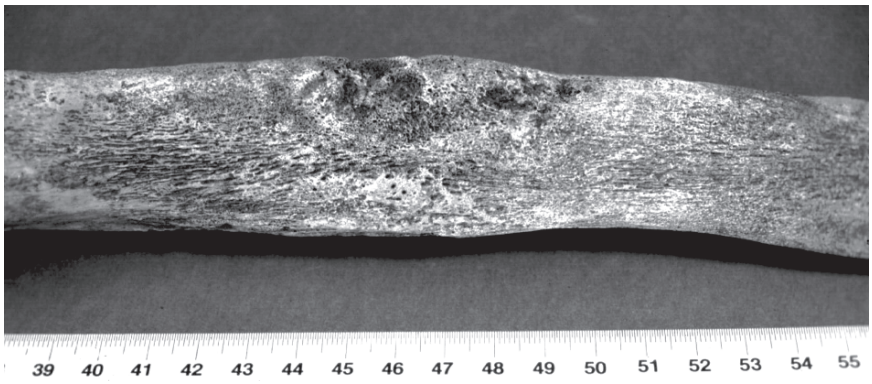

Fig. 7.2 Leprosy-associated osteitis / periostitis of the tibia showing enlargement of the complete bone with strongly irregular bone surface and focal resorption zones. *Scale* cm

diagnostic criterion in distinguishing facial inflammatory destruction not caused by leprosy (Revell 1986). The mandible is normally not affected.

The second body regions with direct skeletal involvement with the bacilli are the long bones, mainly those of the distal lower limbs. These show periostitis with

subperiosteal new bone deposits. Such lepromatous periostitis, which is seen in up to 78% of leprosy cases (Moller-Christensen 1961), produces pitting and irregularity of the surface with fine, longitudinally striated, subperiosteal bone deposition. These features may be seen bilaterally, but may also occur unilaterally, most frequently in the distal third of the tibia, but also in fibulae, femorae and, rarely, in the long bones of the upper extremity.

In contrast, pathological changes in the hands and feet can be due to secondary destruction by non-specific inflammation due to small traumas along with the loss of sensation in the peripheral nerves. This can affect the proximal phalanges, the metacarpals and metatarsals with bilateral, though mostly asymmetric, infliction. The terminal phalanges concentrically erode down to a tapered appearance of the fingers. This deformation is also referred to as "licked candy stick" and finally leads to loss of individual phalanges or only stump-like fingers or toe-tips. In addition, the hands and feet of skeletons may show various degrees of dislocation.

About 15–50% of cases with verified leprosy show diagnostic skeletal pathology which, however, may be modified by superinfection (particularly of the small bones) with associated additional bone destruction. These traces may be uncovered during careful palaeopathological investigation and, accordingly, the certainty with which palaeopathological diagnosis can be made is influenced by the presence, and state of conservation, of the diagnostically relevant bones.

7.5 Literary and Iconographic Evidence of Leprosy in History

7.5.1 Origin and First Descriptions – From Early Dawn Until the Roman Period

The origin and spread of leprosy remain uncertain. The oldest evidence comes from literary and iconographic sources and therefore must be handled with great caution. In this regard, it is of particular significance that the clinical features of leprosy are very distinctive in its advanced stages, but are highly non-specific in its early indeterminate form. As a consequence, the interpretation of historic literary or artifactual evidence of leprosy may be difficult and ambiguous – especially if severe forms of the disease were rare or even absent.

Previously, numerous authors have associated the Hebrew word "tsara'ath" in the Old Testament (book of Leviticus) with leprosy (see Aufderheide and Rodriguez-Martin 1998). Since the Old Testament was probably written about 1500 B.C., "tsara'ath" has been regarded as the earliest written evidence of leprosy in antiquity. However, recent critical reviews raise serious concerns regarding the relationship between biblical descriptions of "tsara'ath" and leprosy. Although the diagnosis of "tsara'ath" was based on skin lesions with obvious hypopigmentation (and probably also with hypaesthesia), and resulted in an expulsion from human community, these leprosy-typical features must be reconsidered, since other symptoms

and features mentioned in the Bible do not correlate well with leprosy. These concerns have been fuelled mainly by biblical records stating that "tsara'ath" was curable. Consequently, it is now more and more accepted that the biblical "tsara'ath" does not refer to leprosy sensu stricto, but rather to a broader range of various skin diseases (Marks 2002).

Similarly, it has become clear that the swelling skin disease described in the ancient Egyptian papyrus Ebers is much more likely to have been gas gangrene than leprosy. In summary, neither biblical nor ancient Egyptian texts provide sufficient evidence for the existence of leprosy at their respective times and regions.

As yet, the oldest reliable literary evidence for the disease can be dated back to the ancient literature of India. Medical texts dating to ca. 600 B.C. provide descriptions of certain features strongly suggestive of leprosy. Accordingly, in the early Indian textbook of "Sushruta Samhita" (Dharmendra 1947; Skinsnes 1973), the skin in leprosy (termed "Kuhthan") is described as being "slightly vermillion-coloured, thin and spreading in its nature. A sort of pricking and piercing pain [is experienced in the affected locality] which loses all sensibility to the touches" (Marks 2002). This description is fairly consistent with a mild, tuberculoid form. Additionally, other clinical pictures of the disease are described, with the most extreme form exhibiting "contraction of the skin, local anaesthesia, a copious flow of perspiration, swelling, and piercing or cutting pain in the affected part together with a deformity of the limbs and hoarseness" (Marks 2002). Other symptoms include "breaking of the local skin…falling off the fingers,…sinking of the nose and ears and redness of the eyes" (Marks 2002). As Marks (2002) suggested, these symptoms are highly suggestive of the lepromatous form. Furthermore, the detailed description and classification of the disease into different stages suggests that it was fairly common in India at the time of the description. Further descriptions of similar pathological conditions are given in the book "Arthasastra", which dates back to ca. 321–296 B.C. This book represents some sort of "manual on the art of government as a guide for kings and the maintainment of the earth" (Marks 2002). Here again, leprous conditions are mentioned along with suggestions for therapy by herbal medication. Marks (2002) suggests that the elaborate inclusion of leprosy into these guidelines strongly supports the presence of the disease at that time – and its presence some considerable time before.

In parallel, there is some evidence that leprosy might have been prevalent in China around 500 B.C. A Chinese document (attributed to Nei Ching Su Weng) attributes an illness to a historically known individual named Pai-Niu, a Confucian teacher. However, the specificity of this disease, termed "li", as leprosy is still a matter of debate (Feeny 1964). Skinsnes (1980) cites a reference from a bamboo book dating back to ca. 250 B.C. that uses the term "li" for a disease characterised by nasal destruction, loss of eyebrows, crippling and fracturing of the legs, anaesthesia of the mucosa and hoarseness – very likely the lepromatous form of leprosy. Accordingly, this description has been regarded as strong support for the accuracy of the diagnosis in Pai-Niu's case. In summary, there is literary evidence suggesting the presence of leprosy in India and China around 500 B.C., although as yet no skeletal – or even iconographic – evidence exists.

Artistic evidence of leprosy in the Greco–Roman period is also extremely uncertain, and an artefact regarded as a leprous hunchback by Grmek (1989) dating to 1300 B.C. and originating from Israel is unconvincing since the figurine fits much better to the dwarfed Egyptian god Bes, who was widely adored in the Egyptian empire.

In summary, literary and artifactual evidence for leprosy dates back to the ninth century B.C. in India and the sixth century B.C. in China. These observations suggest a descent of the disease from the Indian subcontinent (and/or ancient China). However, the origin of the disease is still uncertain.

7.5.2 From the Roman Period Until the Late Middle Ages

The next reliable literary descriptions of leprosy come from two Roman period historiographs: Celsus (25 B.C.–37 A.D.) and Aretaeus of Cappadocia (first century A.D.). Both provide relatively detailed descriptions of leprosy, which was termed "elephantiasis" but not "lepra" (Lechat 2002). Surprisingly, all Greek medical textbooks and all known historiographies before this time provide no description of the disease. This is of note, since, for example, the (significantly earlier) Corpus Hippocraticum contains numerous detailed descriptions of all kinds of contemporary diseases, but none is similar to, or even matches the symptoms of, leprosy (the Greek term "lepra" used by Hippocrates clearly relates to scaling of the skin, such as in psoriasis or fungal skin diseases). It is noteworthy that, almost in parallel, the Chinese surgeon Hua T'o provided a detailed description of a leonine face, thereby indicating that leprosy was still present in East Asia (ca. 150 A.D.; Aufderheide and Rodriguez-Martin 1998).

Pliny the Elder (23–70 A.D.) – a contemporaneous writer to Celus – also describes "elephantiasis" (meaning "leprosy" in our nomenclature) as having been brought to Rome by the returning army of Pompeius around 62 B.C., when he returned from a military campaign against the king of Pont, Mithridates (Lechat 2002). Although the exact attribution to leprosy remains uncertain, and its association with the army returning from Asia is also speculative, these descriptions of a disease possibly representing leprosy coming from the Middle East are of particular note. Recently, Lechat (2002) suggested that leprosy was at that time uncommon in the central Roman Empire since "elephantiasis" was not included in the list of diseases that was used to refuse the sale of slaves, as was the case for "phthisis" (tuberculosis), fevers, eyesores and mental disorders. However, in the period following, the spread of "elephantiasis" in the Roman Empire, e.g. to Gallia and Southern Germania, where Galenus describes a disease of leprous symptoms around 150 A.D., can be assumed (Lechat 2002).

An important observation with the spread of leprosy is the appearance at the beginning of the third century A.D. of special hospitals. These first leprosy hospitals (called "lazar houses") are recorded in Cappadocia and various countries in Central Europe (Ackerknecht 1972). This strongly supports the spread of the disease in the Roman Empire during that time. Furthermore, some descriptions suggest that

socially high-ranking individuals, such as the Emperor Constantine, were also affected by leprosy. However, skeletal evidence and detailed literary description are still lacking.

In subsequent centuries, there is evidence for significant spread of the disease in Europe, but there is also continuous literary evidence for the disease in India and China. Likewise, the Chinese book "*Ch'ien Chin Yao Fang's One Thousand Golden Remedies*" describes typical features of leprosy and includes suggestions for some herbal medications (Skinsnes 1973). Meanwhile, the erection of lazar houses is increasingly documented in several European locations, such as England (638 A.D.) and Constantinople, but also in Japan (Wells 1964). Furthermore, leprosy was spread to Northern European countries by the Vikings, reaching Scandinavia in the tenth century. Here also, the disease affected individuals of various social classes. For example, in 1413, an Icelandic bishop was dismissed from his service since his leprosy-associated deformities prevented him from celebrating holy Mass (Lechat 2002).

The later medieval period is characterised by a continuous increase in the prevalence of leprosy in Europe, as evidenced by the number of lazar houses, until by the thirteenth century the presence of approximately 19,000 such special hospitals had been documented (Roberts 1986). The diagnosis of leprosy – as reported in various documents – was of great significance and was usually established by a special commission that contained specifically trained personnel, including infected members of lazar houses. As a consequence, it is not surprising that about 70% of leprosaria's occupants revealed the typical skeletal manifestations of leprosy (Moller-Christensen 1961). This strongly supports the concept that a diagnosis of leprosy was established carefully and was correct in a considerable percentage of suspected patients. Nevertheless, it is still a matter of strong debate whether the number of lazar houses indeed reflected infection rates by leprosy, and it has been claimed that the real prevalence of the disease was much lower than would be expected from the number of leprosy hospitals.

An important issue during this time period is the claimed association between the spread of leprosy and the crusades. It has repeatedly been hypothesised that leprosy was brought back by the knights of various crusades in the twelfth and thirteenth centuries. As yet, the only evidence for this hypothesis is the rapid concomitant increase in the number of lazar houses in Central Europe during this time period, suggesting increased disease prevalence. Without doubt, the Near Eastern region, including the Holy Land, was affected by leprosy, and there are even excellent descriptions of the disease affecting high-ranking persons, such as King Balduin of Jerusalem who died in 1185 at the age of 23 (Mitchell 2002). The "clinical" description of this case is very typical of his contracting borderline tuberculoid leprosy as a child, first noticed as an area of skin that had lost sensation on his right arm. With increasing age, he seems to have developed the lepromatous form of the disease, with typical mutilation, blindness and hoarse voice (Mitchell 2002). During that period, leprosy-infected crusaders founded a specially formed military order called the "Order of St. Lazarus", which enabled the infected to fight in the king's army despite being separated from the rest of the population.

Thus, although there is good evidence for leprosy in the crusader population, there is no proof for an active role of the crusades in the spread of the disease across Europe. Accordingly, although it is conceivable that many soldiers with signs and symptoms of leprosy took the disease with them on their return home having contracted it in the Near East, the already significant number of existing lepers in Central Europe makes it unlikely that they were the only ones to bring leprosy to Europe (Mitchell 2002).

7.5.3 From the Late Middle Ages Until Modern Times

Having reached a significant number in the Middle Ages, as mentioned above, a strong decrease in the number of lazar houses is noted by the sixteenth century. The reason for this remains an open question. Previously, Chaussinard (1948, 1953, 1966) suggested that a certain degree of cross-immunity between different mycobacteria caused reduced leprosy prevalence along with the increasing spread of tuberculosis. However, as yet there is no proof that the frequency of tuberculosis indeed increased considerably within the time frame in question. Furthermore, critical re-evaluation of the disease frequencies of leprosy and tuberculosis in modern day populations failed to reveal significant cross-interaction between these two diseases (Wilbur et al. 2002). Alternatively, it has been hypothesised that a novel strain of leprosy bacilli, which developed a much less aggressive clinical course, might have superseded the former strain. Finally, the separation of infected patients from the surrounding population might have led to a continuous decrease in the load of infectious sources, leading to a reduction in the number of new infections. However, why leprosy infections were selectively reduced, while other infectious diseases, such as tuberculosis, were not, remains unclear.

In Middle Europe, leprosy had disappeared almost completely by the end of the eighteenth century. However, endemic foci of the disease remained in Baltic and Scandinavian countries. Even today, isolated cases of leprosy occur in European countries, mostly imported from current hot spots where leprosy is endemic. However, in some cases the incubation periods seem to be extremely long and may have been missed upon superficial examination (Gierke et al. 2000).

7.5.4 Leprosy and the New World, Australia and Oceania

The Spanish conquest of Mesoamerica seems to have brought leprosy to the New World. At least there is no convincing evidence that the disease already existed in Pre-Columbian America. Similarly, the spread to the Pacific Islands seems to have been the result of European and/or Chinese colonisation. The first reference to leprosy in Hawaii was in 1823; not more than two generations later, almost 5% of the Hawaiian population suffered from leprosy (Ackerknecht 1972).

Recently, however, osteoarchaeological evidence has shaken this concept of modern day spread of leprosy in the Pacific area. Bone findings suggest that leprosy might have been present in Western Micronesia already between the seventh and fifteenth centuries A.D. (Trembly 1995, 2002), but may have been "overshadowed" by the later spread of leprosy during Western colonisation (see also Sect. 7.6).

7.6 Palaeopathological Findings in Leprosy Research

7.6.1 First Osteoarchaeological Evidence

Besides literary and iconographic evidence, the strongest evidence for leprosy comes from the methodical palaeopathological analysis of human remains, i.e. the bones surviving from burials at various places and from various time periods. As mentioned above, this holds true only for the lepromatous leprosy stages as only these produce the typical pathognomonic features of the disease that allow a concise diagnosis. All cases of the tuberculoid form will elude this type of analysis.

Currently, the oldest skeletal evidence of leprosy comes from a very recent palaeopathological analysis of a Celtic burial in Northern Italy, where Mariotti and co-workers (2005) identified a fourth–third century B.C. skeleton with some typical signs of leprosy, such as rhinomaxillary syndrome and typically malformed fingers. Archaeological evidence suggests that the adult male individual was a warrior who might have been involved in the Eastern Mediterranean wars and thus may have had contact to Near Eastern foci of leprosy. The authors speculate that leprosy spread rapidly to the Western world around the third–fourth century B.C. as single cases, apparently without producing an epidemic, since the affected skeleton was the only one out of 71 adults and 23 sub-adults.

The next skeletal evidence comes from the Ptolemeic (Greek) period in Egypt. In 1980, Dzierzykray-Rogalski described two skulls dating to approximately 200 B.C. found in the oasis of Dakhleh in the Western Desert, which demonstrated the typical lesions of facies leprosa. Recently, Molto (2002) described four further cases – also from the Egyptian desert oasis of Dakhleh and dated to the early-to-mid fourth century A.D. – with typical evidence of leprosy, here seen not only in the skulls but also in the typical malformations of the small bones of the fingers and toes. Covering only a short time period later, Wood-Jones (1908) described a further skull from a Nubian cemetery (fourth–seventh centuries) with destruction of the nasal bones, nasal septum and turbinates that also fits well with a diagnosis of leprosy; Moller-Christensen and Hughes (1966) reviewed and confirmed the diagnosis in this case. In addition, they identified a further skull from this Nubian series that also revealed signs of facies leprosa. A further early case dating to ca. 300–600 A.D. from Bet Guvrin in the Holy Land (Hershkovitz et al. 1992, 1993) was initially suggestive of leprosy, but on subsequent palaeomicrobiological analysis turned out to be a mixed infection with *M. leprae* ancient DNA along with non-specific

inflammation (Spigelman and Donoghue 2001). A further case of leprosy from this region, however, was seen by Zias (1991, 2002) in seventh–tenth century material.

In parallel to this skeletal evidence for leprosy in the Near Eastern / Mediterranean region, first skeletal findings typical of leprosy in western European regions have been discovered in Britain, where a Romano-British skeleton from the fourth century A.D. presented with typical features of leprosy (Reader 1974). Further isolated cases suggestive of leprosy were described in a sixth century adult male skeleton from Gloucestershire (Wells 1962) and a seventh century male skeleton from Cambridgeshire (Moller-Christensen and Hughes 1962). A recent extensive survey of skeletal evidence of leprosy in Britain (Roberts 2002) on a total of 8,253 skeletons revealed 128 affected individuals. This survey covered 1,500 years, with 2 affected sites from the Romano-British period, 12 sites from the Anglo-Saxon period (fifth–eleventh centuries) and 27 sites from later periods (twelfth–seventeenth centuries). This suggests that there was an increase in leprosy over time, which correlates with the historical data. The first cases of leprosy in individual European countries have also been published as case reports: in France, two cases have been recorded from the Roman period of the fifth century (Blondiaux et al. 2002); the first case in Hungary was dated to 1082 A.D. (Palfi et al. 2002), in the Czech Republic 1293 A.D. (Dokladal 2002), and in Finland 1355 A.D. (Vuorinen 2002).

7.6.2 The Mediaeval Rise in Leprosy Prevalence

In parallel to the literary evidence outlined above, skeletal evidence of leprosy during the Mediaeval period is also increasing. Much information has come from extensive palaeopathological investigations of lazar house cemeteries – such as those performed by Moller-Christensen in the 1950s–1970s, and much more recently by Boldsen and co-workers (Boldsen 2001, 2005; Boldsen and Mollerup 2006). Such studies provide not only details of the typical osteopathological features of skeletons affected by leprosy, but form the basis for an estimation of the palaeoepidemiology of leprosy in distinct time periods. At present, this information is available only for Danish cemeteries, which have provided an excellent database for such estimates. However, one has to remember that leprosy infection rates in other countries – and also different time frames – may have been completely different.

In a first extensive palaeoepidemiological approach in 2001, Boldsen determined the rates of burials with signs of leprosy in three distinct settings. The Sanct Jorgen cemetery in Odense was the burial place of a lazar house and harboured 1,507 burials, of which 924 complete skeletons and 239 isolated skulls were present. This cemetery was in use between the thirteenth and the mid-seventeenth centuries. At least two-thirds of the people buried in this cemetery suffered from leprosy, which correlates well with previous findings by Moller-Christensen in a leper cemetery in Naestved, Denmark (1961). These data were compared with the findings in 200 adults from the cemetery of St. Jörgen in Malmö. Although this

cemetery was in use between 1320 and 1520 A.D., the 200 burials under examination covered the late burial period (i.e. presumably after 1450 A.D.); not more than 10% of individuals were affected by leprosy. The third cemetery was that of a mediaeval village population from Tirup dating from the twelfth to the fourteenth century A.D. In the relatively small population of 61 adult skeletons analysed, ca. 35% of individuals showed features of leprosy. The frequency of leprosy in these three burial populations strongly suggests the following:

1. That leprosy was present in a significant proportion of the population in mediaeval Denmark, and was restricted not only to lazar houses, but also affected the rural population of small villages to a very considerable extent.
2. In later periods (ca. fifteenth/sixteenth centuries), the leprosy rates seem to have diminished considerably.
3. Both archaeological and literary evidence suggests that leprosy had disappeared from Denmark by the middle of the sixteenth century.

As a further interesting finding of this study, it turned out that the facial symptoms of leprosy (rhinomaxillary syndrome) were seen almost exclusively in the burials of lazar houses, while cases with minimal facial but more extensive peripheral osteopathology typical of leprosy dominated the leprosy cases in the Tirup sample. Furthermore, this study provides some evidence that people with leprosy symptoms died at a younger age than people without evidence of leprosy.

Subsequent studies by Boldsen (2005) and Boldsen and Mollerup (2006) determined the leprosy rates in four further cemeteries in central Denmark, covering various time frames between 1060 and 1818. All populations were of a considerable size, ranging between 66 and 372 well preserved adult skeletons. These populations revealed the prevalence of leprosy to have been between 13% and 23% in burials between 1060 and 1400, and 1–4% in material between 1200 and later than 1536. Accordingly, the prevalence of leprosy causing skeletal changes in the Early (1000–1200 A.D.) and High (1200–1400 A.D.) Middle Ages was very high, but was low in later burials. This independent study confirms the high prevalence of leprosy also in non-specialised cemeteries, thus confirming the aforementioned high prevalence of the disease found in skeletal remains from other sources.

7.6.3 The Post-Mediaeval Decline in Leprosy Frequency in Europe

A highly important phenomenon in the history of leprosy is the remarkable decline in the disease frequency in the post-mediaeval time period in Europe. This is evidenced both by the strong reduction in osteoarchaeological findings typical of leprosy and the considerable reduction in the number of leprosy hospitals. Thus, the number of “lazar houses” diminished after the fifteenth century. For example, in England, from a peak number of 200 lazar hospitals around the early fourteenth century, only very few were still recorded in the fifteenth and sixteenth centuries

(Manchester 1984). Similar figures have been reported from other countries and regions, suggesting a more general phenomenon. In only a few Northern European regions, such as western Norway and the Baltics, did leprosy remain an epidemic disease, maintaining a low-level prevalence rate in the population, until finally – following the identification in 1873, by the Norwegian doctor Amauer Hansen, of *M. leprae* as the infectious agent – the disease was extinct also in those regions (1955).

In parallel to the decline in the number of lazar hospitals, skeletal evidence also indicated that the rate of leprosy strongly declined in all European regions investigated. Detailed figures from the best analysed region to date – several cemeteries in Denmark (Boldsen 2001, 2005; Boldsen and Mollerup 2006) – were presented in Sect. 7.6.2.

In contrast to this decline in Europe, in other regions of the world a significant spread and increase in the disease has been noted, which parallels the literary evidence for the spread of leprosy in various regions (see also above).

7.6.4 Potential Reasons for the Extinction of Leprosy in Europe

The significant decline of leprosy in post-mediaeval Europe has been attributed to several factors, the effects of which, however, remain uncertain as yet. Currently, it is widely accepted that the segregation of lepers into lazar hospitals represents one important factor that reduced infection rates by the disease. However, taking into account the very long incubation periods (up to several years) and the high frequency of affected individuals during the peak incidence period (at least as documented by the "normal" village cemetery of Tirup, Denmark, with 25–50% leprosy-infected burials; Boldsen 2001), it is unlikely that the separation of the most severely affected lepers would have been sufficient to wipe out the disease.

As a further important factor, it has been claimed that leprosy-infected individuals were more susceptible to other epidemic diseases so that the great plague – which hit Middle Europe severely in 1348 and then repeatedly almost every 10–20 years – may have affected lepers more than the rest of the population. This may have led to a selective reduction in the number of lepers. Although this hypothesis is very interesting, it remains unclear why leprosy-infected individuals should have been affected more frequently, while at the same time the rate of tuberculosis infections increased. Tuberculosis obviously was not much affected by other epidemic diseases, although we have recently obtained molecular proof that the rate of tuberculosis infections was high in a group of plague victims (Zink et al. 2007).

As a further hypothesis, it has been claimed that cross-immunity between *Mycobacterium tuberculosis sive bovis* and *Mycobacterium leprae* might have led to the decline in leprosy, since increased tuberculosis rates seem to have paralleled the decline of leprosy (Chaussinard 1948, 1953, 1966). This interference hypothesis was based on the idea that while infection by *M. tuberculosis* offered some cross-immunity against leprosy, the converse was not true. As a consequence, tuberculosis wiped out leprosy. Experimental observations seem to support this

idea. Additionally, this cross-immunity hypothesis has gained much support from epidemiological estimations (Lietman et al. 1997). Furthermore, the existence of cases with co-infection with leprosy and tuberculosis has been noted previously (Manchester 1984).

Recently, however, on the basis of recent endemic tuberculosis and leprosy data from Texas in the United States, Wilbur et al. (2002) suggested that the two diseases did not influence each other much, and that the rise and decline in one disease was paralleled by the same movement in the other. Very recently, in the largest molecular study on leprosy and tuberculosis to date, Donoghue et al. (2005) showed high levels of co-infection with both diseases in a selected population between the first and the fourteenth centuries, which was interpreted as a further indicator of an interaction between the two diseases. However, a recent large molecular study performed in our own laboratory on a mediaeval to modern day population from South Germany (Nerlich et al. 2007) found only a very low co-infection rate with both diseases. This issue will be discussed in more detail in Sect. 7.7.

In summary, the reason for the evident decline in leprosy around the fifteenth–sixteenth centuries remains as yet very unclear. Besides a multifactorial interaction involving changes in climate, separation of infected individuals, interfering epidemics with high mortality and potential cross-immunity, it may also be speculated that changes in *M. leprae* strains, with the appearance of strains with a much less aggressive clinical performance and concomitant "overgrowth" of the earlier *M. leprae*, might have led to the disappearance of the disease in most parts of Europe.

7.7 Analysis of Ancient *M. leprae* DNA

7.7.1 Methodological Remarks

The identification of *M. leprae* ancient DNA (aDNA) is facilitated by the fact that *M. leprae* has (like all bacilli of the mycobacteria group) an acid-fast cell wall that seems to protect the DNA from extensive diagenetic damage. Nevertheless, as for all aDNA studies, the target size is critical to any molecular analysis. For the specific identification of *M. leprae* DNA, different segments of the two repetitive elements RLEP1 and RLEP3 have most often been used for amplification by PCR (Yoon et al. 1993; Jamil et al. 1994), since these products are specific for *M. leprae* aDNA. Accordingly, PCR products of various size have been generated, in some cases surprisingly large fragments, e.g. in a study by Haas et al. (2000a), fragments of 372 bp and 320 bp were obtained for RLEP1 and RLEP3, respectively. Although fragments of this size may be criticised in terms of target length, both the aforementioned evident protection of the aDNA and the unambiguously positive results make RLEP1 and RLEP3 valuable targets in terms of aDNA research. Recently, Donoghue et al. (2001) identified and used primer pairs generating, on nested amplification, an outer amplification product of 136 bp and an inner product of 110 bp in length. Accordingly, this primer set covers a significantly smaller, but

specific, *M. leprae* DNA segment and thus extends the possibilities available for aDNA research investigating leprosy. Indeed, own recent study on archival paraffin-embedded tissue material from a leprosy patient (which is a comparably "poorly" preserved historic tissue material) yielded a positive result with the Donoghue primer pair, but failed on both RLEP1 and RLEP3 amplifications (Nerlich et al. 2007).

7.7.2 Ancient DNA Analysis of Skeletal Remains – Reports from Isolated Cases or Small Series

The first successful molecular study on the identification of *M. leprae* was performed by Rafi et al. (1994a, 1994b) who positively identified *M. leprae* aDNA in the case of a seventh century leper from the Jordan River in Palestine. Using a protocol that investigated a 439 bp fragment of *M. leprae* DNA, they detected a specific positive amplification product in a severely destroyed first metatarsal bone. However, despite some clinical evidence of leprosy, two further samples in this series yielded negative results (Table 7.1).

The next report on the successful amplification of *M. leprae*-specific aDNA came from our own analysis of human remains from mediaeval- to modern-period skeletons (1400–1800 A.D.) from a small town ossuary in southern Germany (Haas et al. 2000a). Two skulls with typical rhinomaxillary syndrome, and therefore strongly suggestive of leprosy, tested unambiguously positive for both RLEP1 and

Table 7.1 Molecular results in ancient DNA (aDNA) leprosy research

Date (A.D.)	Number of positive cases	Provenance	Author	Publication year
First century	1	Israel	Donoghue et al.	2005
Fourth century	8	Dakhleh Oasis, Egypt	Donoghue et al.	2005
Fourth–seventh centuries	1	Israel	Spigelman and Donoghue	2001
Seventh century	1	Palestine	Rafi et al.	1994
Tenth century	1	Hungary	Haas et al.	2000
Tenth century	4	Hungary	Donoghue et al.	2001, 2005
Eleventh century	1	Hungary	Donoghue et al.	2005
Tenth–thirteenth centuries	1	Sweden	Donoghue et al.	2005
Mediaeval	1	Poland	Donoghue et al.	2001
Twelfth century	3	Spain	Montiel et al.	2003
Thirteenth–fourteenth centuries	1	Scotland	Taylor et al.	2000
Fifteenth–nineteenth centuries	5	South Germany	Haas et al.	2000
			Nerlich et al.	2007
Fifteenth century	1	Hungary	Donoghue et al.	2005

RLEP3 sequences. This was further confirmed by direct sequencing. In parallel, samples from two tenth-century burials from a Hungarian cemetery (Sarretudvari-Hizoföld) were investigated. While no leprosy-specific aDNA was amplifiable from the foot bones of either skeleton, the skull bone residues available from one individual tested positive. This study not only confirms the presence of leprosy in both populations, but suggests even more clearly that the bacillary load is significantly higher in rhinomaxillary lesions than in hand and foot bones with their presumed secondary infections.

Almost in parallel to the latter study, Taylor and co-workers (2000) reported a successful aDNA study on *M. leprae* in an individual from a Christian cemetery in the Orkney Islands, Scotland, dating to A.D. 1218–1370. The individual exhibited the typical rhinomaxillary features of severe lepromatous leprosy, and again aDNA was found only in skull bone samples, but not in those from other regions of the skeleton. The primer pair used covered a 153 bp segment of RLEP; the results were confirmed by direct sequencing.

Donoghue's highly specific nested primer pairs for the detection of leprosy (described above) have also been tested on archaeological material. Out of six samples, three, which were attributed to a nasal specimen from a mediaeval burial from Suraz, Poland, coming from a 40- to 50-year-old male with characteristic rhinomaxillary syndrome and severe mutilation of the fingers and toes, revealed positive amplification results. A positive amplification product was also found in a nasal specimen from two tenth–eleventh century Hungarian burials from Püspövladany, but no leprosy aDNA was seen in a metatarsal sample from another Hungarian cemetery (Opusztaszer-Monostor).

A further case of leprosy was identified by Spigelman and Donoghue (2001) in a 300–600 A.D. skeleton from Bet Guvrin, Israel, which presented with severe mutilation. Application of the Donoghue et al. (2001) primers in this case revealed a positive amplification result in a sample from the affected foot, thereby confirming leprosy in this individual.

The molecular analysis by Montiel et al. (2003) of skeletal remains in four adult skeletons from a twelfth century cemetery in Seville, Spain, showed a positive result for leprosy aDNA in three samples. In all cases, clinically affected metacarpal bone specimens were analysed using RLEP sequences generating 149 bp and 97 bp nested PCR products. This latter report was the first to show positive aDNA results in various members from an obvious leper community.

7.7.3 Ancient DNA Analysis of Skeletal Remains – Palaeoepidemiological Approaches

Following the above-listed reports on isolated cases or small series of molecularly proven leprosy, the first papers based on a molecular estimation of the palaeoepidemiology of leprosy in specific historic populations have started to appear. In part, these include previously published isolated cases, but some new cases are now

included. Table 7.1 presents all the data available to date on molecularly identified cases of historic leprosy.

In 2005, Donoghue et al. presented positive molecular data on 16 cases of leprosy, 2 of which had previously been described by her group (Donoghue et al. 2001). Out of 30 additional individuals, 14 more positive leprosy aDNA cases were identified, covering a time period between the first century and the fourteenth–sixteenth centuries. The material came from Israel (first century, *n*=3, one leprosy positive); the Dakhleh Oasis, Egypt (fourth century; *n*=11; eight positive cases); Püspökladeny, Hungary (tenth century; *n*=5; four positive, of which two had been described previously); Szekesfehervar, Hungary (eleventh century; *n*=2, one positive); Björned, Sweden (tenth–thirteenth centuries; *n*= 3; one positive); Szekesfehervar, Hungary (fourteenth century; *n*=1, none positive); and Szombathely, Hungary (fifteenth century; *n*=3; one positive).

In this series, the rate of *M. tuberculosis* infection was molecularly tested in parallel in order to determine the rate of co-infection. Interestingly, a high frequency of *M. tuberculosis*-positive cases was also identified, with 18 positive cases out of a total number of 32 cases. Even more importantly, the rate of cases with co-infection was high, with 10 out of 24 cases revealing infection by both bacilli. Hence, the authors claim that co-infection (or even "superinfection" of leprosy by the more aggressive tuberculosis) caused an increased mortality rate in lepers, leading to the stepwise extinction of leprosy. This effect may have been aggravated by the socio-economic impact of segregation of leprosy patients, who were – at least in the serious clinical cases of lepromatous leprosy – readily identifiable by their facial mutilation (facies leprosa). Although this is the first study on a larger series of cases providing highly important and relevant data, it suffers from one major caveat: most leprosy cases in these study populations originated from lepers dating between the first and the tenth century, when the infection rate with leprosy was on an extreme incline (see Sects. 7.5 and 7.6), and not from the period when leprosy was wiped out at around the fifteenth–sixteenth century (only 4 of the 32 cases cover this time-period, with only one case testing positive for leprosy, two for tuberculosis, but none for co-infection). Accordingly, despite the significant value of this study, little can be concluded about the reduction in leprosy prevalence during the late Middle Ages and the beginning of modern times.

In order to potentially fill this gap, we have recently extended our own previous study on the molecular analysis of leprosy skulls (see Haas et al. 2000a) with a study on molecular leprosy identification in long bones with signs of chronic infection in a mediaeval to modern population dating from 1400 to 1800 A.D. (Nerlich et al. 2007). Out of a total population of at least 2,547 individuals (minimum individual number), 59 long bones with more-or-less clear morphological evidence of potential chronic infection were tested in parallel for the presence of *M. leprae* and *M. tuberculosis* aDNA. Sufficiently well preserved aDNA could be retrieved in 24 cases, with 10 cases containing *M. tuberculosis* DNA and 5 cases *M. leprae* aDNA (the latter included the two previously tested cases with rhinomaxillary lesions). Despite these significant infection rates for both mycobacterioses, only one case presented with co-infection.

This first methodical palaeopathological and molecular study analysed tuberculosis and leprosy by investigation of mycobacteria specific for tuberculosis and leprosy in the time period between the late Middle Ages and modern times (1400–1800 A.D.). Thereby, we provide evidence of significant infection by both infectious diseases in this population; however, the rate of co-infection in the study group was surprisingly low, thus this observation does not confirm the previously described high co-infection rate. Consequently, these first molecular observations do not support the idea that tuberculosis "wiped out" leprosy due to its more aggressive and destructive growth pattern. Moreover, it is conceivable that, after a period of (more-or-less peaceful) co-existence between leprosy and tuberculosis over ten centuries, either the leprosy strain or the environmental conditions for leprosy changed significantly leading to a reduction in the disease frequency. Finally, this recent study does not lend support to the previous cross-immunisation hypothesis proposed by Chaussinard and others, but takes into account rather more the critical observations of Wilbur et al. (2002). Nevertheless, until a novel proof for any hypothesis arises, this issue remains to be clarified.

7.8 Conclusions and Perspectives

Ancient DNA research and palaeomicrobiology have opened new debates about the origin, spread, and disappearance of leprosy in Europe, as well as in other regions of the world. In this regard, it is important to remember that although certain infectious diseases can manifest with characteristic pathological bone alterations, clinically milder infections – such as the early indeterminate or even the tuberculoid types of leprosy, will remain unidentified by such means.

At present, numerous molecular studies have identified *M. tuberculosis* in various tissue samples from diverse regions and different time periods (e.g. Salo et al. 1994; Nerlich et al. 1997; Haas et al. 2000b; Zink et al. 2003a, 2003b, 2004, 2005; Donoghue et al. 2004, 2005). Far less data exist on *M. leprae*, although several protocols have been established for the successful amplification and identification of its DNA in ancient bone samples. Along with this increasing knowledge, the first studies providing molecularly proven insights into the spread and prevalence of the disease are beginning to appear. However, considering the findings of Boldsen (Boldsen 2001, 2005; Boldsen and Mollerup 2006), which suggest a very high prevalence rate of leprosy in leper communities, but also in "normal" village burials (up to 25–50% of individuals affected), the two most recent molecular studies contain only a few and obviously very selected cases. Consequently, these preliminary data, though valuable, do not at all reflect the "clinical" reality of mediaeval leprosy.

Nevertheless, first insights into basic data on leprosy are emerging from different sources. Literary, osteoarchaeological and comparative molecular analysis of recent *M. leprae* strains from different countries worldwide strongly suggest that the disease originated in Central Africa, India and/or Central China, with subsequent spread westward (and possibly eastward). The advent of literary and

palaeopathological evidence of the disease in the Mediterranean region around 300–500 B.C. suggests possible spread of the disease by warfare or commercial exchange. However, the apparently low prevalence rates at that time may suggest a "less harmful" bacillus or a more favourable host–pathogen interaction between humans and the mycobacteria at that time. This may also be reflected in the surprisingly high co-infection rates with leprosy and tuberculosis.

The pattern reveals significant changes during the Middle Ages, with almost an explosion of infections, together with specifically targeted measures to control the disease (in special hospitals or "lazar houses"), and the occurrence of concurrent epidemics of highly lethal bacilli such as the Black Death. The obvious increase in the numbers of infected persons may have been the consequence of either a novel and more aggressive bacillus strain (as yet unidentified) or a weakened host–pathogen reaction.

The reason for the dramatic decrease in the disease in the fifteenth–sixteenth centuries in Central Europe – despite its persistence in isolated Northern European spots – is also not clear at present and deserves further investigation. Both recent epidemiological (Wilbur et al. 2002) and molecular studies raise serious concerns regarding the hypothesis that cross-immunisation between *M. tuberculosis* strains and *M. leprae* may have been the reason for this decline. Other mechanisms, such as a novel change in the leprosy bacillus strain pattern or other features may be more plausible, although as yet unproven.

Accordingly, the molecular investigation of *M. leprae* in historic tissue material is now, more than 10 years after the first successful palaeomicrobiological identification (Rafi et al. 1994a, 1994b), still in its infancy. Ongoing studies are urgently required to shed more light on the palaeobiology of this unusual pathogen, which was (particularly in the pre-antibiotic era) one of the biggest predators of mankind.

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