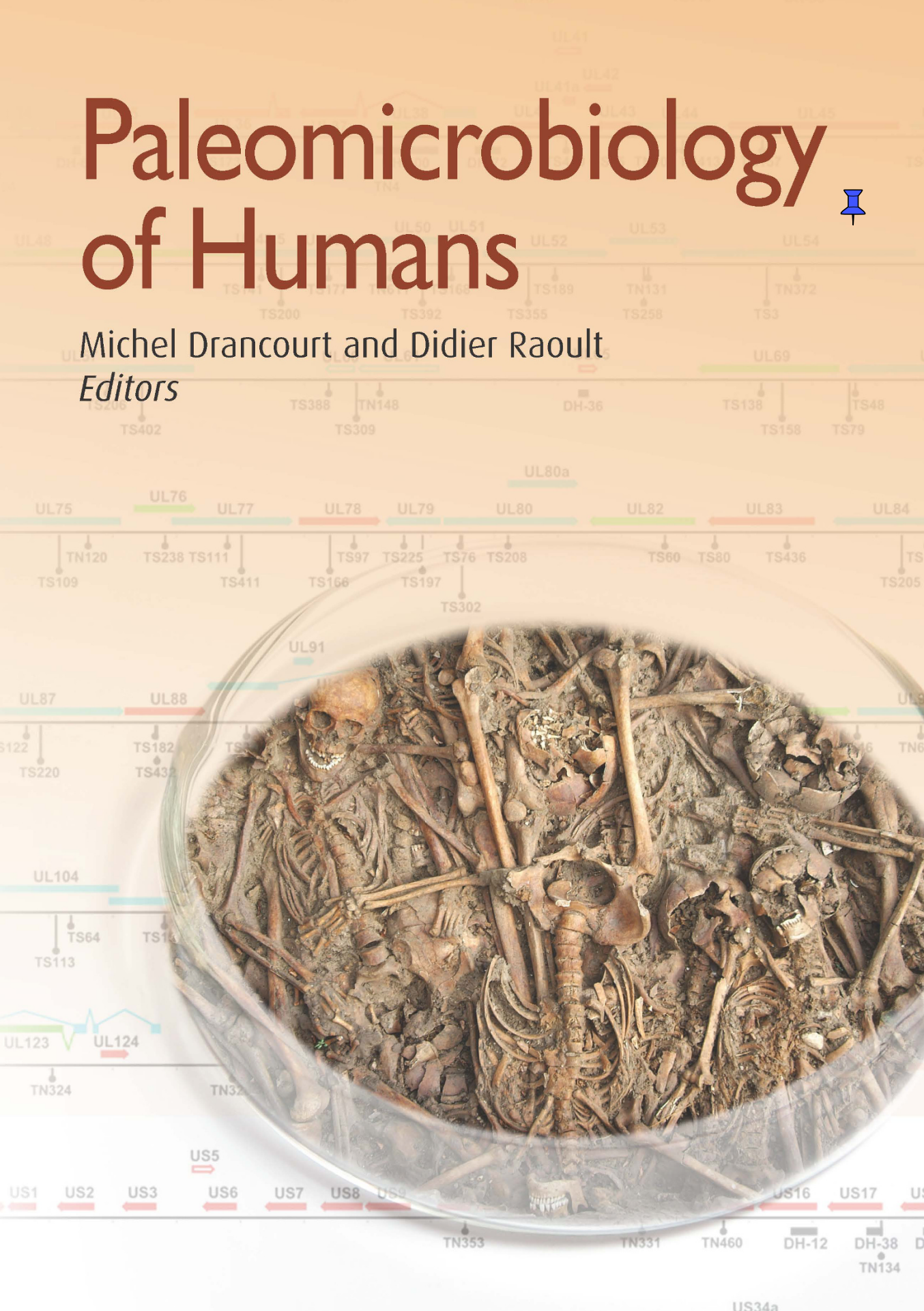
Paleomicrobiology of Humans

Michel Drancourt and Didier Raoult
Editors



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Introduction

Paleomicrobiology is a new field that aims to identify past epidemics at the crossroads of different specialties such as anthropology, medicine, molecular biology and microbiology. Paleomicrobiology is facing several types of problems that are discussed in this book. On the one hand the recognition of human remains associated with epidemic outbreaks, the graves associated with disasters, demographic structures revealing the presence of epidemic moment (Chapters 1 and 2). On the other hand, paleomicrobiology, the history of epidemics, helps to understand the evolution of the history of human beings since now we can find the genetic markers associated with humans, like the gene HLA.LILR inherited from archaic hominids (Neanderthal or Denisovan man) in some populations that presumably have survived due to their resistance to some epidemic pathogens. Paleomicrobiology also helps to track the human migrations (3,4). The materials which can be used to make the diagnosis in paleomicrobiology include soft tissue when it comes to mummies, the arthropods, especially lice, bones and teeth. Use of the dental pulp as a source of genetic material was first used in paleomicrobiology before being used in human genetics (5). The utilization of the dental pulp as a source of DNA research by PCR molecular techniques was initially the subject of a controversy about the authenticity of the results. This controversy, which lasted more than 10 years, is resolved now. The polemics about the initial results concerning plague led to a general reflection on the plague pandemics, which in its turn led to a conclusion that the plague pandemics were probably provoked by the outbreaks of lice. Paleomicrobiology presents the evidence of common epidemics provoked by Bartonella quintana (which is known to be transmitted by lice) and Yersinia pestis which have perfectly demonstrated its role in epidemics and which was confirmed by contemporary plague cases (6). The dispute over the results of the paleomicrobiology led to outlining the identification and interpretation criteria (7). Then, paleomicrobiology developed in different research areas, particularly in the analysis of human coprolites (8), the identification of antibiotic resistance in ancient samples that preceded by several million years the use of antibiotic (9), the history of epidemic typhus (10), of Bartonelose (12) tuberculosis (13), leprosy (14), former intestinal parasites (15), malaria (16), smallpox (17), cholera (18) and finally the history of human lice (19). As a final point, the anatomical analysis of ancient samples also plays a role in the identification of past disease. Altogether, this is the first complete comprehensive book updating (reporting on) the approach of a new multidisciplinary scientific field.

Acknowledgments

The Editors acknowledge their assistant Olga Cusack, IHU Mediterranée Infection, for the expert technical assistance in preparing this edition.

1 Demographic Patterns Distinctive of Epidemic Cemeteries in Archaeological Samples

DOMINIQUE CASTEX[1] and SACHA KACKI[1]

Some ancient burial grounds are valuable testimonies of past epidemics. For both funerary archaeologists and paleobiologists, such archaeological sites offer a remarkable research framework to reveal unfamiliar aspects of these historical events, especially those that occurred during periods for which very few or no written sources exist. The multiplicity of congresses, articles, and syntheses on this topic in recent years illustrates the interdisciplinary research that has progressively emerged over the past two decades.

The detection of an ancient mortality crisis generally relies on archaeological evidence such as mass graves—that is, pits where numerous bodies were buried at the same time (1). However, retaining the hypothesis of an epidemic crisis also requires some paleobiological investigations. First of all, a careful examination of the skeletal remains must be undertaken to make sure that they do not exhibit any traumatic lesions evocative of violent death. Such stigmata would be expected in the case of mass graves related to war or massacre, so that their absence favors the epidemic hypothesis. However, a paleopathogical study cannot demonstrate an epidemic context because the highly virulent pathogens involved kill individuals before any skeletal lesion can develop. Other methods of analysis are therefore needed to ascertain the precise nature of an epidemic. Besides paleomicrobiological studies, analysis of the age and

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sex composition of skeletal assemblages can provide some valuable arguments. Indeed, the age- and sex-specific mortality rates within a population or part of a population at a given time and place may differ depending on the pathogen involved. The study of these biological parameters is particularly relevant to a discussion of the cause of an epidemic mortality crisis.

The scope of this article is voluntarily limited to the demographic analysis of burial grounds related to plague epidemics. Through the study of several of these sites, we illustrate the specificities of mortality by age and sex in such a context. We also discuss some demographic differences between several epidemic outbreaks.

MATERIALS AND METHODS

This work is based on a study of seven European burial sites of plague victims (Fig. 1). With the exception of the Hereford site, all of these burial grounds were used exclusively to bury the victims of plague epidemics. We therefore included in the analysis every skeleton recovered, whether from individual burials or multiple burials. For the Hereford cemetery, which was also used out of the epidemic context over a long period, we studied only the skeletons recovered from three multiple burials, which were proved to be the skeletons of the victims of a plague epidemic.

Two of the sites investigated (Sens and Poitiers) are contemporaneous with the first plague pandemic, known as Justinian's plague. This pandemic began in the 6th century and continued intermittently in Europe until the mid-8th century. Three other sites (Dreux, Hereford, and Barcelona) are contemporaneous with the Black Death—that is, the first outbreak of the second plague pandemic in the mid-14th century. Plague then waxed and waned in Europe until the late 18th century, and the last two sites we analyzed (Les Fédons and Dendermonde) are related to resurgences of the disease at the end of the 16th century. For every one of these archaeological burial

Sites	Chronology	References
1 - Sens (France)	6th c.	2
2 - Poitiers (France)	6th c.	3
3 - Hereford (England)	14th c.	4
4 - Dreux (France)	14th c.	2
5 - Barcelona (Spain)	14th c.	5
6 - Les Fédons (France)	16th c.	6
7 - Dendermonde (Belgium)	16th c.	7

FIGURE 1 Names and locations of the seven European burial sites used for the demographic analysis.

grounds (Fig. 2), the cause of death is attested to by historical sources (Poitiers, Dendermonde) or by DNA analysis (Sens, Dreux, Hereford, Barcelona, Les Fédons).

The first step of the study of each site was to estimate the age at death and determine the sex of all the exhumed individuals by using reliable skeletal indicators (for a critical review of current methods and their application, see reference 8). Estimation of the age at death of non-adults was based primarily on the degree of dental maturation (9–11). When teeth were absent or fully mineralized, we additionally considered the diaphyseal length of the long bones (12) and the stage of fusion of secondary ossification centers (13, 14). Sex determination was carried out only for adult individuals, with reliance primarily on the morphoscopic and morphometric characteristics of the hip bones (15, 16). In order to reduce the number of individuals whose sex could not be determined, we also performed, whenever possible, a secondary sex determination based on the extrapelvic dimorphism of each skeletal assemblage (17). These data were then used to define the composition of the population as well as possible. The mortality profile and the rate of masculinity (i.e., ratio of the number of men to the number of men and women) of each skeletal assemblage were critically discussed within the framework of a comparative analysis,

FIGURE 2 Examples of burials with simultaneous deposits (i.e., mass graves): Poitiers, Poitou-Charentes, France (a); Dreux, Eure-et-Loir, France (b); Dendermonde, Belgium (c).

with reliance on historical data and various standard life tables. Data were compared with an archaic/pre-Jennerian mortality pattern, with the variability of mortality and the confidence intervals of the probabilities of death taken into account. The model life tables of Ledermann (18) were used to make the comparisons. Such an approach has an objective distinct from those of traditional paleodemographic analyses, as it does not aim to determine the demographic characteristics of living populations (e.g., life expectancy at birth, average age at death) but rather to detect anomalous demographic patterns that can be interpreted as evidence of burial selection or abnormal mortality (2). If one assumes that the reference pattern(s) reflect a "natural" demography (i.e., deaths over a long period of time with no major mortality crises), the discrepancies between the reference and the paleodemographic data can reflect change in mortality parameters due to a peculiar event.

Given the poor accuracy of the methods of estimating adult age from the skeleton (8), mortality profiles were analyzed only for immature subjects. As in the model life tables used for comparison, the immature individuals were distributed into 5-year groups (apart from the first two groups, which were 1- and 4-year groups) of attained age. The mortality profile of the subjects from each site was then established by calculating the mortality quotients (Table 1) and was compared with the theoretical mortality pattern (18). The mortality quotient takes the form aQx, where x is the age of entry into an age group and a is the duration in years of the age group. aQx is the ratio of the number of people in an age group who die to the number of people alive at the start of that age group. It thus represents the probability of dying within a precise period of life. It differs from the rate of death, which is the proportion of deaths within an average population. The mortality quotient is more pertinent for comparing a theoretical natural mortality with the mortality profile obtained from an archaeological sample. The life tables of Ledermann (18) allow calculation of the 95% confidence interval of the mortality quotients (illustrated by a range of values in the graphs). The model life tables used in the current study are for a life expectancy at birth ranging from 25 to 35 years; these parameters lie between the 20- and 40-year life expectancy range that characterizes known pre-Jennerian populations (19, 20).

Rates of masculinity were calculated only for adults, as there is currently no reliable method of sex determination for sub-adults. Values were compared with a theoretical rate of 50%.

DEMOGRAPHIC CHARACTERISTICS OF THE PLAGUE BURIAL SITES

The results described below point out the demographic characteristics of each site. Com-

TABLE 1 Demographic data (number of individuals, age, sex, mortality quotients, juvenility index) for each plague site

	Sens		Poitiers		Hereford		Dreux		Barcelona		Les Fédons		Dendermone	
	N	aQx	N	aQx	N	aQx	N	aQx	N	aQx	N	aQx	N	aQx
0	0	0.00	0	0.00	5	27.03	2	28.98	3	25.00	4	30.08	0	0.00
1–4	3	41.10	1	18.87	23	127.78	15	223.88	12	102.56	19	147.29	5	50.51
5–9	10	142.90	4	76.92	28	178.34	10	192.31	16	152.38	24	218.18	22	234.04
10-14	8	133.30	9	187.50	14	108.53	5	119.05	8	89.89	19	220.93	17	236.11
15–19	7	134.60	8	205.13	9	78.26	5	135.14	11	135.80	7	104.48	11	200.00
20 and +	45		31		106		32		70		60		44	
Total males	14		10		40		11		6		30		20	
Total females	17		9		52		15		7		26		18	
Total number	73		53		185		69		120		133		99	
D5-14 / D20+	0.40		0.42		0.40		0.47		0.37		0.72		0.89	

parisons among the sites reveal similarities in mortality profiles specific for plague mortality, as well as some inter-site differences.

Proportion of Immature Individuals

According to the model life tables of Ledermann (18), the proportion of immature individuals in an archaic population theoretically ranges from 45% to 64%, for a life expectancy at birth of between 25 and 35 years. The values in the skeletal assemblages show some variation (Fig. 3). For three of the sites, the proportions recorded are compatible with the one expected in a context of natural mortality. For the four other sites, the values are slightly above the theoretical range. There is a small deficit of subjects who died before 20 years of age.

At first sight, the proportion of non-adults appears more important over time, from the first wave of Justinian's plague in the 6th century to the resurgences of the second pandemic in the 16th century. However, two sites from the 14th century (Hereford and Barcelona) differ from this general pattern. The differences in the proportion of immature individuals for the same time period may reflect geographical variations in the epidemiological characteristics of plague, resulting in a lower mortality rate in the youngest in Barcelona (41.7%) or a higher rate in Dreux (50.7%). However, we cannot exclude a potential bias that would have led to an underestimation of the number of immature individuals in the English and Spanish sites.

Two important biases can be mentioned. The first one concerns the Hereford cemetery. For this site, only individuals from mass graves were taken into account. Several studies have demonstrated that various burial methods were adopted during epidemics and that single graves may coexist with mass graves (6, 21). Thus, a consideration of only mass graves may yield misleading results. The second bias concerns the plague pit of Barcelona. Several of the 120 recovered skeletons consisted only of lower limb bones and were categorized as probable adults according the bone dimensions. This arbitrary choice may have resulted in an underestimation of the number of adolescents and consequently of the number of immature individuals as a whole.

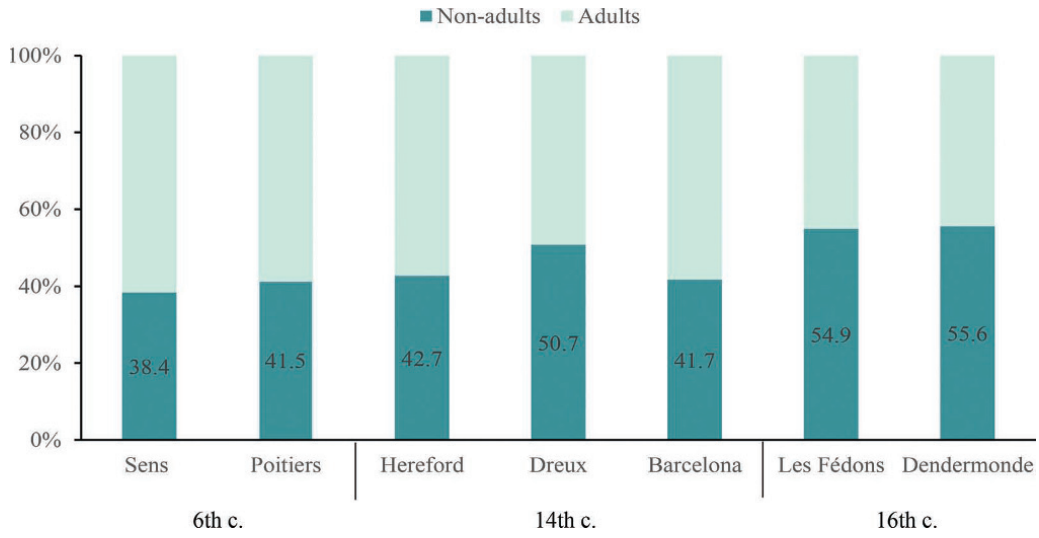
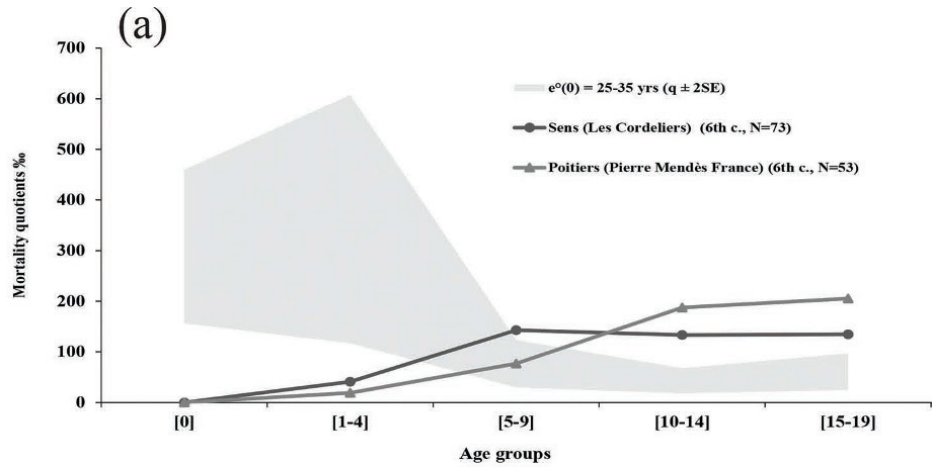
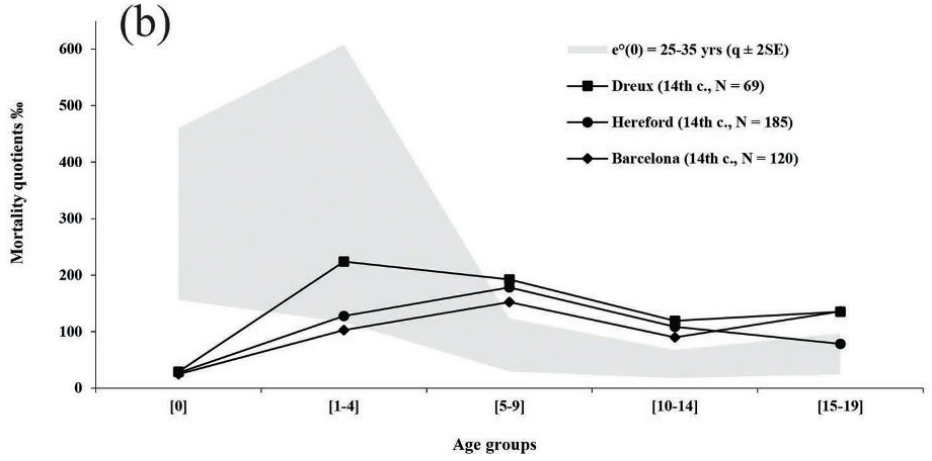
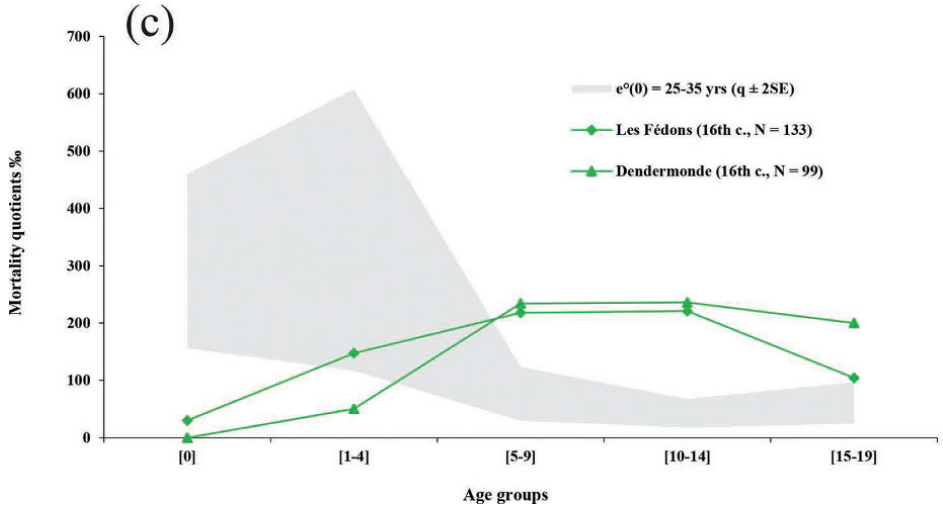


FIGURE 3 **Proportion of non-adults for each plague site.**


(a)
Mortality quotients ‰
700
600
500
400
300
200
100
0
e°(0) = 25-35 yrs (q ± 2SE)
Sens (Les Cordeliers) (6th c., N=73)
Poitiers (Pierre Mendès France) (6th c., N=53)
[0]
[1-4]
[5-9]
[10-14]
[15-19]
Age groups


(b)
Mortality quotients ‰
600
500
400
300
200
100
0
e°(0) = 25-35 yrs (q ± 2SE)
Dreux (14th c., N = 69)
Hereford (14th c., N = 185)
Barcelona (14th c., N = 120)
[0]
[1-4]
[5-9]
[10-14]
[15-19]
Age groups


(c)
Mortality quotients ‰
700
600
500
400
300
200
100
0
e°(0) = 25-35 yrs (q ± 2SE)
Les Fédons (16th c., N = 133)
Dendermonde (16th c., N = 99)
[0]
[1-4]
[5-9]
[10-14]
[15-19]
Age groups

Based on the data currently available, the lowest representation of non-adults in the most recent plague burial sites deserves to be pointed out but cannot be interpreted as clear evidence of an evolution of plague epidemiological characteristics. Further studies would be needed to reach a conclusion on this question.

Mortality Quotients by Age Groups

The mortality profiles were established by calculating the mortality quotients for all age groups. They were then compared with a theoretical mortality model (18). Regardless of their chronology, the skeletal assemblages show recurrent anomalies (Fig. 4). In each case, there is a deficit in the number of children younger than 5 years of age. The infantile (younger than 1 year) mortality quotient and the mortality quotient of the 1- to 4-year age group are low in comparison with those characterizing a natural mortality. The most significant deficit affects the first age group. Whatever the site, the mortality quotient values of children younger than 1 year of age never exceed 30‰, whereas the theoretical values range from 156.3‰ to 459.8‰. For the two sites linked to Justinian's plague (Sens and Poitiers), infants are totally missing (Fig. 4a).

The relative constancy of the infantile mortality quotient values suggests that this deficit is specific to plague mortality. We cannot ignore that a cultural bias may have contributed to this anomaly, as very young children may have been buried in places other than the plague cemeteries. However, the anomaly is so recurrent that it is more likely that it is a distinguishing demographic characteristic of plague. This particularity should be investigated further in future research.

The mortality quotient of the 1- to 4-year age group is much more variable. Its value should range between 116.9‰ and 607.6‰. It is considerably lower for the two sites contemporaneous with Justinian's plague and for the Dendermonde cemetery, whereas it is close to the lower boundary of the theoretical interval for Barcelona, Hereford, and Les Fédons and has a median value for Dreux. Except for the last site, this mortality quotient never exceeds 147.3‰.

Conversely, the mortality quotients in the 5- to 9-year, 10- to 14-year, and 15- to 19-year age groups are noticeably higher than expected. Moreover, the proportions of individuals in all of these three age groups differ from the proportion for a natural mortality, which is characterized by a minimum number of deaths in the 10- to 14-year age group. These anomalies are obvious on the mortality profiles. The over-representation of older children and adolescents is also clearly demonstrated by the high values of the juvenility index ($D_{5\text{-}14}/D_{20+}$), which systematically exceed the range of 0.1 to 0.3 expected in an archaic mortality pattern (22). The juvenility indices are notably similar for plague burial sites from the 6th and 14th centuries, with values between 0.37 and 0.47 (Table 1). This anomaly is much more pronounced for the 16th century sites, which have very high juvenility indices.

Numerous individuals between the ages of 15 and 19 years also died. Although the mortality quotients for this age group vary depending on the sites, the values obtained are systematically close to the upper boundary of the theoretical interval (Hereford, Les Fédons) or far beyond it. This systematic surplus reveals that older adolescents were not spared during plague epidemics. No differences were observed according to the chronology of the epidemics.

FIGURE 4 Distribution of ages at death for immature subjects. Comparisons with theoretical values of Ledermann (1969): Justinian's plague, 6th century (a); Black Death, 14th century (b); resurgences, 16th century (c).