



Dental caries at Lapa do Santo, central-eastern Brazil: An Early Holocene archaeological site

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ABSTRACT

The origin and dispersion of the first Americans have been extensively investigated from morphological and genetic perspectives, but few studies have focused on their health and lifestyle. The archaeological site of Lapa do Santo, central-eastern Brazil, has exceptionally preserved Early Holocene human skeletons, providing 19 individuals with 327 permanent and 122 deciduous teeth dated to 9,250 to 7,500 years BP. In this study, we test whether the inhabitants of Lapa do Santo had high prevalence of dental caries as previous studies of Lagoa Santa collection have indicated, using individual and tooth as units of analyses. The results show a high prevalence of dental caries in the permanent dentition (5.50%, n=327 teeth; 69.23%, n=13 individuals) compared to other samples of hunter-gatherers worldwide. In addition, dental caries in deciduous teeth start occurring as early as 3 to 4 years old, suggesting an early start to caries. Compared with other samples from Lagoa Santa, Lapa do Santo shows statistically similar prevalence of overall caries but different caries location pattern. We believe that a subsistence adaptation to a tropical environment rich in sources of carbohydrates, such as fruits, is the best explanation for the overall caries prevalence.

Key words: bioarchaeology, Brazilian prehistory, dental pathologies, Lagoa Santa, oral health, paleoamericans.

INTRODUCTION

Hundreds of human remains from Early Holocene have been found in the rock shelters of the Lagoa Santa region, since the first excavations of Peter Lund in the 1830s and 1840s (Araujo et al. 2012, Holten and Sterll 2011). Many studies have used the Lagoa Santa assemblage to reconstruct

population history of these first inhabitants of the Western Hemisphere (e.g., Neves and Hubbe 2005). In contrast, few studies have focused on aspects of health and behavior of these inhabitants (e.g., Da-Gloria and Larsen 2014). Unfortunately, the majority of the Lagoa Santa human collection is not associated with an archaeological context, limiting chronological and contextual inferences. In this study, we report dental caries prevalence of a well-excavated and extensively dated

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archaeological site in the region, named Lapa do Santo (Figure 1). Several reasons qualify this sample as unique in local and continental contexts. Firstly, differing from other sites in the region, the Lapa do Santo rock shelter was excavated using modern archaeological techniques, such as the use of a total station, providing a detailed spatial record of the archaeological remains. Secondly, this site was extensively dated using both indirect (52 dates on charcoal) and direct radiocarbon dates (13 dates on bone and tooth). Thirdly, the careful exhumation and curation of human skeletons at Lapa do Santo resulted in a high number of teeth per mouth (25 teeth per individual compared to just 10 teeth for the other sites of Lagoa Santa), allowing a more complete understanding of the oral health of each individual.

Here, we investigate the occurrence of dental caries at Lapa do Santo. Cavities are the result of demineralization of enamel, dentine, and/or cement caused by organic acids formed during the fermentation of carbohydrates by plaque bacteria. Cavities are the late stage of a process that starts underneath the surface and progressively causes the formation of a visible lesion (Hillson 2001). Sugar in the diet has the strongest association with the caries process (Larsen 2015). Worldwide surveys of the bioarchaeological literature have shown that hunter-gatherers have better oral health than agriculturalists (Cohen and Crane-Kramer 2007, Larsen 2015, Turner 1979), but the foragers of Lagoa Santa seem to be an exception among hunter-gatherers. Da-Gloria and Larsen (2014) showed poor oral health at Lagoa Santa sites compared to other hunter-gatherers from the Americas. Thus, we expect that the Lapa do Santo assemblage to show high prevalence of caries compared to other hunter-gatherer samples.

LAPA DO SANTO SITE

Lapa do Santo site is within the archaeological region of Lagoa Santa, state of Minas Gerais,

which is located in eastern-central Brazil, some 400 km from the Atlantic coast (Figure 1). The region is dominated by karst, which includes hundreds of rock shelters in limestone outcrops (Neves and Piló 2008). The current climate of the region is tropical, characterized by high humidity, marked seasonality, an average precipitation of 1,286.5 mm and a mean annual temperature of 21.4 °C (Patrus 1998). The main biome of the region is the Brazilian savannah (*cerrado*), dotted with patches of deciduous and semi-deciduous forests (IBGE 1992), which were present throughout the Holocene (G.Q. Freire, unpublished data). A more humid and stable climate was detected in the Early Holocene through pollen (Raczka et al. 2013) and geoarchaeological data (Araujo et al. 2013).

The Lapa do Santo site (UTM 23K 600926 E – 7845965 N; datum SIRGAS 2000) consists of a sheltered area of 1,300 m² with its mouth opening towards the west and a steep slope to the north. From 2002 to 2012, systematic archaeological interventions excavated an area of 50 m². The faunal assemblage contains deer (*Mazama* sp.), armadillos (e.g., *Dasypus novemcinctus*), pacas (*Cuniculus paca*), peccaries (*Tayassu* sp.), cavies (*Cavia* sp.), Brazilian rabbits (*Silvylagus brasiliensis*), reptiles (e.g., *Ameiva ameiva*), among other species (C.P. Perez, unpublished data). In terms of botanical remains, the archaeological sites of Lagoa Santa are rich in the fruits araticum (*Annona classiflora*), pequi (*Caryocar brasiliense*), jatobá (*Hymenaea* sp.), and palm nut (*Syagrus* sp.) (Nakamura et al. 2010). The variability of plants and of medium to small-sized faunal remains suggest that early Holocene inhabitants consumed a generalized diet at Lapa do Santo. Concerning chemical analyses, T. Hermenegildo (unpublished data) undertook an isotopic analysis using Early Holocene human remains recovered from the site of Lapa do Santo. His results found relatively low levels of $\delta^{15}\text{N}$, suggesting a diet based on plants and primary consumers such as deer and cavies.

TABLE I
Sex, age-at-death, number of carious teeth, and caries prevalence per skeleton at Lapa do Santo.

Skeletons	Sex ^a	Age	Mean age	Teeth	Carious teeth	%
St-SepI ^b	1	35-50	42.5	26	1	3.85
St-SepII ^b	2	20-25	22.5	15	1	6.67
St-SepIII ^b	1	25-35	30	25	0	0.00
St-SepIV ^c	-	6	6	13	0	0.00
St-SepV ^b	2	45-60	52.5	10	1	10.00
St-SepVI ^c	-	4	4	16	0	0.00
St-SepVII ^c	-	4	4	15	1	6.67
St-SepX ^b	2	>18	35	15	5	33.33
St-SepXI ^b	1	20-25	22.5	17	4	23.53
St-SepXV ^b	1	35-45	40	29	1	3.45
St-SepXVI ^b	2	18-21	19.5	31	0	0.00
St-SepXVIIa ^b	1	20-30	25	30	1	3.33
St-SepXIX ^c	-	3	3	18	0	0.00
St-SepXX ^c	-	4	4	18	16	88.89
St-SepXXI ^b	1	40-50	45	28	0	0.00
St-SepXXII ^b	1	30-40	35	26	0	0.00
St-SepXXIV ^b	-	25-40	32.5	22	2	9.09
St-SepXXVI ^b	1	30-40	35	32	2	6.25
St-SepXXVII ^c	-	4-5	4.5	19	0	0.00
Loose Teeth ^b	-	-	-	21	0	0.00
Loose Teeth ^c	-	-	-	23	1	4.35

^a 1 = male; 2 = female; ^b permanent teeth; ^c deciduous teeth.

BP. The first one, burial II, provided two dates: 790 ± 40 years BP (BETA 253497) and $8,331 \pm 44$ (S-EVA 24491). The second date is consistent with the site stratigraphy and was obtained from a rib bone fragment securely associated with the burial (Strauss 2016). The second one, burial V, provided a minimum date of $5,990 \pm 40$ years BP (BETA 215195). This age was obtained from a sample containing low amount of collagen, which necessitated dispensing of a pre-treatment based on a full series of alkali extractions carried out by BETA Analytic Laboratory (BETA), increasing the possibility of sample contamination. Its stratigraphic position suggests that the actual date of burial V is older than its reported age. In short, the Lapa do Santo assemblage is considered here as belonging to the Early Holocene period, probably between 9,250 to 7,500 years BP. Dating of the Lapa do Santo skeletons do not allow chronological

divisions within this interval. Thus, we treat the Lapa do Santo sample as a single sample. In fact, there are no archaeological and biological elements supporting biocultural divisions of this period. The Lapa do Santo sample consists of the largest Paleoamerican sample for a single site in the Americas with archaeological context and well-preserved human skeletons.

Cariou lesions were scored when a cavity was observed through visual inspection with no optical aids and no use of probe. We recorded caries position and pulp exposure by caries in order to better characterize the carious lesions. Position of the lesions was accessed through five categories: gross, occlusal, interproximal, root/cement enamel junction (CEJ), and buccal/labial/lingual. Gross carious lesions are large cavities occurring in more than one position with no identifiable point of initiation (Hillson 2001). In this article,

the prevalence of each category of carious lesion position was calculated in relation to the total number of carious lesions. A caries correction factor was calculated using the prevalence of posterior and anterior dentition in order to correct by differential tooth loss (Erdal and Duyar 1999). A second caries correction factor was calculated using the proportion of teeth with pulp exposure by caries and number of teeth lost antemortem (AMTL) for each sex (Lukacs 1995). AMTL was scored in cases in which the alveolus was resorbed at the extent of becoming evident the tooth loss. Sex estimation of adult Lagoa Santa skeletons was based on traits of the cranium and pelvis (Buikstra and Ubelaker 1994). Subadult age estimation relied on dental development (Ubelaker 1989) and epiphyseal closure (Buikstra and Ubelaker 1994), while adult age estimation was based on cranial suture closure (Meindl and Lovejoy 1985), pubic symphysis morphology (Brooks and Suchey 1990), and auricular surface morphology (Buckberry and Chamberlain 2002). The integration of these methods was carried out by one of us (PD) depending on the preservation of each skeleton. The age used for the statistics was the mean age of the interval estimated (see Table I).

Two methods were used in this investigation: tooth/alveolus and individual counts (Lukacs 1989). In the first method, the prevalence of caries was computed by dividing the total number of teeth/alveoli affected by the total number of teeth/alveoli. In the second method, the number of individuals with at least one observable tooth/alveolus affected was divided by the total sample of individuals. The statistical method used to compare caries prevalence was the chi-square test of heterogeneity in 2 x 2 tables, while t-tests were applied to age-at-death comparisons. Levene's test was applied for testing equality of variances in t-tests (McKillup 2005). Alpha was set at 0.05, and the statistical tests were run in SPSS 18.0.

In order to have a regional view of the caries prevalence at Lagoa Santa, we compared the caries

prevalence of Lapa do Santo and an assemblage of 16 other Early Holocene archaeological sites from the same region. The Lagoa Santa sample used in this comparison was analyzed by one of us (PD) in 2009 and 2010 from public institutions in Brazil (see Da-Gloria and Larsen 2014 for details), while the Lapa do Santo sample was analyzed by the same researcher (PD) in 2014. This comparison aimed to create a contrast between the Lapa do Santo sample and a less contextualized Lagoa Santa sample.

RESULTS

The prevalence of dental caries using teeth as the units of analysis is 5.50% (18/327) for permanent teeth (Table II). When individuals are the unit of analysis, Lagoa Santa inhabitants show 69.23% (9/13) of affected individuals with permanent dentition, and 33.33% (2/6) of affected individuals with deciduous dentition. The number of permanent carious teeth per individual ranges from 0 to 5, and their relative caries prevalence range from 0% to 33.33% (Table I). Females (9.86%, n=77) show statistically similar prevalence of caries as males (4.23%, n=213) when teeth are the units of analysis ($\chi^2=3.179$; $p=0.07$). The lack of significance is also observed when individuals are the unit of analysis. When the prevalence of caries is corrected by differential preservation of posterior and anterior

TABLE II
Prevalence of dental caries by tooth type using permanent teeth as the unit of analysis.

Tooth Type	Caries		
	Teeth	Affected	%
I1	33	0	0.00
I2	40	1	2.50
C	41	0	0.00
PM1	45	1	2.22
PM2	45	1	2.22
M1	44	2	4.55
M2	42	6	14.29
M3	37	7	18.92
Total	327	18	5.50

teeth, the sex difference increases slightly. When the prevalence of caries is corrected by antemortem tooth loss, it accentuates even more the difference between females and males (Table III and IV). When these corrected results are compared statistically ($\chi^2=7.829$, $p<0.01$), females show significantly more carious lesions (13.92%; 11/79) than males (4.55%; 10/220). The sex difference at Lapa do Santo is not caused by age bias, since mean age-at-death for males (34.38 years old; $n=8$) and females (32.38 years old; $n=4$) are statistically similar ($t=0.307$, $p=0.77$; Levene's test: $W=1.3004$, $p=0.28$; Figure 2; Table I).

Table V shows the prevalence of dental caries position in the permanent dentition. From 18 teeth affected by caries at Lapa do Santo, the most af-

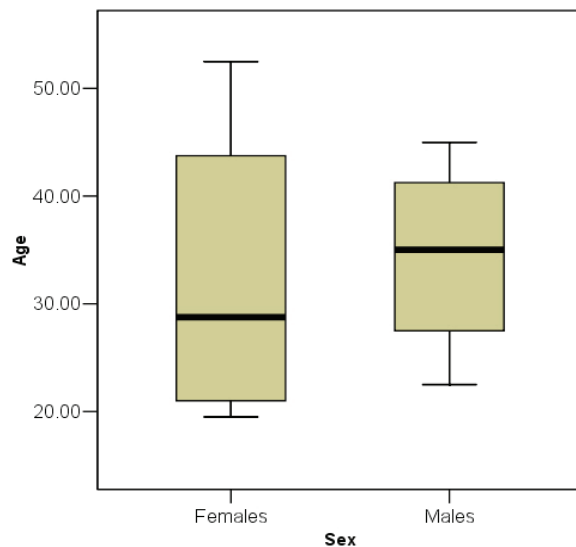


Figure 2 - Distribution of the mean age-at-death by sex of skeletons at Lapa do Santo (see the colors in the online version).

TABLE III
Permanent teeth lost antemortem by skeleton at the Lapa do Santo site.

	Sex	Alveoli	Teeth lost antemortem	%
St-SepI	1	31	2	6.45
St-SepII	2	16	0	0.00
St-SepIII	1	28	0	0.00
St-SepV	2	27	8	29.63
St-SepX	2	17	0	0.00
St-SepXI	1	28	0	0.00
St-SepXV	1	32	2	6.25
St-SepXVI	2	31	0	0.00
St-SepXVIIa	1	32	0	0.00
St-SepXXI	1	31	3	9.68
St-SepXXII	1	31	0	0.00
St-SepXXVI	1	32	0	0.00
Total	-	336	15	4.46

TABLE IV
Prevalence of caries corrected by tooth loss.

	Males		Females		Total	
	Prevalence ^a	%	Prevalence ^a	%	Prevalence ^a	%
Posterior	8/139	5.76	7/43	16.28	17/213	7.98
Anterior	1/74	1.35	0/28	0.00	1/114	0.88
Total	9/213	4.23	7/71	9.86	18/327	5.50
Erdal and Duyar ^b	-	4.10	-	10.17	-	5.32
Lukacs ^c	10/220	4.55	11/79	13.92	21/342	6.14

a = teeth affected by caries divided by total teeth. **b** = percentage of caries in posterior dentition multiplied by (5/8) + percentage of caries in anterior dentition multiplied by (3/8), see Erdal and Duyar (1999); **c** = see Lukacs (1995).

TABLE V
Lapa do Santo prevalence of dental caries position by sex in permanent dentition.

	Males		Females		Total	
	Cariou lesions	%	Cariou lesions	%	Cariou lesions	%
Gross	0	0.00	1	14.29	1	5.56
Occlusal	7	77.78	6	85.71	14	77.78
Interproximal	0	0.00	0	0.00	0	0.00
Root/CEJ	0	0.00	0	0.00	1	5.56
Buccal/labial/lingual	2	22.22	0	0.00	2	11.11
Total	0	0.00	0	0.00	18	100.00

ected position is the occlusal face (77.78%, n=14), followed by the buccal/labial/lingual (11.11%, n=2), root/CEJ faces (5.56%, n=1), and gross caries (5.56%, n=1). The prevalence of caries causing pulp exposure is 16.67% (n=3), indicating some degree of severity. The gross caries lesion and two teeth with pulp exposure were found in female dentitions.

Regarding the regional comparison, the overall rates of caries (5.50%, 18/327) at Lapa do Santo is statistically similar ($\chi^2 = 2.4675$; $p = 0.12$) to the Lagoa Santa sample (8.26%, 57/690). The proportion of root/CEJ caries is lower ($\chi^2 = 4.9512$; $p = 0.03$) at Lapa do Santo (5.56%, 1/18) than at Lagoa Santa (31.67%, 19/60), while the prevalence of occlusal caries is higher ($\chi^2 = 8.6398$; $p < 0.01$) at Lapa do Santo (77.78%, 14/18) than at Lagoa Santa (38.33%, 23/60). Mean age-at-death (Lapa do Santo=33.71 year-old, n=12; Lagoa Santa=28.29 year-old, n=108) does not seem to explain these differences.

Lapa do Santo shows 14.75% (18/122) of deciduous teeth affected by caries (Table VI). These carious lesions, however, are concentrated in one individual with 16 carious lesions. Two individuals affected by dental caries were 4-year-old, while one carious lesion was found in a loose tooth of a 3-year-old individual. The individual with 16 caries likely suffered from some congenital disease, which an ongoing study is investigating. Excluding this individual, the caries prevalence in deciduous teeth at Lapa do Santo is 1.92% (2/104).

TABLE VI
Prevalence of dental caries by tooth type in the deciduous dentition at Lapa do Santo.

	Deciduous dentition		
	Teeth	Affected	%
i1	24	5	20.83
i2	26	5	19.23
C	29	4	13.79
dm1	26	2	7.69
dm2	17	2	11.76
Total	122	18	14.75

DISCUSSION

General trends among pre-historic hunter-gatherer skeletal samples are important references to interpret local populations. Lapa do Santo dental caries prevalence using teeth (5.50%) as unit of analysis is higher than dental caries from global (1.72%; Turner 1979) and Western Hemisphere (3.58%; Da-Gloria and Larsen 2014) samples of hunter-gatherers, standing closer to mixed economies (4.37%; Turner 1979). Using individuals as units of analysis, Lapa do Santo prevalence of dental caries (69.23%) is higher than prevalence in hunter-gatherers from the Western Hemisphere using similar methodology (31.58%; Da-Gloria and Larsen 2014). The reasons a hunter-gatherer population show unexpectedly high prevalence of caries is a relevant question here. Such exceptions have been reported in other populations worldwide. A high prevalence of caries (14.3% of occlusal caries, n=182 lower molars) was reported at Moita in the Mesolithic period in Portugal (Lubell et al.

1994), in the Lower Pecos region of Texas (14%, n=421 teeth; Hartnady and Rose 1991), in coastal California (13.3%, Canada Verde, n=1718 teeth; Walker and Erlandson 1986), on the Brazilian Atlantic Coast (10.7%, n=1219 teeth; Turner and Machado 1983), and in Late Pleistocene period in Morocco (51.7%, n=344 teeth; Humphrey et al. 2014). These populations all have in common the likely consumption of foods with high carbohydrate content, such as dried fruits in Portugal, cactus, agave and wild legumes in Texas, wild roots and tubers in coastal California and Brazil, and acorns and pine nuts in Morocco. Indeed, Lapa do Santo population seems to be similar to them in having a highly cariogenic diet. Among the botanical remains found at Lapa das Boleiras in the Lagoa Santa region, wild pequi and jatobá have the highest carbohydrate content by 100g of dry fruit (Nakamura et al. 2010). Although these plants are non-domesticated, plant management of pequi occurs in native populations from Central Brazil (Smith and Fausto 2016). This practice seems to be very old in the tropical regions of South America (Erickson 2008). These fruits are the best candidates to explain the high prevalence of caries at Lapa do Santo.

The comparison of dental pathologies at Lapa do Santo by sex shows females with more carious lesions when the prevalence is corrected by antemortem tooth loss (AMTL). In bioarchaeology, poor oral health in females is traditionally attributed to the sexual division of labor, in which females tend to gather plants, prepare food, and consume carbohydrates more often than males (Larsen 2015). Recently, Lukacs (2008, 2011) stressed the importance of biological factors (and its behavioral implications) in female oral health, such as the role of fertility and hormones in caries prevalence. At Lapa do Santo, however, isotopic data suggests older age-at-weaning (T. Hermenegildo, unpublished data), implying low fertility for that population. Indeed, we believe that

dietary habits relating to the high consumption of carbohydrates by females is the best explanation for this difference. Da-Gloria and Larsen (2014), using a larger sample of Lagoa Santa teeth, showed that both tooth wear and caries prevalence increase with age. Therefore, we do not believe that tooth wear significantly affects the observed prevalence of caries.

The regional comparison shows no significant difference of Lapa do Santo with other Lagoa Santa sites regarding overall caries. On the other hand, there are more occlusal caries than root caries at Lapa do Santo, while the Lagoa Santa sample shows the opposite pattern. Relatively more occlusal caries may be related to low tooth wear or to less exposure of the roots at Lapa do Santo (Hillson 2001). Further investigation of these factors in the Lagoa Santa collection may provide an answer.

Studies with caries prevalence using deciduous teeth are not common in the literature. As one exception, Sciulli (1997) analyzed sites from the Ohio Valley dated to the Late Archaic period, reporting prevalence ranges from 0% to 1.65% from 11 archaeological sites, resulting in the total of 0.88% (7/793). This value is statistically similar to the Lapa do Santo prevalence ($\chi^2=1.002$; $p=0.32$), excluding the possibly diseased individual (1.92%, n=104). Although these Late Archaic populations focused their subsistence on wild resources, their diet appears to have had some contribution from domesticated plants, suggesting a mixed economy. That is, the prevalence of caries in the deciduous dentition in the Lapa do Santo sample seems to be similar to those in mixed economy populations. In addition, occurrence of carious lesions in individuals 3 to 4 years old at Lapa do Santo indicates a cariogenic diet since their first introduction to solid food. The food eaten by these young individuals probably included the wild fruits, described above, which have a high carbohydrate content.

Ecological factors seem to be related to the availability of carbohydrates to human

consumption. Ströhle and Hahn (2011) showed that hunter-gatherer societies living in desert and tropical grasslands consumed the highest quantity of carbohydrates (ca. 29%-34% of the total energy) in a worldwide database of 63 hunter-gatherer societies living in different ecological conditions. As an example, the Hadza hunter-gatherers living in the African savannah eat carbohydrates regularly, such as tubers and fruits (Marlowe 2010). In fact, the results shown here suggest that the diet of the Early Holocene inhabitants of Lagoa Santa was based on foods with relatively high carbohydrate content.

CONCLUSIONS

The dental pathologies found in the teeth of the Lapa do Santo assemblage reveal a population with relatively high prevalence of dental caries, which started as early as 3 to 4 years old. These results corroborated our expectation that the Early Holocene inhabitants of Lagoa Santa have unusually poor oral health compared to other hunter-gatherer populations, especially the females. We believe that dietary habits are the main factors explaining the unusual prevalence of dental pathologies at Lapa do Santo. Zooarchaeological and paleobotanical material recovered from Lapa do Santo suggest a diversified subsistence strategy including small-to medium-sized animals and non-domesticated plants, such as fruits. In short, the dental markers and the archaeological context suggests that Lagoa Santa inhabitants had a subsistence based on a relatively high carbohydrate content diet which may characterize a lifestyle adapted to a mosaic of tropical environments.

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