

Well supplied in life, set aside in death: A multi-isotope study of Justinian plague victims from Saint-Doulchard (France, 7th-8th centuries AD)

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RESEARCH ARTICLE



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Well supplied in life, set aside in death: A multi-isotope study of Justinian plague victims from Saint-Doulchard (France, 7th-8th centuries AD)

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Abstract

Objectives: Justinian plague and its subsequent outbreaks were major events influencing Early Medieval Europe. One of the affected communities was the population of Saint-Doulchard in France, where plague victim burials were concentrated in a cemetery enclosure ditch. This study aimed to obtain more information about their life-histories using the tools of isotope analysis.

Materials and Methods: Dietary analysis using carbon and nitrogen isotopes was conducted on 97 individuals buried at Le Pressoir in Saint-Doulchard, with 36 of those originating from the enclosure ditch. This sample set includes all individuals analyzed for plague DNA in a previous study. Mobility analysis using strontium isotope analysis supplements the dietary study, with 47 analyzed humans. The results are supported by a reference sample set of 31 animal specimens for dietary analysis and 9 for mobility analysis.

Results: The dietary analysis results showed significantly different dietary behavior in individuals from the ditch burials, with better access to higher quality foods richer in animal protein. 87Sr/86Sr ratios are similar for both studied groups and indicate a shared or similar area of origin.

Discussion: The results suggest that the ditch burials contain an urban population from the nearby city of Bourges, which overall had a better diet than the rural population from Saint-Doulchard. It is implied that city's population might have been subjected to high mortality rates during the plague outbreak(s), which led to their interment in nearby rural cemeteries.

KEYWORDS

 87 Sr/ 86 Sr, Early Middle Ages, Justinian plague, δ^{13} C, δ^{15} N

Dominique Castex and Petr Velemínský have equal positions.

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1 | INTRODUCTION

The Plague of Justinian, the first historically documented plague epidemic, was an impactful event for the societies of Early Medieval Europe. Despite its historical importance, it has been the subject of only few bioarcheological studies (e.g., Castex, 2008; Helmuth & Ankner, 1996; Holleville et al., in press; Staskiewicz, 2007), none of which included isotopic methods. Due to this, many aspects of the lifestyle of the affected communities and the epidemic, such as diet, mobility and the relationship between lifestyle and mortality, are still poorly understood. By analyzing carbon, nitrogen and strontium isotopes of bone collagen and tooth enamel in a skeletal sample derived from the site of Le Pressoir in Saint-Doulchard, near Bourges, in Central France, where plague victims were previously identified (Keller et al., 2019), this study aims to contribute to a better understanding of these aspects and provides an opportunity to discuss the complex relationship between epidemic mortality and these aspects of lifestyle. To the best of our knowledge, no such isotopic study was so far undertaken in the Justinian plague context of Early Medieval Europe.

The Justinian plague, a bacterial infection caused by Yersinia pestis, struck Europe during the reign of the Eastern Roman Emperor Justinian in the 6th century AD (Retief & Cilliers, 2006; Schamiloglu, 2016). The pandemic probably originated in Egypt in 540 AD, arriving in Constantinople a year later and quickly spreading throughout Europe, reaching even rural and remote areas (Sarris, 2022). Subsequent recurrences of the epidemic, although smaller in scale, continued to plague the continent over the following centuries (Biraben & Le Goff, 1969; McCormick, 2015; Retief & Cilliers, 2006; Schamiloglu, 2016; Stathakopoulos, 2004), finally ceasing in the middle of the 8th century (Retief & Cilliers, 2006: Schamiloglu, 2016). The Justinian plague is estimated to have killed around 100 million people (Wagner et al., 2014), although some doubts about its impact exist (e.g., Mordechai et al., 2019, but see Sarris, 2022). Genetic traces of the causative bacteria Y. pestis have been reported in human remains from Early Medieval cemeteries across Europe (Feldman et al., 2016; Harbeck et al., 2013; Keller et al., 2019; Wagner et al., 2014).

Carbon and nitrogen stable isotope analysis is a well-established tool in bioarcheology, used for the dietary reconstruction of past populations; it utilizes isotope fractionation of carbon and nitrogen, resulting in their variable distribution throughout biosphere 2008; Peterson & Fry, 1987; Schwarcz & Schoeninger, 1991). For carbon, major differences arise at the base of the trophic system during carbon fixation by plants, based on their respective metabolism (Lee-Thorp, 2008; Peterson & Fry, 1987; Schwarcz & Schoeninger, 1991). Dominant in temperate European environment, C3 plants display notably lower δ^{13} C values than C4 plants (Smith & Epstein, 1971), represented mainly by millet. This distinction is further carried on to their consumers, with only a minor increase of δ^{13} C by approximately 0%–1%. Marine food sources can be detected by intermediate δ^{13} C values, as marine ecosystems derive their carbon mainly from dissolved carbonate (Katzenberg, 2008; Lee-Thorp, 2008). Differences in $\delta^{15}N$ stem primarily from nitrogen

metabolism in animals (and humans). The lighter ^{14}N isotope is preferentially excreted by organisms, and $\delta^{15}N$ therefore grows approximately 3%–5% with every trophic step (Bocherens & Drucker, 2003; Hedges & Reynard, 2007). In humans, diets with greater quantities of meat and dairy products will therefore display the highest $\delta^{15}N$ values. Longer trophic chains in aquatic ecosystems allow for detection of fish consumption as well (Katzenberg, 2008). The exact $\delta^{15}N$ and $\delta^{13}C$ value, however, can be influenced by multiple factors and cultural practices such as manuring, breastfeeding or legume consumption (Katzenberg, 2008; Reitsema, 2013).

Strontium isotopes are used for assessing mobility within populations and identifying nonlocal individuals. This method has been reviewed in great detail elsewhere (see e.g., Bentley, 2006; Montgomery, 2010). In short, as strontium-87 is created by the radioactive decay of rubidium-87, its distribution varies depending on the local bedrock, with respect to its age and initial Rb content. As rocks erode, Sr is released and substitutes for calcium in organic tissues. These tissues then reflect the ⁸⁷Sr/⁸⁶Sr ratios of strontium sources available for the local biosphere during the time of their creation. By analyzing human tooth enamel, an individual that spent their early childhood in an area isotopically different from the studied site can be identified by different ⁸⁷Sr/⁸⁶Sr.

2 | MATERIALS AND METHODS

The studied dataset originates from an Early Medieval cemetery at Le Pressoir in Saint-Doulchard (GPS: 47° 6' 12.773" N 2° 21' 31.291" E), shown on Figure 1a,b. The cemetery, shown on Figure 1c. was established at the end of the 6th century, within an area defined by an enclosure ditch; in the following centuries it developed into a densely occupied burial area. After the 8th century a decrease in burial intensity is apparent, but the cemetery remained in use until the 12th century. During the 7th-8th centuries, the cemetery enclosure ditch, after being filled in, was intersected by numerous burials, including a high proportion of double and triple graves, which are absent in the regular cemetery. Such simultaneous interments of several bodies in the same pit are indicative of the concomitant deaths of several individuals, and are therefore likely related to an event of increased mortality. Considering the dating of these burials, the absence of perimortem trauma on the skeletons, and the paleodemographic composition of the ditch skeletal sample, which shows similarities to those of proven plague cemeteries (a reduced number of individuals under 1 year old and abnormally high number of children between 5 and 14 years old, older adolescents and young adults; Castex, 2008; Castex & Kacki, 2016), it was hypothesized that the ditch burials were linked to the Justinian plague. This hypothesis was later confirmed by DNA analysis which revealed the presence of Y. pestis DNA in 11 out of 25 tested ditch burials (Keller et al., 2019).

The reference sample set for dietary analysis consists of 31 bone samples of local archeological fauna, contemporary to the human burials, and includes 10 *Bos taurus* specimens, 11 *Sus scrofa domestica*

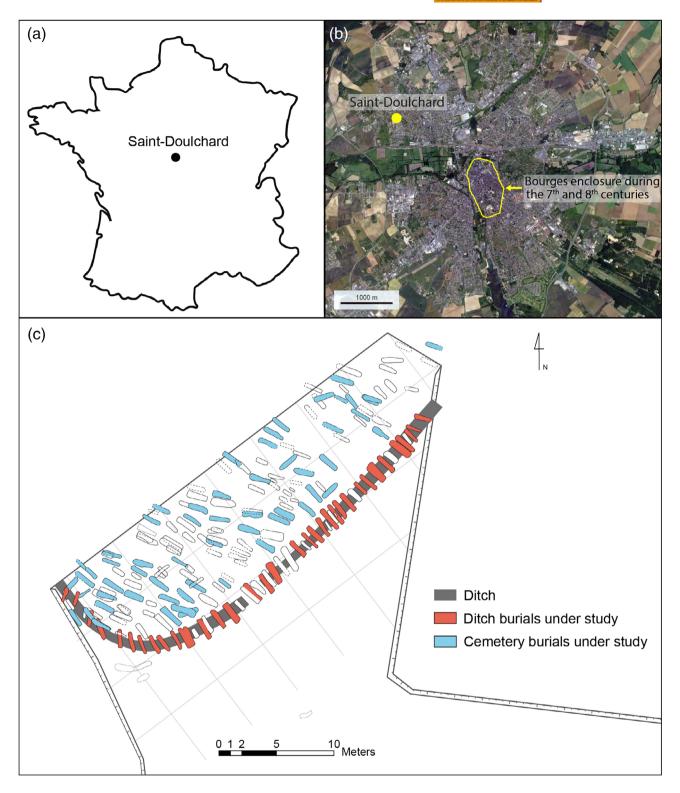


FIGURE 1 Location of the Saint-Doulchard burial site on the French territory (a) and with respect to Early Medieval Bourges (b), and map of the burial ground showing the ditch and cemetery burials included in the isotope analysis (c).

and 10 *Caprinae* individuals, 3 of those being assessed as *Ovis aries*. The reference sample set used for mobility analysis comprises tooth enamel from nine archeological specimens of *Sus scrofa domestica*, excavated in the close vicinity of Bourges, at Saint-Doulchard, Séracourt, Le Grandes Varennes, Vouzay, and L'Angoulaire.

The human dataset for dietary analysis contains 97 individuals from the 7th to 8th centuries AD. In total, 36 ditch burials were analyzed, consisting of 18 males, 9 females, and 7 unsexed individuals, all over 15 years of age, with two additional subadults aged <15 years. All 25 skeletons that underwent the DNA study were included. The

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regular cemetery is represented by 61 adults (31 males, 30 females). Sex and age at death estimations were preformed using methods described in Owings Webb and Suchey (1985), Brůžek (2002), Schmitt (2005, 2008), Brůžek et al. (2017), Coqueugniot et al. (2010), and Moorrees et al. (1963). Individuals under study have been classified into five age groups: Infans II (7-14 years), Juvenis (15-19 years), Adultus (20-34 years), Maturus (35-49 years), and Senilis (over 50 years). Sr analysis was performed on 47 human individuals, using tooth enamel from the first or second molars. The sample set consists of 24 ditch burials including 10 plague DNA positive cases and 23 burials from the regular cemetery.

For dietary analysis, approximately 1 g of compact bone was sampled from each of the studied individuals, preferentially from a rib; where unavailable, long bone fragments were sampled instead. Ribs were selected for sampling to minimize the destructive impact of the analysis on the skeletons, as well as due to their faster collagen turnover (Fahy et al., 2017), which means that their isotopes average the diet over a shorter time frame prior to the individual's death. In order to remove possible contaminants, the surfaces of the collected bones were mechanically abraded, and the sample was ultrasonicated in deionized water for at least 25 min. After drying, samples were manually crushed into fragments not exceeding 0.7 mm. Approximately 250 mg of those fragments were used for collagen extraction, which followed the standard protocol by Longin (1971), as modified by Bocherens (1992). The samples were consecutively demineralized in 1 M HCl for 20 min, filtered, immersed in 0.125 M NaOH for 20 h, filtered again and denatured at 100°C in 0.01 M HCl for 17 h. After final filtration, the extracted gelatin fraction was freeze-dried. Isotope ratios of the freeze-dried samples were measured by EA-IR mass spectrometry at Iso Analytical Ltd., Crewe, UK, The VPDB and AIR scales using IAEA-CH-6 and IAEA-N-1 inter-laboratory comparison standards were used to calibrate the stable carbon and nitrogen isotopic compositions. The routine analytical error for %C and %N was 2% in RSD (relative standard deviation) terms and the measurement uncertainty was less than 0.1% (1SD) for carbon and 0.2% for nitrogen.

The enamel samples (first molar, or second molar when M1 was unavailable) for Sr analysis were prepared as follows: the teeth crown surfaces were mechanically abraded to remove surface contaminants. Afterwards, teeth were ultrasonicated for 30 min in deionized water, followed by 30 min ultrasonication in 1 M acetic acid, followed by another ultrasonication for 10 min in deionized water. Bulk enamel samples were collected by drilling into the tooth crown. The released powder was collected in sealed microtubes. Sr separation was performed in a clean laboratory at the Geological Department of the Czech Academy of Sciences, using the modified protocol by Pin et al. (2014). To summarize, samples were dissolved in concentrated HNO₃ at 70°C for approximately 18 h; the solution was then dried down and re-dissolved in 1 mL of 1 M HNO₃. Strontium was isolated using a Srspecific resin (Triskem) and the ⁸⁷Sr/⁸⁶Sr ratios were measured with a Thermo Triton Plus thermal ionization mass spectrometer (TIMS), using W filaments with the presence of a Ta activator. An ⁸⁸Sr/⁸⁶Sr of 8.3752 was used for mass fractionation correction. NIST SRM 987 yielded an 87 Sr/ 86 Sr of 0.710251 ± 0.000014 (2SD, n = 39) during the course of this study.

RESULTS

All collagen samples proved to be sufficiently well preserved, with an average collagen weight percentage of 7.77% ± 4.48% (±1SD; 4.81% ± 2.53% and 8.71% ± 4.57%, for animal and human samples, respectively), fulfilling the collagen quality criteria (>1%) as defined by Ambrose (1990) and van Klinken (1999). Measured carbon and nitrogen content averaged 39.6% ± 2.6% and 14.7% ± 1.1% (1SD), with an average C:N ratio of 3.15 ± 0.05 (fauna: 3.21 ± 0.07 ; humans: 3.13± 0.03), ranging from 3.05 to 3.38.

Faunal samples averaged $-21.3\% \pm 0.4\%$ in δ^{13} C and 7.4% \pm 1.3% in δ^{15} N. For Bos taurus samples, the carbon averaged $-21.5\% \pm 0.4\%$ and nitrogen 7.8% $\pm 1.1\%$. In Caprinae the values were similar, with δ^{13} C of $-21.2\% \pm 0.3\%$ and δ^{15} N of 7.0% \pm 0.9%. Sus scrofa specimens showed values of $-21.1\% \pm 0.5\%$ and 7.5% \pm 1.6% in δ^{13} C and δ^{15} N, respectively. No differences were noted between the diets of all the analyzed animal species (ANOVA, δ^{13} C: p = 0.075; δ^{15} N: p = 0.401).

Measured human isotope values are shown on Figure 2. δ^{13} C values averaged $-19.8\% \pm 0.2\%$, ranging from -20.3% to -19.3%. δ^{15} N reached values from 8.8% to 12.6%, on average 11.0% \pm 0.8%. Inhumations in the regular cemetery reached -19.9% \pm 0.2% on average (1SD) for δ^{13} C and 10.8% \pm 0.8% for δ^{15} N. Ditch burials had δ^{13} C values between -20.3 and -19.3%, with an average of $-19.8\% \pm 0.3\%$ (1SD). Nitrogen ranged from 10.0% to 12.6%, averaging 11.4% ± 0.6% (1SD). In the 25 individuals analyzed for plague DNA, no differences were found either in carbon or nitrogen values between those tested positive and negative (Welch t-test, p = 0.7232 for carbon, p = 0.6936 for nitrogen). Similarly, plague DNA positive individuals showed no difference in either carbon (Welch t-test, p = 0.6519) or nitrogen (Welch t-test, p = 0.8345) isotope values when compared to all other ditch burials. Significant differences were, however, present between the ditch inhumations and the regular cemetery in both $\delta^{15}\mbox{N}$ (Wilcoxon rank sum test, p < 0.001) and δ^{13} C (Welch t-test, p = 0.04177), as shown on Figures 3 and 4. Spacing between the average isotopic values of humans and herbivorous fauna (Bovids and Caprinae) reached 1.5 $(\delta^{13}C)$ and 3.6 $(\delta^{15}N)$ %. When the ditch and the regular cemetery are compared separately, the human-fauna spacing differs as well $(\delta^{13}C: 1.6\% \text{ vs. } 1.5\%; \delta^{15}N: 4.0\% \text{ vs. } 3.4\%).$

A significant difference in nitrogen values between males and females was present in the dataset (Wilcoxon rank sum test, p = 0.00483), but the significance was lost when ditch and cemetery were tested separately. This is most likely a statistical artifact, introduced by males constituting twice as many sexed individuals in the ditch than females (18 vs. 9), combined with an overall higher $\delta^{15}N$ in the ditch burials. For carbon significant age-based differences were present between the ditch and the regular cemetery (Kruskal-Wallis, p = 0.02082). This, however, is likely a statistical artifact as well, as

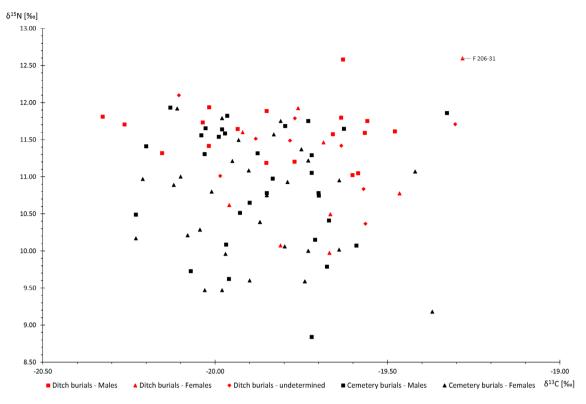
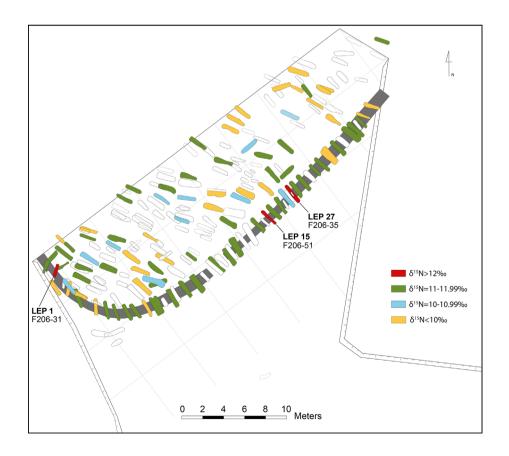
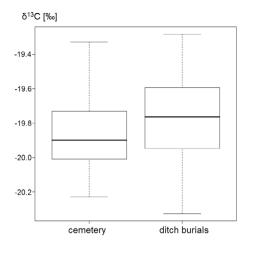


FIGURE 2 Measured human δ^{13} C and δ^{15} N values from Le Pressoir in Saint-Doulchard (France). Individual from grave F206-31, the carrier of a distinct plague strain, is shown.

FIGURE 3 Schematic plan of the cemetery at Saint-Doulchard with measured $\delta^{15}N$ values.





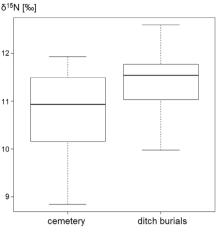


FIGURE 4 Boxplot comparison of δ^{13} C and δ^{15} N values of the regular and ditch burials at Le Pressoir in Saint-Doulchard (France).

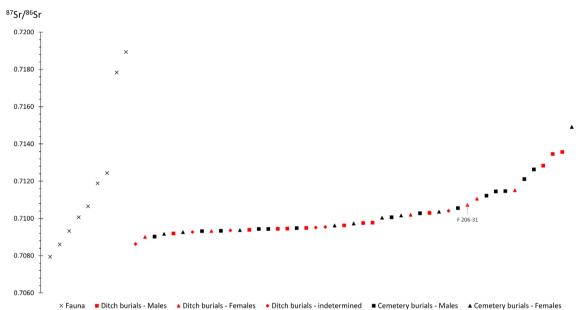


FIGURE 5 Measured ⁸⁷Sr/⁸⁶Sr ratios from Le Pressoir in Saint-Doulchard (Bourges, France). Grave F206-31, carrier of a distinct plague DNA strain and a dietary outlier, is shown.

the Ditch Adultus category displays higher $\delta^{13}C$ values than the rest of the dataset and consists of only three individuals, including the dietary outlier F206-31 (LEP 1). No other statistically significant relationship between isotopic values and age at death or sex was noted in the dataset.

Measured strontium isotope ratios are shown on Figure 5. Pig $^{87}{\rm Sr/^{86}Sr}$ ratios proved to be rather variable, ranging from 0.70795 to 0.71893 with an average of 0.71197 \pm 0.0037. Human $^{87}{\rm Sr/^{86}Sr}$ ratios ranged from 0.70863 to 0.71491 with an average of 0.71028 \pm 0.00136. No statistically significant differences in $^{87}{\rm Sr/^{86}Sr}$ were noted between ditch and cemetery burials (Wilcoxon test, p=0.7438), indicating that both groups originated from a similar isotopic background.

Two strontium reference samples (87Sr/86Sr of 0.71893 and 0.71784) are clear outliers and most likely originated from a different area, as animals can be subjected to various forms of mobility.

Regrettably, it was not possible to collect and analyze more environmental samples from the studied site. If the local biostrontium is established using the remaining seven reference samples, the resulting range is 0.7070-0.7132. This is notably wider than expected for the prevailing geological conditions: The local bedrock is made up of a mixture comprising mainly marls and, in lesser quantities, limestone, while the wider area surrounding Saint-Doulchard (ca. 15 km) is rather uniform, dominated by limestones and other carbonate sedimentary rocks with expected ⁸⁷Sr/⁸⁶Sr ratios of 0.7072-0.7115 (EDGI, 2022; Willmes et al., 2018). According to an isoscape model based on modern plant and soil samples, the expected 87Sr/86Sr should lie roughly between 0.7111 and 0.7139 (Willmes et al., 2018). Nonetheless, the analyzed area was not sampled in the isoscape study, and is based only on statistical modeling, which carries a risk of potential prediction error (Willmes et al., 2018). The use of human enamel data trimmed for outliers for local range estimation has been suggested for sites

with unavailable reference material (Bentley et al., 2004; Scaffidi & Knudson, 2020). If outliers with the highest ⁸⁷Sr/⁸⁶Sr ratios (LEP 3, 4, 20, 62, 63, 68) are excluded at Saint-Doulchard, the "local" ⁸⁷Sr/⁸⁶Sr range based on human enamel data would result in 0.7084-0.7113. This range fits within the expected ratios of the underlying geology but contradicts the isoscape prediction. Only the aforementioned outliers and samples LEP 26, 54, and 65 fall outside this range, consisting of an equal number of ditch and cemetery burials (4 and 5, respectively). This group consist of six males in the senilis age category, one maturus male (LEP 3) and two females (LEP 26, senilis; LEP 68, maturus). It can be argued that these nine individuals originated from an area with a different isotopic background; due to the discrepancies in the local range estimations, however, definite conclusions on the local/nonlocal origin of the analyzed humans should be avoided with the current data.

4 | DISCUSSION

Animal diet at Saint-Doulchard was based predominantly on C3 plants and did not differ significantly between analyzed species. Although the isotope results from the Saint-Doulchard fauna appear analogous to the limited dataset from Merovingian Norroy-le-Veneur in the Metz region (Vytlačil et al., 2018), δ¹⁵N values are generally higher than reported at Late Roman Age Îlot de la Boucherie in northern France (Mion et al., 2016) and Early Medieval Missignac-Saint Gilles le Vieux in south-eastern France (Mion et al., 2019). The elevated $\delta^{15}N$ could reflect general ecological variability (Goude & Fontugne, 2016) or result from the consumption of manured fodder at Saint-Doulchard, as manuring has been shown to increase the $\delta^{15}N$ of plants (Bogaard et al., 2007; Fraser et al., 2011). The similarity of the measured δ^{13} C and δ^{15} N values of Suids and other herbivorous species at Saint-Doulchard suggests that omnivorous pigs were fed a plant-based diet rather than human refuse, and could have been pastured in e.g. forests, as was probably the case in other Early Medieval contexts in France and elsewhere (Mion et al., 2016; Mion et al., 2019; Vytlačil et al., 2018).

Like the animals, the diet of the humans buried at Saint-Doulchard was based mainly on C3 plants with little to no significant input of C4 plants such as millet. Animal protein consumption in the form of meat or dairy was relatively common in general, as the human-herbivore spacing of average $\delta^{15}N$ is within a commonly referred trophic step increase (3%-5%, Bocherens & Drucker, 2003; Hedges & Reynard, 2007). The range of human $\delta^{15}N$ values, 3.8%, is, however, rather wide, which signifies substantially different access to animal protein food sources within the population. The ditch burials in particular display significantly higher $\delta^{15} N$ values and greater humanherbivore spacing (4.0%). When compared to other contemporary sites in today's France (Mion et al., 2016, 2019; Vytlačil et al., 2018), nitrogen ratios and human-herbivore spacing appear elevated at Saint-Doulchard in general, which could be attributable to an increased consumption of animal protein and possibly manured grains. Freshwater fish probably did not comprise a major segment of the

at Saint-Doulchard is of special interest. The isotopic results are consistent with a more diverse diet, with an increased animal protein intake, than the diet detected in the regular cemetery. Manuring was unlikely to have been the cause of the elevated $\delta^{15}N$ in the ditch burials, as in order to cause this distinction it would require two funthe population, which is improbable in Early Medieval France. The age-related dietary differences are a similarly unlikely cause due to the lack of significant age-based differences in the isotopic values, and the ditch $\delta^{15}N$ values being consistently elevated across all age categories. Increased $\delta^{15}N$ has often been linked with a prominent status, or a generally higher social standing in a stratified society (e.g., Błaszczyk et al., 2021; Czermak et al., 2006; Kaupová et al., 2019; Knipper et al., 2015; Yoder, 2012). For Early Medieval Gaul, however, no dietary distinction of elites has been proven in nitrogen values (Vytlačil et al., 2018), although dietary data availability for Early Medieval France is still severely limited. Furthermore, the ditch burials at Saint-Doulchard itself do not display any distinctive elements allowing the social level of their occupants to be qualified. Moreover, the ditch's primary role was demarcation of the funerary space, and the presence of inhumed bodies within it is therefore rather unusual. A clear connection between the $\delta^{15}N$ and prominent status in the ditch burials therefore cannot be made. Further, the ditch burials were carried out in an epidemic or emergency context linked to plague (Keller et al., 2019), and they should contain its (direct or indirect) victims. Thus, a temporal change in the dietary structure is also an improbable cause for the higher $\delta^{15}N$ in the ditch burials. Although a more refined chronological separation to analyze dietary shifts is not possible for the cemetery burials, the ditch burials likely occurred within a short period of time. The plague did not lead to the abandonment of the cemetery and as it cannot be precisely pinpointed in time, the dataset likely contains cemetery burials that occurred after the outbreak. A dietary change would, therefore, need to happen shortly prior to, and possibly be linked to, the plague in some way.

There are several possible explanations for the increased $\delta^{15}N$ values in a plague context. The direct influence of a rapidly progressing plague infection on bone isotope values is highly unlikely. Due to the bone turnover rate, isotope values in bulk collagen are representative of a long-term average of over a decade (Hedges et al., 2007), and even long-lasting systemic diseases like leprosy have not been proven to influence isotope values (Linderholm & Kjellström, 2011). Other indirect characteristics of plague mortality, such as for example malnutrition (DeWitte & Wood, 2008), are unlikely to be the reason behind the higher $\delta^{15}N$ in the ditch burials as well. Starvation is

The dietary behavior of the ditch individuals more likely reflects different food preferences or availability for a population subgroup on a perhaps smaller, local, or regional scale. Urban populations have been shown to display distinctive diet in medieval contexts with higher δ¹⁵N values compared to rural areas (Aguraiuja-Lätti & Lõugas, 2019; Kaupová et al., 2018; Pérez-Ramallo et al., 2022; Schats et al., 2022). As the studied site is in very close vicinity to the city of Bourges, the different dietary structure of the ditch burials could reflect an urban diet with higher availability of meat or dairy, possibly through greater food choice via markets or a higher socio-economic status of the townsfolk. Additionally, if the ditch individuals were residents of Bourges, they would be inhabiting a practically identical territory to the population from Saint-Doulchard, and would therefore display indistinguishable ⁸⁷Sr/⁸⁶Sr ratios, as can be seen in the presented results. This explanation fits from the archeological perspective as well: plague victim burials generally tried to follow the standard rite, but when the casualties of a raging plague epidemic exceeded funerary capacities, the affected populations had to result to improvised funerary measures such as burials in mass graves or nonfunerary structures, for example cisterns, orchards, or ditches, following no general rule (Castex & Kacki, 2022; Gutsmiedl-Schümann et al., 2018; Kacki et al., 2022; McCormick, 2015). As Gregory of Tours lists Bourges among the cities depopulated by plague, similar fate might have befallen the city: plague could have led to an overcrowding of available cemetery spaces, and the dead might have been transported to neighboring sub-urban or rural cemeteries such as Le Pressoir at Saint-Doulchard. There they would, due to the already excessive number of deaths and lack of available space, be buried in the cemetery ditch. Although in Bourges itself no disaster burials from this period have been found so far, this indirectly implies that the impact of Justinian plague on the city might have been greater than suggested from available data. Stable carbon and nitrogen isotope analysis of the bone collagen of

indeed an often-documented aftereffect of the Justinian plague outbreaks (Retief & Cilliers, 2006). Acute starvation can increase $\delta^{15}N$ values through the catabolic effect, when the body starts to catabolize its own tissues (Reitsema, 2013). Such an increase in $\delta^{15}N$ is often accompanied by decreased values of $\delta^{13}C$ (Beaumont & Montgomery, 2016; Neuberger et al., 2013), which however were not seen in the ditch burials. Furthermore, it is questionable whether an individual could survive with such a protein-insufficient diet over the several years needed for it to influence bulk collagen (Beaumont et al., 2013; Reitsema, 2013; Walter et al., 2020); even in identified famine burials, catabolically elevated $\delta^{15}N$ values have not been reported in the bulk bone collagen (Beaumont et al., 2013; Walter et al., 2020). If extreme long-term protein deficiency is not considered for the isotope results at Saint-Doulchard, the high $\delta^{15}N$ values are contra-indicative of sub-optimal nutrition, suggesting rather a diet bountiful in animal protein and arguably better than that in other contemporary populations in France. Increased consumption of animal products could have occurred when plague survivors had to supplement insufficient crop production, disrupted due to the lack of an available workforce-but the availability of domesticated animals might be expected to be influenced by this as well. Moreover, the high prevalence of Y. pestis DNA within the ditch indicates that these burials contain predominantly the direct victims of the plague, rather than victims of the subsequent famines, even though other causes of death cannot be ruled out in some individuals.

An explanation for the elevated $\delta^{15}N$ might be found in a case where the group buried in the ditch at Saint-Doulchard does not directly belong to the local rural community. This hypothesis may be supported by Gregory of Tours (1963), who mentions individuals fleeing their home regions during plagues. Additionally, Saint-Doulchard takes its name from Dulcardus, a 6th century saint who founded a hermitage on the site of the current town church, and to whom miraculous healing powers were attributed. However, the church does not appear before the 7th century, and the reception of the sick is mentioned rather allusively in reference to the miracles that Dulcardus is said to have accomplished (Head, 1990; Jarossay, 1902). Moreover, there is no archeological evidence so far to support this legend or to suggest any cultural differences of the ditch burials influencing the diet. While personal preferences in food choices cannot be ruled out, a notable difference in ecological baselines that could affect $\delta^{15}N$ values in the case of different areas of origin for the ditch individuals would be expected over substantial geographical distances, which is not supported by the ⁸⁷Sr/⁸⁶Sr data. Although mobility cannot be excluded in some individuals, both groups, as well as potential nonlocals, originated from a similar isotopic background. It is noteworthy that the young adult female from grave F206-31 (LEP 1) displays the highest δ^{13} C and δ^{15} N values in the whole dataset, and is also the only carrier of one of the two distinct strains of Y. pestis detected at Saint-Doulchard (Keller et al., 2019). Such outlying isotopic values could be explained by the different origin of such an individual (e.g., Hakenbeck et al., 2010), who could have been infected by plague elsewhere and then transported a distinct strain of the bacterium with her to Saint-Doulchard. However, F206-31 does not represent an

outlier in the Sr data and fits well within the rest of the dataset, making this interpretation tentative.

CONCLUSION

the Justinian plague victims buried in a cemetery ditch at Saint-Doulchard in France identified a diet different from the inhumations in the regular cemetery, with an overall higher intake of animal protein, reflected by higher δ¹⁵N values. The generally similar ⁸⁷Sr/⁸⁶Sr values of the tooth enamel in both groups suggest isotopically similar areas of origin. While other explanations were explored, the results suggest that the individuals buried in the cemetery ditch might be inhabitants from the nearby city of Bourges, as diet in urban environments usually differs from that in rural areas, and the increased presence of animal derived foods in the diet of urban populations has been documented in isotope studies of other contexts. If this was the case, the results imply that the impact of Justinian plague on the city might have been greater than suggested from the available data. It is possible that mortality during a plague outbreak exceeded the

funerary capacities of the town cemeteries and the plague victims had to be interred in the nearby rural areas. As a direction for further studies, a comparison with isotopic data originating directly from Bourges would be helpful in examining this issue, as would a more general comparison of diets in urban and rural areas of Early Medieval France.

AUTHOR CONTRIBUTIONS

Zdeněk Vytlačil: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); visualization (equal); writing - original draft (lead). Raphaël Durand: Conceptualization (equal); data curation (equal); investigation (equal); resources (equal); supervision (equal); validation (equal); visualization (equal); writing - review and editing (equal). Sacha Kacki: Conceptualization (equal); data curation (equal); formal analysis (equal); supervision (equal); visualization (equal); writing - review and editing (equal). Marion Holleville: Formal analysis (supporting); investigation (supporting); writing - review and editing (supporting). Sylva Drtikolová Kaupová: Investigation (supporting); methodology (supporting); supervision (equal); validation (equal); writing - review and editing (equal). Jaroslav Brůžek: Conceptualization (supporting); formal analysis (supporting); resources (supporting); supervision (equal); writing - review and editing (equal). Dominique Castex: Conceptualization (equal); funding acquisition (lead); project administration (lead); resources (equal); supervision (equal); writing - review and editing (equal). Petr Velemínský: Conceptualization (equal); data curation (equal); funding acquisition (lead); project administration (equal); resources (supporting); supervision (equal); validation (equal); writing - review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information of this article.

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