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Dental Health in the Aqllakuna from Farfán (Peru): A New Perspective on an Inca Female Institution (ca. 1470-1532 A.D.) Using Micro-CT and Histological Analysis

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Abstract

This research aims to explore the lifeways of an underrepresented subgroup of females while reducing the use of destructive methods in bioarchaeology. The excavation of Farfán on the North Coast of Peru revealed a rare *aqlla* cemetery from the Late Horizon (1470-1532 A.D.). The *aqlla* was an Inca religious institution where young females were sequestered to brew *chicha* and weave in their *aqllawasi*. According to ethnohistorical sources, these "Chosen Women" were expected to represent a homogenous and advantaged subset of the population. This hypothesis is assessed by comparing their dental lesions to the general population using macroscopy, micro-CT, and histology. The dental analysis found similar caries, calculus, and wear and more linear enamel hypoplasia and interglobular dentin in the *aqlla* suggesting these females were not as described in ethnohistorical documents. The multi-modal approach supports micro-CT as a non-destructive imaging technique providing novel information to complement macroscopy and histology.

Keywords:

Bioarchaeology; Biocultural approach; Micro-CT; Histology; Dental health; *Aqlla/ Aclla*; Female institution; Interglobular Dentin; Andes; Farfán

Résumé

Cette recherche vise à explorer le mode de vie d'un groupe de femmes sous-représentées en plus de promouvoir l'utilisation de méthodes non destructives en bioarchéologie. L'excavation du site archéologique de Farfán a révélé un rare cimetière *aqlla* sur la côte nord du Pérou. *L'aqlla* était une institution religieuse durant l'Empire inca (1470-1532 EC.) où des « Femmes Choisies » étaient confinées dans une *aqllawasi* pour tisser et préparer la boisson *chicha*. Selon les documents ethnohistoriques, elles représentaient un sous-groupe avantagé et homogène de la population. Cette hypothèse est évaluée en comparant leurs lésions dentaires à celles de la population générale par macroscopie, histologie et micro-CT. L'analyse dentaire a révélé une prévalence similaire en caries, calcul dentaire et attrition ainsi que plus d'hypoplasie linéaire de l'émail et de dentine interglobulaire dans *l'aqlla.* Ces résultats *s*uggèrent que ces femmes n'étaient pas telles que décrites dans les documents ethnohistoriques. L'approche multimodale encourage l'utilisation du micro-CT comme technique d'imagerie non destructive pouvant complémenter l'information obtenue par microscopie et histologie.

Mots-clés:

Bioarchéologie; Approche bioculturelle; Micro-CT; Histologie; Santé dentaire; *Aqlla/ Aclla*; Institution de femmes; Dentine interglobulaire; Andes, Farfán

Resumen

Esta investigación tiene como objetivo explorar las formas de vida de un subgrupo de mujeres subrepresentadas mientras se reduce el uso de métodos destructivos en bioarqueología. La excavación de Farfán en la Costa Norte de Perú reveló un raro cementerio de *aqllas* del Horizonte Tardío (1470-1532 d.C.). El *aqlla* fue una institución religiosa durante el Imperio Inca donde las mujeres jóvenes eran secuestradas para elaborar *chicha* (bebida de maíz fermentada) y tejer en su *aqllawasi*. Según fuentes etnohistóricas, se esperaba que estas "Mujeres Elegidas" representaran un subconjunto homogéneo y favorecido de la población. Esta hipótesis se evalúa comparando sus lesiones dentales con las de la población general utilizando macroscopía, micro-CT e histología. El análisis dental evidenció similar presencia de: caries, cálculo y desgaste dental y más hipoplasia lineal del esmalte y dentina interglobular en el grupo de las *aqllas*. Estos resultados sugieren que estas mujeres no eran como se describen en los documentos etnohistóricos. El enfoque multimodal apoya el micro-CT como una técnica de imagen no destructiva que proporciona información novedosa para complementar la macroscopía y la histología.

Palabras clave:

Bioarqueología; Enfoque biocultural; Micro-CT; Histología; Salud dental; *Aqlla/ Aclla*; Institución de mujeres; Dentina interglobular; Andes, Farfán

Summary for Lay Audience

This research generates bioarchaeological evidence to a subgroup of Inca females underrepresented in the literature which is particularly important considering the biased and incomplete portrait of these females in ethnohistorical accounts. During the Late Horizon period (1470-1532 A.D.), the Inca created the *aqlla* institution [pronounced "akya"] where females were selected before puberty to weave, cook, and brew *chicha*, a beverage from fermented maize for ceremonial and economical purposes. The information known about these "Chosen Women" relies on a group of ethnohistorical documents called the Spanish Chronicles. According to these documents, these females are expected to represent a homogenous and advantaged subset of the population. This hypothesis can be assessed by comparing their dental lesions, physiological stress, and diet to individuals from the general population. The analysis for this research on 45 teeth from 32 individuals buried at Farfán on the North Coast of Peru aims to examine the veracity of the Spanish Chronicles regarding the *aqlla* and promotes the use of non-invasive and non-destructive methods to analyse teeth. Teeth are particularly interesting to investigate past populations since they can record information on diet, mobility, disease, and childhood stress. Cavities and dental wear were assessed here to yield information about maize consumption while developmental defects on the outer and inner surface of the tooth provided information about episodes of illness and malnutrition during childhood. More specifically, cavities, dental wear, and developmental defects (e.g. linear enamel hypoplasia and interglobular dentin) were recorded through macroscopy, histological analysis (study of tissue with microscopy), and a non-invasive technique called micro-computed tomography (micro-CT). The latter technology, particularly important with the increasing concern about destructive analysis, relies on X-rays to reconstruct a virtual 3D volume of the tooth that can be manipulated to be cut

through and observed from different angle. The detailed analysis suggest that these females experienced slightly fewer cavities and more episodes of childhood illness than the general population from this site. Thus, dental evidence disagrees with the description of these females in the Spanish Chronicles. The multi-modal approach supports micro-CT as a non-destructive imaging technique providing novel information to complement macroscopy and histology.

Résumé pour public non-spécialiste

Cette recherche produit des données bioarchéologiques pour un groupe de femmes incas sous-représentées dans la littérature; ce qui est important compte tenu du portrait biaisé et incomplet de ces femmes dans les documents ethnohistoriques. L'Empire inca (1470-1532 EC) a créé l'institution *aqlla* [prononcé « akya »] où de jeunes femmes étaient sélectionnées avant la puberté pour tisser, cuisiner et préparer la *chicha*: une boisson à partir de maïs fermenté. Les connaissances sur ces « Femmes Choisies » reposent principalement sur une collection de documents ethnohistoriques appelés les Chroniques espagnoles. Selon ces documents, ces femmes devraient représenter un sous-groupe avantagé et homogène de la population. Cette hypothèse peut être évaluée en comparant leurs lésions dentaires à la population générale. L'analyse sur 45 dents provenant de 32 individus enterrés à Farfán sur la côte nord du Pérou vise à examiner la véracité des Chroniques espagnoles sur l'*aqlla* et promouvoir l'utilisation de méthodes d'analyse non destructive sur les dents archéologiques. Les dents sont particulièrement intéressantes pour explorer les populations passées puisqu'elles peuvent conserver des informations sur l'alimentation, la mobilité et les stress physiologiques vécus durant l'enfance. Les caries et l'attrition dentaire fournissent de l'information sur la consommation de maïs alors que les anomalies développementales permettent d'obtenir de l'information sur les épisodes de maladies et de malnutritions durant l'enfance. Plus spécifiquement, les caries, le niveau d'attrition dentaire, le calcul dentaire et les anomalies développementales (hypoplasie linéaire de l'émail et dentine interglobulaire) ont été observés à l'œil nu (macroscopie), en étudiant les tissus sous microscope (histologie) et avec une technique d'imagerie non invasive appelée microtomographie (micro-CT). Cette dernière

technologie reconstruit à partir de rayons X un modèle virtuel 3D d'une dent qui peut être manipulé, coupé et observé depuis différents angles ce qui est important considérant le désir croissant de minimiser les analyses destructives. L'analyse détaillée effectuée suggère que ces femmes présentent légèrement moins de caries et plus d'épisodes de maladies durant l'enfance que la population générale. Autrement dit, l'analyse dentaire de ces femmes ne correspond pas aux attentes des Chroniques espagnoles. L'approche multimodale supporte le micro-CT comme une technique d'imagerie non destructive qui permet d'obtenir des informations additionnelles pour complémenter la macroscopie et l'histologie.

Resumen para público no especializado

Esta investigación genera evidencia bioarqueológica de un subgrupo de mujeres incas subrepresentadas en la literatura. Este tema es particularmente importante considerando el retrato sesgado e incompleto de estas mujeres en los relatos etnohistóricos. Durante el período del Horizonte Tardío (1470-1532 d.C.), los incas crearon la institución aqlla donde las mujeres eran seleccionadas antes de la pubertad para tejer, cocinar y elaborar chicha, una bebida de maíz fermentado con fines ceremoniales y económicos. La información que se conoce sobre estas "Mujeres Elegidas" se basa en un conjunto de documentos etnohistóricos llamados Crónicas Españolas. Según estos documentos, se espera que estas mujeres representen un subconjunto homogéneo y favorecido de la población. Esta hipótesis se puede evaluar comparando sus lesiones dentales, estrés fisiológico y dieta con individuos de la población general. En el marco de esta investigación se analizaron 45 dientes de 32 individuos enterrados en Farfán, en la costa norte de Perú con el objetivo de examinar la veracidad de las Crónicas Españolas sobre el aqlla y a su vez, promover el uso de métodos no invasivos y no destructivos para analizar los dientes. Los dientes son particularmente interesantes para investigar poblaciones pasadas, ya que pueden registrar información sobre dieta, movilidad, enfermedades y estrés infantil. Más específicamente, las caries, el desgaste dental y los defectos del desarrollo (p. ej., hipoplasia lineal del esmalte y dentina interglobular) se registraron

mediante macroscopía, análisis histológico (estudio del tejido con microscopía) y una técnica no invasiva llamada microtomografía computarizada (micro-CT). Esta última tecnología, particularmente importante con la creciente preocupación por el análisis destructivo, se basa en rayos-X para reconstruir virtualmente en volumen 3D el diente, el cual se puede manipular para cortarlo y observarlo desde diferentes ángulos. El análisis detallado sugiere que estas mujeres experimentaron un poco menos de caries y más episodios de enfermedades infantiles que la población general de este sitio. Por lo tanto, la evidencia dental no está de acuerdo con la descripción de los individuos femeninos en las Crónicas españolas. El enfoque multimodal respalda al micro-CT como una técnica de imagen no destructiva que proporciona información novedosa para complementar la macroscopía y la histología.

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During my undergrads, I jumped on every opportunity I could to get closer to biological anthropology including a semester at the University of Leicester in England where I was lucky enough to hold my first human bone: a femur. After that, there was no coming back. I discovered the world of palaeopathology. I knew without a doubt that I had to take my chance in this field. It paid off.

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Chapter 1 - Introduction

1.1 Background

In bioarchaeology, past cultures are investigated through remains and their surrounding context. Skeletons are dynamic processes that incorporate information during life keeping a record of an individual's history. Bioarchaeologists focus on the skeleton to retrieve this information to understand past populations. Among others, sex, stature, bone chemistry, and pathological lesions can be obtained through the analysis of skeletons. These characteristics can address questions such as demography of past populations, reconstruction of diet and migration, infectious disease, violence, disparities within population, and more (Larsen, 2002).

Individuals are entangled with their environment and social identity, therefore, past populations need to be considered in their broader context to interpret the information collected on the skeleton (Gowland, 2015). For example, in exploring health disparities in an Inca population from Machu Picchu, Turner and Armelagos (2012) consider the impact of social status, diet, and mobility. Similarly, this thesis will investigate bioarchaeological evidence in their cultural context with a focus on dental material to gain a better understanding of past population during the Inca Empire (1470-1532 A.D.).

1.1.1 Farfán & the *Aqlla*

Farfán, on the Peruvian North Coast (Figure 1.1), was an important administrative and ceremonial site in the Jequetepeque Valley. It was marked by the influence of three civilisations: the Lambayeque (1100-1300 A.D.), the Chimú (1300-1470 A.D.), and the Inca (1470-1532 A.D.) (Mackey & Nelson, 2020). The Incas build the largest and most heterogeneous empire in Pre-Columbian societies through their military power and political acumen. Their empire, *Tawantinsuyu*, started in Cuzco during the Late Intermediate Period (ca. 1000-1438 A.D.) and extended from Chile to Ecuador by the Late Horizon (ca. 1438-1532 A.D.) including over ten million inhabitants from multiple social groups with different beliefs and social organisations (D'Altroy, 2015).

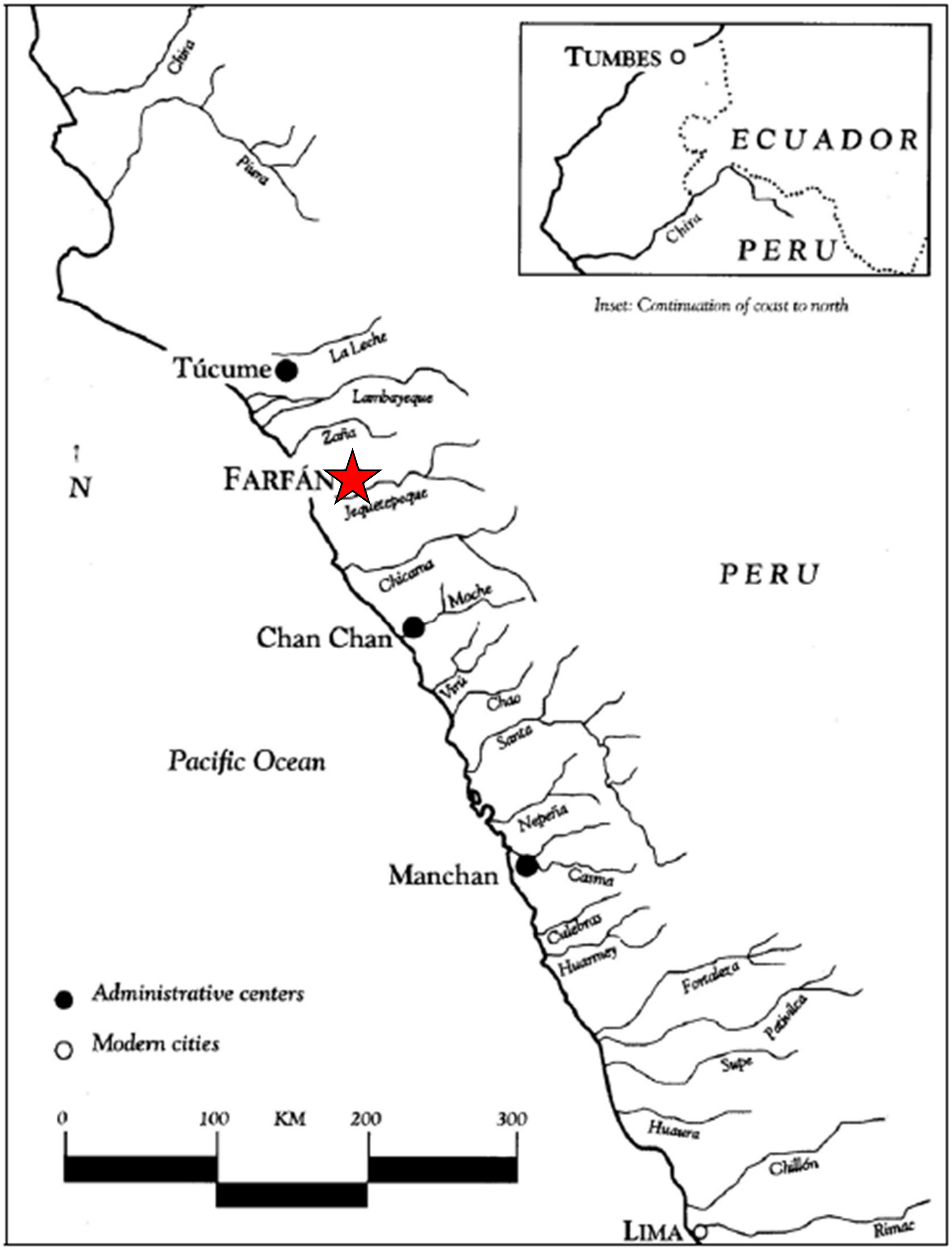


Figure 1.1. Map of the North Coast showing Farfán in the Jequetepeque Valley and other administrative centers nearby after Figure 1.2 from Mackey & Nelson (2020: 16).

The Inca Empire fell with the arrival of Spaniards under the command of Francisco Pizarro in 1532 A.D. The Inca culture remained alive in *Tawantinsuyu* until the strict reforms imposed by the Viceroy Francisco de Toledo in 1570-1572 A.D. which destroyed most of the remaining practices. Since the Incas did not have a writing system, the only written records available of their empire are the ethnohistorical sources written between the 16th and 17th centuries, after the Spanish Conquest, by European scholars and religious men. They recorded information about the military structure, political strategies, and ceremonial practices of the Incas leading to multiple documents regrouped under the Spanish Chronicles (D'Altroy, 2015). These historical documents written by Spanish and *mestizos* (Spanish-Andean) chroniclers are the basis of ethnohistorical research in the Andes. They represent a primary source of information for Andean archaeologists regarding the *aqlla* institution as archaeological evidence has been sparse. Details about the *aqlla* differ from one source to another but overall, they describe the *aqllakuna* as being beautiful and high-status virgins chosen at a young age to be devoted to religious and economic purposes for the state until they reach approximately fourteen years of age. Then, they could be given (or taken) to marry high-status men, become concubines, serve as another Inca's wife, be sacrificed, or stay in the *aqllawasi* to train new *aqllakuna* (Cobo, 1979[1653]; Sarmiento, 1967[1572]). The description of the *aqlla* in the Spanish Chronicles is explored further in chapter 2.

The exact number of girls involved in the institution is unknown as no defined limit of the size of an *aqllawasi* have been reported in the literature. Each province had an *aqllawasi*, leading to an estimate of 80 of these houses throughout the Empire (D'Altroy & Schreiber, 2004). Considering the Inca population size, there might have been between 6,900 and 15,500 "Chosen Women" living in *aqllawasi* based on estimates from the Spanish Chronicles (Surette, 2008). However, very few of these houses have been clearly identified at archaeological sites. Recognized samples include the site of Tomebamba in Equator (Idrovo Urigüen, 2000), and the sites of Cuzco (Eaton, 1916), Huanuco Pampa (Morris & Thompson, 1985), Pachacamac (Uhle, 1903), Túcume (Heyerdahl *et al.*, 1995), and Farfán (Mackey & Nelson, 2020) in Peru.

At Túcume, the only other site on the North Coast believed to have an *aqllawasi*, Toyne (2002) reported a wider age range, poorer health, and more trauma from interpersonal violence, in these females compared to the general population. Her conclusion suggests a more violent life than was expected from the highly regarded *aqllakuna* described in the literature. However, Toyne (2002) did not have a large comparative sample. Mackey and Nelson's (2020) analysis of the *aqllakuna* from the Farfán collection also found evidence that does not support the ethnohistorical accounts. They suggest that the *aqllakuna* are not different from the rest of the population and have more variability in terms of age range, health, and geographic origins than the general population. However, a detailed dental analysis has not yet been done.

1.1.2 Dental Analysis in Bioarchaeology.

Teeth represent a unique angle to investigate past populations. They can provide precious information about affiliation, diet, mobility, disease, childhood stress, and age-at-death (Hillson, 1996; Kelley & Larsen, 1991). Dental tissues are denser than bone so often preserve well in the archaeological record. Their direct interaction with the environment can alter their surfaces permanently. This combination of preservation and influence of environment on their development and integrity during an individual's life makes teeth the ideal sample for bioarchaeological analyses such as assessment of palaeopathological lesions (Hillson, 1996).

Each tissue has its unique properties and can be associated with specific features and pathological lesions to provide precious information on an individual's lifeways. For example, carious lesions (also known as cavities) caused by the destruction of dental tissues by bacteria, are often associated with a diet high in carbohydrates like maize (Hillson, 1996). In other words, teeth provide a unique window of investigation to assess health and diet in a population like the one from Farfán. The relationship between dental pathological lesions and lifeways is discussed further in chapter 3.

Previous studies have investigated health in past populations by focusing on a few dental features at a time, such as linear enamel hypoplasia (e.g. Temple, 2014), or carious lesions and antemortem tooth loss (e.g. Williams & Murphy, 2013). However, since each feature provides different information, a thorough examination combining multiple methods will reveal a more accurate portrait regarding the types and prevalence of dental diseases.

1.2 Research Objectives

This thesis aims to provide a better understanding of the *aqlla* institution through a detailed dental analysis of the material from the Inca site of Farfán while contributing to comparative methodological discussions between destructive histological analysis and non-invasive computed tomographic (CT) analysis. A total of 45 teeth from 32 individuals collected during the Farfán Project between 1999 and 2006 are analysed in detail including 12 primary burials from the *Huaca* Burial Platform. The teeth are subjected to macroscopic observations, micro-computed tomography (micro-CT), and histology to comprehensively assess the dental health of these individuals.

The site of Farfán on the North Coast of Peru revealed a unique burial context with the *Huaca* Burial Platform where a high proportion of females were buried with sacrificial items, and weaving and spinning artefacts. This specific Inca platform from the Late Horizon (ca. 1470-1532 A.D.) is interpreted as the burial site of members of an *aqllawasi*, a house of "Chosen Women", known as an *aqlla* (Mackey & Nelson, 2020). Despite being one of the main topics of interest in Peruvian bioarchaeology (e.g. Costin, 1998; Surette, 2008; Toyne, 2002) little is known about this female institution. The lifeways of females were not a main topic of interest to male chroniclers leading to a biased and incomplete description of these females. This observation highlights the need to explore the institution further. According to ethnohistorical sources, these females were inducted into the institution around 8 to 12 years of age based on their beauty, rank, or skills. Their main responsibilities included spinning, weaving, brewing *chicha*, and assisting ceremonies for the state (Acosta, 2011 [1590]; Cieza de León, 2010 [1553]; Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Garcilaso de la Vega 2010 [1609]; Polo de Ondegardo, 1873 [1572]). *Chicha*, a beverage from fermented maize, required women to masticate the kernels before spiting the pulp in jars with water and let the concoction ferment. These sources, although informative, have been criticised and likely did not capture all the complexity of the *aqlla* institution (e.g. D'Altroy, 2015; Rowe, 1946;

Surette, 2008). Bioarchaeological evidence could confirm or refute the ethnohistorical descriptions, but very few *aqllawasi* have been identified on archaeological sites.

Based on their described honoured status and lifeways, it could be hypothesised that bioarchaeological evidence should present females with few dental indicators of childhood stress, and a high level of dental caries due to brewing activity, while their grave goods would include higher status items like fine textiles, valuable ceramics, and weaving materials (following Toyne, 2002). Previous research at the Inca administrative site of Farfán by Dr Andrew Nelson revealed more variability in terms of age range, cranial modification, isotope values, and health conditions including vertebral damages and infections than expected in a sample of *aqlla* (Mackey & Nelson, 2020). A detailed dental analysis of these individuals has not been done.

Variation in oral health is sometimes thought to reflect socio-economic organisation with elites having fewer pathological lesions than commoners (Farnum, 2002; Klaus, 2008). This statement assumes that higher status confers greater access to high-quality foods, which often includes animal protein than commoners who possibly consumed more carbohydrates (Farnum, 2002; Klaus, 2008; D'Altroy, 2002; Moeseley, 2001). A better diet and fewer episodes of malnutrition could lead to a stronger immune system making such individuals less susceptible to pathogens and nutritional deficiencies. During the Inca Period, most of the population was commoners, but ethnohistorical sources claim that Inca society had a hierarchical social organisation (D'Altroy, 2002; Moseley, 2002). Thus, we, might expect differences in dental health in Inca populations partly related to socio-economic status as observed in a Maya population (White *et al.,* 2005). If the *aqllakuna* were drawn from higher status families, they may have fewer dental lesions associated to childhood illness.

This thesis investigates the identity of the *aqllakuna* through their dental health to assess their variability within the *Huaca* Burial Platform in comparison to other cemeteries at Farfán. The following hypotheses on the "Chosen Women" drawn from the ethnohistorical sources are tested:

1) As these females supposedly brewed *chicha* regularly, which involves mastication of maize to start the fermentation process, they should have a higher frequency of carious lesions and higher wear than the general population who experienced a regular diet and occasional chicha brewing.

2) As these females were supposedly venerated and considered as part of the elite, they may have had access to better food and cultural conditions resulting in generally good health, so they should have a lower prevalence of physiological stress lesions than the general population.

3) As these females were supposedly drawn mostly from local elite families, which would have shared similar lifeways, they should have a large degree of uniformity in their dental health when compared to the general population in Farfán.

4) Micro-CT reveals as many features as traditional histological methods without destroying teeth and thus can be used as an alternative, or complement, macroscopic and histological methods.

The fourth hypothesis focuses on the methodological approach used for this project. Indeed, in bioarchaeology, many researchers rely on macroscopy, microscopy, or histology to analyse dental defects. While histology, the study of tissues at a microscopic level, can provide a lot of data at the cellular level, the creation of thin sections destroys part of the tooth, which is a major concern in bioarchaeology (Abel *et al.*, 2012; Forshaw, 2014; Roberts, 2016). The emergence of computed tomography (CT), an X-ray imaging technique allowing 3D reconstruction, offers an alternative method of analysis to visualise internal structures. The development of high-resolution CT imaging, like micro-CT, represents an ideal non-destructive and non-invasive method (Cox, 2015) to investigate teeth. Micro-CT has been used as an alternative to examine caries (Rossi *et al.*, 2004; Tomczyk *et al.*, 2014; Towle *et al.*, 2019), enamel defects (Marchewka *et al.*, 2014; Xing *et al.*, 2016), and interglobular dentin (IGD), a mineralisation defect in dentin usually associated with vitamin D deficiency (Colombo *et al.*, 2019; Veselka *et al.*, 2019). No thorough examination with micro-CT of multiple features associated with dental health is known to the author.

Colombo *et al.* (2019) were the first to examine interglobular dentin detection in an archaeological sample using 2D images from micro-CT and histology. Their results suggest detection is possible with both techniques, but some information is lost with micro-CT (also see Veselka *et al.,* 2019). Their study did not utilize the 3D volume of the micro-CT. Thus, my project will contribute to the current discussion of this novel approach joining 2D mapping with a 3D volume. This registration of 2D images such as histology onto 3D volumes created by tomography (CT, micro-CT, MRI) (Burnett *et al.*, 2014; Slater *et al.*, 2017) is also known as correlative tomography or "multi-modal imaging". Similar to correlative microscopy where the registration of images from two or more 2D imaging modalities or resolutions are merged into a single image, the objective is to take advantage of the properties of different imaging modalities such as colour, density, and resolution, to highlight specific features (Loussert Fonta & Humbel, 2015). The combination of images taken at different resolutions is not new, as it has long been used for "scout and zoom" strategies for finding regions of interest (ROIs) at low resolution for interrogation at higher resolution with another imaging modality (Burnett *et al.*, 2014; Caplan, *et al.*, 2011). However, the extension of the concept into the 3rd dimension, and the increasingly sophisticated image processing and registration techniques that have recently become available have led to a great expansion in the field in the last decade.

1.3 Material and Methods

From 1999 to 2006, the Farfán Project revealed five cemeteries including the *Huaca* Burial Platform believed to be representative of the *aqlla* institution (Mackey & Nelson, 2020). The osteobiographies from 68 primary burials analysed by Dr Nelson were considered to investigate the lifeways of the aqlla. The focused sample for the detailed dental analysis undertaken here includes a total of 45 teeth from 32 individuals including 20 teeth belonging to 12 females from the *Huaca* Burials Platform.

This focused sample was analysed using non-destructive imaging (micro-CT) and destructive analyses (histology). Different components of dental health are considered including pathological lesions such as caries, attrition (dental wear), dental calculus, and developmental dental abnormalities such as linear enamel hypoplasia (LEH) and interglobular dentin (IGD) to better understand the studied population (Table 1.1).

Table 1.1. Features of interest in the detailed dental analysis of individuals from Farfán using three methods.

Dental Features	Osteobiography	Macroscopy	Micro-CT	Histology	Meaning
Abscesses	X				Bacterial infection of the dental pulp
Antemortem Tooth Loss	X				Advanced infection, trauma
Caries	X	X	X	X	Carbohydrate consumption
Occlusal Wear	X	X	X	X	Food processing, extra-masticatory activities
Dental Calculus	X	X	X	X	Protein consumption, cultural practices (e.g. excessive chewing)
Linear Enamel Hypoplasia	X	X	X	X	Non-specific childhood stress
Interglobular Dentin			X	X	Dietary deficiency (Ca, vitamin D) and fluorosis
Hypercementosis	X	X	X	X	Excessive wear and malocclusion

1.4 Theoretical Framework – Biocultural Anthropology

The biocultural approach which combines biological and cultural perspectives can be characterized by the "linking of demographic, biological, and cultural processes within an ecological framework" (Martin *et al.*, 2013: 10). This holistic approach will be applied in this research by considering the osteobiographies of the individuals and their cultural contexts, as initially done in Saul and Saul (1989). This method links skeletal markers to environment and culture to reconstruct individuals' lifeways with a focus on palaeopathology. This biocultural framework is especially relevant to assess health in past populations considering the multifactorial variables influencing an individual's well-being. Thus, with a thorough analysis of teeth using micro-CT and histology, this thesis assesses the dental health of the *aqlla* from Farfán. The biological and cultural contexts obtained from the ethnohistorical sources, the osteobiographies, archaeological evidence (Mackey & Nelson, 2020), and isotope values are considered to gain insight into, and demystify, the social status, diet, and mobility of the *aqllakuna*.

The archaeological context of Farfán will be considered to interpret the dental material in a broader context. Notes and photographs for all individuals as well as details on the Farfán site have been collected by Dr Andrew Nelson and Dr Carole Mackey during the Farfán Project (1999-2006) providing information on cultural practice, general health, and social status. Moreover, stable isotopic analysis ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) of bones and teeth from selected individuals amongst the five cemeteries of Farfán was performed, which provided information on diet and mobility. The results of these observations are reported in Mackey and Nelson (2020) and White *et al.* (2020).

In brief, this project on the Andean site of Farfán in Peru aims to reduce the use of destructive methods for bioarchaeological materials in addition to investigating the peculiar context of the *aqlla* institution during the Inca Empire. This research will test if these women were from a relatively homogenous and advantaged subset of the population as suggested by ethnohistorical sources evidenced by better and more homogeneous dental health than the general population from the site. The dental analysis offered by this project will provide a better understanding of the living conditions in an *aqlla* during the Inca Empire which will contribute to the "growing body of research investigating the intersections of diet, mobility, and differential health in Andean contexts" (Turner & Armelagos, 2012: 72) and expand knowledge on this female institution. Moreover, the accuracy and precision of the detection of dental features with micro-CT could represent a valuable substitution to destructive histological analysis, which will be an important methodological contribution to the field of dental anthropology.

1.5 Organisation of the Thesis

The second chapter of this thesis offers a critical view of the description of the *aqllakuna* in the ethnohistorical sources. Chapter 3 provides a short overview of the dental pathological lesions recorded in this research. Chapter 4 presents a literature review on dental research in Peruvian archaeological materials covering what has been analysed, how it has been analysed, and the main results. Chapter 5, Materials, covers the material excavated by the Farfán project with a focus on the main difference between the five cemeteries and focusing on the primary burials. Chapter 6, Methods, explains all the analyses performed including macroscopic observation, micro-CT, histology, and statistical analyses. Moreover, it explains the main advantages and inconveniences of each technique and how a comparative approach can contribute to the advancement of knowledge about the *aqlla* identity. Chapters 7 and 8, respectively, report the results and a discussion of the main findings. Finally, chapter 9 concludes the thesis with a brief overview of the research project and offers future directions for this project.

Notes

Aqllawasi / *acllawasi*/ *acllahuasi* to design the 'House of the Chosen Women'; *aclla*/*aqlla* (singular); *aqllakuna*/ *acllacona*/ *acllacuna*/ *acllas* (plural) are all spelling encountered in the literature depending on the language prioritised (Spanish or Quechua). For this thesis, the spelling used in D'Altroy (2015), closer to the Quechua form, is followed except for direct citations. A glossary of all the foreign words used can be found in Appendix A.

Chapter 2 - *Aqlla* in the Spanish Chronicles

Aqllakuna: Quechua word combining the word *aqlla* – pronounced: "akya" – meaning 'Chosen Women' and *kuna* – pronounced: "kouna" - which indicate the plural form of a noun or a member of a class (Betanzos, 1996 [1576]).

The Spanish Chronicles include over 50 ethnohistorical documents written by Spanish and *mestizos* (mix Spanish and Andean ancestry) during the 16th and 17th centuries following the Conquest. There are no written records of Inca society prior to the Spanish Chronicles since the Incas transmitted their culture through oral traditions. The first reports on the Incas were written by soldiers, religious officials, bureaucrats, and scribes when the Spanish arrived in the Andes in 1532 under the command of Francisco Pizarro (D'Altroy, 2015). The early ethnohistorical authors such as Cieza de León (2010 [1553]) noted descriptive observations focusing on their arrivals, the geography, the religion, and the military and political strategies. Later sources, from the end of the 16th century, incorporated more cultural information, but they did not rely on eyewitnesses as much as the ones from the mid-16th century. They relied on oral transmission and previous historical sources. Thus, except for Father Cobo's manuscript (1979 [1653]) which was based on lost documents, the latter chroniclers are considered less reliable (Rowe, 1946) and therefore are not the focus of this chapter.

Despite their direct interactions with the Incas, there is a lot of variation and confusion in the Spanish Chronicles regarding the identity and role of the *aqlla*. The institution is identified under many appellations including "virgins of the Sun," "virgins of the Inca," "*mamakuna,*" and "*aqlla.*" In general, the *aqllakuna* are described as "Chosen Women" selected before puberty by the *apupanaca* (provincial governor) and confined in a house called the *aqllawasi* where they learned to cook, weave, spin, and brew *chicha* for the state and religious ceremonies. Once their training was completed, they could become *mamakuna* (old women in the institution who preserved their virginity and trained the youngers), become secondary wives or concubines of the Inca or other male elites, or serve as sacrifices for the upcoming year. This chapter aims to untangle the various description surrounding the *aqlla*.

It is important to note that this chapter does not include all the sources from the Spanish Chronicles, but instead covers the main authors to offer a critical view of their writings while unravelling the *aqlla* institution. The ethnohistorical sources cannot reveal all the subtleties of the institution, but this chapter aims to provide a more in-depth description of the *aqlla* than is generally provided. The main characteristics of the institution are grouped in this chapter in sub-categories including the perception of the religious institution throughout the Inca Empire, the *aqllakuna*'s virginity, the selection process, the organisation within the *aqllawasi*, what happened after their training. The chapter concludes with a critical view on the chroniclers. This approach describes the *aqlla* institution while highlighting the variability of the information reported in Cieza de León (2010 [1553]), De Las Casas (1892 [1552]), Pizarro (1921 [1571]), Polo de Ondegardo (1873 [1572]), Sarmiento De Gamboa (1898 [1572]), Betanzos (1996 [1576]), Acosta (2011 [1590]), Garcilaso de la Vega (2010 [1609]), Guamán Poma de Ayala (2009 [1615/1616]), and Cobo (1979 [1653]). These individuals were commoners witnessing the change in polity (Cieza de León, Pizarro, Sarmiento De Gamboa), an administrator (Polo de Ondegardo), individuals related to the Incas (Betanzos, Garcilaso de la Vega, Guamán Poma de Ayala), and ecclesiastics (De Las Casas, Acosta, Cobo).

2.1 *Aqllawasi* As a Religious Institution Throughout the Empire

Under the Inca polity, each province had an *aqllawasi* (Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Polo de Ondegardo, 1873 [1572]) modelled after the one in Cuzco which housed more than 1,500 females' blood-related to the Inca (Garcilaso de la Vega, 2010 [1609]). The exact number of females in the provincial houses is unknown but they likely housed fewer individuals. All these females were sustained by the State for their needs in food, lands, and materials (Betanzos, 1996 [1576]; Garcilaso de la Vega, 2010 [1609]).

The *aqlla* institution was strongly associated with deities in all the ethnohistorical sources with the Sun as their main god figure (Acosta, 2011 [1590]; Betanzos, 1996 [1576]; Cieza de León, 2010 [1553]; Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Garcilaso de la Vega, 2010 [1609]; Guamán Poma de Ayala, 2009 [1615/1616]; Pizarro, 1921 [1571]; Polo de Ondegardo, 1873 [1572]). In addition to the Sun, they also venerated the moon (Guamán Poma de Ayala, 2006; 2009 [1615/1616]), Thunder, and other gods (Cobo, 1979 [1653]). The *aqllawasi* in Cuzco was dedicated strictly to the Sun (Garcilaso de la Vega, 2010 [1609]) while the provincial houses had to perform their daily sacrifices to feed the Sun and other local *huacas* (Betanzos, 1996 [1576]; De Las Casas, 1892 [1552]; Polo de Ondegardo, 1873 [1572]). The *huacas* were sacred things associated with the worship of nature and ancestors such as a mountain, a lake, the moon, an animal, a person, a structure, and so on.

Other houses with *mamakunas*, virgins, and daughters of lords were built in addition to the house dedicated to the Sun. These females were recognized as "wives of the Inca" (Betanzos, 1996 [1576]). Although Betanzos (1996 [1576]) claims that there were provincial houses for the "wives of the Sun," and others for the "wives of the Inca," he is the only author consulted who separated them into different houses. In opposition, Garcilaso de la Vega (2010 [1609]), affirms that the "wives of the Sun" were only in Cuzco while the provincial ones were considered "wives of the Inca." This confusion between the "Virgins of the Sun," and the "Virgins of the Inca" is also mentioned in Pizarro (1921 [1571]). Since the Inca is often compared to the Sun in the Spanish Chronicles, there might have been confusion between the two especially when we consider the translation and interpretation from Quechua to Spanish to English. Considering that the house in Cuzco was associated with royalty, the author believes it was likely considered closer to the Sun than the provincial houses.

2.2 Sequestered Virgins

One of the main characteristics of the female institution encountered in the ethnohistorical sources is the importance of virginity. The *aqllakuna* were identified as "Virgins of the Sun" or "Virgins of the Inca" in Cieza de León (2010 [1553]), Garcilaso de la Vega (2010 [1609]), and Polo de Ondegardo (1873 [1572]). It was such an important trait that the girls were selected at a young age to ensure their virginity (Garcilaso de la Vega, 2010 [1609]). Once in the *aqllawasi*, according to most sources, they lived in seclusion to protect their virginity and not be made common. The *aqllakuna*, and possibly the *mamakunas*, were highly guarded by *mamakunas* (Sarmiento De Gamboa, 1898 [1572]) or guards to avoid interaction with men (Betanzos, 1996 [1576];

Cieza de León, 2010 [1553]; Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Garcilaso de la Vega, 2010 [1609]; Polo de Ondegardo, 1873 [1572]). The men guarding the door or serving the *aqlla* were eunuchs; they were castrated for precaution (Betanzos, 1996 [1576]). All interactions were closely watched; only the *Coya* (the queen) and her daughters could visit the *aqlla* (Garcilaso de la Vega, 2010 [1609]). Although it is unclear if this referred only to the *aqllakuna* in Cuzco or all of them. Guamán Poma de Ayala pushes the restrictions further where "not even the Inca and his high priests would dare speak to these virgin *acllacona*" (2009 [1615/1616]: 247).

The *mamakunas,* chosen to stay in the "convent" after their training, were expected to remain permanently cloistered and virgins (Betanzos, 1996 [1576]; Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Garcilaso de la Vega, 2010 [1609]; Guamán de Poma, 2006 [1615/1616]). Pizarro (1921 [1571]) contradicts this statement by writing that the women dedicated to the Sun could go out during the day. Since he wrote from memory, he might have confused the *mamakunas* with the virgin servants of the *Coya* or another group of females. Indeed, many women of royal blood vowed chastity and a similar lifestyle to the *aqllakuna* but they kept their freedom and lived in their own house (Garcilaso de la Vega, 2010 [1609]). These women might have been the ones described by Pizarro.

Breaking their vow of chastity was punished by a death sentence. Both parties involved were hanged (Cobo, 1979 [1653]; Guamán Poma de Ayala, 2009 [1615/1616]; Sarmiento De Gamboa, 1898 [1572]), buried alive (Acosta, 2011 [1590]; Cieza de León, 2010 [1553]), stoned to death (Betanzos, 1996 [1576]), or burned alive (Garcilaso de la Vega, 2010 [1609]). In the most extreme scenario, the sentence was extended to everybody related to the man including his wife, children, kinsmen, flock, and servants. This sentence was never applied as no one ever broke the law (Garcilaso de la Vega, 2010 [1609]) although Pizarro saw males facing the death penalty for leaving the "convent" at night. Pizarro (1921 [1571]) also claimed that these women were lying about their chastity and were in fact involved with servants and guards. Moreover, Betanzos (1996 [1576]) mentioned that children secretly born from *mamakunas* were raised by women who had lost their children. Thus, some *mamakunas* probably broke their vow and stayed alive despite the death sentence they were supposed to face.

The concept of virginity is controversial in ethnohistorical sources, as the perception is contradictory amongst the authors. For some, virginity was respected and a symbol of purity among the Incas (Garcilaso de la Vega, 2010 [1609]) but it was also reported as being "very offensive to [the Incas]" (Cobo, 1979 [1653]: 29; Cieza de León, 2010 [1553]). It was a sign that the woman had never been loved before and therefore was not well seen. A man could take a woman on a trial basis to be his concubine and serve him and marry her if she satisfied him. However, Guamán Poma de Ayala (2006 [1615/1616]) wrote that no attention was paid to "fallen woman" and they would likely not get married.

2.3 Selection Process

In addition to their virginity as a prerequisite, the *aqlla* had to be selected by the *apupanaca*, a provincial commissioner named by the Inca in each province with the responsibility to manage the *aqllawasi*. He chose young girls around 8-12 years old, although, the age varies amongst the sources (see Table 2.1), based on their beauty or good disposition (Acosta, 2011 [1590]; Cieza de León, 2010 [1553]; Cobo, 1979 [1653]; Garcilaso de la Vega 2010 [1609]; Polo de Ondegardo, 1873 [1572]). While some authors claim that parents' ranks were not considered (Acosta, 2011 [1590]; Cobo, 1979 [1653]; Garcilaso de la Vega, 2010 [1609]; Pizarro, 1921 [1571]; Polo de Ondegardo, 1873 [1572]) others specified that their nobility could be a criterion (Cieza de León, 2010 [1553]; De Las Casas, 1892 [1552]; Garcilaso de la Vega, 2010 [1609]). Betanzos does not specify the rank of the girls, but he mentioned that "virgins and daughters of lords" (1996 [1576]: 110) can be called wives of the Inca. According to other sources, daughters of common people (Garcilaso de la Vega, 2010 [1609]), and *Mitimaes* from other provinces (communities who had been relocated following Inca's arrivals) could also be chosen (Cieza de León, 2010 [1553]).

Table 2.1. Age for selection of girls by the *apupanaca* to send in the *aqllawasi*.

Sources	**Girls' age for selection**
De Las Casas (1892 [1552])	≥ 10 years
Pizarro (1921 [1571])	10 years
Sarmiento De Gamboa (1898 [1572])	≥ 12 years
Betanzos (1996 [1576]); Polo de Ondegardo (1873 [1572])	8-9 years
Acosta (2011 [1590]); Garcilaso de la Vega (2010 [1609])	≤ 8-9 years
Cobo (1979 [1653])	≤ 8 years

The age varies slightly amongst the sources, but none of them could be absolute. Indeed, the Incas had a well-developed annual calendar with the same number of days for yearly and monthly activities as the Gregorian calendar, but they did not have a multi-year one. In fact, "they did not count their age in years […] there was never an Indian who knew his age" (Cobo, 1979 [1653]: 252-253). Thus, the *apupanaca,* or witnesses, could not know how old the chosen girls were. They probably looked young and were likely chosen before the rite of passage marking their puberty, but the exact age cannot be accurate in the ethnohistorical sources. It is interesting to note that earlier sources are associated with older ages while in later sources the age for selection tends to be younger. This highlights the complexity and subjectivity of age estimation in the Incas and the potential bias in the ethnohistorical sources.

There are many discordances between the sources. The girls were probably chosen before puberty based on their beauty or skills by the *apupanaca*. While the *aqllawasi* in Cuzco required royal rank, the provincial *aqllawasi*, such as the one in Farfán, more likely included girls from all ranks with a preference toward daughters of elite males. After their selection, the girls were brought to the *aqllawasi* where *mamakunas* trained them (Acosta, 2011 [1590]; Cobo, 1979 [1653]; Polo de Ondegardo, 1873 [1572]).

2.4 Hierarchy and Roles Within the *Aqllawasi*

2.4.1 Structure

Once in the *aqllawasi*, the girls were trained for different tasks. The exact social structure within the institution is unclear. There was no hierarchy described by Cieza de León

(2010 [1553]), but some structure was implied in Garcilaso de la Vega as the "girls had their *mamacunas* of the same caste as themselves" (2010 [1609]: 294) while Pizarro wrote that "ten *acllas* were under a mistress, ten mistresses under an abbess who was directly under the authority of the vicars" (1921 [1571]: 521). The *aqlla* were classified by their age, status, or dedication. Among Guamán Poma de Ayala, Pizarro, and Polo de Ondegardo the only common group was the "*aqlla* of the Inca" who became concubines of the Inca. They accomplished tasks usually associated with the *aqlla* institution while the other groups are barely mentioned in the Chronicles.

2.4.2 *Aqlla's* Activities

The structure within the *aqlla* might not be explicit in most of the Spanish Chronicles, but the authors elaborate on the multiple tasks performed by the *aqllakuna* in the *aqllawasi*. Even if none of the authors were able to see their daily activities, they were mostly concordant. The most commonly described activities include spinning, weaving, cooking, and brewing *chicha* (Figure 2.1) (Betanzos, 1996 [1576]; Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Garcilaso de la Vega, 2010 [1609]; Guamán Poma de Ayala, 2009 [1615/1616]; Pizarro, 1921 [1571]; Polo de Ondegardo, 1873 [1572]). The *aqlla* in Cuzco dressed in clothes and headwear the Inca and the *Coya*, made the clothes sacrificed to the Sun, and brewed special *chicha.* The clothes made by the "Wives of the Sun" in Cuzco were reserved for the Inca since common mortals including captains and chiefs, were not allowed to use divine things. However, clothes made in the provincial *aqllawasi* were distributed among the royal family and elite males (Garcilaso de la Vega, 2010 [1609]). Their work was sent to Cuzco for *Raymi* festival (Polo de Ondegardo, 1873 [1572]).

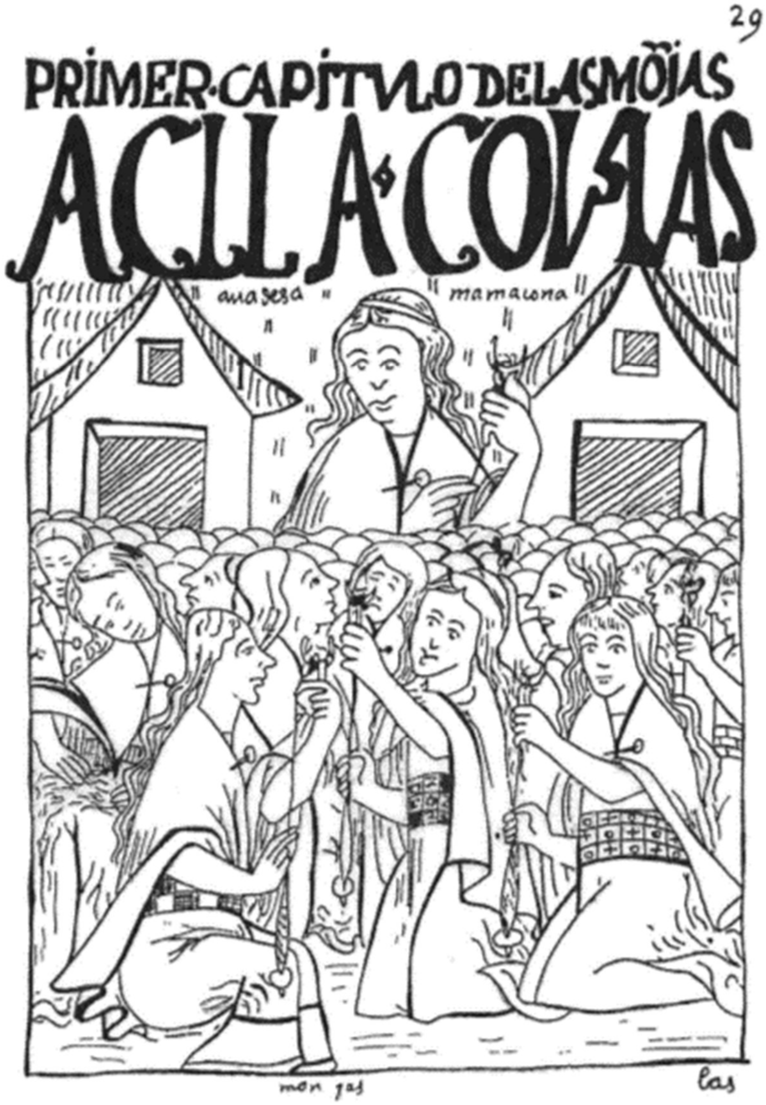


Figure 2.1. ***Aqllakuna*** **spinning in the courtyard of the Cuzco** ***aqllawasi*** **(Reproduced from Guamán Poma de Ayala, 2009 [1615/1616]: 235).**

Other activities have been reported, but they are not unanimous among the Spanish Chronicles. The *aqllakuna* could sing (Guamán Poma de Ayala, 2009 [1615/1616]), make the ceremonial bread called *çancu* (Garcilaso de la Vega, 2010 [1609]), oversee ceremonies to the Sun (Betanzos, 1996 [1576]), work in fields, or take care of *tambos* (Betanzos, 1996 [1576]; Cieza de León, 2010 [1553]; Guamán Poma de Ayala, 2009 [1615/1616]). The *tambos* were stations along the road to provide food for lords and soldiers traveling (Betanzos, 1996 [1576]; Cieza de León, 2010 [1553]; Guamán Poma de

Ayala, 2009 [1615/1616]). It is difficult to understand how these activities could be executed without interacting with men.

2.4.3 *Mamakunas*

Like the *aqllakuna*, the *mamakunas* were responsible for weaving and brewing *chicha*. Despite being their only task in Cieza de León (2010 [1553]), most authors agree that the *mamakunas* had more responsibilities than the *aqllakuna* including overseeing the *aqllawasi* and training the younger girls (Acosta, 2011 [1590]; Betanzos, 1996 [1576]; Cobo, 1979 [1653]; Garcilaso de la Vega, 2010 [1609]; Polo de Ondegardo, 1873 [1572]). They also made sacrifices every day to the statue of the Sun and shooed away flies that might rest on the statues (Betanzos, 1996 [1576]). The *mamakunas* have been reported in charge of guarding the *aqllakuna* (Sarmiento De Gamboa, 1898 [1572]), being wet nurses for orphans (Guamán Poma de Ayala, 2006 [1615/1616]), looking after the supplies, and becoming abbesses or portresses (Garcilaso de la Vega, 2010 [1609]).

2.4.4 Other Females in the *Aqllawasi*

Other individuals could be found in the *aqllawasi* in addition to the *aqllakuna*, *mamakunas*, and guards. For the house in Cuzco, virgins and elite daughters served the “Chosen Women”. They could not be considered as “Wives of the Sun” since they were not blood-related to the Inca (Garcilaso de la Vega, 2010 [1609]). In all *aqllawasi*, women who had been raped could also become servants of the *aqllakuna* (Guamán Poma de Ayala, 2009 [1615/1616]). Moreover, based on Guamán Poma de Ayala (2006 [1615/1616]) there were three of the ten possible general paths for women based on their age and ability that could have been related to the *aqllawasi*: old women weaving clothes for the community and serving noble women and *aqlla;* beautiful young maidens of marriageable age who could be selected to serve the temples without being *aqllakuna*; and girls between 5 and 9 years of age serving the *Coya*, noble women, virgins, or *mamakunas* (Guamán Poma de Ayala, 2006 [1615/1616]).

2.5 After Being *Aqllakuna*

Once the *aqllakuna* reached approximately 13 or 14 years of age, the *apupanaca* brought them to Cuzco for *Raymi* festival where the Inca dictated their fate. It is unclear in the Spanish Chronicles whether only the most beautiful females were taken to the Inca center or all of them. Four paths could be assigned: 1) *mamakuna*; 2) servants or concubine of the Inca; 3) secondary wives of captains and prestigious men as a reward from the Inca (Acosta, 2011 [1590]; Cobo, 1979 [1653]; Pizarro, 1921 [1571]; Polo de Ondegardo, 1873 [1572]); or 4) be sacrificed during the year (Acosta, 2011 [1590]; Cobo, 1979 [1653]; Polo de Ondegardo, 1873 [1572]).

Amongst the females destined to become *mamakunas*, some were assigned to the "convent" where they remained chaste and worked for the Sun temple in Cuzco. Other *mamakunas* stayed in the *aqllawasi* to oversee young girls' training, prepare food, *chicha*, and clothes for ceremonies and sacrifices (Betanzos, 1996 [1576]; Polo de Ondegardo, 1873 [1572]). The number of females promoted to *mamakunas* varied between years, the main objective was to replace the ones that had died (Cobo, 1979 [1653]).

The Inca kept the most beautiful girls to become his concubines and serve him (Cobo, 1979 [1653]; Garcilaso de la Vega, 2010 [1609]). He also gave many girls as extraordinary favour to reward elite men such as captains, soldiers, and lords (Cobo, 1979 [1653]; Sarmiento De Gamboa, 1898 [1572]). It was the only way men could accept a second wife. Despite, the commonly described possibility for the *aqllakuna* to be offered as second wives to elite men (Cobo, 1979 [1653]; Pizarro, 1921 [1571]), Garcilaso de la Vega (2010 [1609]) disagrees with the other chroniclers on the topic. He wrote that the *aqllakuna* were honoured and venerated as wives of the Sun/ Inca. Marrying someone from a lower status, even if he was highly esteemed by royalty would have been a disgrace for the *aqlla*, the Inca, and religion. It was better to become a servant of the Inca than the wife of "common" men. Garcilaso de la Vega (2010 [1609]) suggests the confusion might come from the daughters of captains or *Curacas* and sometimes illegitimate girls of royal blood who were given to other elite men as an honour from the Inca. They were "virgins" but not *aqllakuna*.

Sacrifices were frequent and happened for a variety of reasons including natural phenomena such as earthquakes and eclipses or in the name of the Inca, before a war, for his health, or to accompany him in the afterlife (Cobo, 1979 [1653]; Polo de Ondegardo, 1873 [1572]). The souls of these sacrificed virgins were believed to rest in great peace (Acosta, 2011 [1590]; Cobo, 1979 [1653]; Polo de Ondegardo, 1873 [1572]). The *aqllakuna* selected for this purpose could be sacrificed any time throughout the year.

Not surprisingly, the end of life in the *aqlla* institution is barely mentioned in the Spanish Chronicles. Apparently, most of these females died in their function, but some might have returned to their province. According to Garcilaso de la Vega (2010 [1609]), once they were a concubine of the Inca, they could not return to the *aqllawasi* and therefore they became servants of royalties, or they could return to their province with a house and land provided. The *mamakunas* could also return to their province once they were very old or they could remain in the *aqllawasi* until death (Garcilaso de la Vega, 2010 [1609]). Cieza de León (2010 [1553]) also affirms they stayed in *aqllawasi* until they "became old" but he does not specify if they returned to their province or died in the "convent", while for Guamán Poma de Ayala (2006 [1615/1616]), they died in the *aqllawasi*.

2.6 The Authors of the Ethnohistorical Sources

The information extracted on the *aqlla* in the main ethnohistorical sources varies among authors. It is difficult to assess their accuracy, especially regarding the *aqlla* institution. Indeed, the institution was probably disintegrated when the Spaniards arrived in the Andes and as a female institution, it was not their primary focus of interest. Many of them emphasized how the *aqlla* were strictly guarded to avoid contact with all men (Betanzos, 1996 [1576]; Cieza de León, 2010 [1553]; Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Garcilaso de la Vega, 2010 [1609]; Polo de Ondegardo, 1873 [1572]; Sarmiento De Gamboa, 1898 [1572]) therefore the authors could not have directly interacted with these females in the eventually they were even interested in their perspective. The little interest and lack of direct interactions with the *aqllakuna* might partially explain the confusion on the institution in the Spanish Chronicles. It is also important to acknowledge the background of the authors and their informants (Table 2.2) to assess the reliability of the information collected.

Table 2.2. The authors of the ethnohistorical sources, their background, informants, and the main information they provide on the *aqlla* institution.

Sources	Author's Background	Main Informants	*Aqlla* Information
De las Casas (1892 [1552])	Dominican priest Never visited the area	Reports from others	Chosen > 10 years old from elites Serve the Sun in the Temple Guarded
Cieza de León (2010 [1553])	Common soldier	From direct observations Interviews with Inca aristocracy	Most beautiful, many daughters of lords Virgins from other provinces selected Weave fine cloth for lords, dedicated to service of the temple, brew *chicha*
Pizarro (1921 [1571])	Cousin of Francisco Pizarro	Personal observations Wrote from memories	Chose at 10 years old from elites 4 classes of *aqlla* Brew *chicha*, spin, weave, cook Go out but enclose at night Marry male elites
Polo de Ondegardo (1873 [1572])	Lawyer Administrator of Cuzco	Personal observations	8-9 years girls from all rank 4 classes of *aqlla* Sew, weave, brew drinks At 13-14 years taken for the Temple to be religious servants, sacrifices, or serve the Inca and other prestigious men
Sarmiento De Gamboa (1898 [1572])	Mariner, geographer, administrator, and explorer	Publicly read to Inca for accuracy	12 years when given in marriage to prestigious men *Mamakuna* guarded the *aqllakuna* Very beautiful virgins
Betanzos (1996 [1576])	Married to the wife of the Inca Atawallpa For the account of Viceroy Mendoza Spoke Quechua	Stories from Inca princess and her family	Chosen 8-9 years old Spinning, weaving, cooking, make *chicha*, in charge of sacrifices Guarded by castrated men 3 types of virgins
Acosta (2011 [1590])	Explorer and Naturalist Jesuit	From direct observations Wrote from memory while being in Spain	Selected < 8 years old from all ranks *Mamakunas* trained the *aqllakuna* 14 years send to Cuzco to serve in sanctuaries, being sacrificed, serves as wives/concubines of Inca or others In the house a certain time than for Inca or gods Bury alive if break chastity
Garcilaso de la Vega (2010 [1609])	*Mestizo* of Spanish Conqueror and Inca princess Christian education Left Peru in 1560 Spoke Quechua	Wrote from memory and correspondents Valera's manuscript	Chosen < 8 years old from all ranks Make bread, brew *chicha*, weave, spin, look after ceremonies Can serve queen, become *mamakuna*, concubine/ second wives of the Inca Beautiful girls
Guamán Poma de Ayala (2009 [1615/16])	*Mestizo* Christian education Spoke Quechua	No eyewitnesses From Memory	Chosen around 10 years old Daughter of elites 6 classes of *aqlla* + 6 classes of "common virgins" work, weave, spin, sing apprentices between 4-10 years old
Cobo (1979 [1653])	Jesuit priest Spent most of his life in Peru	Earlier lost manuscripts	Chosen < 8-9 years old from all ranks 14 years old become *mamakuna*, sacrifices, servant/ concubine Spin, weave, cook, brew *chicha*, other jobs

The diversity of the authors' origins, beliefs, and how they collected their information about the Incas affects the reliability of their descriptions as it can create bias, sometimes highly noticeable such as their religious beliefs in their writings. The religious bias is especially problematic regarding topics such as chastity or the duties of the *aqllakuna*. Acosta (2011 [1590]), Cobo (1979 [1653]), De Las Casas (1892 [1552]), and Valera who is cited by Garcilaso de la Vega (2010 [1609]) were priests or Jesuits while the catholic education of Guamán Poma de Ayala (2006; 2009 [1615/1616]) shines through his writing. Words such as "depraved custom [talking about the disregard for virginity before marriage]" (Cobo, 1979 [1653]: 30), "corrupted concubines" (Guamán Poma de Ayala, 2009 [1615/1616]: 237), or "[they] never knew the splendour and beauty of chastity" (Cobo, 1979 [1653]: 30) left little imagination on the authors' opinions regarding the importance of virginity. Moreover, the comparison of the *aqlla* with "nuns" (Cobo, 1979 [1653]; Garcilaso de la Vega (2010 [1609]; Guaman Poma de Ayala, 2009 [1615/16]; Sarmiento De Gamboa, 1898 [1572]) or other groups of historical virgins such as the vestal virgins of Rome (Acosta, 2011 [1590]; Cieza de León, 2010 [1553]; Cobo, 1979 [1653]) might have influenced the perception of the authors.

The vestal virgins were a group of females in Ancient Rome selected between 6-10 years of age based on their dispositions and socio-economic status who oversaw purification rites, storage, and preparation of harvested grain for food. The concept of virginity was at the center of this religious institution (Wildfang, 2006). This description was similar to the "nuns" in Spanish Convents where girls were sent by their family to the convent to be trained and educated, to learn how to be "a buena esposa" (Albarrán-Estrada *et al.*, 2014; Castelao, 2009; Lopez, 2010). There was a clear association between the nobility and the religious institution considering that most of the females sent were noble daughters (Lopez, 2010) reminding the confusion around the status for the selected *aqllakuna*. (Castelao, 2009). These "virgins" might have tinted the view of the chroniclers regarding the *aqlla.* In Spanish Convents, the girls were selected when they were young, expected to stay chaste, to complete religious and administrative duties, clean, cook, and sew in the institution while training and educating other girls. Similarities in their description with the *aqllakuna* can be noted and might not be the result of a coincidence. The main difference was the dowry present in Spanish Convents from the 16th century, which was absent from the description of the *aqlla* institution. The influences of previous religious institutions on the description of the female institutions from the New World is especially interesting when we explore the similarities in the description of the *aqlla* from the Inca Empire and the Aztec virgins in Mexico also described by Spaniards (Cordova, 2009). However, this is a topic for another project.

There are similarities between the comparison, and it is unfortunately impossible to distinguish if the analogy was made after learning about the *aqlla* or to simplify the institution. Moreover, by relying on previous sources, the misconception could have been carried on by later authors. This chronological phenomenon is visible through the use of Christian words to discuss the *aqlla* institution increasing in the later sources (see Table 2.3). Acosta, Garcilaso de la Vega, Guamán Poma de Ayala, and Cobo, four religious' men, relied on memories or previous sources to write their manuscripts which could have affected their understanding of the *aqlla* institution.

Table 2.3. Religious words referring to the *aqlla* institution in the Spanish Chronicles.

Year	Authors	Religious Terms
1552	Las Casas	virgins
1553	Cieza de León	priest; vestal virgins; virgin
1571	Pizarro	-
1572	Polo de Ondegardo	virgins
1572	Sarmiento De Gamboa	nuns
1576	Betanzos	cult; virgin
1590	Acosta	nuns; priest; vestal virgin
1609	Garcilaso de la Vega	convent; nuns; priestess; virgin
1615	Guamán Poma de Ayala	nuns; priestess; virgins
1653	Cobo	convent; nuns; vestal virgins; virgins

2.6.1 Sources From the 16th Century

The earliest observations on the Incas available date from the mid-16th century after the end of the Inca Empire with the Spanish Conquest. These earlier sources were written by common soldiers and official scribes. They were more descriptive and focused mostly on geography, wealth, and the military (D'Altroy, 2015). The first to offer a general description of Peru was Cieza de León in 1553 (Rowe, 1946), a common soldier who visited the northern Andes in 1547-1550. He went to school in Spain until 13-14 years of age when he left for the New World. Even without a higher education, his curiosity and habit to take detailed and objective notes, make him one of the most reliable chroniclers (D'Altroy, 2015). He wrote his manuscripts based on his direct observations and interviews with the Inca aristocracy in Cuzco (with the help of translators). However, he paid little attention to the *aqlla* institution referring only to *mamakuna* with no distinction within the Temple and briefly mentioned their principal duties.

The Dominican priest, De Las Casas (1892 [1552]), another early writer, is also brief on the *aqlla* institution. He does not identify them other than guarded virgins dedicated to the Sun. It is important to note that De Las Casas never visited Peru. He carefully collected reports from other authors which many have disappeared (D'Altroy, 2015; Rowe, 1946). This contrasts with Cieza de León and Pizarro who relied on their own observations.

Pizarro, the cousin of Francisco Pizarro who led the Spaniards to the Andes in 1532, witnessed the end of the Inca Empire. His insight is interesting, but he wrote his manuscript 40 years after his arrival based on memories, similar to Garcilaso de la Vega. Therefore, even if he was able to reflect on his observations and go beyond descriptions it is difficult to evaluate how much of his writings were influenced by time (D'Altroy, 2015). Memories are unfortunately not the most reliable sources as they can be "colored by time, political, and economic objectives, and wariness of Spanish repression" (D'Altroy, 2015: 18). For example, Pizarro (1921 [1571]) is the only author discussed in this chapter who said that the *aqllakuna* were not cloistered all the time in their house. Is it accurate or did he simply mix servants of the *Coya* with the *aqllakuna*? Moreover, like Cieza de León (2010 [1553]), he only refers to the females in the institution as *mamakunas* (Pizarro, 1921 [1571]) making it difficult to assess the difference between *aqllakuna* and *mamakunas.*

The interest in Inca culture grew toward the end of the 16^{th} century, and thus more information on Inca institutions was reported. Captain Sarmiento De Gamboa was mandated to write an official account on Inca history by the Viceroy Francisco de

Toledo. His work is considered mostly reliable as Sarmiento De Gamboa publicly read his account to a group of Incas to confirm the accuracy of his writings (D'Altroy, 2015). He included many details on the Incas lifeways, but the *aqllakuna* were likely not consulted directly and are only briefly mentioned in his books. He mentioned the distinction between the *aqllakuna* and *mamakunas*, and the different roles they had based on an outsider's perspectives. He did not write many details on the institution, but he covered the selection process, the guarding role of *mamakunas*, and the process to be given in marriage to lords and captains.

Polo de Ondegardo, who was the administrator of Cuzco between 1561 and 1571 wrote more details about the *aqlla* institution as he paid particular attention to the different Inca institutions. His work is heavily used by the Spanish Jesuit Acosta and Father Cobo (Rowe, 1946).

All these sources are considered mostly reliable amongst the Spanish Chronicles, but they were written by Spaniards, a critique often encountered in the earlier sources. They offer an outsider's perspective except for Betanzos who had connections with the royal Inca family. Betanzos was married to Dona Angelina Yupanque, the last wife of the Inca Atawallpa, and thus his writings are based on the Inca princess and her family with a bias in favour of Atawallpa (D'Altroy, 2015). Unfortunately, he wrote very little about the *aqlla*. Even in these early manuscripts relying on direct sources, it is difficult to assess the accuracy of the *aqlla* description.

2.6.2 Sources From the 17th Century

After the 1600s, there were no eyewitnesses of the flourishing Inca Empire or the *aqlla* institution. Thus, the authors often relied on previous sources or on oral transmissions to write their manuscripts. They are considered less reliable than the sources from the 16th century with a few exceptions like Father Cobo (Rowe, 1946). Indeed, even if the Inca culture was still active, the interviews and reports were based on memories, interpretations, and beliefs of storytellers. Despite this downside, some provide new information on the Incas by including sources that were subsequently lost, rectifying earlier interpretations, or adding visual support. Regarding the *aqlla*, these sources provide more details on the institution and its organisation. No sources other than Cobo (1979 [1653]), Garcilaso de la Vega (2010 [1609]), and Guamán Poma de Ayala (2006, 2009 [1615/1616]) have been considered in this chapter since the others relied mostly on previous materials.

Cobo was a Jesuit priest who spent most of his life in Peru. His manuscript was only published in the mid-17th century. He relied mostly on previous documents including Polo de Ondegardo (1873 [1572]), and many others who have been lost through time (Rowe, 1946). This association might explain the similarities of the information collected in Cobo (1979 [1653]) and Polo de Ondegardo (1873 [1572]) about the *aqlla*. Cobo's work is one of the most cited sources mostly due to his detailed and well-organised writing on Inca culture (D'Altroy, 2015).

Another advantage of the sources from the early 17th century was the Andean lineage of some of the authors such as Garcilaso de la Vega and Guamán Poma de Ayala who were *mestizos*. They spoke Quechua fluently and were raised in a mixed culture with close contact with Inca culture and traditions.

Criticizing the work of Spaniards who did not speak Quechua (e.g. Polo de Ondegardo), Garcilaso de la Vega reviewed previous information recorded. He also included material from the lost *Historia Occidentalis* written by the esteemed Jesuit Valera who was also *mestizo* (D'Altroy, 2015). One of the most important elements he corrected about the *aqlla* was the misconception of previous sources on how they were given as secondary wives to elite males. He provides more details than any other source about the "Chosen Women". However, the accuracy of all the information should be considered moderately. According to Rowe (1946), Garcilaso de la Vega is considered reliable for daily life information and description of biodiversity following the Colonial Period which he eyewitnesses, but his reports on Inca history and religion are 'rose-coloured'. His possible insight from his mother, an Inca princess, makes him more reliable than other sources since she was probably familiar with the *aqlla* institution, but he also relied on his memories and correspondences to write his manuscripts. Therefore, his description of the *aqlla* is highly valuable but should not be considered as the ultimate truth.

Finally, Guamán Poma de Ayala, one of the best-known Inca Chroniclers, is especially recognized for the hundreds of drawings included in his writings (e.g. Figure 2.1). These pictures are the only visual we have from this period. In some cases, they reveal more than the writing itself (D'Altroy, 2015). However, Guamán Poma de Ayala's work is considered unreliable when it comes to history, especially regarding his chronology (Rowe, 1946).

2.7 Conclusion

The ethnohistorical documents known as the Spanish Chronicles represent a unique insight into Inca society. However, there are clear biases from the authors of the ethnohistorical sources regarding the *aqlla* institution. First, as mentioned earlier in this chapter, the focus of interest from these males was not a female institution when they arrived in the Andes and the institution was dismantled under the Spanish polity leading to limited direct observations. Second, most authors had a Christian or Jesuit education even if they were not priests (e.g. Cobo; De las Casas, Guamán Poma de Ayala) creating a clear religious bias. The *aqlla* were often compared to nuns or the vestal virgins of Rome (Acosta, 2011 [1590]; Cieza de León, 2010 [1553]b; Cobo, 1979 [1653]; Garcilaso de la Vega (2010 [1609]; Guaman Poma de Ayala, 2009 [1615/16]; Sarmiento De Gamboa, 1898 [1572]). The similarities between the Vestal Virgins (Wildfang, 2006) or the nuns from Spanish Convent (Albarrán-Estrada *et al.*, 2014; Castelao, 2009; Lopez, 2010) with the *aqllakuna* are visible. Even if it is impossible to evaluate the degree of the knowledge of Cieza de León regarding the Vestal Virgins of Rome, they could have influenced his perception of the *aqlla* institution. The same concern applies to other authors. There almost certainly has been a lack of objectivity toward the religious institution of *aqlla*. Moreover, the vow of chastity might not have been as important as highlighted in these sources. The vow could have been a way to preserve the *aqlla*'s focus on the institution and their duties by avoiding pregnancy. The importance of the "religious" vocation of the *aqlla* institution might have been overestimated. The institution could have been an educational tool instead of a religious one. Garcilaso de la Vega (2010 [1609]) reports that noble boys were sent to Cuzco for a four-year training where they learned Quechua, Inca religion, governmental laws, using arms, *khipu* accounting, and so on. The *aqlla* could have been a similar concept with a focus on ceremonies instead of military.

Overall, the ethnohistorical sources agree that the "Chosen Women" were perpetual virgins selected before puberty, around 8-12 years of age, by the *apupanaca* based on their beauty, skills, or rank. Once selected, they lived in their local *aqllawasi* where they learned to cook, brew *chicha*, weave, and spin clothes. These prepared foods and clothes were given in sacrifices for the Sun and other *huacas.* After their training, when they were around 14 years of age, they were assigned to the role of *mamakuna*, concubine or second wife of the Inca and other elite males, or sacrifices. They stayed in their function until they died. While this description is the most often encountered, the *aqlla* institution presents some discordance amongst the authors regarding the social status of these females, the hierarchy within the *aqllawasi*, and the other tasks performed by these females. The confusion between the different groups of virgins (*aqllakuna*, gifts from the Inca, servants) might explain some of the variations recorded.

The main question when studying the "Chosen Women" through the Spanish Chronicles is how much can we rely on these manuscripts to demystify the *aqlla* institution? There are many inconsistencies between the authors and even within the same manuscript as highlighted by this chapter. The earlier sources are considered more reliable as they are based on eyewitnesses of the Inca Empire, but they cover the *aqlla* institution very briefly while the later sources from the 17th century paid more attention to the *aqlla* but were more influenced by people's interpretation. The ethnohistorical documents offer one perspective, but archaeological records offer another viewpoint on the Incas. Linking the ethnohistorical descriptions with archaeological data is the best way to assess the accuracy of the *aqlla* during the Inca Empire. This thesis offers a unique perspective on the *aqllakuna* found at Farfán to uncover their lifeways through a detailed dental analysis and attempt to untangle the information reported in the Spanish Chronicles.

Chapter 3 - Pathological Lesions in Teeth

Dental anthropology offers a unique perspective to analyse the dynamic changes of a population through the teeth and jaws. Similar to general health, the oral health of an individual is affected by many factors including growth and development, physiological stress, diet, and cultural behaviours (Figure 3.1). Thus, by investigating pathological lesions in teeth, much information can be retrieved. This chapter briefly presents tooth formation and the different pathological lesions evaluated in the Farfán population including caries, abscesses, antemortem tooth loss (AMTL), occlusal wear, enamel defects, interglobular dentin (IGD), dental calculus, and hypercementosis.

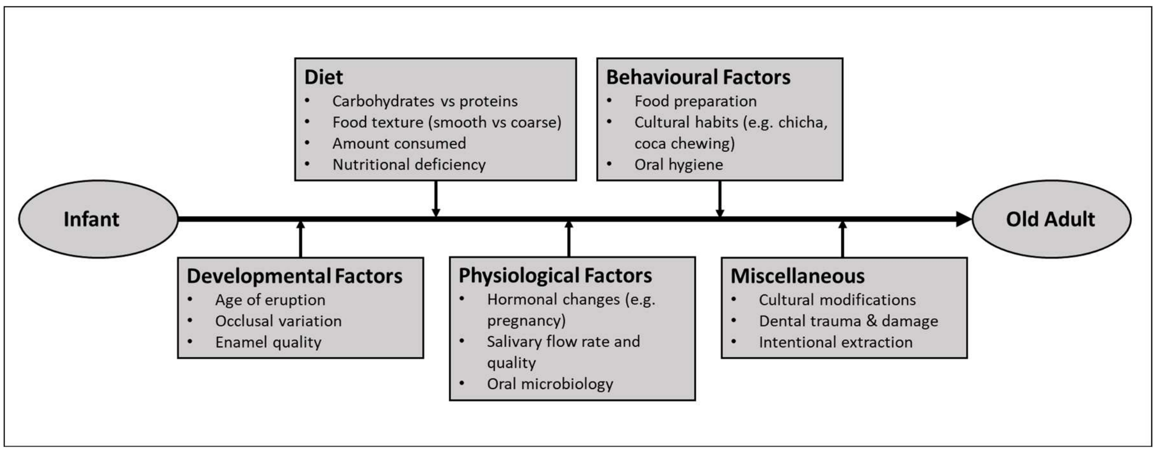


Figure 3.1. Factors affecting oral health throughout the lifetime of individuals after Figure 2.1 in Tran (2016: 16).

3.1 Tooth Formation

Teeth are composed of three main tissues that are mineralised during growth and development: dentin at its core, enamel coating the crown, and cementum coating the root. Junctions between tissues are identified by their location, the enamel-dentin junction (EDJ), the cement-dentin junction (CDJ), and the cement-enamel junction (CEJ) (Figure 3.2). Each tissue has its particularities and can be associated with specific features and pathological lesions.

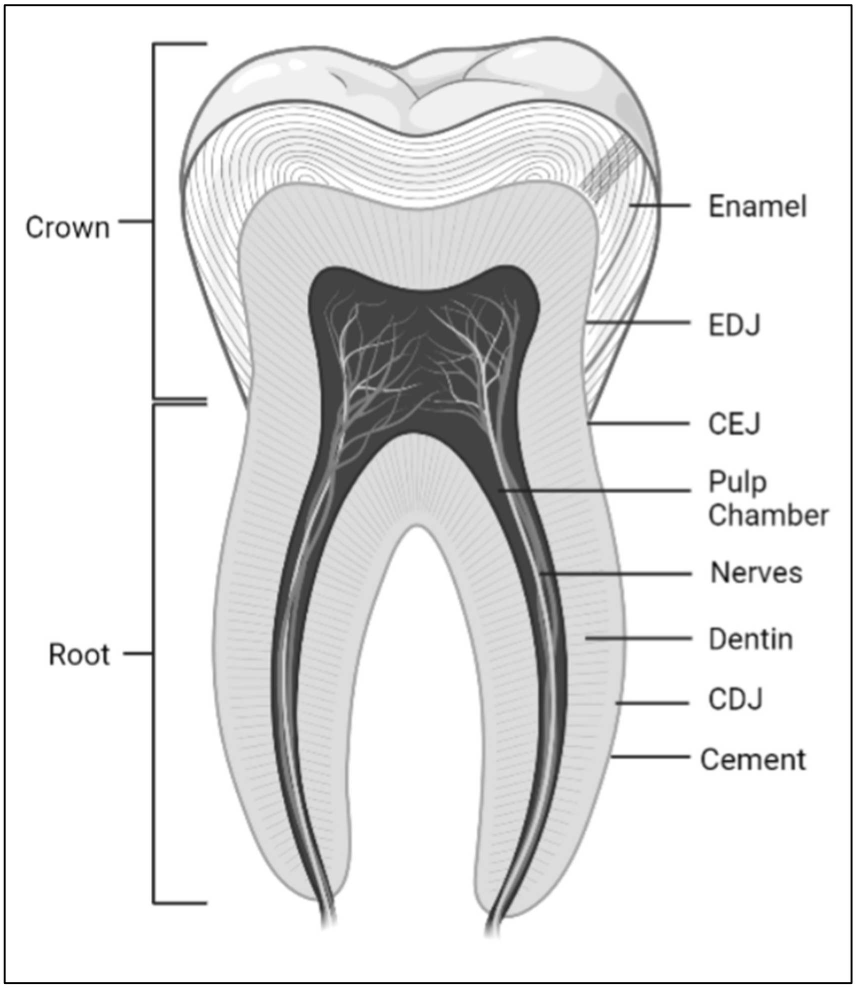


Figure 3.2. Structure of a molar as seen from a bucco-lingual plane. Figure created in BioRender. EDJ: enamel-dentin junction; CEJ: cement-enamel junction; CDJ: cement-dentin junction.

The growth of teeth allows them to record changes during development following a specific timeline. Tooth development follows a specific pattern starting in utero for deciduous incisors and first molars, with the first layers of dentin deposited by odontoblasts quickly followed by the formation of enamel by specialised cells called ameloblasts. The layers of dentin continue to be deposited by odontoblasts from the inside of the tooth until only the pulp chamber remains. Once the crown is completed the tooth erupts and the root pursues its formation. Cementum growth is initiated at the beginning of root formation by cells known as cementoblasts. Contrarily to dentin and enamel, layers continue to form throughout an individual's life and remodelling is possible in damaged areas (Hillson, 1996). Eventually, the deciduous dentition is replaced by the permanent one. The permanent dentition begins forming 3 to 4 months after birth with eruption beginning around 5-8 years of age with the first molars and incisors, followed by canines, premolars and second molars between 9.5 and 12.5 years (Hillson, 2013) (Figure 3.3). Third molar formation is less predictable. Their formation is usually completed during the late teens or early twenties, but the initiation of formation varies between 7 and 13 years of age (Hillson, 2013). A few standards charts have been developed (e.g. AlQahtani *et al.,* 2010; Kasper *et al.*, 2009; Mincer *et al.*, 1993) over the years to use third molars for age estimation despite the wide developmental window.

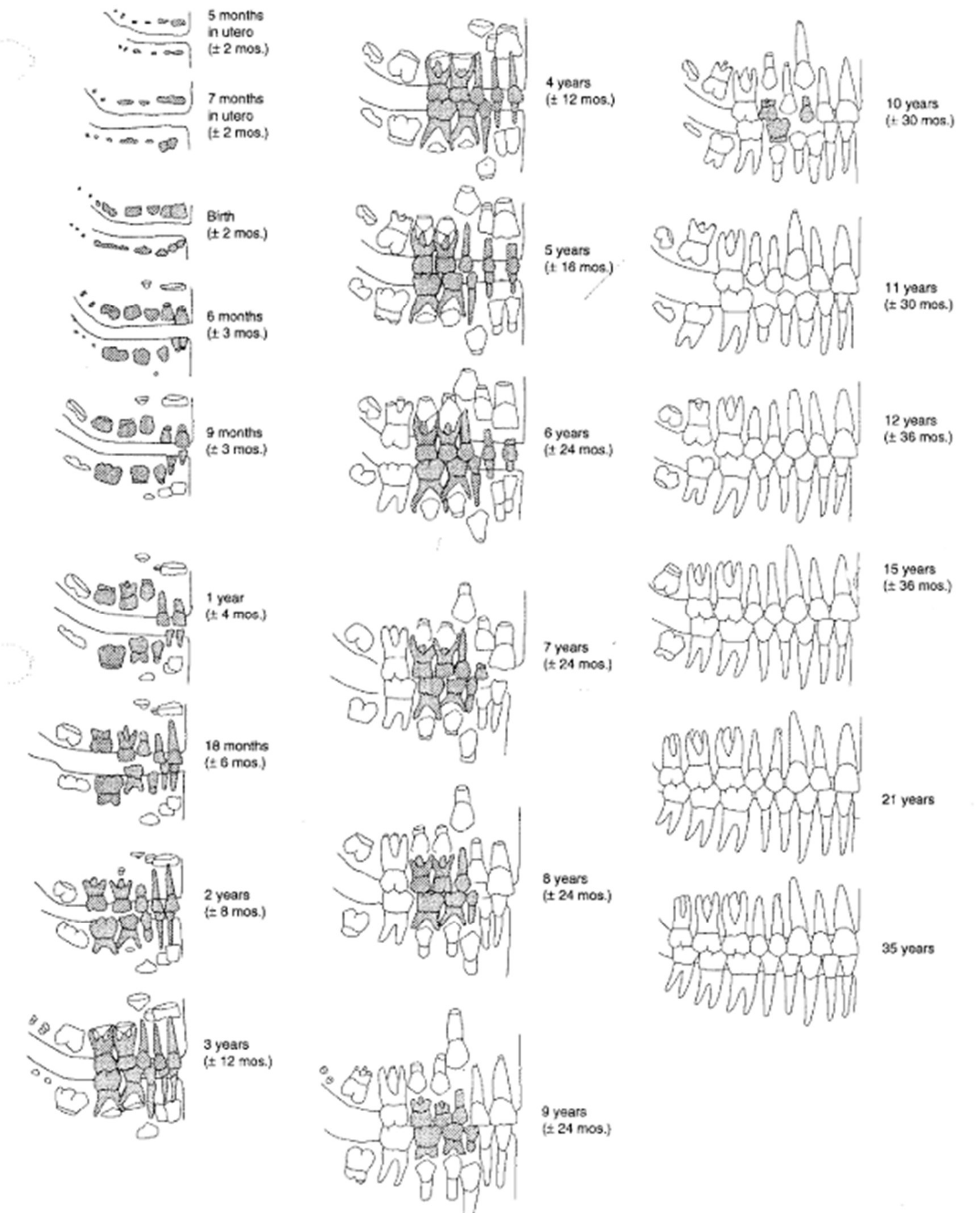


Figure 3.3. Charts of dental eruption after Figure 24 from Buikstra and Ubelaker (1994: 51).

The exact age of development tends to be population specific (Hillson, 2013). Gaither (2004) built a chart on Andean population anchoring the timeline on premolars' eruptions. She noticed some difference between European and Peruvian population with the latter showing more advanced eruption and formation by as much as 1.5-2 years relative to the Ubelaker's model (Gaither, 2004). However, Nelson used the Ubelaker's charts derived from European and North American populations for his bioarchaeological analysis to establish individual ages based on dental development (Mackey & Nelson, 2021). Considering the difference reported by Gaither (2004), a slight overestimation of age in subadults is likely present in the Farfán population.

3.2 Carious Lesions

Caries refers to the destruction of the enamel, dentine, and cementum due to acidity. Bacteria found in dental plaque, mostly of the streptococcus family, ferment carbohydrates releasing acidic products which alter dental tissues (Cottrell, 1996). It is recognized macroscopically by discolouration and the cavitation (indentation) it creates in the tooth as seen in Figure 3.4. Bioarchaeologists usually do not score caries based on only discolouration because there are many causes of staining and discolouration in the postmortem (e.g. burial) environment. If the dentin is exposed, carious lesions can lead to complications such as abscesses and antemortem tooth loss (Cottrell, 1996).

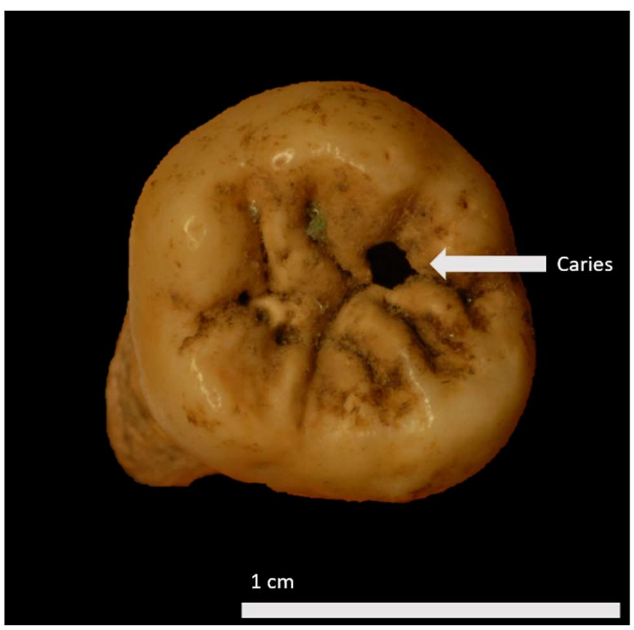


Figure 3.4. Example of occlusal caries as seen on the upper left third molar from individual J25 T8 from Farfán. Picture taken by Émy Roberge.

Three main factors can influence caries progression: pathological agents (e.g. type of bacteria), the environment (e.g. diet), and host susceptibility (e.g. tooth morphology, saliva, pregnancy) (Hillson, 1996). Teeth like molars have more irregular surfaces and are therefore more prone to caries while a diet high in carbohydrates and sugar decreases the oral pH increasing the risk of carious lesions (Cottrell, 1996).

3.3 Coca Lesions

An unusual carious lesion has been recorded in the Andes and is associated with the cultural practice of coca chewing caused by the lime used to release the alkaloids in the coca. Coca is a leaf chewed for its anaesthetic effect. Among its effects, coca chewing decreases the secretion of saliva increasing the caries rate especially at the CEJ. It is recognized by a characteristic triangular carious lesion at the CEJ associated with brown staining on the buccal surface of the molars (Indriati & Buikstra, 2001). The caries are located where the coca quid sits in the mouth with the lime inserted into it. The characteristic carious lesions represent the main feature of coca chewing, but other consequences of the practice have been noted including heavy calculus (Klepinger *et al.*, 1977), and AMTL (Langsjoen, 1996).

3.4 Abscesses

Once dentin is exposed, bacteria and their products are more likely to reach the dental pulp leading to inflammation, pulp death, and the formation of a granuloma full of pus at the apical foramen (Cottrell, 1996). If the infection is left untreated, which would be the case in pre-Columbian Peru, the accumulation of pus would initiate bone resorption to allow drainage into soft tissues to relieve the pressure (Cottrell, 1996). This tunnel can go through the bone and create a cavity exposing the apex of the root (Figure 3.5) and affecting the integrity of the socket, potentially leading to antemortem tooth loss (AMTL) or systemic infection. Dental infection was likely a leading cause of mortality in ancient societies (Clarke, 1999).

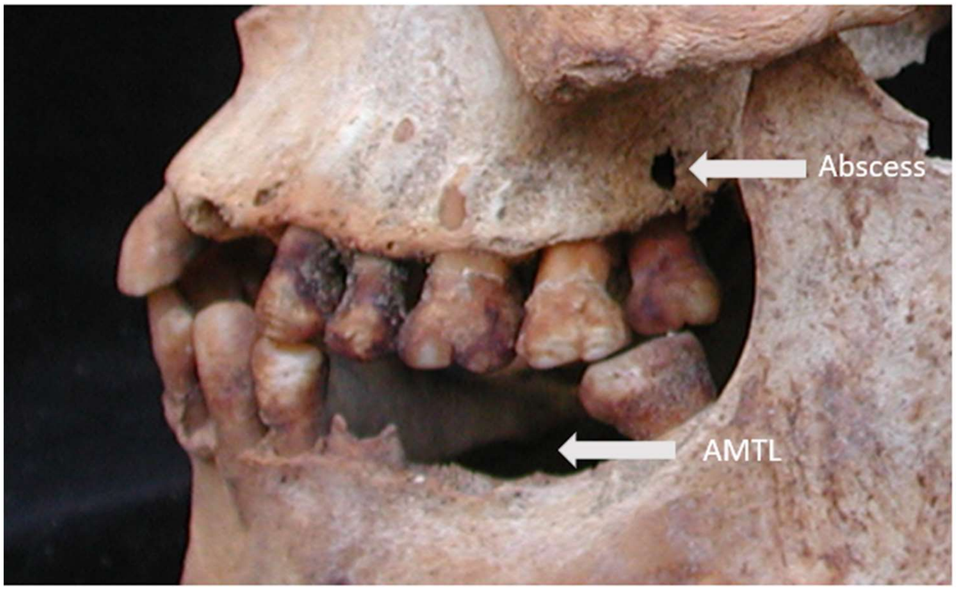


Figure 3.5. Example of abscess on the upper left third molar and AMTL on lower left second premolar to lower left second molar from individual I10 T2 from Farfán. Picture taken by Dr Andrew Nelson.

3.5 Antemortem Tooth Loss

AMTL is a consequence of advanced infections, trauma, or extreme bone loss from periodontal disease. In these circumstances, the affected tooth might be intentionally extracted to relieve pain. However, without intervention, damage to the pulp chamber and root canal force the body to exfoliate the tooth leading to involuntary AMTL (Hillson, 1996, 2008). The alveolar bone around the empty socket eventually remodels (Figure 3.5). It is important to distinguish AMTL, determined by some remodelling in the alveolar socket, from postmortem tooth loss (PMTL) as they have different implications when evaluating oral health (Hillson, 1996). While the first is associated with poor dental health, the second only affects the availability of the dental material to the osteologist.

3.6 Occlusal Wear

Wear includes tooth-to-tooth (attrition) as well as tooth-to-food (abrasion) contacts. It reflects the degree of degradation of the dental crown over time (Figure 3.6). It is mostly affected by age and diet. Older individuals and those with a coarse diet tend to have more advanced occlusal wear than younger individuals (Hillson, 1996). Wear is often evaluated in relationship with caries since the flattening of the occlusal surface decreases the possibility of carious lesion formation on irregular surfaces. However, severe wear can also lead to dentin and pulp exposure increasing the risk of carious infection in teeth (Hillson, 1996).

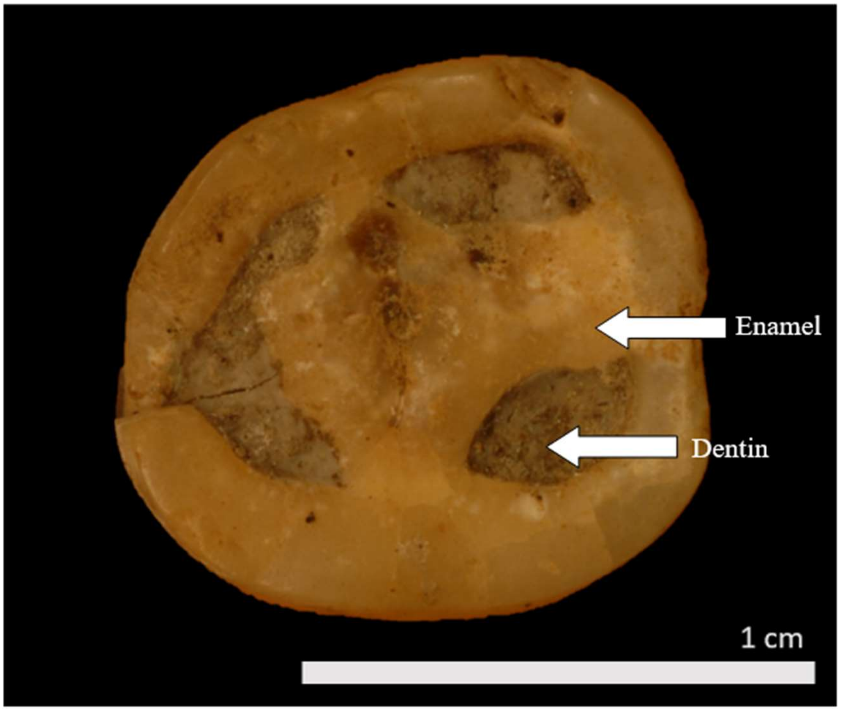


Figure 3.6. Occlusal surface showing an example of moderate occlusal wear level exposing dentin on the lower right third molar from individual J25 T6 from Farfán. Picture taken by Émy Roberge.

3.7 Enamel Defects

The aetiology of enamel defects like linear enamel hypoplasia (LEH) is nonspecific, but it has been associated with nutrient deficiencies and diseases that disrupt the enamel mineralisation process (Goodman & Rose, 1990). During tooth formation, enamel is formed (called amelogenesis) by the secretion and deposition of the enamel matrix in concentric bands (Hillson, 1996). If stress such as birth, nutritional deficiency, trauma, acute diarrheal disease, or weaning problems occur the corticosteroids released by the adrenal cortex disrupt the cells leading to permanent enamel defects that would be visible if the individual recovered (Goodman & Rose, 1990).

Enamel hypoplasia can take many forms including missing enamel, or horizontal grooves (Figure 3.7) which are recognized together as LEH (Goodman & Rose, 1990). Pits and vertical grooves can also be observed as a consequence of enamel hypoplasia (Goodman & Rose, 1990). By measuring the distance of the enamel defect from the CEJ and using a formula such as the ones established by Goodman and Rose (1990) and Cares-Henriquez and Oxenham (2019), or a growth chart like the one by Reid and Dean (2006), it is possible to estimate the age of occurrence of the physiological stress event.

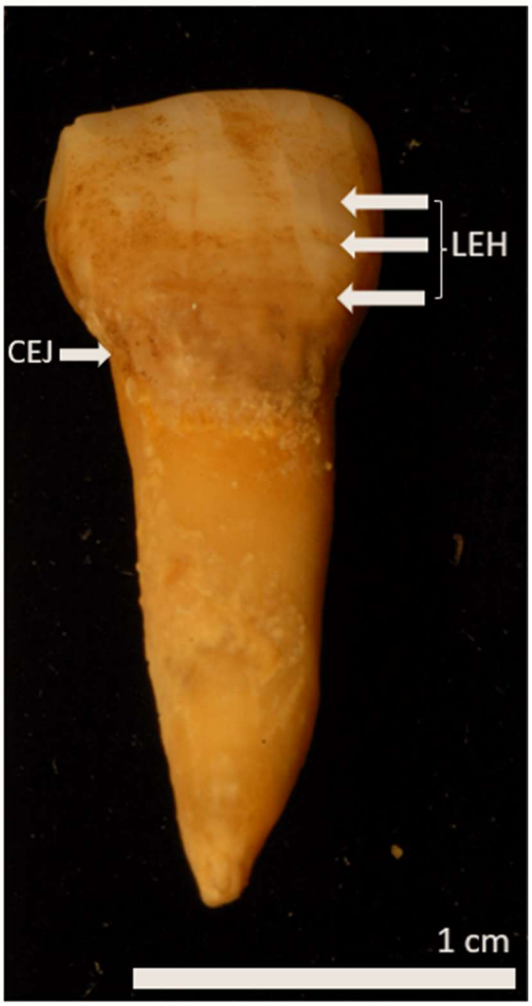


Figure 3.7. Horizontal grooves identified as LEH on the labial surface of the upper right first incisor of individual I10 T2 from Farfán. Picture taken by Émy Roberge.

LEH is one of the most commonly recorded lesions associated with systemic metabolic disruptions in dental anthropology (Hillson, 1996). However, other enamel defects can occur. Although not discussed thoroughly for this project, defects such as brown discolouration (Toyne, 2002), Wilson bands (Garland *et al.*, 2016; Lacerte, 2019), and crypt fenestration enamel defects (Thomas *et al.*, 2019) are also indicative of physiological stress and have been recorded in Andean populations.

3.8 Interglobular Dentin

Similar to LEH, interglobular dentin (IGD) is a defect that occurs in teeth during development, but focuses on dentin instead of enamel. It is recognized by small patches of unmineralised calcospherites in the dentin usually near the dentino-enamel junction (Figure 3.8) due most commonly to vitamin D deficiency (Mellanby, 1928). This defect has also been reported with low phosphate (Nikiforuk & Fraser, 1979; Murayama *et al.*, 2000) or calcium levels (Yoshiki & Yanagisawa, 1974), and in cases of fluorosis (Fejerskov *et al.*, 1979; Kuijpers *et al.*, 1996; Schour & Smith, 1934). The age of an individual during periods of vitamin D deficiency can be determined based on charts on dentin formation (Veselka *et al.*, 2019). These patches can only be recorded by examining the internal structure of teeth using histology, or micro-CT.

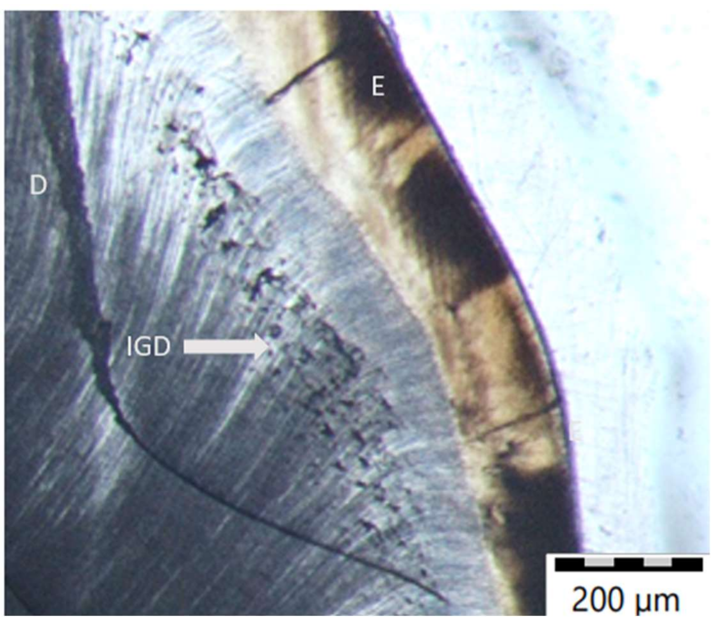


Figure 3.8. Interglobular Dentin on the buccal side of the upper right first incisor from individual E T1 from Farfán as seen under a light microscope, lens 12.5X. D refers to dentin and E refers to enamel.

Vitamin D is an important hormone for humans, yet, its deficiency is currently one of the most widespread health conditions worldwide (Holick, 2007). In bioarchaeology, vitamin D deficiency is usually recognized through inadequate bone mineralisation and deformation in the skeleton as seen in rickets and osteomalacia, which takes time to become visible (Ortner, 2003). When the bowing develops during growth period, healing can occur, erasing evidence of the deficiency. IGD allows for the recording of less severe or resorbed cases of vitamin D deficiency (Brickley *et al.*, 2020). It has not been recorded in bioarchaeological samples from the Andes so far.

3.9. Hypercementosis

In bioarchaeology, cementum has been investigated in association with age as it continuously grows over time. Cement thickness varies between 100-600 µm in adult (Cottrell, 1996). In some individuals, it is possible to observe an overproduction of cementum referred to as hypercementosis. It can be recognized by the bulbous and irregular-looking root as seen in Figure 3.9. The exact aetiology is unknown, but it can be caused by repeated micro-trauma if the tooth is loosened in the socket. To stabilize the tooth, the body responds by laying down more cement. Hypercementosis can also occur in unerupted teeth suggesting that there are other possible causes involved (Hillson, 1996).

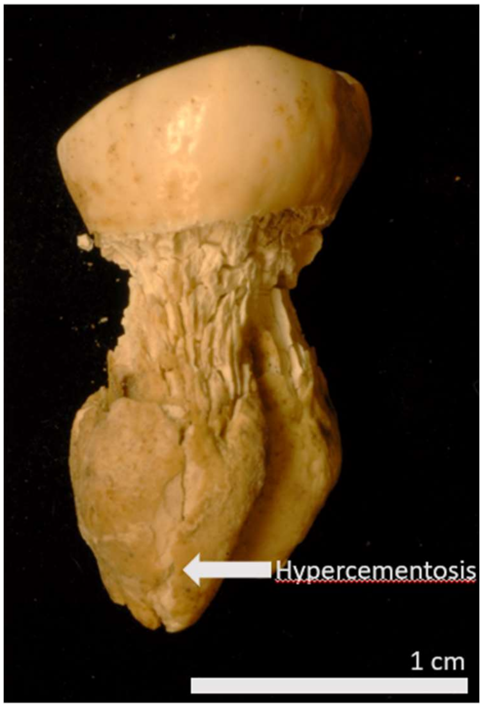


Figure 3.9. Hypercementosis observed in the upper left second molar from individual III T1 from Farfán. Picture taken by Émy Roberge.

3.10 Dental Calculus

The minerals in oral fluids precipitated forming dental plaque, which when not removed, calcifies to become calculus (Hillson, 1996) (Figure 3.10). Calculus is particularly interesting from a bioarchaeological perspective for its potential to trap and preserve microscopic plant and animal remains as well as DNA microbiomes permitting analysis of an individual's diet (Gagnon, 2019). In bioarchaeology, the focus on calculus is often toward its association with diet (Lieverse, 1999). While calculus is not associated with a specific diet, a high consumption of protein tends to lead to a more alkaline oral pH than carbohydrate leading to more calculus. Indeed, the composition of saliva and an oral environment alkaline (pH >7), favouring the precipitation of minerals, are the main factors contributing to the formation of dental calculus (Cottrell, 1996). Other factors can affect calculus growth including cultural practices such as chewing which increases saliva flow, and coca leaf chewing which increases the saliva flow and, the combination with lime (calcium oxide) introduces more minerals in the oral environment promoting lculus formation (Klepinger *et al.*, 1977). Although dental calculus is not a direct pathological condition, the bacteria accumulated can lead to periodontitis, caries, and other health issues due to the bacteria accumulated in the plaque.

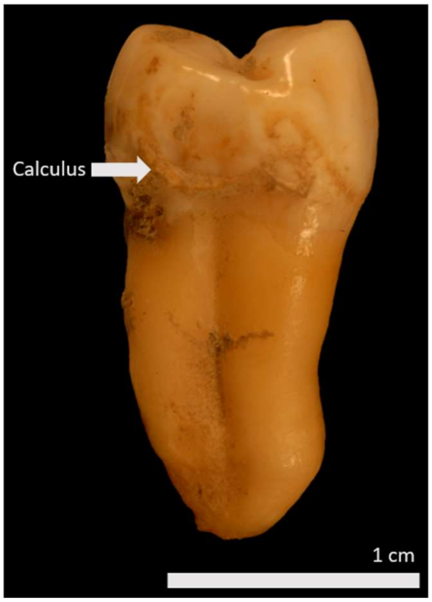


Figure 3.10. Slight calculus on the mesial side of the upper left second premolar of individual CH 1b from Farfán. Picture taken by Émy Roberge.

3.11 Conclusion

Teeth represent a fascinating material to investigate past populations in bioarchaeology. Since the dental tissues do not remodel throughout life, it allows a recording of the diet, physiological stress, environment, and cultural factors faced by an individual. Carious lesions, dental calculus, and wear increase with age and thus yield information about the years before death. Due to their direct interaction with the environment, pathological lesions on teeth reveal precious information about an individual's lifeways. For example, carious lesions are often associated with a diet high in carbohydrates like maize (Hillson, 1996); cervical-root caries on the buccal side of molars are associated with coca-leaf chewing (Indriati & Buikstra, 2001); advanced wear may reflect a coarse diet or an activity such as grinding maize (Hillson, 2003); dental calculus may suggest a diet rich in protein (Hillson, 1996, 2003; Kelley & Larsen, 1991). Defects occurring during development like LEH and IGD provide information on childhood stress from the prenatal period to early adolescence (Hillson, 1996).

Chapter 4 - Dental Health in Peru during the Late Intermediate Period, Late Horizon, and Colonial Period (ca. 1000-1780 A.D.)

The Andean regions have faced multiple changes of polity such as the Lambayeque, the Incas, and the Spanish (Figure 4.1). Each had its own dynamic affecting the population's social structure, diet, and distribution of resources. Thus, each culture can induce variation in its population's health in different ways. For example, a higher rate of dental lesions was recorded in the Morrópe population after the Spanish Conquest compared to Inca times which correlates with an increase in population density and greater consumption of carbohydrates (Klaus, 2008).

<table>
<tr><th>Time Scale</th><th>Period/ Horizon</th><th colspan="2">North Coast</th><th>North Highland</th><th colspan="2">Central Coast</th><th>Central Highland</th><th>South Coast</th><th>South Highland</th></tr>
<tr><td>1532</td><td>Colonial</td><td colspan="2">Colonial</td><td>Colonial</td><td colspan="2">Colonial</td><td>Colonial</td><td>Colonial</td><td>Colonial</td></tr>
<tr><td>1450</td><td>Late Horizon</td><td colspan="2">Inca</td><td>Inca</td><td colspan="2">Inca</td><td>Inca</td><td>Inca</td><td>Inca</td></tr>
<tr><td>1350</td><td rowspan="4">Late Intermediate Period</td><td rowspan="2">Chimu</td><td></td><td rowspan="6"></td><td rowspan="2">Chancay</td><td rowspan="7">Lima</td><td rowspan="3"></td><td rowspan="5">Ica</td><td rowspan="3">Kilke</td></tr>
<tr><td>1250</td><td rowspan="4">Lambayeque/ Sican</td></tr>
<tr><td>1150</td><td rowspan="3"></td><td rowspan="3"></td></tr>
<tr><td>1000</td><td rowspan="4">Wari</td><td rowspan="15"></td></tr>
<tr><td>800</td><td rowspan="2">Middle Horizon</td></tr>
<tr><td>600</td><td colspan="2" rowspan="4">Moche</td><td rowspan="2">Wari</td><td></td></tr>
<tr><td>400</td><td rowspan="3">Early Intermediate Period</td><td rowspan="3">Recuay</td><td rowspan="3">Nazca</td></tr>
<tr><td>200</td><td colspan="2" rowspan="11"></td><td rowspan="2">Huarpa</td></tr>
<tr><td>AD/ BC</td></tr>
<tr><td>200</td><td rowspan="2">Early Horizon</td><td colspan="2">Galllinazo</td><td rowspan="2">Chavin</td><td rowspan="9"></td><td rowspan="3">Paracas</td></tr>
<tr><td>400</td><td colspan="2">Salinar</td></tr>
<tr><td>600</td><td rowspan="3">Initial Period</td><td colspan="2">Cupisnique</td><td rowspan="7"></td></tr>
<tr><td>800</td><td colspan="2" rowspan="6"></td><td rowspan="6"></td></tr>
<tr><td>1000</td></tr>
<tr><td>2000</td><td rowspan="2">Preceramic</td></tr>
<tr><td>3000</td></tr>
<tr><td>4000</td><td rowspan="2">Lithic Period</td></tr>
<tr><td>10000</td></tr>
</table>

Figure 4.1. Andean chronology after D'Altroy (2015) and Moseley (2001).

Only a few researchers have explored Andean populations through their teeth. Among all the accessible studies on dental anthropology focusing on dental health in Peru, 25 were associated with the Late Intermediate Period (LIP, ca. 1000-1470 A.D.), the Late Horizon (ca. 1470-1532 A.D.), and the beginning of the Colonial Period (ca. 1532-1780 A.D.). Most of these studies, using macroscopic observations, record only a few pathological lesions following Buikstra and Ubelaker's (1994) standard. A few studies have gone beyond the traditional method by using fluorescent magnifier light (Klaus & Tam, 2010; Turner, 2015) or scanning electron microscope (SEM) (Lacerte, 2019). Ortiz *et al.* (2016) used a multi-method approach using radiography, computed tomography (CT), and SEM for analysis, but their sample includes only two individuals.

This chapter will present a summary of the socio-cultural context and the variability in pathological lesions among the dental research accessible between the LIP and the Colonial Period in Peru. The exploration of the periods before and after the Late Horizon contributes to understanding the context surrounding the dental material from Farfán. The articles include archaeological sites from different regions of Peru and represent multiple cultures. Among them, 15 articles have data derived from the LIP, 13 from the Late Horizon, and three from the Colonial Period. The dental lesions discussed include caries including coca chewing lesions, abscesses, antemortem tooth loss (AMTL), dental wear, enamel defects with a focus on linear enamel hypoplasia (LEH), and dental calculus.

4.1 Late Intermediate Period (ca. 1000-1470 A.D.)

The rise of the Lambayeque (ca. 1000-1300 A.D.) followed by the Chimú civilization (ca. 1300-1470 A.D.) initiated social and economic changes on the Peruvian North Coast. A new religious system focusing on the worships of ancestors instead of temple gods contributed to restructuring the society. The *ayllu*, or family unit, became the main social structure. Elite members were buried with more valued items (e.g. metals, spondylus, and sacrifices) than commoners. The status difference also affected resource distribution (Quilter, 2014). Elite members had prioritized access to high-valued food such as camelid meat, chili peppers, maize, and coca while commoners relied mostly on wild plants and marine proteins for their diet (Gumerman, 2002). This disparity among social strata might also be reflected in their dental health. The development of irrigation canals and agricultural fields played an important role during the LIP. Resources and valued items were traded through the road systems consolidated under the Chimú by increasing connectivity between different communities (Quilter, 2014). The LIP is characterized by the presence of various regional identities. In addition to the Lambayeque and Chimú polities, many distinct regional cultures were present such as the Chancay (producing

Chancay-style ceramics and textiles) and Ychsma (known for their ceramics) on the Central Coast, the Chincha who were merchants and the Ica on the South coast. LIP was a period of changes characterised by increased interactions between ethnicities in the Andes (Quilter, 2014).

Among the articles found on dental health, 11 were associated with the LIP. They include Lambayeque polity in La Leche Valley (Bader, 2012; Shimada *et al.* 2004), Yschma from Rinconada Alta (Lacerte, 2019); Moche polity from the Chapel of San Pedro de Morrópe (Klaus & Tam, 2009; 2010), Tiwanaku and Esquina from Tumilaca la Chimba (Lowman *et al.*, 2019), Chancay from Los Pinos (Lanfranco & Eggers, 2010) and Pasamayo (Okumura, 2014), Ica population in Southern Peru (Elzay *et al.*, 1977), Inca from Laguna de Los Condores (Pezo-Lanfranco, *et al.*, 2017), and unspecified pre-hispanic cultural groups from Los Pinos on the Central Coast (Pezo-Lanfranco *et al.*, 2017), Cuzco in the Southern Highlands (Ortiz *et al.*, 2016), and 4 sites on the Southern Coast (Indriati & Buikstra, 2001).

4.2 Late Horizon (ca. 1470-1532 A.D.)

The Late Horizon is associated with the prosperous period of the Inca Empire. While Inca influences started as early as 1400 A.D. in the southern highlands of Peru, the Inca Conquest reached the North Coast around 1470 A.D. (Quilter, 2014). Despite the trend to unify the four parts of *Tawantinsuyu* under the predominant Inca culture, more than 200 separate ethnic groups have been identified (Kolata, 2013). Indeed, many regional ethnicities from the LIP remained present as the Inca incorporated, local elements of many of the conquered cultures instead of imposing an entirely new system.

In continuity from the LIP, the LH was a time of flourishing expansion in the Andes, with temples and fortresses of stones, an improved road system, and important agricultural expansion with hydraulic structures including canals and aqueduct systems (Kolata, 2013; Quilter, 2014). Food, especially maize and coca, was intrinsically associated with wealth. Ranks were also important under the Incas. Chroniclers reported a highly structured society with numerous divisions within each community in addition to the social separation between regions (Quilter, 2014). In addition, to commoners and nobles, the Inca created specialist categories of servants such as the *aqlla*, highly valued, and the *yanakunas*, people from lower status who served as labourers and servants (D'Altroy, 2015). It is unclear how it affected their access to resources.

Most of the 13 articles during the LH consider Inca ethnicity with research at Machu Picchu (Turner, 2008, 2015; Turner & Armelagos, 2012; Verano, 2003), Ica department (Elzay *et al.*, 1977), Puruchuco-Huaquerones (Murphy, 2004; Murphy & Boza, 2012; Williams & Murphy, 2013), Rinconada Alta (Salter-Pedersen, 2011), Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017), and Túcume (Toyne, 2002). The other articles on the Chachapoya (Tran, 2016) and Ychsma from Maranga (Boza Cuadros, 2010) were under Inca influence as well.

4.3 Colonial Period (ca. 1532-1780 A.D.)

The Spanish Conquest, in 1532 A.D. represented a turning point in Andean history with the collapse of the Inca Empire and the reconfiguration of the political and social structures. The following decades were marked by disease, starvation, and war (Quilter, 2014). While inequalities were present before the European arrival, the unequal access to land and impoverished diets were novel (Cueller, 2013). The impoverishment might have been enhanced by the transition in agriculture from a focus on maize toward one on sugar cane. While some colonial policies such as the eradication of *chicha* production and consumption failed, others such as the sanctioning of private ownership of lands for indigenous elites enhanced pressure on indigenous communities (Cueller, 2013).While the impacts of colonialism are important, it is not the main focus of investigation and therefore it will only be briefly covered with three research articles on the North Coast from the Chapel of San Pedro de Morrópe (Klaus & Tam, 2010), Morrópe (Thomas *et al.*, 2019), and a site at Eten in the Lambayeque Valley (Garland *et al.*, 2016).

4.4 Inconsistencies in Methodology

As seen in Table 4.1 summarizing the dental pathological lesions recorded, one of the main challenges encountered when comparing dental studies is the unit of analysis. For some, the evaluation of dental health is broad and qualitative such as in Verano (2003) who acknowledged the presence of pathological lesions but did not specify the number or type of teeth affected. In other studies, focusing on dental material, the results are more quantitative. Data can be presented as the average value in the population (Lacerte, 2019), the prevalence per teeth available (e.g. Pezo-Lanfranco & Eggers, 2010), or more commonly, per individual (e.g. Lowman *et al.*, 2019). Each unit presents advantages and inconveniences important to consider. This inconsistency represents a challenge for inter-site comparison, and it can also create a bias in the analysis. The prevalence per individual can be overestimated or underestimated in small sample sizes and may not represent a population. For example, 80% of individuals were identified as coca chewers at Algodonal during the LIP (800-1350 A.D.) (Indriati & Buikstra, 2001), but this population included only five individuals, therefore, the coca chewers might be overrepresented. Comparatively, analysis per tooth is biased by the number of teeth remaining in their socket. Many teeth can be lost antemortem or postmortem which could lead to an underestimation of the number of lesions in an individual as highlighted in Thomas *et al.* (2019) who observed enamel defects on a sample of 119 canines from 63 individuals. While it was not possible to unify the unit of analysis due to the methodological inconsistencies, the following section aims to compare the results from all studies for each dental pathological lesion.

Table 4.1. Dental pathological lesions in Peru between the Late Intermediate Period and the Colonial Period (1000-1780 A.D.).

Sites	Region	Period	Year	Culture/ Ethnicity	Sample Size	Abscesses	Wear	AMTL	Caries	Coca	Calculus	LEH	Method	Sources
Algodonal	Southern coast	LIP	800 - 1350 AD	-	5	uncommon	slight to moderate	-	54.5% of teeth	80.0% of ind.	slight to moderate	-	M	Indriati & Buikstra 2001
Chen-Chen	Southern coast	LIP	800 - 1350 AD	-	46	uncommon	slight to moderate	-	42.1% of teeth	37.0% of ind.	slight to moderate	-	M	Indriati & Buikstra 2001
Chiribaya Alta	Southern coast	LIP	800 - 1350 AD	-	26	uncommon	slight to moderate	-	45.3% of teeth	42.3% of ind.	slight to moderate	-	M	Indriati & Buikstra 2001
Yaral	Southern coast	LIP	800 - 1350 AD	-	9	uncommon	slight to moderate	-	30.1% of teeth	55.6% of ind.	slight to moderate	-	M	Indriati & Buikstra 2001
Laguna de Los Condores	Northern Highlands	LIP	800 - 1470 AD	Inca	55	43.6% of ind.	moderate	11.66% teeth	30.6% of teeth; 66.6% of ind.	60.0% of adults	2.12 ± 0.65 mm	-	M	Pezo-Lanfranco *et al.* 2017
La Leche Valley	North Coast	LIP	900 - 1100 AD	Lambayeque	50	-	slight	-	9% of teeth	-	-	0.7 episode/ anterior teeth	M	Shimada *et al.* 2004
La Leche Valley	Northwestern	LIP	900 - 1100 AD	Lambayeque	4	1.8% of socket	-	25% of ind.	21.7% of posterior teeth	-	-	75% of ind.	M	Bader 2012
Tumilaca la Chimba	Southern	LIP	950 - 1250 AD	Tiwanaka	20	35% of ind.	-	20% of ind.	5% of ind.	-	-	0% of ind.	M	Lowman *et al.* 2019
Los Pinos	Central Coast	LIP	1000 - 1470 AD	Chancay	196	-	moderate	11.5% teeth	22.1% of teeth	-	-	-	M	Lanfranco & Eggers, 2010
Los Pinos	Central Coast	LIP	1200 - 1300 AD	-	200	56% of ind.	moderate	11.6% teeth	22.2% of teeth; 61.5% of ind.	41% of adults	2.37 ± 0.80 mm	-	M	Pezo-Lanfranco *et al.* 2017
Pasamayo	Central Coast	LIP	1200 - 1450 AD	Chancay	78	4.9% of sockets; 26.9% of ind.	-	15.5% of sockets; 50% of ind.	16.1% of teeth; 17.9% of ind.	-	-	-	M	Okumura 2014

Site	Region	Period	Year	Culture/ Ethnicity	Sample Size	Abscesses	Wear	AMTL	Caries	Coca	Calculus	LEH	Method	Sources
Ica department	Southern	LIP	1200 - 1450 AD	Ica	9	-	moderate	25% of teeth	4.2 caries per ind.	-	moderate 42% ; heavy 29%	-	M	Elzay *et al.* 1977
Tumilaca la Chimba	Southern	LIP	1250 -1476 AD	Estuquiña	23	0% of ind.	-	9% of ind.	19% of ind.	-	-	17% of ind.	M	Lowman *et al.* 2019
Rinconada Alta	Central Coast	LIP/ LH	900 - 1532 AD	Yschma	26	1.4 ± 1.8 abscesses per ind.	-	3.1 ± 4.7 teeth per ind.	1.8 ± 1.7 caries per ind.	-	-	96.1% of ind.	SEM	Lacerte 2019
Armatambo	Central Coast	LIP	1300 - 1470 AD	-	25	48.0% of ind.	moderate	26.7% teeth	20.5% of teeth; 50% of ind.	61.1% of adults	1.98 ± 0.69 mm	-	M	Pezo-Lanfranco *et al.* 2017
Chapel of San Pedro de Morrope	North Coast	LIP/ LH	900 - 1532 AD	Mochica	203	1.3% of teeth	slight to moderate	7.7% of teeth	9.7% of teeth	-	12.9% of teeth	-	M	Klaus & Tam 2010
Cuzco	Southern Higlands	LIP/ LH	1400 - 1532 AD	pre-Hispanic	2	10.9% of sockets; 100% of ind.	moderate to advance d	13% of teeth; 50% of ind.	16% of teeth; 100% of ind.	-	-	-	Rad., CT, SEM	Ortiz *et al.*, 2016
Machu Picchu	Southern Higlands	LH	1438 - 1532 AD	Inca	70	9.5% of teeth	moderate	52% of ind.; 17.7% of teeth	16% of ind.	-	-	-	M	Turner 2015
Machu Picchu	Southern Higlands	LH	1450 - 1532 AD	Inca	74	-	-	71% of ind.	-	-	-	71% of anterior teeth	M	Turner & Armelagos 2012
Ica	Southern	LH	1450 - 1532 AD	Inca	16	-	moderate	11% of teeth	0.56 caries per ind.	-	slight 88%; moderate 12%	-	M	Elzay *et al.* 1977
Puruchuco-Huaquerones	Central Coast	LH	1450 - 1535 AD	Inca	207	7.1% of teeth; 39.6% of ind.	-	15% of sockets; 71.3% of adults	12.7% of teeth; 54.7% ind.	16.9% of adults	-	52.6% of ind.	M	Murphy, 2004

Site	Region	Period	Year	Culture/ Ethnicity	Sample Size	Abscesses	Wear	AMTL	Caries	Coca	Calculus	LEH	Method	Sources
Maranga	Central Coast	LH	1470 - 1532 AD	Ychsma under Inca influences	15	-	-	33.3% of ind.	53.3% of ind.	-	-	20% of ind.	M	Boza Cuadros 2010
Puruchuco-Huaquerones	Central Coast	LH	1470 - 1532 AD	Inca	90	5.9% of sockets; 57.8% of ind.	-	13.3% of teeth; 73.3% of ind.	15.6% of teeth; 84.4% of ind.	-	-	-	M	Williams & Murphy 2013
Rinconada Alta	Central Coast	LH	1470 - 1532 AD	Inca	116	10.3% of teeth; 47.5% of ind.	-	16.9% of socket; 67.3% of ind.	27.6% of teeth; 76.2% of ind.	5.9% of ind.	-	76.0% of ind.	M	Salter-Pedersen 2011
Tucume	North Coast	LH	1470 - 1532 AD	Inca	21	52.4% of ind.	slight	1.8% teeth per ind.	8.3% of teeth, 63% of ind.	0% of ind.	slight to moderate	72.7% of ind.	M	Toyne 2002
Laguna de los Condores	Northern Highlands	LH	1470 - 1532 AD	-	23	39.1% of ind.	moderate	20.9% teeth	30.2% of teeth; 66.6% of ind.	31.6% of adults	1.99 ± 0.82 mm	-	M	Pezo-Lanfranco *et al.* 2017
Machu Picchu	Southern Higlands	LH	1470 - 1532 AD	Inca	74	52% of ind.	slight to moderate	71% of ind.	66% of ind.	-	-	71% of anterior teeth	M	Turner 2008
Machu Picchu	Southern Higlands	LH	1470 - 1532 AD	Inca	180	3.9% of ind.	-	11.7% of ind.	5% of ind.	-	-	2.2% of ind.	M	Verano 2003
Kuelap, Chachapoyas	North Coast	LH	1470 - 1535 AD	Chachapoya	106	58 sockets; 39.4% of ind.	slight to moderate	23% of teeth	15.9% of teeth	1.1% of adults	22% of teeth	-	M	Tran 2016
Morrope	North Coast	Colonial	1532 - 1750 AD	Muchik	63	-	-	-	-	-	-	-	M	Thomas *et al.* 2019
La Capilla Santa Maria Magdalena de Eten	North Coast	Colonial	1625 - 1760 AD	Muchik	15	-	-	-	-	-	-	-	M	Garland *et al.* 2016

Method: M, macroscopy; rad., radiography

4.5 Carious Lesions

The high consumption of maize makes caries one of the dental pathological lesions the most investigated in Peru. Carious lesions were reported in 21 out of 25 studies allowing observation of the evolution of caries over time and regions in the country.

During the LIP, the caries frequency is quite variable in Peru. Research has documented 31.2% of individuals on the southern coast (Indriati & Buikstra, 2001) with at least one carious lesion. The frequency varies between 17.9% of individuals from Pasamayo (Okumura, 2014) and 61.5% of individuals at Los Pinos (Pezo-Lanfranco *et al.*, 2017) on the Central Coast. It continues to increase in the North, where carious lesions were present in 66.6% of individuals from Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017) and in 100% of individuals in the La Leche Valley (Bader, 2012). The percentage of the population was not reported in Morrópe, but at least one caries was present in 9.7% of teeth from the site (Klaus & Tam, 2010).

During the Late Horizon, carious lesions seemed slightly more frequent. At Machu Picchu, caries was documented in 66.0% of individuals (Turner, 2008) while on the Central Coast, it varies from 33.3% of individuals from Maranga (Boza Cuadros, 2010) to 76.2% of individuals from Rinconada Alta (Salter-Pedersen, 2011). On the North Coast, it was reported in 63.0% of individuals from Túcume (Toyne, 2002). The prevalence among the population was not always available as seen in Table 4.1. The frequency on teeth varies around 10.0-15.0% of teeth are affected on the Central Coast and North Coast (Murphy, 2004; Murphy & Boza, 2012; Toyne, 2002; Tran, 2016) supporting the trends toward more carious lesions during LH compared to LIP. This elevated incidence is probably related to an increase in carbohydrate-rich diets.

Little is known about the impact of the Spanish contact on the caries rate in populations. Only one study from Klaus and Tam (2010) reported a difference in caries rates in post-contact populations at Morrópe. A higher prevalence of caries was found in post-contact group compared to the pre-hispanic population.

While there is a slight trend toward an increase in caries rate through time, it is difficult to distinguish major changes in people's lifeways between periods. There is a lot of variability between, as well as within, populations based on geography and socio-demographic profile. For example, despite the expectation of a higher prevalence of carious lesions in high-status individuals due to premium access to maize, the impact of social status is unclear. Murphy (2004) did not record a significant difference in caries rate between individuals based on their social status, but Shimada *et al.* (2004) recorded a lower caries frequency in high-status individuals. Most studies do not acknowledge the difference in social status between individuals.

4.6 Coca Lesions

The peculiar carious lesion associated with the cultural practice of coca chewing is reported in seven articles. Coca leaf chewers were identified in a broad range varying between 37.0% and 80.0% of the adults on five different sites on the South Coast during LIP (Indriati & Buikstra, 2001). The frequency was within this range in the northern highlands with 60.0% of adults at Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017) and on the Central Coast of Peru with, respectively, 41.0% and 61.1% of adults at Los Pinos and Armatambo (Pezo-Lanfranco *et al.*, 2017).

Coca lesions occur at lower frequencies in sites following the Inca Conquest suggesting a decrease in the cultural practice under the Inca. They were identified in 16.9% of adults at Puruchuco-Huaquerones (Murphy, 2004) and 5.9% of individuals at Rinconada Alta (Salter-Pedersen, 2011) on the Central Coast. The northern regions had the highest prevalence with 31.6% of adults from Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017), but also the lowest with 1.1% of adults from Chachapoyas (Tran, 2016), and absent in individuals from Túcume (Toyne, 2002). This disparity might be the result of a difference in cultural practices in the highlands compared with coastal regions. Coca was grown and prepared in the *chaupiyunga* region, in the highlands, by specialized workers before being trade (Julien, 1998) which could explain the differential access favouring the highlands over the coastal regions. No comments were found regarding the social status of these individuals. For both periods, no significant difference was recorded between the sexes (Indriati & Buikstra, 2001; Murphy, 2004). As expected, like carious lesions, the lesion was more prevalent in older individuals (Indriati & Buikstra, 2001).

4.7 Abscesses

The number of abscesses is reported as the prevalence among the population or per socket making the comparison between studies challenging. Overall, following carious lesions, the presence of this pathological lesion is quite variable in Peru during all periods. For the LIP, at Tumilaca la Chimba, abscesses were present in 35.0% of individuals from early LIP (ca. 950-1250 A.D.), but in 0% of individuals during late LIP (ca. 1250-1470 A.D.) (Lowman *et al.*, 2019). The reason for this lower prevalence on the southern coast is unknown but considering the caries rate which is also low (< 20% of the population) it might be associated with some cultural or environmental factors leading to fewer dental infection. In this situation, the reduction in abscesses is secondary to a reduction in carious lesions. Abscesses were also considered as "uncommon" on the southern coast in Indriati and Buikstra (2001). In the North, the prevalence of abscess increased with at least one abscess present in 43.6% of individuals from Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017) and 42.9% of individuals from La Leche Valley (Bader, 2012). Rates are similar on the central coast. In Pasamayo, Los Pinos, and Armatambo, respectively 26.9% (Okumura, 2014), 56.0%, and 48.0% (Pezo-Lanfranco *et al.*, 2017) of individuals had at least one abscess.

Prevalence tends to be higher during the Late Horizon with 52.0% of individuals at Machu Picchu (Turner, 2008), 39.6% of individuals from Puruchuco-Huaquerones (Murphy, 2004), and 47.5% of individuals from Rinconada Alta (Salter-Pedersen, 2011). Northern sites show a slight decrease with 39.1% of individuals from Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017), 39.4% of individuals from Chachapoyas (Tran, 2016), and 52.4% of the individuals from Túcume (Toyne, 2002). These results are consistent with an increase in the prevalence of carious lesions.

The frequency of abscesses usually increased with age (Williams & Murphy, 2013) but the oldest age group does not always have the highest number of abscesses. No specific age difference has been reported in the Andes, but Salter-Pedersen *et al.* (2011) recorded the highest number of abscesses in middle-aged adults (68.5% of individuals). Toyne (2002) recorded more abscesses on average in late adolescent females (1.7 abscesses per individual) than the other females from the *Huaca* Larga. No significant differences in prevalence were found between social status or sexes (Murphy, 2004), but this pathology seems to be slightly more frequent in males (Okumura, 2014; Williams & Armelagos, 2013). Interestingly, the abscesses in the highland populations are mostly related to caries while in the coast population they were more frequently associated with dental wear (Pezo-Lanfranco *et al.*, 2017) supporting a more abrasive diet due to sand and grit.

4.8 Antemortem Tooth Loss

AMTL is encountered in most of the populations in the Andes (see Table 4.1). During the LIP, in the South, 25.0% of teeth were lost at sites in the Ica Department (Elzay *et al.*, 1977), and 13.0% of teeth from Cuzco (Ortiz *et al.*, 2016). Similar values were documented on the coasts, with 11.6% of teeth from Los Pinos (Pezo-Lanfranco *et al.*, 2017), 15.5% of teeth from Pasayamo (Okumura, 2014), 7.7% of teeth from Morrópe (Klaus & Tam, 2010); and in the northern highlands with 11.6% of teeth from Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017).

During the Late Horizon, there is a tendency toward more AMTL than during LIP which agrees with the increase in carious lesions and abscesses. It was present in 17.7% of teeth from Machu Picchu (Turner, 2015). An increased rate was also observed in the North with 20.9% of teeth loss antemortem at Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017) and 23.0% of teeth from Chachapoyas (Tran, 2016). Although, at Ica, the AMTL prevalence decreased to 11% of teeth (Elzay *et al.*, 1977). On the Central Coast sites, it represents 15% of teeth (Murphy, 2004) at Puruchuco-Huaquerones, and 16.9% of teeth from Rinconada Alta (Salter-Pedersen, 2011).

No clear trends were found in post-contact populations except a slight decrease in AMTL post-contact when compared with pre-Hispanic populations (Klaus & Tam, 2010).

Overall, the rate of tooth loss between social status or sexes is not statistically significant (Murphy, 2004; Tran, 2016), but a higher AMTL rate was recorded in females from

Maranga (Boza Cuadros, 2010) and Rinconada Alta (Lacerte, 2019; Salter-Pedersen, 2011). At Puruchuco-Huaquerones more males than females presented AMTL (Murphy & Boza, 2012) but females had more AMTL per individual (Williams & Armelagos, 2013). Thus, this observation might support the assumption of more caries in females.

4.9 Occlusal Wear

In Peru, dental wear is usually documented qualitatively following the standardised scale provided by Buikstra and Ubelaker (1994). For the sites during LIP, wear level is described, on average, as slight in La Leche Valley (Shimada *et al.*, 2004), slight to moderate on the southern coast (Indriati & Buikstra, 2001) and Morrópe (Klaus & Tam, 2010), moderate in Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017), Armatambo (Pezo-Lanfranco *et al.*, 2017), Ica region (Elzay *et al.*, 1977), and Los Pinos (Lanfranco & Eggers, 2010; Pezo-Lanfranco *et al.*, 2017). A higher level, moderate to advanced, was only recorded in Cuzco (Ortiz *et al.*, 2016). During the Late Horizon, the description is similar with a slight level at Túcume (Toyne, 2002), slight to moderate in Machu Picchu (Turner, 2008) and Chachapoyas (Tran, 2016), and moderate in Machu Picchu (Turner, 2015), Ica department (Elzay *et al.*, 1977), and Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017).

No clear difference in occlusal wear levels occurred throughout periods, but pre-Hispanic populations tend to show slightly more severe wear levels than populations from the Colonial Period (Klaus & Tam, 2010). Geographically, coastal populations leaned toward a slightly more severe wear level than the highlands due to sand and grit accidentally incorporated in their food (Pezo-Lanfranco *et al.*, 2017). No significant difference has been noticed between sexes in Shimada *et al.* (2004) and Turner (2015) but their samples include a larger number of females. The only strong association recorded is with older individuals expressing more advanced wear (Klaus & Tam, 2010; Toyne, 2002; Tran, 2016) suggesting that wear levels are age-related in the Andes.

4.10 Enamel Defects

Episodes of malnutrition and disease during development documented through enamel defects like LEH are also commonly mentioned in dental anthropology reports in Peru.

LEH was reported in 13 articles. Similar to the other dental pathological lesions discussed, the prevalence of LEH is quite variable in the Andes.

During LIP, at Tumilaca la Chimba, LEH was not identified in any individuals in a Tiwanaku population (ca. 950-1250 A.D.) and only in 17.0% of individuals from Estuquina culture (ca. 1250-1470 A.D.) (Lowman *et al.*, 2019). In comparison, in the Lambayeque Valley, Klaus and Tam (2009) recorded LEH in 63.9% of individuals while it was present in 96.1% of the Yschma subsample population from Rinconada Alta (Lacerte, 2019), and 75.0% of individuals from La Leche Valley (Bader, 2012).

This high variation in LEH frequency continued during the Late Horizon. In the Southern Highlands, it was recorded in only 2.2% of individuals at Machu Picchu (Verano, 2003), but Verano (2003) did not focus on dental material in his bioarchaeological analysis. Other analyses reported a much higher frequency of LEH on teeth (Turner, 2008; Turner & Armelagos, 2012). On the Central Coast, at Maranga, LEH was recorded in 20.0% of individuals from Ychsma culture (Boza Cuadros, 2010); a prevalence lower than in other Inca populations on the Central Coast with 52.6% of individuals at Puruchuco-Huaquerones (Murphy, 2004) and 76.0% of individuals from Rinconada Alta (Salter-Pedersen, 2011). The prevalence of LEH at Rinconada Alta is similar to the one at Túcume where it was present in 72.7% of individuals (Toyne, 2002).

LEH was not extensively reported in articles on populations during the Colonial Period. Although the exact prevalence was not presented, a significant decrease in LEH was reported in post-colonial populations most likely due to an increase in childhood mortality (Klaus & Tam, 2009).

There was no significant difference between males and females in terms of LEH expression at Rinconada Alta (Lacerte, 2019; Salter-Pedersen, 2011) suggesting both genders were treated similarly during their childhood. The impact of social status is unclear as most studies do not clearly distinguish commoners from elites, but Shimada *et al.* (2004) noticed a higher frequency of LEH in lower social status individuals in the La Leche Valley.

4.11 Dental Calculus

Dental calculus is only reported in seven articles, although not uniformly with some articles recording the presence, the levels or the thickness as seen in Table 4.1. The level of dental calculus does not vary drastically across the Andes through time. It is usually described as being slight to moderate in populations. However, there seems to be regional variation with changes of polity suggesting differential access to protein consumption.

During the LIP dental calculus was reported as slight to moderate in Southern and Central Coast populations (Indriati & Buikstra, 2001; Pezo-Lanfranco *et al.*, 2017). Calculus was considered as more extensive in the Ica Department with 42% of individuals exhibiting a moderate level of dental calculus (< 1 mm) and 29% of individuals with a heavy level (> 1 mm) (Elsay *et al.*, 1977).

In comparison, during the Late Horizon, a decrease of dental calculus was observed in Ica population under the Inca Empire where it was reported as slight (88.0% of individuals) or moderate (12.0% of individuals) (Elzay *et al.*, 1977). This trend was also seen at Laguna de Los Condores (Pezo-Lanfranco *et al.*, 2017). On the Central Coast, 15.6% of adults exhibit dental calculus (all degree combined) (Murphy, 2004) while the North Coast, at Túcume, recorded slight to moderate dental calculus in 91.0% of the individuals with females more likely to have moderate deposit although, the sample from Túcume contains more females (3 males, 19 females) which can create a bias (Toyne, 2002).

4.12 Conclusion

Few articles have been published on dental health in Peru exploring material from the LIP, the LH, or the Colonial Period. In addition, they usually focus on a few features at the time. Toyne (2002) is the only study found that investigate abscesses, AMTL, caries and coca lesions, dental calculus, and enamel defects. In general, dental health is poor or moderate in the Andes with a high frequency of caries, AMTL, abscesses, and LEH while dental calculus is usually slight; all of which reflects a diet high in carbohydrates. The archaeological populations investigated present variable prevalence of dental pathological lesions supporting the heterogeneity observed between populations and regions in Peru.

Although the differences in dental health are not pronounced between the different regions, periods, or socio-demographic profiles, general trends have been noted.

Wear-associated caries and carious lesions were more frequent in coastal populations agreeing with a more coarse and abrasive diet in this region (Pezo-Lanfranco *et al.*, 2017). However, a higher number of caries per individual were found in highland populations. Northern populations tend to exhibit more LEH and caries than southern populations probably reflecting more maize in their diet. While geographic factors are important, cultural factors can also affect the prevalence of caries. The Incas tend to exhibit more caries than other cultural groups such as the Ychsma at Maranga during the Late Horizon (Boza Cuadros, 2010) and other diverse ethnicities during the LIP.

Not many studies consider the social status of the individuals even if "most variation on oral health falls along lines of social class" (Klaus & Tam, 2010: 596). Elite members are expected to present fewer pathological lesions to support their access to a higher-quality diet which would include more proteins while commoners would rely more on carbohydrates (Klaus, 2008). Accordingly, Shimada *et al.* (2004) recorded a lower frequency of caries in higher social status individuals in the Lambayeque Valley. A significant difference was found regarding the frequency of carious lesions and abscesses among adults from different social strata, with Sicán elite burials showing slightly better health than commoners from earlier Moche culture (Bader, 2012). The health disparities between social status were less distinct than anticipated at Puruchuco-Huaquerones during Late Horizon (Murphy, 2004) and Chachapoyas (900-1535 AD) (Tran, 2016).

These previous studies rely mostly on macroscopic observations following the standards presented by Buikstra and Ubelaker (1994). Alternative methods such as micro-CT have not been used yet despite the amount of information it can provide for analysis. Lacerte (2019), Ortiz *et al.* (2016), and Tran (2016) are the only ones using microscopy or histology. Even in those studies, dentin defects like interglobular dentin (IGD) have not been noted. Thus, the current project on Farfán offers an uninvestigated perspective to bring additional information on dental health across the Andes.

Chapter 5 - Material

The Farfán Project was directed by Dr Mackey from 1999 to 2006 on the namesake site in Peru (Mackey & Nelson, 2020). In total, six rectangular compounds up to 250 meters in length containing rooms and plazas of variable sizes were excavated. Five cemeteries were uncovered: Compound VI Cemetery, Cemetery J, Mound G Cemetery, Cemetery I, and the *Huaca* Burial Platform. Despite the poor state of preservation, 98 individuals from the Late Horizon (1470-1532 A.D.) were excavated. The notes, photographs, and osteobiography of each individual were compiled by the project bioarchaeologist Dr Nelson and made available for the current project.

The osteobiography includes information such as sex, age-at-death, stature, completeness of the skeletons, cranial modification, pathology, and general observations on the dentition (Appendix B). Adult age categories were assessed based on the fusion of the medial clavicle, fusion of basisphenoid, conformation of the pubic symphysis (Brooks & Suchey, 1990; Buikstra & Ubelaker, 1994) as well as the consideration of dental decay and spinal degeneration. In subadults, dental development (Buikstra & Ubelaker, 1994; Moorrees *et al.*, 1963) and epiphyseal fusion (Buikstra & Ubelaker, 1994) were considered. Sex in adults was determined based on pelvic morphology (Buikstra & Ubelaker, 1994; Phenice, 1969) and cranial morphology (Buikstra & Ubelaker, 1994). The median stature was estimated using long bone lengths and equations in Del Angel and Cisneros (2004) (adapted from Genovés, 1967) commonly used for Mesoamerican and South American populations (see chapter 2 in Mackey & Nelson, 2020).

This information is supplemented by isotope values (carbon, nitrogen, and oxygen) previously obtained using the facilities of the Laboratory of Stable Isotope Science at UWO (see White *et al.*, 2020) to build the contextual and environmental framework for this site. Stable carbon isotope values approximate carbohydrate consumption including maize, nitrogen isotope values evaluate protein consumption, and oxygen isotope values give insight into the individual's geographic location during teeth and bones formation. If the value is collected on bones, it provides information into the last ten years of the individual's life. On dental material, isotope values reveal information from childhood and adolescence depending on the tooth analysed. Details on isotope values can be found in Appendix C.

This chapter covers the archaeological context of Farfán, and the major bioarchaeological findings to help reconstruct the lifeways on the site. The focus is on the 68 primary burials excavated. Details on these individuals and their associated grave goods can be found in Appendix D. Additional information on all the remains and the site including illustration of the positions of burials can be found in Life, Death and Burial Practices during the Inca Occupation of Farfán on Peru's North Coast (Mackey & Nelson, 2020). This chapter also includes an overview of Túcume, the only other cemetery on the North Coast of Peru believed to include burial *aqllakuna,* which is used as a comparative sample for this project. The chapter concludes with an overview of the dental material available in the focused sample (N=45 teeth) for the multi-method analysis.

5.1 Farfán Archaeological Context

Farfán follows the valley margin for three kilometres along the mountain of Cerro Faclo on the North Coast of Peru (Figure 5.1). The site is advantageously located at the crossroads to reach Cajamarca, the northern Inca capital. Moreover, its location between the Jequetepeque River and the smaller Chamán River provides riverine resources and irrigation for agricultural plants such as corn (*Zea mays*), beans (*Phaseolus* sp.), and chili peppers (*Capsicum frutescens*) (Cutright, 2009). The region is usually characterised by a dry climate except during *El Niño* or ENSO events caused by changes from the ocean's current bringing heavy rain, floods, erosion of agricultural lands, and disruption in the marine ecosystems (Dillehay & Kolata, 2004). This prime location at the centre of trade and cultural interactions promoted the development of the site as a religious, cultural, economic, and administrative centre (Mackey & Nelson, 2020).

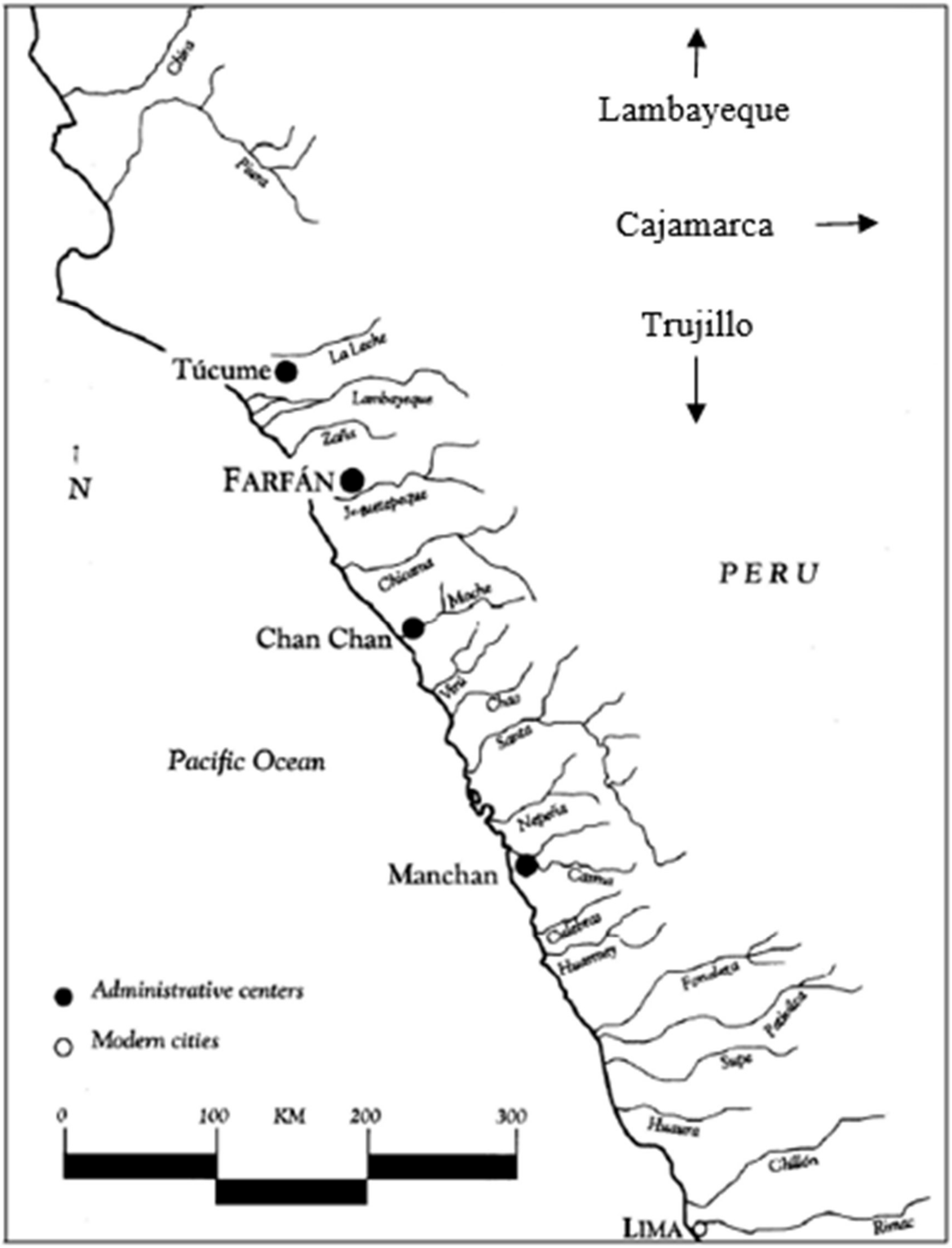


Figure 5.1. Map of the North Coast of Peru showing the location of Farfán after Mackey & Nelson (2020:16).

Farfán is considered "the largest funerary sample associated with grave goods" on the North Coast of Peru (Mackey & Nelson, 2020: 2). Along the Cerro Faclo, Compound VI Cemetery was found at the northernmost extremity of Farfán. At the centre of the site is found the *Huaca* Burial Platform. Moving toward the South are located respectively Mound G Cemetery, Cemetery I, and Cemetery J (Figure 5.2). Compound VI Cemetery, Mound G, and Cemetery I are above-ground while Cemetery J is subterranean. The *Huaca* Burial Platform was a solid adobe mound covered in sand. The site was not well preserved due to long-term looting, destruction from *El Niño*, run-off from the adjacent mountain, and the construction of the Pan-American highway. This is observable particularly in Compound VI Cemetery where the integrity of the remains has been compromised by water leaching the collagen out of the bones (Mackey & Nelson, 2020).

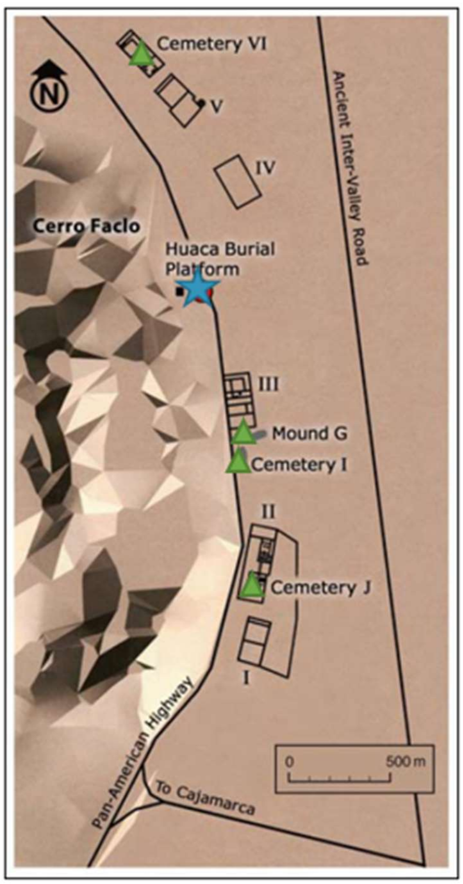


Figure 5.2. Overview of the archaeological site of Farfán showing the location of the six extant compounds after Figure 3.1 from Mackey & Nelson (2020: 87).

The remains uncovered are a biased sample, they do not include all deaths that occurred at the site. Among the 68 primary burials, more females and younger individuals were interred than expected in a population (20 females, 9 males, and 39 subadults) (Mackey & Nelson, 2020). Despite this bias, the primary burials were used to shed light on the life at Farfán during the Late Horizon. This peculiar demographic bias is best observed at the *Huaca* Burial Platform which is believed to be the cemetery of sacrificed *aqllakuna*. The other cemeteries were associated with administrators (Cemetery J, Compound VI Cemetery). Two cemeteries (Mound G and Cemetery I) included individuals affiliated with the Lambayeque ethnicity. Previous analysis suggests that there is no consistent difference between cemeteries as no significant differences have been found in terms of age distribution, spinal degeneration, trauma, population traits, or isotope values between the cemeteries. This population of administrators and *aqllakuna* seems homogeneous, but each cemetery encompassed its own characteristics (Mackey & Nelson, 2020).

5.1.1 Compound VI Cemetery

Compound VI is divided into two areas: one dedicated for food and drinks and one, on the southeast side, for the burials. The three-level cemetery contains 11 primary burials (4 males, 3 females, and 4 subadults) of individuals who probably lived and worked at the compound. They were all oriented toward the East except for I T4. The bodies, mostly in a seating position, were wrapped in shrouds before being interred with fine ceramics, jewellery, and metals. The richest tomb was an old adult female (II T2) buried on the second level with copper rings and necklaces (Mackey & Nelson, 2020).

Compound VI Cemetery includes more males than females compared to the other cemeteries at Farfán. This is not surprising considering its association with administrative duties. This cemetery also has the lowest average stature on the site but exhibits low childhood stress, low level of trauma, and the lowest incidence of infectious disease. There is no evidence of sacrifice, and therefore these individuals probably died of natural causes. They represent a fairly homogeneous group in terms of diet and activity (Mackey & Nelson, 2020).

Since the collagen has leached from the bones, stable carbon and nitrogen isotope values are only available in a neonate (III T3). Oxygen isotope values from bones were collected in eight individuals. The Compound VI Cemetery had the individuals with the highest oxygen isotopes values suggesting that they spent time in a coastal region other than the Jequetepeque valley (White *et al.*, 2020).

5.1.2 Cemetery J

Cemetery J, the other cemetery associated with administrative duties, is on the opposite side of the site, at the southern end. It contains the interments of eight adults (5 females, 3 males), and nine subadults for a total of 17 burials separated in three main areas. The biggest area, J25 (N=9), on the west side, is built on three levels while J14 (N=3), on the north, and J26 (N=4) on the east are both on two levels. The additional female, JE19 T1 was not included in the monography but is considered here in the focused sample. Individuals were interred in a seated position with their legs crossed and knees apart facing toward different cardinal points. No obvious osteological differences were found to separate the three areas. All individuals were interred with offerings. Infants and children were generally buried with a piece of copper and shell beads while vessels were found with adults. Weaving material was only found with the woman J25 T9.

Individuals from Cemetery J exhibit multiple childhood stress episodes (LEH) and a very high rate of dental pathology. Based on their stature, they had relatively good health and nutrition during their adolescence. Signs of trauma were recorded on many individuals suggesting moderately active lifestyles. Degenerative changes of the spine including vertebral collapse and age-related osteoporosis were present in eight individuals.

Based on oxygen isotope values all the individuals in this cemetery lived in the Jequetepeque Valley for at least the last ten years before death. Two individuals (J25 T6 and J25 T9) most likely spent time elsewhere during their childhood as evidenced by their dental values. The stable carbon and nitrogen isotope values present a wide variation within the cemetery with J25 T6 having a high proportion of maize and marine protein in his diet (highest C level on the site) while other individuals had a lower proportion of maize and protein consumptions with J25 T9 having the lowest $\delta^{15}N$ value on the site.

5.1.3 Mound G Cemetery

Mound G Cemetery is the smallest cemetery at Farfán (N=7). It contains some individuals affiliated with the Lambayeque ethnicity as suggested by the presence of cranial modification Form C. Forms B and D were also expressed, but in opposition to other cemeteries Form A (non-modified) was not present (see chapter 2 in Mackey & Nelson, 2020 for details). One of the main particularities is the unusual configuration of the cemetery where six individuals (2 females, 4 subadults) are positioned around an elderly high-status female suffering from congenital hip dysplasia consistent with cerebral palsy (G T11). She had the most personal grave goods in Mound G Cemetery including algarroba wood poles, vessels, rings, silver beads, gourds, llama feet, guinea pig bones and weaving materials. In comparison, the middle-aged female buried at the entrance of the cemetery (G T3a) was interred with only a few vessels, undecorated stone spindle whorls, copper beads, a piece of copper, and camelid textile.

These individuals expressed a low level of childhood stress and overall good dental health. The three adult females exhibit some spinal pathology with minor degenerative changes in the lower spine. This cemetery also has the highest prevalence of infectious disease in Farfán with six of these individuals with periostitis. The only trauma reported were healed stress fractures on the fibula in the female with cerebral palsy (G T11).

Isotope analysis was done on two of the three females in Mound G. Oxygen isotope values suggest that G T11 was local while G T4 grew up locally but spent some time in a different coastal area during the last ten years of her life. Based on stable carbon and nitrogen isotope values, G T4 had a diet low in maize and a fairly typical level of protein intake while G T11 had a fairly typical maize consumption combined with an elevated level of protein consumption.

5.1.4 Cemetery I

In total five primary burials (1 female, 1 male, and 3 subadults) separated into two sectors were excavated in Cemetery I. This cemetery includes mostly single interments except for I3 T1a/ b which include an infant buried with an adult male. Two ethnic affiliations can be identified based on cranial modifications. Sector A associated with individuals of

Inca ethnicity (cranial modification Form F) had grave goods such as vessels, gourds bowls, spondylus shells, weaving material, and cooking ollas. Sector B includes individuals displaying Lambayeque ethnic affiliation (cranial modification Form C) buried with vessels, llama parts, and copper spindle whorls.

These individuals were not in good health. Similar to Compound VI Cemetery, they present conflicting characteristics. They had the highest average stature and lowest prevalence of spinal pathology, but the highest prevalence of LEH and dental pathological lesions. They also had evidence of trauma and infection.

Oxygen isotope values for individuals from Sector A associated all of them with the Jequetepeque Valley while I10 T3 in Sector B had higher bone and enamel values indicating time spent in another coastal region. Stable carbon and nitrogen isotope values, only available in bones from I3 T1a, suggest a diet significant in maize and protein intake likely from marine sources.

5.1.5 *Huaca* Burial Platform

At the base of the Cerro Faclo was located the biggest and most interesting cemetery of Farfán: the *Huaca* Burial Platform. It contained 46 interments including 27 primary burials distributed among four tombs identified as North Tomb 1, East Tomb, Center Tomb 2 and Center Tomb 11, and two burial chambers (Chamber 1 and Chamber 2). All the primary burials with recognizable sexual dimorphism were identified as females except for one male (CH 1a). The subadults were also most likely females based on their associated grave goods. The bodies were positioned and interred directly on the tomb floors in single interments with a non-random distribution of age. Considering the setting and population bias in terms of age and sex these females were most likely sacrificed (Eeckhout & Owens, 2008) even if no cut marks or broken hyoids were documented. They were buried with many grave goods including weaving equipment and quantities of finely finished textiles supporting the assumption that they were sacrificed *aqllakuna*.

5.1.5.1 North Tomb 1

In North Tomb 1 located on the north side of the platform, three individuals (2 female and 1 infant) were covered by a unique textile atop a woven reed mat. The older female (N T1b) was in a seated position facing North with the infant laying on her legs while the adolescent (N T1a), also in a seating position, was facing West. The presence of many grave goods, cinnabar on their face, and three complete llamas with age parallel to those of humans support the assumption that this was a rich tomb.

The social status of these individuals did not shield them from pathology. The infant N T1c has evidence of blood-borne infection, the adult N T1b has vertebral lesions most likely associated with Pott's spine or spinal tuberculosis, and the adolescent N T1a demonstrates spondylolysis. Isotope values suggested that the three individuals were from the Jequetepeque Valley and had a diet relying highly on both maize and protein.

5.1.5.2 East Tomb

The biggest tomb (N=7) identified as the East Platform (4 m X 1.5 m X 1.5 m) was divided into three levels located on the southeast side of the *Huaca* Burial Platform. The lower level counted five remains including two adult females. They were excavated with large textile bundles, ceramic vessels, and plates. Among these individuals, was found the seemingly highest-status individual interred at Farfán during the Late Horizon based on her offerings. This old female (E T7) was probably a *mamakuna* based on the quantity and quality of her grave goods. Among her grave goods was found an ear spool representing quarter moons similar to the one found at the site of Túcume (Heyerdahl *et al.*, 1995) and a needle case of deerskin reminiscent of the one made of reed found in the Cemetery of the Sacrificed Women at Pachacamac (Tiballi, 2010). One adolescent was found on each of the upper levels. The adolescent on the second level (E T4) was interred with the most ceramic vessels and weaving swords of algarroba wood. She also had a small wooden figurine of a female kneeling which might be a representation of *Zaramama*, the mother of maize (MacCormack, 1991). She was facing East while all the other females were facing North. Personal grave goods were interred with all females, but weaving implements were only found with the two adults (E T5 and E T7) and the adolescent E T4.

These individuals exhibit some health issues with evidence of periostitis possibly associated with a systemic disease like scurvy was recorded in three juveniles (E T6, E T9, and E T10) and pathological remodelling of the left femoral head and neck of E T5. They mostly had good spinal health although E T1 potentially had Scheuermann's kyphosis, E T5 had Schmorl's nodes, and degenerative changes were noted on E T7. Their dental health was mostly poor but slightly better in the adolescents E T1 and E T4.

Oxygen isotope values suggest that these women originated from different regions throughout Peru but stayed in the Jequetepeque Valley during their adulthood. They grew up locally (E T1, E T4, E T7, and E T9), in the highlands (E T6 and E T5), and in other coastal regions (E T10). A very high value in nitrogen was also recorded in E T7 while E T6 presented the lowest value at Farfán.

5.1.5.3 Central Tomb 2

The upper part of the central tomb (C T2) contains multiple interments between 6 to 30 years of age with numerous communal offerings (see Appendix D for details). The burials are separated by spondylus shells with adolescent and young adults on the east side and juveniles on the west side. These individuals were in relatively good health evidenced by the absence of signs of infection or spinal degeneration other than a butterfly vertebra in young adult (C T2C). Due to water leaching, oxygen isotope values could not be analysed. Stable carbon and nitrogen isotopes were only done on two individuals both suggesting a diet high in maize and in marine proteins (Mackey & Nelson, 2020).

5.1.5.4 Center Tomb 11

Center Tomb 11 (C T11), located below Center tomb C T2, contains only four burials each oriented toward a cardinal point. This tomb had a higher number of personal and communal grave goods than the tomb above. Interestingly, the hair of two of these females (C T11C and C T11D) was covered in mud before being wrapped in shrouds suggesting ceremonial interments. *Chicha* was poured on the bundles which accelerated their destruction.

No evidence of infectious disease was documented. They mostly had poor or very poor dental health except for the adolescent (C T11C) who is considered with very good dental health. She, however, has bowing of fibula (associated with vitamin D, dysplasia, or congenital) and a distortion of the spine indicating scoliosis and an angular kyphosis. In adults (C T11A/ B), age-related osteoporosis on the spine and degeneration of joints around the sternum (C T11D) were found. Their isotope values suggest they were from various regions and had a diet relying on maize and marine proteins.

5.1.5.5 Looted Chambers

On the west side, two looted primary burial chambers and their grave goods were excavated. The first chamber (CH) included looted primary burials of seven individuals interred with ceramics, fragments of metal and copper discs, and camelids and bird bones. None of these individuals presents evidence of infection. The only primary burial male (CH 1a) interred at the *Huaca* Burial Platform shows signs of osteoporosis and collapse of lumbar vertebrae. He presents characteristics reminiscent of the *Dos Cabezas* giants (Donnan, 2001) thought to be *eunuchs* (pre-pubertal castration). The second chamber is a small chamber with multiple niches and llama remains. The chamber also includes four secondary burials on the exterior of the east wall and grave goods such as fired ceramic vessels and small unfired cups, jars, copper ornament, and copper rings.

5.1.6 Summary

Overall, each cemetery contributes to the reconstruction of the history of Farfán and its population. As seen above, a lot of information is available regarding the individuals whose teeth are investigated in detail with this research. Five females were identified as being particularly high-status based on their grave goods (G T4, G T11, E T4, E T7, and N T1b). They were all from the Jequetepeque region, had some health issues, and had a typical diet. Teeth for detailed analysis for this study were available only from the three females from the *Huaca* Burial Platform (E T4, E T7, N T1b).

Despite the assumptions from the Spanish Chronicles regarding the *aqlla*, the *Huaca* Burial Platform presents the greatest variability in any cemetery from Farfán regarding ages, statures, childhood stress, cranial modifications, health, and isotope values, adding to their mystery. All these females lived in the Jequetepeque Valley toward the last years of their lives, but they originated from various regions including local area, the highlands, and other coastal sites. No specific pattern was found in their diet. Their stable carbon and nitrogen values range from the lowest and highest end of the spectrum found at Farfán. This cemetery includes juveniles and many adolescents and adults. At least one of these adults (E T7) was identified as a *mamakuna* as suggested by her high-status and grave goods. A high variability, challenging the description from the Spanish Chronicles, was also found in the *aqlla* from Túcume.

5.2 Comparative Sample: Túcume

5.2.1 Archaeological Context

The only other *aqlla* site with bioarchaeological data on the North Coast of Peru is Túcume in La Leche Valley which offers an inter-site comparison to the *aqlla* at Farfán. The coastal climate, like Farfán, is mainly dry but encounters wet seasons during *El Niño* (Heyerdahl *et al.*, 1995). The site of almost 220 ha was excavated between 1990 and 1992 through the Túcume Archaeological Project. Túcume has a long and complex history with evidence from the Lambayeque, Chimú, Inca, and Colonial periods which are documented through the architecture and ceramics (Heyerdahl *et al.*, 1995). Among the different structures excavated, the Stone Temple, on the northeast side of the largest human-made pyramid *Huaca* Larga (700 m X 280 m X 30 m) was associated with religious practices. Within the temple high-status female burials were found with weaving and spinning paraphernalia who could represent an *aqlla* community (Narváez, 1995).

5.2.2 The Stone Temple

The Stone Temple contained four chambers called *Recintos*. *Recinto* One had three high-status males in mummy bundles. *Recinto* Three contained 19 high-status females, which were possibly *aqllakuna,* including one in a mummy bundle. *Recintos* Two and Three contained many offerings such as human (secondary burials from the pre-Inca period) and camelid remains while *Recintos* One and Four were filled with ceramic sherds, camelid bones, and *crisoles* (finger pots, often unfired). The site was altered by repetitive rainfall associated with *El Niños* and the destruction of the Stone Temple which was covered with a special fill and burned probably following the arrival of Spaniards (ca. 1532-1540 A.D.) (Narváez, 1995).

The 22 remains were originally buried in a seated and flexed position but some slumped forward due to taphonomic processes (Baitzel, 2019). The two middle-aged males exhibit evidence of healed minor trauma. One of them might have been the last Inca governor in Túcume. He was wrapped in decorated layers of textiles with multiple signs of status including valuable metal artefacts, jewels, gourds, spoons, pots, and painted textiles. The other middle-aged and the young adult had few artefacts with little or no offerings recorded around them (for details, see Narváez, 1995: 93-96). All three males had evidence of multiple childhood stress episodes and relatively good dental health. The dental material from the Stone Temple was studied by Toyne (2002).

The 19 females of various ages (1 juvenile, 3 adolescent, 12 young adults, 3 middle-aged adults) were buried within five group pits. They were buried with many weaving artefacts found in reed baskets, pottery (Inca and Chimú), personal adornments, and offerings reflecting a high-status (Narváez, 1995). In opposition to other *aqlla* sites, no evidence of *chicha* brewing vessels was found in the Stone Temple. Five females had evidence of physical trauma, and all of them presented at least one sign of childhood stress (harris line, cribra orbitalia, porotic hyperostosis, or enamel defects). A high rate of dental pathological lesions was reported including numerous cases of carious lesions, periodontal disease, dental wear, and antemortem tooth loss (Toyne, 2002) (Appendix E).

Based on the single interment event, the ceremonial importance of the site, the high status of the remains, and the bias in population demographic, these females are thought to have been *aqllakuna*. Similar to the general observations of Farfán, their bioarchaeological data do not fit the description offered by the Spanish Chronicles. Their age range is older than what is expected in newly recruited *aqllakuna* (8-12 years old). Moreover, they exhibit many stress markers and some healed trauma contrasting with the high and "honoured virgins" status *aqllakuna* were believed to have had.

5.3 Tooth Sample

To deepen the information from the osteobiographies, in this study, a detailed analysis of teeth from Farfán is performed. Samples of anterior and posterior teeth from primary burials and complete secondary burials were collected, when available, and brought back to Western University. Due to time constraints, only teeth sampled from primary burials were considered for the detailed dental analysis. The focused sample includes 45 teeth from 32 primary burials. More specifically, it includes 20 teeth from 12 individuals from the *Huaca* Burial Platform (*aqlla* cemetery), seven teeth from Compound VI Cemetery, one tooth from Mound G Cemetery, three teeth from Cemetery I, and 14 teeth from nine individuals from Cemetery J. The sample is composed mainly of molars (N=25) and incisors (N=17), but also includes two canines and one premolar (Table 5.1). Among the 12 subadults and 20 adults considered, five are males while 19 have been identified as females. The eight individuals remaining were too young to determine their sex but are presumed to be females based on the context and their associated artefacts. Thus, the focused sample supports the sexual bias observed at Farfán. In general, the teeth were relatively well preserved despite the effect of *El Niño* and looting on site except for Compound VI Cemetery which was more deteriorated possibly due to exposure to the elements. A similar observation was also made by Feilen (2004).

Table 5.1. Information from samples from Farfán available for dental observations.

Skeleton ID	Cemetery	Sex	Age	Stature Estimate (cm)	Tooth sample 1	Tooth sample 2	Isotopic Data
G T3a	G	F	MA	143.8	-	LLM1	N
I10 T2	I	F	MA	149.9	URI1	-	Y
I10 T3	I	U	CH	-	lli1	-	Y
I3 T1a	I	M	MA	157.4	-	LLM2	N
J14 T2	J	F	YA	144.7	URC	-	N
J25 T9	J	F	YA	146.0	-	LLM3	Y
J25 T2	J	U	NEO	-	uxi2	-	N
J25 T4	J	U	IN	-	lli2	llm1	N
J25 T6	J	M	MA	158.1	-	LRM3	Y
J25 T8	J	F	YA	144.6	ULI1	ULM3	N
J26 T1a	J	U	CH	-	lli1	LLM1	N
J26 T1b	J	U	JUV	-	urm2	URM1	N
JE19 T1	J	F	OA	149.4	LLI1	LLM1	N
CH 1b	Platform	F	YA	150.4	-	ULP4	N
C T11A	Platform	F	OA	140.3	URI1	URM3	Y
C T11B	Platform	F	OA	140.8	URI2	URM3	Y
C T11D	Platform	F	OA	139.3	-	LRM2	Y
E T1	Platform	F	AD	151.9	URI1	URM3	Y
E T4	Platform	F	AD	143.7	ULI1	ULM3	Y
E T5	Platform	F	MA	149.7	ULI1	ULM3	Y
E T6	Platform	U	JUV	-	URI1	URM2	Y
E T7	Platform	F	OA	151.5	LLI1	LLM3	Y
E T10	Platform	U	JUV	-	URI1	-	Y
N T1a	Platform	F	AD	141.0	ULI1	LRM3	Y
N T1b	Platform	F	OA	139.2	-	LRM3	Y
I T3	VI	M	AD	157.7	LLC	-	N
II T1	VI	F	MA	144.7	-	LRM3	N
II T2	VI	F	OA	142.6	-	LLMX	N
II T4	VI	M	MA	-	-	LLM2	N
III T1	VI	F	OA	146.0	-	ULM2	N
III T2	VI	M	MA	157.7	-	LRM1	N
III T3	VI	U	NEO	-	-	xxm1	N

For Sex column: F refers to females; M refers to males; U indicates unknown or indeterminate. Age: NEO, neonate, 0-0.9 years; IN, infant, 1-2.9 years; CH, child, 3-5.9 years; JUV, juvenile, 6-11.9 years; AD, adolescent, 12-19.9 years; YA, young adult, 20-29 years; MA, mid adult, 30-39 years; OA, old adult, 40+ years. For Tooth sample: the first letter refers to the upper (U), lower (L), or unknown (X); the second letter refers to right (R), or left (L); the third letter indicates tooth type whether incisor (I), canine (C), premolar (P), or molar (M); the digit indicates which tooth specifically based thus for one side, in an adult, will have I1, I2, C, P3, P4, M1, M2, M3; lower case letter indicates deciduous teeth. For Isotopic Data: Y indicates that isotopic information is available for this individual while N is not present.

Chapter 6 - Methodology

Many components were incorporated in this project to demystify the *aqllakuna* through their dental health while comparing multiple analytical methods. The osteobiographies filled in by Dr Nelson were compiled to compare the dental health of the individuals between the *Huaca* Burial Platform and the general population which included individuals from Cemetery I, Cemetery J, Mound G Cemetery, and Compound VI Cemetery. The methodological comparison was done on a subsample of 45 teeth identified as the focused sample. The *aqlla*'s dental health is evaluated through the number of abscesses, antemortem tooth loss, caries, wear level, calculus, and hypercementosis. The presence of physiological stress during childhood is evaluated with linear enamel hypoplasia (LEH) and interglobular dentin (IGD). Table 6.1 summarise the features of interest with each method.

Table 6.1. Features of interest in the dental analysis of individuals from Farfán.

Dental Features	Osteobiography	Macroscopy	Micro-CT	Histology	Meaning
Abscesses	X				Bacterial infection of the dental pulp
Antemortem Tooth Loss	X				Advanced infection, trauma
Caries	X	X	X	X	Carbohydrate consumption
Occlusal Wear	X	X	X	X	Food processing, extra-masticatory activities
Dental Calculus	X	X	X	X	Protein consumption, cultural practices (e.g. excessive chewing)
Linear Enamel Hypoplasia	X	X	X	X	Non-specific childhood stress
Interglobular Dentin			X	X	Dietary deficiency (Ca, vitamin D) and fluorosis
Hypercementosis	X	X	X	X	Excessive wear and malocclusion

This chapter describes further the information collected in the osteobiographies from the Farfán population as well as the three methods applied for the detailed dental analysis which are macroscopy, micro-CT, and histology. Moreover, the 45 teeth that underwent micro-CT were also scanned with a clinical CT scan at St-Joseph's hospital and three thin-sections undergone backscattered scanning electron microscopy (BSE-SEM). While the focus of the project was not on these two methods, details can respectively be found in Appendices F and G. Finally, this chapter concludes with a description of the statistical analyses and outlines the recording of the dental pathological lesions.

6.1 Osteobiographies

The information on the Farfán population available in the pre-existing osteobiographies documented by Dr Andrew Nelson was first collected (Nelson, pers. comm., 2020; Mackey & Nelson, 2020). These data include demographic variables such as sex, age-at-death, and stature. Regarding dental health, the data collected include the number of teeth present (and thus the number lost antemortem and postmortem), the number of abscesses, number of carious lesions, occlusal wear level, the number and location of LEH, the presence of dental calculus, and hypercementosis. Other dental anomalies such as peg-shaped or shovel-shaped incisors, discolouration, and enamel pearls and the presence of additional skeletal conditions, such as infection and spinal degeneration were also collected (see Appendix H). The isotope data previously analysed (White *et al.*, 2020) were included to inform on diet ($\delta^{13}C$, $\delta^{15}N$) and geographic origin ($\delta^{18}O$) of individuals. This information contributes to the individual's profile, providing a more accurate portrait of lifeways and biocultural context in Farfán than would be possible from a single tooth or even all the teeth. Thus, for hypotheses #1 to #3 (see p.7), the dental health from the osteobiographies represents the core of the comparative analysis between cemeteries.

6.2 Macroscopic Observations

Macroscopy is the most commonly used method to record dental pathological lesions as seen in Chapter 4. This method is easily accessible and requires little analytical equipment. For this project, the 45 teeth in the focused sample were observed macroscopically by the author based on Buikstra & Ubelaker's (1994) standard. A 10X magnification loupe was used when necessary. The macroscopic data included the number of carious lesions, the occlusal wear levels, the number of LEH and their distance from the cemento-enamel junction (CEJ), the presence of dental calculus, and the presence of hypercementosis as well as any other relevant observations. The data collection form can be found in Appendix I.

In addition, photographs of each tooth were taken with a Nikon D200 (10.2 megapixels) camera with a 90 mm macro lens prior to destructive analysis. Photographs were taken of the occlusal view, lingual, buccal, and mesial or distal sides with a focus on observable dental pathological lesions.

6.3 Micro-CT

Computed tomography (CT) is a non-invasive method relying on the collection of projection images of an object with X-rays to construct a 3-dimensional volume. Images are taken without destroying the material while allowing the observation of the internal structure of an object. CT is especially appreciated in research due to its simplicity, reproducibility, and preservation of the material analysed. The latter represents an important advantage compared to destructive analyses such as light microscopy or scanning electron microscopy (SEM). Images can be manipulated, sectioned, dissected, and measured (Abel *et al.*, 2012).

Clinical CT scans are more accessible as they are usually found in hospitals, however they present poor resolution for the purpose of this study (see Appendix F). The development of high-resolution technology like micro-CT scanners achieves greater resolution. This enhanced resolution comes from the smaller focal spot combined with the high-resolution detector and geometric magnification of the object onto the detector (Conlogue *et al.*, 2020). The voxel (3D pixel) size can be much thinner than the thickness achieved with histology (Davis & Wong, 1996) reaching as small as 5 µm for a tooth crown. Moreover, the rotation of the object instead of the X-ray source and detector allows the reduction of the mechanical vibrations which also improves the resolution (Luedemann, 2007). The accuracy of all measurements depends on the quality of the scanning and the virtual reconstruction (Abel *et al.*, 2012).

Micro-CT scans have been used in dental research for the past 30 years (Luedemann, 2007). However, research specifically on dental health is less common, a topic even more sparse when focusing on archaeological material. Most of the research on ancient samples is performed to identify dental pathological lesions, measure enamel thickness in relation to hominoid evolution, or investigate LEH (Table 6.2). Thus, micro-CT is a method with a lot of untapped potential to investigate teeth. However, factors such as the preservation of the material, which affects its density, can affect the quality of imaging (Villa & Lynnerup, 2012). No strict protocol is followed by researchers. Thus, micro-CT settings are variable between researchers. For the purpose of this project, our own parameters were developed to maximise the resolution of dental image quality for the Farfán sample.

Table 6.2. Voltage, current, and filter used for micro-CT settings based on the features investigated. Includes clinical and archaeological research investigated one or more of the features of interest for this project using micro-CT.

Features	Research	Tooth Types	Voltage	Current	Filter	Authors
caries	C	incisor	60 kV	133 uA	-	(Kamburoğlu *et al.*, 2008)
caries	A	molar, premolar	100 kV	100 mA	-	(Tomczyk *et al.*, 2014)
caries (dentin)	C	molar, premolar	50 kV	800 uA	-	(Özkan *et al.*, 2015)
caries (dentin/ occlusal)	C	molar	50 kV	800 uA	-	(Kamburoglu *et al.*, 2011)
caries (incipient)	A	molar	50 kV	-	2mm Al	(Arnaud *et al.*, 2017)
caries (incipient)	C	molar, premolar	50 kV	800 uA	1mm Al	(Rovaris *et al.*, 2019)
caries (occlusal)	C	molar	60 kV	60 uA	-	(Kawato *et al.*, 2009)
caries (proximal)	C	molar, premolar	100 kV	100 uA	1mm Al	(Boca *et al.*, 2017)
caries (proximal)	C	molar	100 kV	100 uA	1mm Al	(Soviero *et al.*, 2012)
caries (proximal)	C	molar, premolar	100 kV	98 mA	1mm Al	(Mitropoulos *et al.*, 2010)
caries (proximal)	C	molar, premolar	50 kV	800 uA	1mm Al	(Rovaris *et al.*, 2018)
dentin defect	C	mix	70 kV	-	0.5mm Al	(Ribeiro *et al.*, 2015)
enamel caps	A	molar	50 kV	NA	2mm Al	(Giuffra *et al.*, 2014)
enamel hypoplasia	A	mix	140 kV	120 uA	-	(Xing *et al.*, 2016)
enamel hypoplasia	A	canine, molar	80 kV	100 uA	-	(Marchewka *et al.*, 2014)
enamel thickness	A	molar	100 kV	-	Al + Cu	(Smith *et al.*, 2009)
enamel thickness, mineral density	P	mix	50 kV	160 uA	-	(Olejniczak & Grine, 2006)
interglobular dentin	A	mix	140 kV	110 uA	0.1mm Cu	(Colombo *et al.*, 2019)
interglobular dentin	A	molar, canine	100 kV	100 ua	0.1mm Cu	(Veselka *et al.*, 2019)
mineral density	C	molar	70 kV	114 mA	0.5mm Al	(He *et al.*, 2011)
mineral density	C	premolar	100 kV	100 mA	-	(Huang *et al.*, 2007)
mineral density	C	molar	89 kV	100 mA	Cu	(Neboda *et al.*, 2017)
mineral density	C	molar	100 kV	100 mA	-	(Neves *et al.*, 2015)
mineral density	C	molar	100 kV	100 mA	-	(Neves *et al.*, 2016)
mineral density	C	premolar	60 kV	120 uA	-	(Lashgari *et al.*, 2015)

Research: A= Archaeology, C= Clinical, P = Primatology

For this project, each tooth was embedded in florist foam for stability and stacked vertically in a 4.5-cm-diameter cardboard tube next to a water calibrator (Figure 6.1).

This design allowed the scanning of three teeth sequentially in program mode for a total duration of 12h30min (4h10min each). The images were captured with the software Inspect-X v.4.4. Preliminary scans indicated that the signal-to-noise ratio by frame averaging was optimal with 4-hour long scans. A homemade calibrator with a carbon fiber tube blocked with cork and filled with water was inserted in the middle for calibration. The samples were scanned with 1500 projection, 8 frames averaged per projection using a molybdenum (MO) target at 82 kV, 150 µA, or at 82 kV, 148 µA with an aluminium (Al) filter of 0.5 mm. The aluminium filter was added after the filament was changed resulting in a change in brightness calibrators requiring adjustment to keep the same overall brightness throughout (see Appendix J). A resolution of 12.5 µm was achieved for each tooth.

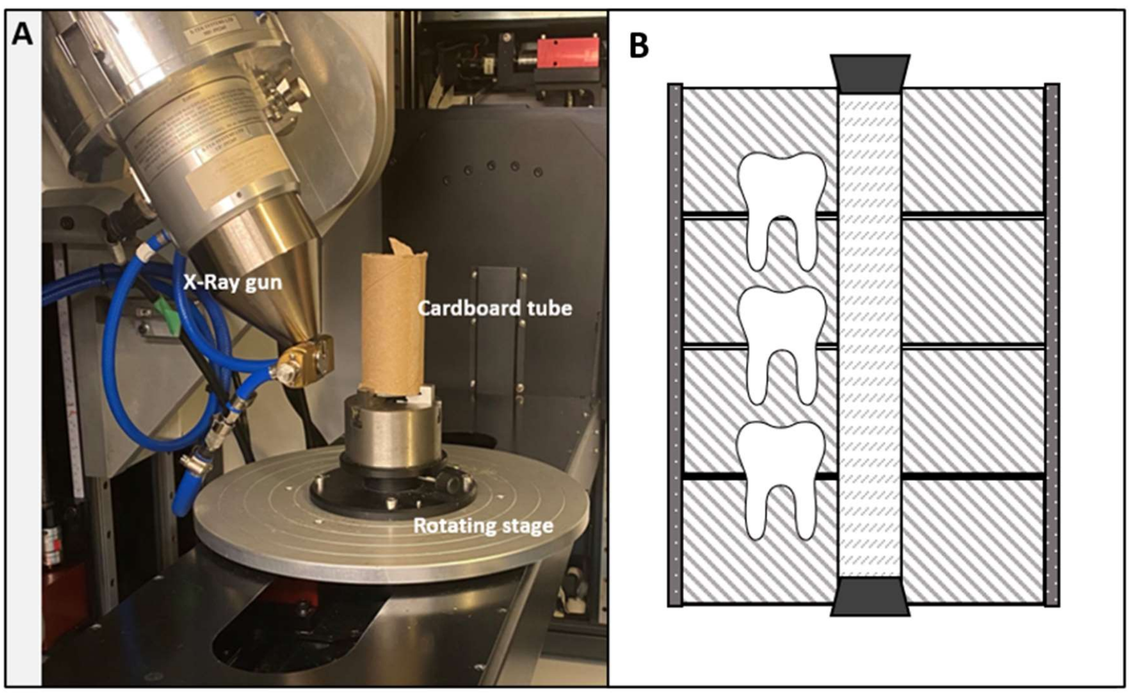


Figure 6.1. Set-up for micro-CT scan. On the left, picture within the micro-CT at the Museum of Archaeology of London (Ontario). On the right, schema illustrating the set-up in the cardboard tube (dark gray) with the water calibrator (grey wave) ending with cork (black) while the dashed rectangles represent florist foam. Picture taken by Émy Roberge.

The micro-CT and CT data were visualised using Dragonfly 2020.2 (theobjects.com), a free and user-friendly specialized software for CT imaging. Many tools are available for analysis including lookup tables used here to improve visualisation of dental pathological lesions (Appendix K). To account for the size of the micro-CT voxels (12.5 µm) and the thickness of histological thin-section (between 60-100 µm), the "slab" function have been used on two teeth (Appendix L).

6.3.1 High-Resolution Scans

After initial analysis, ten teeth suspected to have interglobular dentin (IGD) were scanned again without the calibrator to achieve a higher resolution. Those teeth were, similarly, embedded in a small plastic tube with florist foam to reduce movement (Figure 6.2). The exposure parameters used were the same as the other scans whether 82 kV, 148 µA, 0.5 Al filter. A resolution of 5.5 or 6.75 µm depending on the size of the tooth (Table 6.3) could be reached by moving the sample closer to the source without the water calibrator. These teeth were stacked vertically to run four sequential scans in program mode.

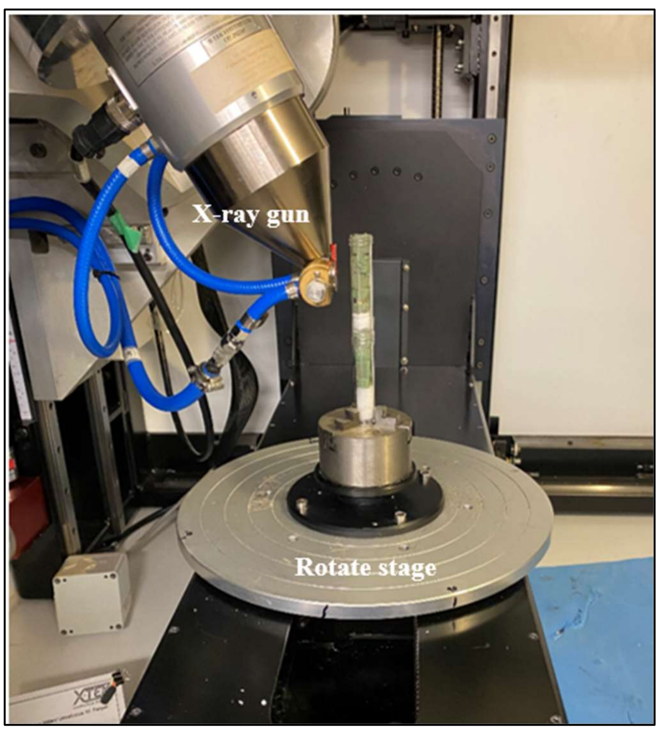


Figure 6.2. Set-up to scan four teeth in the micro-CT with a resolution of 5.5 or 6.75 µm. Picture taken by Émy Roberge.

Table 6.3. Teeth from Farfán analysed at elevated resolution with micro-CT.

ID	CH 1b	E T1	E T7	E T7	E T10	I10 T2	J14 T2	J25 T8	J26 T1a	I T3
Tooth	ULP4	URI1	LLI1	LLM3	ULI1	URI1	URC	ULI1	LLM1	LLC
Voxel size (μm)	5.5	6.75	5.5	6.75	5.5	6.75	5.5	5.5	6.75	6.75

6.3.2 Reconstruction

After scanning, the images were reconstructed using CT Pro software v.4.4. The volume reconstruction was done with a beam hardening correction of 3 and a median filter (number of pixels average together) of 3X3 before doing the calibration. The median filter was added to reduce noise with the calibrator. On a slice with a lower level of noise, a circle with a diameter of approximately 2.5 cm at the centre of the calibrator was drawn in the "Calibration" tab. The mean values for the water needed to be normalized to a mean grayscale value of 1,000. The software uses this grayscale value of 1,000 for water and a value of 0 for air to calculate a linear calibration for the grayscale values throughout. The median filter was removed before reconstructing the volume in CT Pro to maximise the level of details seen in the teeth.

6.4 Histology

Histology is a method that provides information on tissues at the cellular level, but it requires the destruction of the sample to create thin-sections for observation. Due to time constraints and poor preservation, a subsample of 15 out of 45 teeth was selected for histological analysis. The teeth were selected based on their type (anterior vs posterior), their preservation to avoid any that were very damaged, and the presence of interesting features seen with the micro-CT, with a focus on IGD. Priority was given to the individuals from the *Huaca* Burial Platform. As seen in Table 6.4, the sample included ten teeth (5 incisors, 5 molars) from the *Huaca* Burial Platform, and five teeth from the general population (2 incisors, 2 canines, 1 molar). The molar of individual E T7 was embedded and cut, but it was removed from the sample due to artefacts during histological preparation that affected the visibility of the thin-section.

Table 6.4. Samples selected for histological analysis.

Individual	Cemetery	Anterior Tooth	Posterior Tooth
C T11A	HBC	URI1	-
C T11B	HBC	URI2	-
C T11D	HBC	-	LRM2
CH 1b	HBC	-	ULP4
E T1	HBC	URI1	-
E T10	HBC	ULI1	-
E T4	HBC	-	ULM3
E T7	HBC	LLI1	LLM3
I T3	VI	LLC	-
I10 T2	I	URI1	-
J14 T2	J	URC	-
J25 T8	J	ULI1	-
J26 T1a	J	-	LLM1
N T1a	HBC	-	URM3

Cemetery: HBC = *Huaca* Burial Platform, I = Cemetery I, J = Cemetery J, VI = Compound VI Cemetery. For teeth identifications: the first letter refers to the upper (U), lower (L), or unknown (X); the second letter refers to right (R), or left (L); the third letter indicates tooth type whether incisor (I), canine (C), premolar (P), or molar (M); the digit indicates which tooth specifically

Epoxy embedding before sectioning is recommended on teeth that are not well preserved (FitzGerald, 2006). Since some teeth have already been sampled for previous analysis (e.g. isotopic analysis) and because of their variable state of preservation, all 15 teeth were embedded to ensure their integrity. Following a simplified version of the FitzGerald protocol (2006), EpoThin® resin and hardener were weighed in a disposable cup with a ratio of 1:0.39 (resin to hardener) with a disposable pipette. The solution was mixed with a wooden stick for about a minute with the cup tilted at 45°. A uniform layer approximately 5-mm-thick of the solution was poured in transparent rectangular moulds to avoid the direct contact of the tooth with the bottom of the mould. Once the first layer had hardened, after at least 24h, the moulds were identified using a black sharpie before positioning longitudinally one tooth with an angle of 45° per mould. Following the same ratio, the resin and hardener were mixed again and poured over the tooth until covering it by approximately 5 mm. The embedded teeth were left in the fume hood for at least 24h before proceeding to the next steps.

Once embedded, each tooth was cut toward the middle on the mesio-distal plane using a BUEHLER Isomet Slowspeed saw. A second cut was done to obtain a slice of approximately 1-mm-thick. In some cases, when the tooth was very friable a thin layer of cyanoacrylate glue was applied before performing the second cut (e.g. incisor from J25 T8). The grinding and polishing in sequence were done following the method described in De Boer *et al.* (2013). After obtaining 1-mm-thick slice from the cut, the first side was ground on P220 waterproof sandpaper with distilled water in a circular motion. It was then polished manually in a figure eight path as suggested in the Fitzgerald protocol on P1200 and P2000. Once a thickness of approximately 0.5 mm was obtained, the slice was sonicated for 5 minutes and cleaned with distilled water. The polished side was glued to a microscopic slide with cyanoacrylate glue. To grind and polish the second side, the index, middle, and ring fingertips of the dominant and gloved hand were glued on the back of the microscopic slide (De Boer *et al.*, 2013). The same motion was done than for the previous side with a circular motion on P220 waterproof sandpapers, and a figure eight path on P1200 and P2000 until the section had reached a thickness between 60 µm and 150 µm (Hillson, 1996) as a compromise between material integrity and visibility. The slice was cleaned in distilled water with a drop of soap and rinsed in distilled water before being sonicated for 10 minutes (Fitzgerald, 2006). Once dried, each slice was observed under a polarised light microscope using an OLYMPUS SZX9 microscope at 400X magnification.

6.5 Correlative Tomography

The remaining half of six embedded teeth were scanned on the micro-CT scanner using the similar parameters (82 kV, 148 µA, 0.5 Al filter) used earlier, but 1 frame averaged per projection and no minimize rings for a 26-minute scan. The virtual half-tooth obtained was merged and registered with the previously -and complete- scan of the corresponding tooth on Dragonfly using the “Move” tool. This step allowed to approximate the location of the histological slice to combine the 2D and 3D images. Then the histological image was downloaded in TIF format and registered to the volume. Details for this procedure can be found in Appendix M.

6.6 Statistical Analysis

6.6.1 The *Aqlla* at Farfán

The unit of analysis is a common challenge in dental anthropology. When teeth are the unit, each tooth is considered independently from another. While this approach increases the sample size, it also increased the sample's heterogeneity and does not acknowledge the person as an individual (Gagnon & Wiesen, 2013). When individuals are the unit of analysis, the data can be overgeneralized if there is no distinction between an individual who has one lesion from one with multiple lesions (Hillson, 1996; 2008).

The comparison of frequency by individual was deemed to be the most appropriate option to provide a complete examination of dental health to address the hypotheses on the lifeways in Farfán. This approach considers the individual's profile and maximises, comparability with the published literature. General chi-square tests were used to compare prevalence of each dental pathological lesion between the *aqlla* and the general population (Cemetery I, Cemetery J, Mound G Cemetery, Compound VI Cemetery). The average of lesions per individuals between groups was evaluated through t-tests. To detect confounding variables between the dental features and contextual data including age, sex, stature, and isotope values, Pearson's correlation coefficients were calculated for quantitative variables and chi-square tests for qualitative variables. The variability between the *aqlla* and the general population discussed in hypothesis #3 was evaluated through the standard deviation of each dental feature. Finally, an inter-site comparison was performed using chi-square tests and t-tests. Here, the dental health of the individuals from the *Huaca* Burial platform obtained through their osteobiographies was compared with the data from Túcume (available in Toyne, 2002, see Appendix E), the other known *aqlla* site on the North Coast of Peru. The statistical analyses were done in IBM SPSS Statistics 27.

6.6.2 Intra- and Inter-Observer Variations

The inter-observer variation on recording dental characteristics was evaluated with the weighted kappa coefficient (AlQahtani *et al.*, 2010; Mitropoulos *et al.*, 2010; Rovaris *et al.*, 2018; Soviero *et al.*, 2012). The observations on the 45 teeth compiled by Dr Nelson and obtained from the osteobiographies was compared to the data recorded macroscopically by the author on the focused sample.

To account for intra-observer error, the dental characteristics were recorded twice by the same the author. This happened a couple of weeks apart for macroscopy, and a couple of months apart for micro-CT. The first and second observations were compared with the weighted kappa coefficient (AlQahtani *et al.*, 2010; Mitropoulos *et al.*, 2010; Rovaris *et al.*, 2018; Soviero *et al.*, 2012).

6.6.3 Comparison Between Methods

For the methodological comparisons, each tooth was considered as an independent unit of analysis. Chi-square tests and t-tests were used to compare the data obtained between the different methods based on the observations done by the author on the focused sample using macroscopy and micro-CT (N=45), and from micro-CT and histology (N=14).

All tests were run with a 95% confidence interval and the usually accepted p-value of 0.05. However, since the sample size is small for each cemetery (N<30), there is an increased risk for false positive (type I error) and lower statistical power (experiment's capacity to detect an effect). Approximately 80% of the literature in bioarchaeology used Null Hypothesis Significance Testing (NHST) with a p-value to determine if their results are significant (Smith, 2018). However, this value is often misunderstood and overused (Smith, 2018). Indeed, the p-value is not reliable when the statistical power is below 90%. A p-value indicates the frequency with which we would obtain statistical significance if we repeated our analysis results multiple times in this specific population. It should not be interpreted as a probability that the null hypothesis is true. Indeed, a large difference may be statistically insignificant if the sample size is small, and the opposite is also possible with small differences being statistically significant in large sample (Gardner & Altman, 1986). Thus, while p-values are mentioned, the effect sizes (Cramer's *V* or Cohen's *d*) have been calculated to quantify the difference observed and to provide more information about testing (Smith, 2018). The effect size is calculated from the means and standard deviation of the population to quantify the *magnitude* of the difference. For example, a small effect size of 0.2 indicates that the two groups differ by

0.2 standard deviation. Thus, the difference is minimal between these groups for the calculated test even if the p-value is statistically significant.

6.7 Recording Dental Characteristics

As seen in Table 6.1, each dental feature evaluated can contribute to reconstructing the lifeways of past individuals. Although the information retrieved differs between the three methods, the recording of dental characteristics was done as systematically as possible.

The micro-CT information was obtained using Dragonfly 2020.2 while the histological data were collected with OLYMPUS SENSE software v.3.2.2. The frequency of each lesion was established per group (*aqlla* vs. non-aqlla) and the average per individual was also determined as done in Toyne (2002) to facilitate comparison with the sample from Túcume.

6.7.1 Abscesses

Abscesses are usually associated with carious lesions (Hillson, 1996). Thus, a higher frequency of carious lesions predicts a higher frequency of abscesses in an individual or a population. Abscesses cannot be recorded only based on the tooth and therefore their presence or absence was noted only based on the information documented in the osteobiographies. The average of abscesses per individual and the frequency of individuals presenting abscesses in both groups (*aqlla* vs. non-aqlla) in the Farfán population was established following Toyne (2002).

6.7.2 AMTL and PMTL

Similar to abscesses, antemortem tooth loss (AMTL) and postmortem tooth loss (PMTL) were only considered through the osteobiographies. The distinction between PMTL and AMTL was based on the presence of remodelling of the alveolar bone. AMTL can be an indicator of pathology or carious lesions and therefore its frequency can affect the frequency of other conditions while PMTL can lead to the underestimation of lesions since the material is not available for analysis. The frequency was calculated based on the average number of tooth loss per individual following Toyne (2002).

6.7.3 Caries

Carious lesions were assessed in the osteobiographies and using the three different analytical methods. The recording of caries is not consistent in dental anthropology. One of the main challenges faced is to distinguish carious lesions from diagenesis and non-carious pits on enamel. However, the criteria considered to identify a lesion are variable between studies (see Table 6.5). Coloration, mentioned in Buikstra and Ubelaker (1994) is often considered however, diagenetic changes can mimic the characteristic discoloration (Hillson, 2001) and it is not visible on micro-CT scans. For this project, a carious lesion was identified when at least 3 out of the 7 criteria presented in Table 6.5 were present, two of these criteria needed to be cavitation and irregular edges as only active lesions were acknowledged to avoid confusion with non-carious lesions. Some of these characteristics could only be recorded in one method. Demineralisation could only be assessed with micro-CT since the variation in density could be visualised and measured with Dragonfly.

Table 6.5. Criteria to identify carious lesions.

Characteristics	Sources
Coloration (darkness)	(Boca *et al.*, 2017; Buikstra & Ubelaker, 1994; Pezo-Lanfranco & Eggers, 2010; Towle *et al.*, 2019)
Cavitation	(Buikstra & Ubelaker, 1994; Kawato *et al.*, 2009; Neves *et al.*, 2016; Özkan *et al.*, 2015; Towle *et al.*, 2019)
Irregular edges	(Buikstra & Ubelaker, 1994; ICDAS II; Hillson, 2001)
2-mm depth	(Cucina & Tiesler, 2003; Okumura, 2014; Pezo-Lanfranco & Eggers, 2010; Sakashita *et al.*, 1997)
Demineralisation	(Boca *et al.*, 2017; Neves *et al.*, 2015, 2016; Rovaris *et al.*, 2018; Soviero *et al.*, 2012)
Penetrate dentin	(Cucina & Tiesler, 2003; Kamburoglu *et al.*, 2011; Kawato *et al.*, 2009; Mitropoulos *et al.*, 2010; Okumura, 2014; Özkan *et al.*, 2015; Rovaris *et al.*, 2018)
Appearance (shiny or glossy, matte or non-glossy);	(ICDAS II; Mitropoulos 2010)
Bowled-shape	(Boca *et al.*, 2017; Rossi *et al.*, 2004)

The total number of carious lesions recorded on each tooth was noted. Their characteristics were added in comments. For the osteobiographies, the frequency of caries was calculated per individual as done in Toyne (2002).

6.7.4 Occlusal Wear Level

Dental occlusal wear was assessed using three methods. Buikstra and Ubelaker's (1994) standard was followed based on Smith's (1984) eight stages of wear for incisors, canines, and premolars and Scott's (1979) ten stages of wear for molars. In Scott's (1979) method, molars were divided into four quadrants with each of them being assigned a value between 0 and 10 and then added together for a total score between 4 and 40. However, this dual system does not allow comparison between anterior and posterior teeth, therefore a rank category was designed following Table 6.6. The categories were also a more appropriate method to compare the results obtained by the author with the qualitative wear levels available in the osteobiographies.

Table 6.6. Occlusal Wear Level Categories Followed by the Author.

Category	Smith's (1984)	Scott's (1989)
None	0	0
Slight	1-2	4-8
Slight-Moderate	3-4	9-16
Moderate	5-6	17-24
Moderate-Advanced	7	25-32
Advanced	8	33-40

6.7.5 Dental Calculus

Dental calculus is not commonly recorded in Andean dental anthropology (see Chapter 5). Thus, it was categorized as present or absent in the osteobiographies and on each tooth for the three different analytical methods. The amount of dental calculus was noted in comments as slight, moderate, or heavy based on Buikstra and Ubelaker's (1994) standard. The frequency was calculated with the number of individuals with dental calculus in each group following Toyne (2002).

6.7.6 Linear Enamel Hypoplasia

The presence of LEH was noted from the osteobiographies and on the 45 teeth for macroscopy, micro-CT, and histological analyses. For macroscopy, measurements of LEH were taken at the center of the buccal surface of the lesion from the CEJ (Buikstra & Ubelaker, 1994).

The total number of stress episodes and their ages of occurrence were compared between methods. Goodman and Rose (1990)'s commonly used as a quantitative method available for anterior and posterior teeth to estimate age of occurrence. However, it has been criticized for its limitations regarding cuspal enamel formation (Goodman & Song, 1999). Other formulae with slight variations have been developed such as the ones found in Cares-Henriquez and Oxenham (2019). A comparison of LEH calculation from Goodman and Rose (1990) and Cares-Henriquez and Oxenham (2019) showed a difference of only 1.3 months (p-value = 0.007) (Lacerte, 2019). Thus, the distance between CEJ and LEH on anterior dentition from Cares-Henriquez and Oxenham (2019) was used to establish the age associated to the formation of the enamel defects. For deciduous teeth, the age at which the stress event occurred was determined following Armelagos and Blakey (1985). The frequency of individuals in each group with at least one defect and the average of lines per individual in each group were calculated following Toyne (2002).

6.7.7 Interglobular Dentin

Interglobular dentin is the consequence of a mineralisation defect leading to the formation of patches following incremental lines in the dentin (D'Ortenzio *et al.*, 2018). It is not possible to examine dentin through macroscopic observations. As done in Colombo *et al.* (2019), D'Ortenzio *et al.* (2016), and Veselka *et al.* (2019), the internal structure was evaluated with micro-CT and histology to detect IGD. For each tooth examined (N=14), the total number of defects recorded was noted and categorized as "possible", "probable", or "distinct". To categorise the defect, five characteristics were assessed: 1) unilateral or bilateral occurrence (on the thin-section only) (D'Ortenzio *et al.*, 2018); 2) whether the defect appeared faint, variable, or marked; 3) if the defect was continuous or discontinuous; 4) the shape of the defect patches; and 5) if the defect was present or absent on micro-CT. The approximate ages of occurrence were defined in histological analyses based on dentin development of the tooth based on charts from Brickley *et al.* (2020) and in (AlQahtani *et al.*, 2010).

6.7.8 Hypercementosis

Hypercementosis, was assessed in the osteobiographies, and through macroscopy, micro-CT, and histology. It was documented as present or absent based on the observation of "a bulbous, irregularly bulging root" (Hillson, 1996: 205) or an excessive deposit of cementum which could be easily observed with macroscopy, micro-CT and histology.

6.7.9 Dental Anomalies

Any additional dental anomalies (e.g., enamel chips, discoloration, dental crowding, peg-shaped incisors, dental pearls) were noted and included in a comment section. These anomalies were not the focus of the analysis, but their detection is discussed qualitatively in the Discussion (chapter 8).

6.8 Conclusion

In brief, this study compared the advantages and disadvantages of macroscopic examination, micro-CT, and histological analyses while shedding light on the *aqlla* of Farfán through various dental characteristics to reconstruct their lifeways. The dental examination considered abscesses, caries, AMTL, occlusal wear, LEH, IGD, dental calculus, and hypercementosis. To evaluate if the *aqlla* had more carious lesions, more advanced wear, and fewer episodes of stress during childhood as expected from descriptions in the ethnohistorical documents, statistical analyses were performed between cemeteries using the data collected from the osteobiographies. The comparison of methods to assess the detection of dental pathological lesions through macroscopy vs. micro-CT vs. histology was based on the observations collected on a focused sample of 45 teeth. The statistical analyses included chi-square tests, t-tests, Pearson's correlation coefficients, and effect sizes.

Chapter 7 - Results

This project provides a detailed dental analysis of the individuals excavated at Farfán using a multi-modal approach to complement the osteobiographies. The analyses aim to evaluate if the dental health of the individuals from the *Huaca* Burial Platform is representative of the portrait of the *aqlla* depicted in the Spanish Chronicles. The individuals excavated from the four other cemeteries (Cemetery J, Cemetery I, Mound G Cemetery, and Compound VI Cemetery) at Farfán are used as a comparative sample to represent the "general population" on site. Moreover, this thesis compares the data obtained through macroscopy, micro-CT, and histology on a focused sample of 45 teeth to evaluate the pros and cons of destructive vs. non-destructive analysis.

The following chapter will present the results obtained from the individual osteobiographies to address hypotheses #1 to #3 and the observations collected on the focused sample (using the tooth as the unit of analysis) to address hypothesis #4 (see p.7). The chapter is divided into three main sections. The first section will briefly highlight the differences between the observations collected from the osteobiographies produced by Dr Nelson and the detailed dental analysis performed by the author for the teeth included in the focused sample. The second section aims to evaluate dental health with the information obtained from the osteobiographies to explore lifeways in the *aqlla*. For this section, while there are 68 primary burials with individual osteobiographies, some dental pathological lesions were not observable in all individuals. Therefore, the exact number of observations varies for each lesion. The second section will also compare the data from Túcume (available in Appendix E), the only other known *aqlla* site on the North Coast of Peru, with the *Huaca* Burial Platform for an inter-site comparison. Finally, the third section will focus on the comparison between the methods used to promote non-destructive analysis over destructive analysis. The information compiled from the osteobiographies and obtained through the detailed analysis can be found in Appendix H and Appendix N respectively.

7.1 Inter-Observer Variation Between Osteobiographies and Detailed Dental Analysis

This section highlights the inter-observer variation between the information extracted from the osteobiographies produced by Dr Nelson and the information collected through the detailed dental analysis of the 45 teeth included in the focused sample. A few differences were noted between Dr Nelson and the author's observations (Table 7.1). Overall, more lesions were detected through the detailed dental analysis compared to the osteobiographies except for wear level.

Table 7.1. Comparison of dental pathological lesions recorded in 45 teeth from Farfán by Dr Nelson (through osteobiographies) and the author (macroscopy and micro-CT).

	wear level			**caries**			**number of LEH**			**Dental calculus**	**Hyper-cementosis**
Method	total	mean	sd	total	mean	sd	total	mean	sd	Total Teeth	Total Teeth
Osteobiographies	99	2.25	1.70	3	0.07	0.26	3	0.11	0.32	3	1
Macroscopy	74	1.64	1.37	13	0.29	0.63	23	0.51	0.87	4	4
Micro-CT	77	1.79	1.36	16	0.36	0.80	26	0.58	1.00	6	7

Total of each column represents the cumulative number of each lesion in the individuals included in the focused sample.

For wear level, the information in the osteobiographies was available only at the individual level and thus the discrepancy for a single tooth in the focused sample is not surprising. The author recorded more caries than recorded in the osteobiographies for the corresponding tooth which can be explained by the extensive examination for carious lesions including smaller ones. More LEH was recorded through the detailed analysis mainly due to the presence of LEH on third molars in the focused sample which were not reported in the osteobiographies. The prevalences of dental calculus and hypercementosis were also higher in the observations done by the author which might be the result of the examination of the tooth outside of its socket compared to osteobiographies.

The main difference in recording between observers was the recording of IGD in the focused sample possible through micro-CT which could not be assessed in the osteobiographies. Thus, while the focus for the comparison between cemeteries is on the osteobiographies, the IGD data obtained from the focused sample of 45 teeth is added to the comparison.

7.1.1 Focused Sample vs. Entire Sample of Primary Burials

This sub-section addresses the correspondence of the data between the entire sample of primary burials excavated at Farfán (N=68) and the sample of 32 individuals in the focused sample. The goal is to evaluate if the focused sample is representative of the entire burial population, and thus to assess if interpretations from the focused sample are likely applicable to the whole sample of 68 individuals.

Despite the difference seen in sex (Figure 7.1) and age (Figure 7.2) distributions do not differ significantly between the entire and the focused samples based on chi-square tests (Sex: $\chi^2 = 5.62$, p-value = 0.132, Cramer's $V = 0.24$; Age: $\chi^2 = 5.02$, p-value = 0.658, Cramer's $V = 0.22$). Thus, the sample of 32 individuals selected for further analysis is considered representative of the population of Farfán based on demographic distributions.

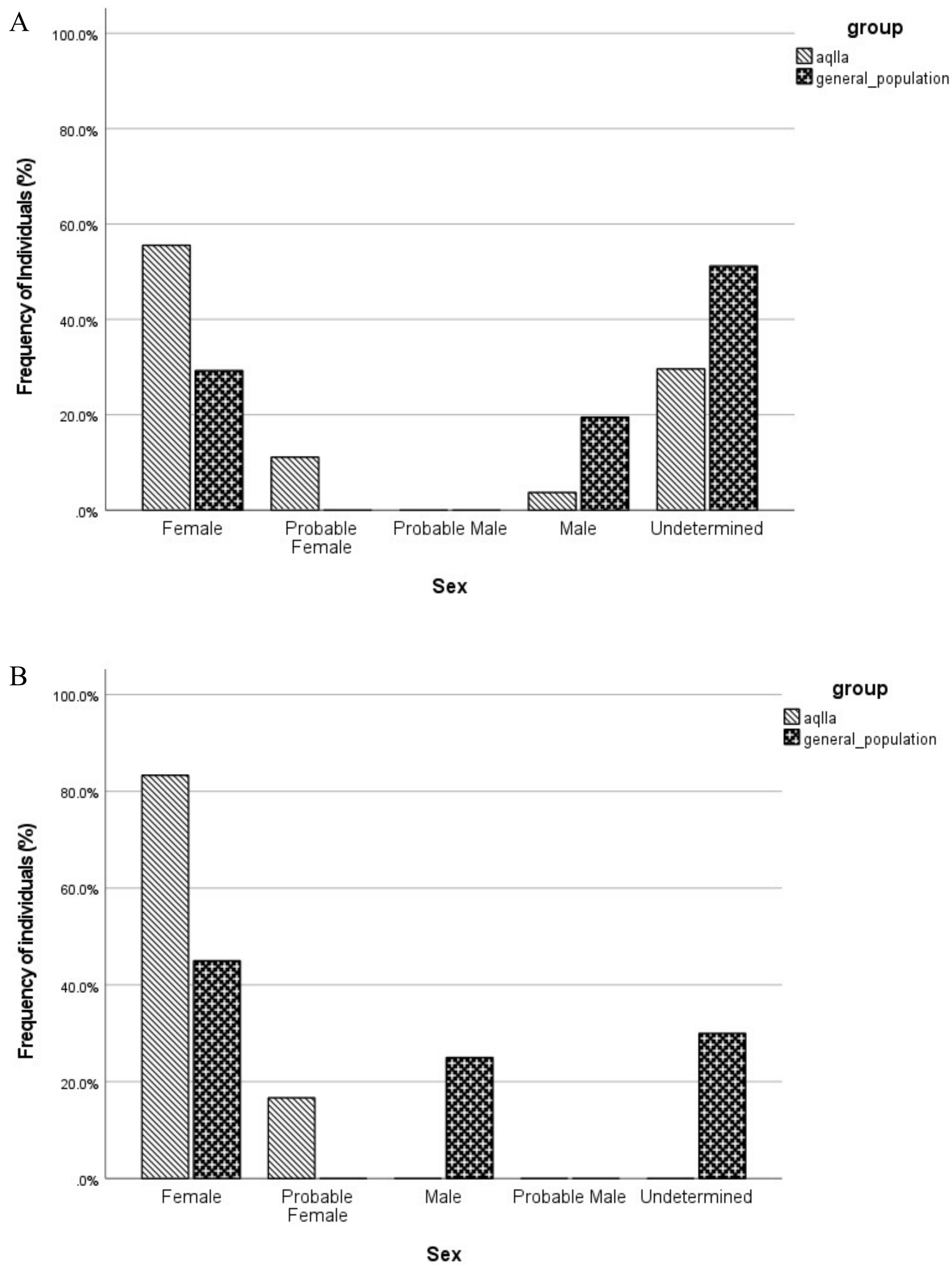


Figure 7.1. Sex distribution of individuals from A) the entire sample of primary burials (N=68) and B) the focused sample (N=32) in Farfán. Undetermined represents non-adults.

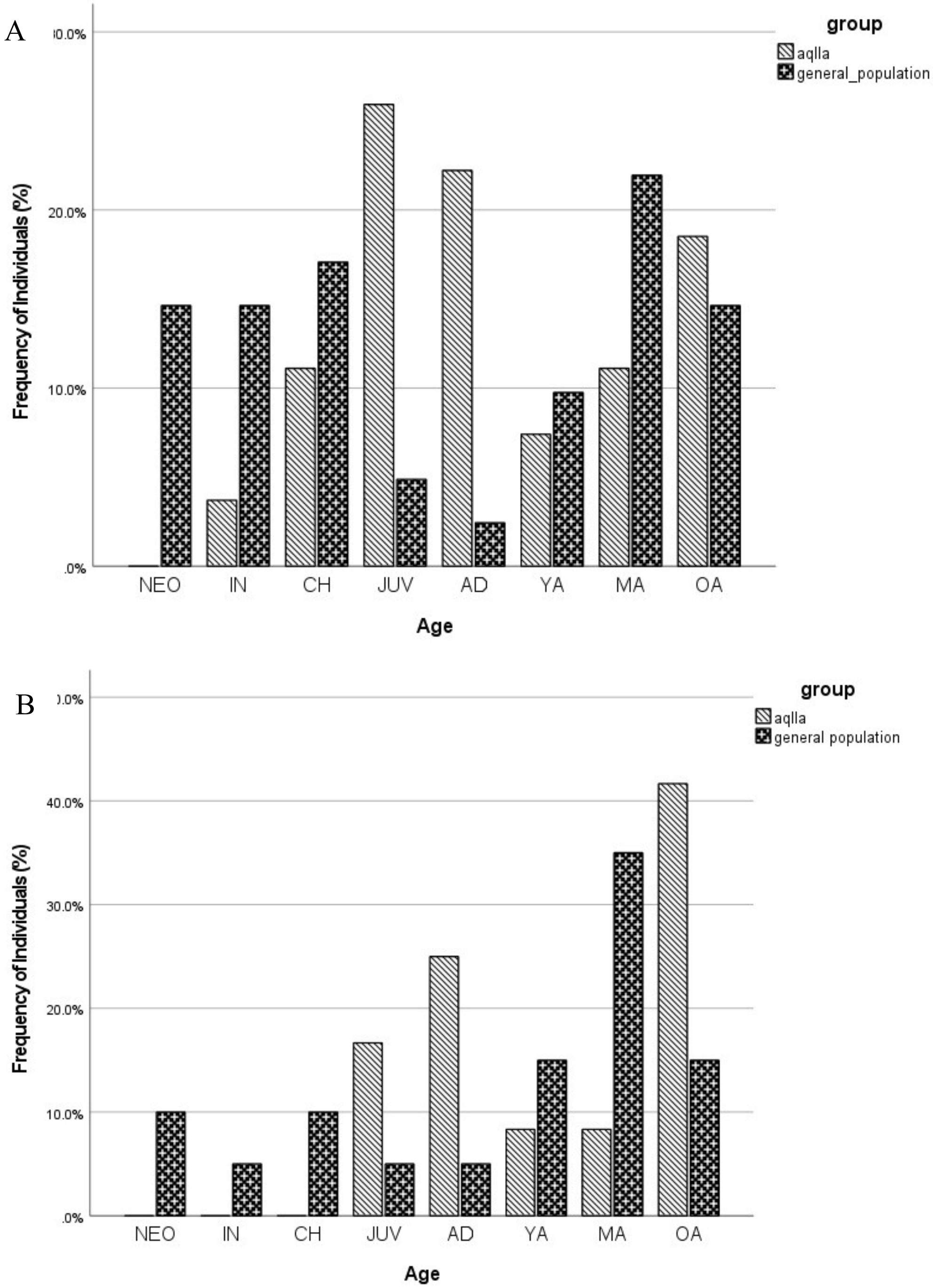


Figure 7.2. Age distribution of individuals from A) the entire sample of primary burials (N=68) and B) the focused sample (N=32) in Farfán. Age: NEO (neonate) between 0 and 0.9 years, IN (infant) between 1 and 2.9 years, CH (child) between 3 and 5.9 years, JUV (juvenile) between 6 and 11.9 years, AD (adolescent) between 12 and 19.9 years, YA (young adult) between 20 and 29 years, MA (mid adult) between 30 and 39 years, OA (old adult) more than 40 years.

As expected, in both the entire sample and the focused sample, the *aqlla* cemetery contains a significantly higher proportion of females than the four other cemeteries combined (χ^2 = 12.24, p-value = 0.007, Cramer's *V* = 0.42) (Figure 7.1). For both the entire sample and the focused sample the age groups differ significantly between the individuals from the *Huaca* Burial Platform and the individuals from the four other cemeteries (χ^2= 19.21, p-value = 0.008, Cramer's *V* = 0.53) (Figure 7.2). The *Huaca* Burial Platform contains a higher proportion of juvenile, adolescent, and old adults than the four other cemeteries. In comparison, the general population has a higher proportion of neonates, infants, children, and middle-aged adults.

Dental health is similar between the entire sample of primary burials (N=68) and the individuals from the focused sample. Compared to all the primary burials, with the exception of the number of teeth loss postmortem, the focused sample presents, a slightly broader range for the number of abscesses, the number of teeth loss antemortem, the number of caries, and the number of episodes of childhood illness as indicated by LEH. Based on t-tests (Table 7.2), the dental health characteristics display statistical similarities (p-value > 0.05). Thus, in most cases, the focused sample selected for detailed analysis in this thesis is reasonably representative of the entire sample buried at Farfán. However, to maximise sample size, the comparison of dental health characteristics between cemeteries prioritised the data, when available, from the entire sample of primary burials.

Table 7.2. Dental health characteristics between the entire sample (N=68) and the focused sample (N=32).

Dental Characteristics	Entire Sample (Mean ± SD)	Focused Sample (Mean ± SD)	t	p-value	Cohen's *d*
Abscess	1.12 ± 2.43	1.41 ± 2.85	-0.498	0.620	0.12
AMTL	1.73 ± 3.34	2.00 ± 3.50	-0.345	0.731	0.08
PMTL	2.65 ± 4.65	1.07 ± 2.24	1.725	0.088	0.40
# Caries	1.98 ± 2.17	2.37 ± 2.30	-0.758	0.450	0.17
Wear Level	2.39 ± 1.82	2.32 ± 1.68	0.172	0.863	0.04
# LEH	1.89 ± 2.12	2.08 ± 2.31	-0.343	0.733	0.08

7.2 Dental Health in Farfán

This section evaluates if the dental health of the *aqllakuna* is different from the general population at the site and if the dental characteristics recorded are representative of the description of these females in the Spanish Chronicles. The dental health recorded at the *Huaca* Burial Platform is compared to Cemetery J, Cemetery I, Mound G Cemetery, and Compound VI Cemetery which are identified as the "general population". This section, based primarily on the information available in the osteobiographies of the entire sample of primary burials (N=68), is divided by pathological lesions starting with 1) cariogenicity including abscesses, AMTL, carious lesions, and occlusal wear; 2) episodes of childhood illness including LEH and IGD; and 3) dental calculus. The comparison of the *aqlla*'s dental health between Farfán and Túcume completes this section.

7.2.1 Cariogenicity – *Aqlla* vs. General Population

7.2.1.1 Abscesses

Based on the osteobiographies, abscesses were present in 35% (N=8/23) of the individuals from the *Huaca* Burial Platform and 31% (N=9/29) of individuals from the general population (χ^2 = 0.08, p-value = 0.775, Cramer's V = 0.04). No association was found between the presence of abscesses and sex (χ^2 = 7.83, p-value = 0.050, Cramer's V = 0.39) or age (χ^2 = 13.04, p-value = 0.071, Cramer's V = 0.50), but the moderate effect size suggests an influence.

The average number of abscesses per individual in the *aqlla* cemetery is 0.96 ± 1.58 (mean ± sd) while in the other cemeteries, the average is slightly higher at 1.24 ± 2.96 (mean ± sd). This difference is mainly influenced by two individuals with a high number of abscesses per individual (7 and 14 abscesses) found in Cemetery J (see Table 7.3). A t-test comparing the average number of abscesses per individual shows no significant difference between the *aqlla* and the general population (t = -0.42, p-value = 0.679). The small effect size (Cohen's d = 0.12) supports the similarity between groups despite the influence of sex and age profiles on the presence of abscesses.

Table 7.3. Number of abscesses per cemetery in the primary burials.

	Number of Abscesses per Individual							
Cemetery	**0**	**1**	**2**	**3**	**4**	**5**	**7**	**14**
Mound G Cemetery	3	1	0	0	0	0	0	0
Cemetery I	2	0	1	0	0	1	0	0
Cemetery J	9	2	1	1	0	0	1	1
Compound VI Cemetery	6	0	0	0	0	0	0	0
Total general population	20	3	2	1	0	1	1	1
Huaca Burial Platform	15	2	2	1	2	1	0	0

7.2.1.2 Tooth Loss

AMTL is present in 39% (N=9/23) of the primary burials from the *aqlla* cemetery and 45% (N=13/29) of individuals from the other cemeteries at Farfán (χ^2 = 0.17, p-value = 0.680, Cramer's V = 0.06). The *aqlla* exhibits lower AMTL per tooth per individual with an average of 0.78 ± 1.53 (mean ± sd) while in the general population, the average is 2.48 ± 4.14 teeth per individual (Figure 7.3). A t-test between these groups detected a significant difference (t = -2.04, p-value = 0.048). The effect size (Cohen's *d* = 0.52) shows that the probability to have AMTL is moderately higher in the general population compared to the *aqlla*. A significant association was found between the presence of AMTL and age (χ^2 = 27.46, p-value < 0.001, Cramer's V = 0.73) and sex (χ^2 = 16.49, p-value = 0.001, Cramer's V = 0.56).

The average of PMTL is 1.96 ± 2.98 per tooth per individual at the *Huaca* Burial Platform representing 48% (N=11/23) of individuals while the four other cemeteries combined exhibit an average of 3.21 ± 5.63 (mean ± sd) per tooth per individual affecting 48% (N=14/29) of the general population. The differences in prevalence (χ^2 = 0.08, p-value = 0.780, Cramer's V = 0.04) and means (t = -1.03, p-value = 0.309) are not significant. Cohen's *d* at 0.27 supports the interpretation that PMTL is similar between groups.

7.2.1.3 Caries

The data retrieved from the osteobiographies reveal a prevalence of 65% (N=15/23) of individuals from the *aqlla* cemetery with caries which is very similar to the prevalence found in Farfán's general population (64% of individuals; N = 16/25) ($\chi^2 = 0.54$, p-value = 0.463, Cramer's $V = 0.10$) (Figure 7.3). The *aqlla* have an average of 1.96 ± 2.06 (mean ± sd) carious lesions per individual while the other cemeteries combined have an average of 2.0 ± 2.3 (mean ± sd) carious lesions per individual. There is no significant difference regarding the average of carious lesion between groups (t = -0.07, p-value = 0.940, Cohen's $d = 0.02$). No association have been found between the prevalence of caries and age ($\chi^2 = 7.72$, p-value = 0.358, Cramer's $V = 0.39$) or sex ($\chi^2= 4.11$, p-value = 0.249, Cramer's $V = 0.28$).

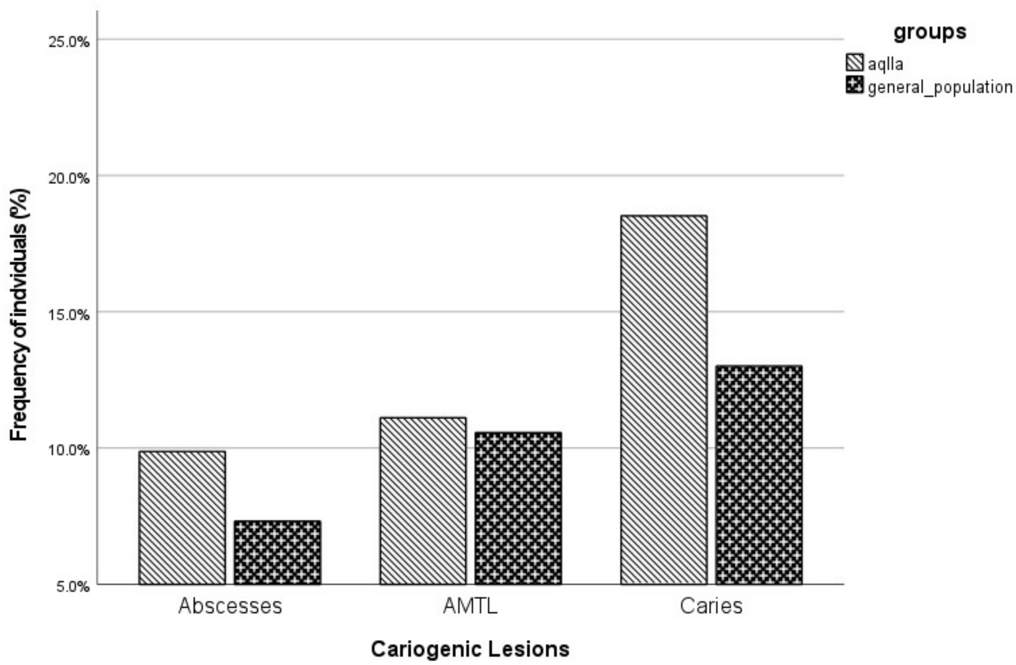


Figure 7.3. Prevalence of lesions associated with caries in the entire sample of primary burials (N=52) in Farfán.

The total number of carious lesions per cemetery seen in Table 7.4 shows the variation across the primary burials. While numerous caries have been found in the *Huaca* Burial Platform, individuals from Cemetery J exhibit a similar range supporting that the *aqlla* individuals are not different in term of carious lesions.

Table 7.4. Distribution of carious lesions per individual in each cemetery.

	Number of Caries per Individual							
Cemetery	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Mound G	1	1	1	0	0	0	0	0
Cemetery I	1	0	1	0	1	1	0	0
Cemetery J	5	1	2	2	1	1	2	1
Compound VI Cemetery	6	0	0	0	0	1	0	0
Total general population	13	2	4	2	2	3	2	1
Huaca Burial Platform	8	4	3	3	1	2	2	0

7.2.1.4 Occlusal Wear

The qualitative occlusal wear level is slightly higher in the *aqlla* cemetery than the general population (Figure 7.4), but the samples do not differ significantly (χ^2 = 5.68, p-value = 0.460, Cramer's V = 0.29). The majority of the primary burials from the *aqlla* cemetery exhibit a slight wear level (32%, N = 7/22) or a moderate-advanced to advanced wear level (50%, N = 11/22). In comparison, the general population exhibits mostly an absent (24%, N = 7/29) or slight (28%, N = 8/29) wear level while moderate-advanced or advanced wear levels combined represent 31% (N = 9/29) of individuals.

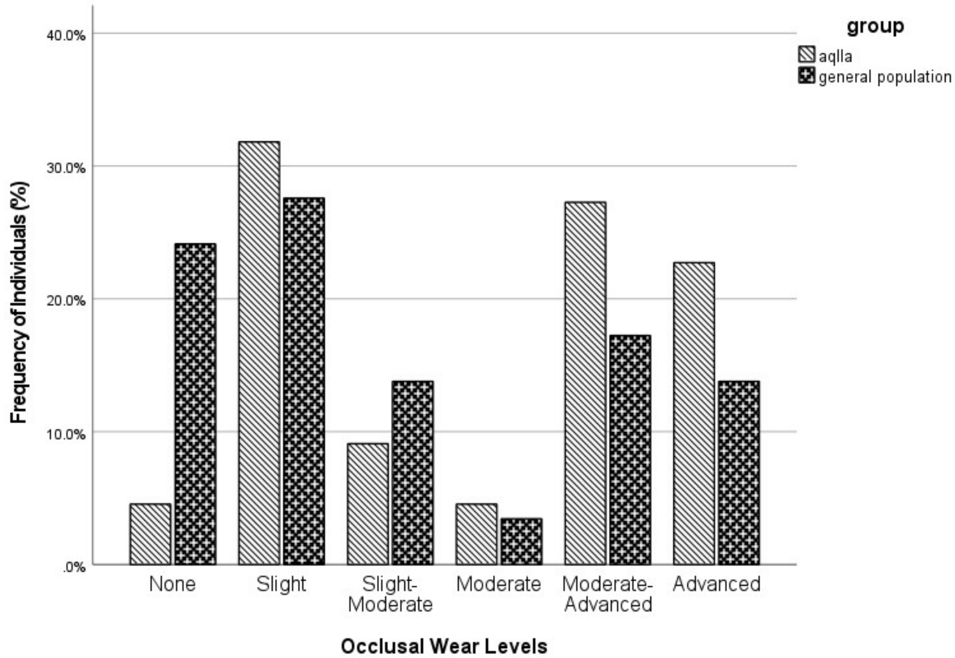


Figure 7.4. Distribution of occlusal wear level encountered in the individuals from the primary burials (N=51) in Farfán.

At the *Huaca* Burial Platform, the higher percentage of slight and advanced wear levels reflect the structure of the population. Indeed, a positive association was found based on chi-square test between wear level and age (χ^2 = 70.90, p-value = 0.001, Cramer's V = 0.53) and wear level and sex (χ^2 = 37.83, p-value = 0.001, Cramer's V = 0.50). The *Huaca* Burial Platform contains both a high proportion of young individuals (6-19.9 years of age) and older individuals (> 40 years of age) compared to the other cemeteries. As seen in Table 7.5, individuals from Cemetery J exhibit mostly a slight or slight-moderate occlusal wear level while individuals from the Compound VI Cemetery exhibit mostly moderate-advanced or advanced wear levels which are representative of age structure.

Table 7.5. Distribution of occlusal wear levels across the cemeteries of Farfán (N=51).

	Wear Levels					
Cemetery	None	Slight	Slight-Moderate	Moderate	Moderate-Advanced	Advanced
Mound G	1	1	1	0	1	0
Cemetery I	1	2	0	0	0	1
Cemetery J	4	3	3	0	2	1
Compound VI Cemetery	1	2	0	1	2	2
Total general population	7	8	4	1	5	4
Huaca Burial Platform	1	7	2	1	6	5

7.2.2 Childhood Physiological Stress – *Aqlla* vs. General Population

7.2.2.1 Non-specific Indicator of Childhood Stress – Linear Enamel Hypoplasia

Based on the information from the osteobiographies, a total of 38 separate LEH episodes were detected in 63% (N = 17/27) of individuals from the *Huaca* Burial Platform for an average of 1.81 ± 1.63 (mean ± sd) episodes per individual. The four other cemeteries combined have a higher number of separate LEH episodes at 50, but the percent of affected individuals is considerably lower at 34% (N = 14/41) with an average of 1.96 ± 2.47 (mean ± sd) episodes per individual. There is no significant difference regarding the average number of episodes of childhood stress between both the *aqlla* and the general population (t = -2.01, p-value = 0.810, Cohen's d = 0.07). Thus, as seen in Figure 7.5, the *aqlla* had a significantly higher prevalence of LEH than the general population (χ^2 = 6.03, p-value = 0.049, Cramer's V = 0.30). Amongst the general population, Cemetery I and Cemetery J have a considerable higher percent of individuals with at least one enamel defect, at respectively 60% and 47% of their population. An association was found based on chi-square tests between the presence of LEH and sex (χ^2 = 22.60, p-value = 0.001, Cramer's V = 0.41) and age (χ^2 = 38.31, p-value < 0.001, Cramer's V = 0.53).

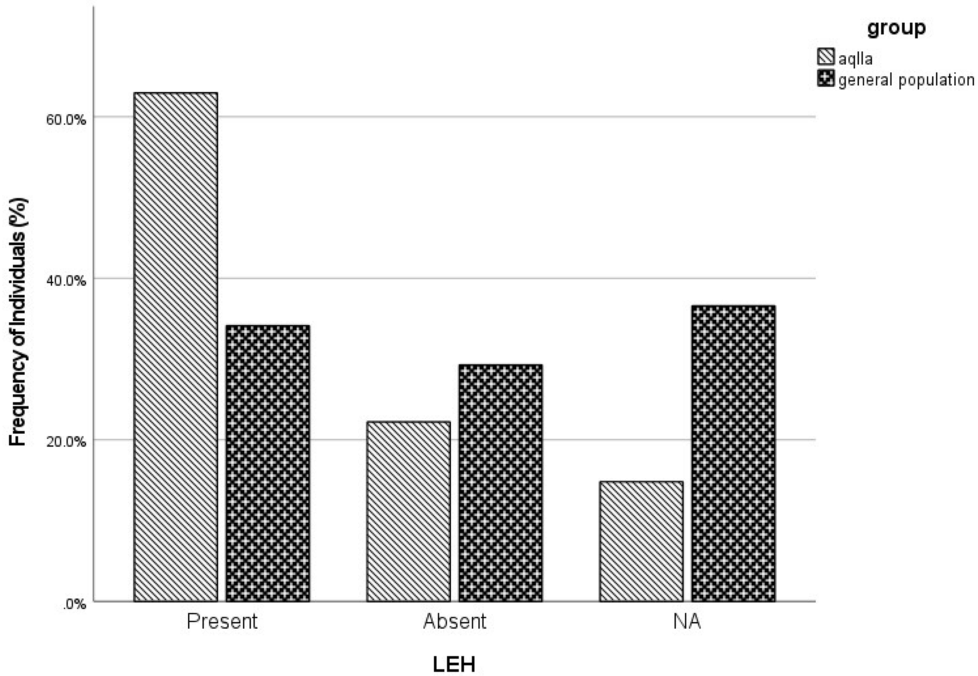


Figure 7.5. Prevalence of LEH in the primary burials (N=49) in Farfán.

The ages of LEH occurrence in the anterior dentition (total of 64 defects) between 0 to 5.5 years of age (Table 7.6). Most episodes occur between 3 to 4.5 years (64% of the episodes) for all cemeteries in Farfán. At the *Huaca* Burial Platform, 68% of LEH developed between 3 to 4.5 years of age compared to 61% in the general population.

Table 7.6. Age Distribution of enamel defects per cemetery. Age ranges were established using Cares-Henriquez and Oxenham's (2019) estimation formula.

Age	Mound G	Cemetery I	Cemetery J	Compound VI Cemetery	**Total general population**	*Huaca* Burial Platform	Total in Farfán
0.5_1.0	0	1	0	0	1	0	1
1.0_1.5	0	0	0	0	0	0	0
1.5_2.0	0	0	2	0	2	3	5
2.0_2.5	0	0	4	0	4	1	5
2.5_3.0	0	1	3	0	4	4	8
3.0_3.5	0	2	5	0	7	8	15
3.5_4.0	1	1	3	0	5	9	14
4.0_4.5	0	0	7	1	8	4	12
4.5_5.0	0	0	2	0	2	1	3
5.0_5.5	0	0	0	0	0	1	1
Total	1	5	26	1	33	31	64

7.2.2.2 Interglobular Dentin

IGD was not assessed in the osteobiographies. However, since the focused sample is likely representative of all the primary burials, the IGD detective should be representative of the entire sample. Based on micro-CT data, IGD was present in most of the focused sample. The defect was observed in 31 teeth from 25 out of 32 individuals including 92% (N = 11/12) of individuals from the *Huaca* Burial Platform and 70% (N = 14/20) of individuals from the general population (Figure 7.6) (including distinct, probable, and possible cases). While the individuals from the *Huaca* Burial Platform exhibit a higher prevalence, the Chi-square test did not detect a significant difference between the two groups ($\chi^2 = 2.06$, p-value = 0.151). The effect size (Cramer's $V = 0.25$) indicates a small association between the cemetery and the presence of IGD in individuals. The presence of IGD tend to be higher in females ($\chi^2 = 9.05$, p-value = 0.029, Cramer's $V = 0.53$) and in adults ($\chi^2 = 11.28$, p-value = 0.127, Cramer's $V = 0.59$).

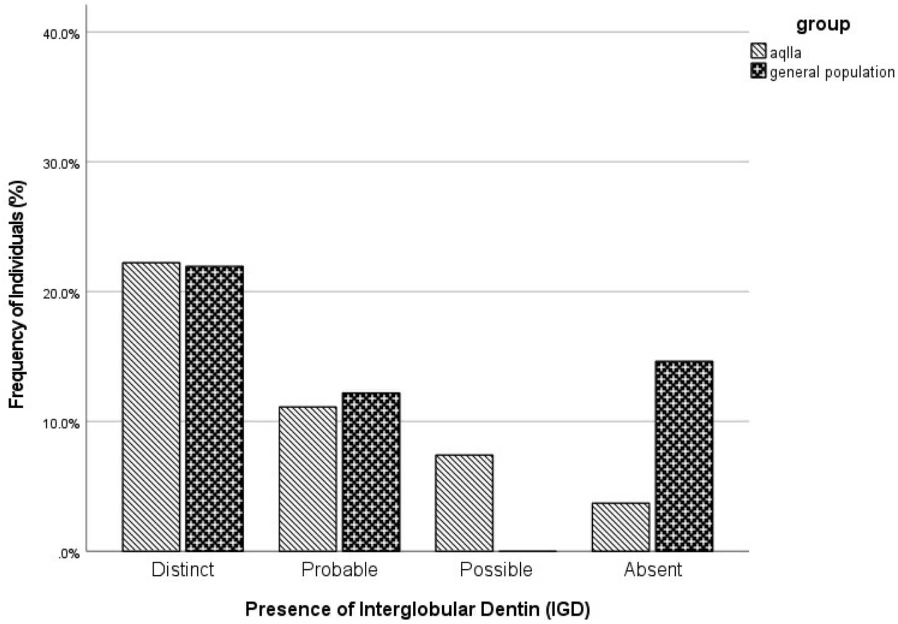
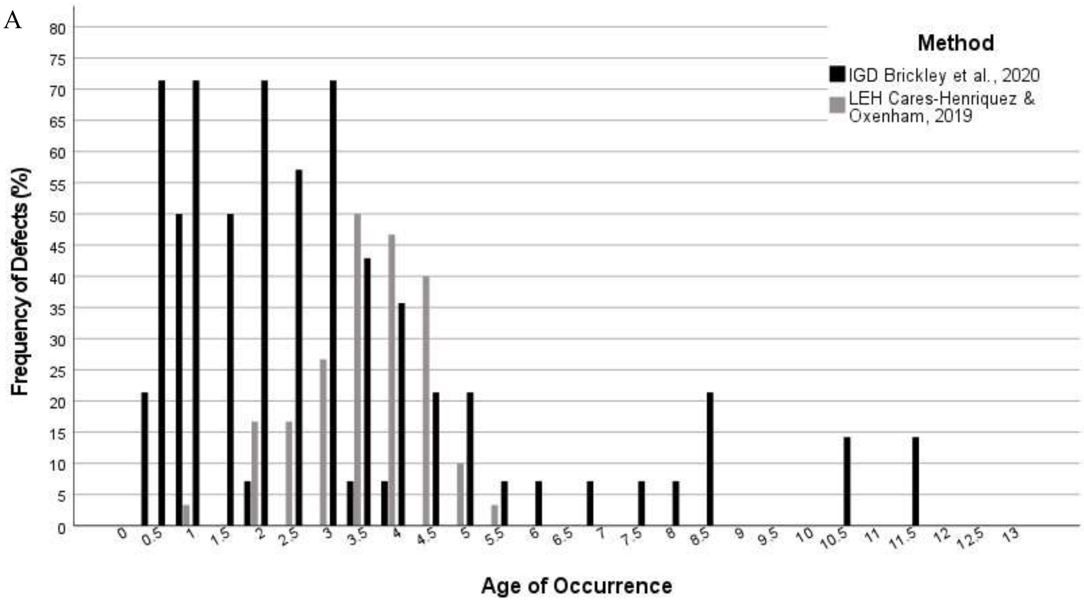


Figure 7.6. Prevalence of interglobular dentin in the focused sample based on micro-CT observations (N=32 individuals).

Micro-CT does not exhibit as many details about IGD as histology (see section 7.3.2.4). Based on micro-CT observations in the 45 teeth, a total of 1.75 ± 1.37 (mean ± sd) episodes of IGD was found in at the *Huaca* Burial Platform while the general population had 1.88 ± 2.32 (mean ± sd) episodes (t = -0.22, p-value = 0.826, Cohen's d = 0.07). These averages are far below the number of defects recorded with histology; thus, the number of episodes and ages of occurrence are based on the histological analysis (N=14). The five teeth from the general population selected for histological analysis exhibit 8.20 ± 2.17 (mean ± sd) episodes of IGD while the nine teeth from the *Huaca* Burial Platform exhibit 6.11 ± 1.69 (mean ± sd) episodes. This analysis revealed no significant difference between groups based on p-value (t = -2.01, p-value = 0.068, Cohen's d = -1.12), but the large effect size suggests there is a difference. All episodes occurred before 13 years of age, occurring approximately every 4 to 6 months. Most episodes occurred between 1 and 5 years of age similar to the ages of occurrence of LEH (Figure 7.7). The tables from AlQahtani *et al.* (2010) has an estimated ages of occurrence in IGD 1.49 years older than the charts from Brickley *et al.* (2020) (4.38 ± 2.66 vs. 2.89 ± 2.58), a significant difference based on t-test (t = -3.98, p-value < 0.001, Cohen's d = 0.57) .

A



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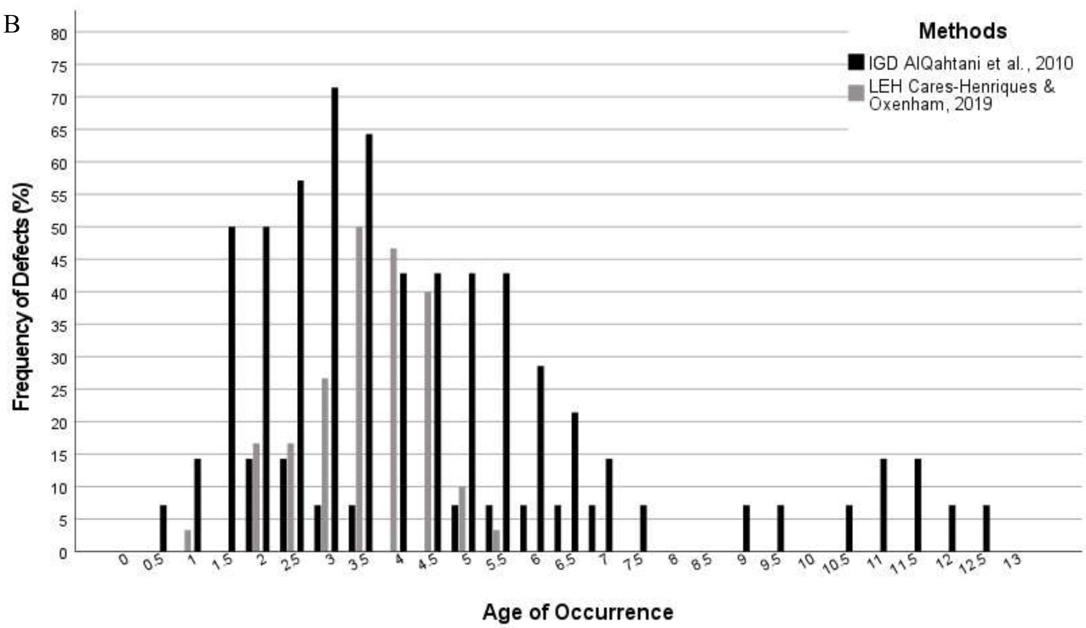

Figure 7.7. Age of occurrence of childhood stress in Farfán comparing IGD (N=14 individuals) and LEH (N=30 individuals). A) Age of IGD established from charts from Brickley *et al.*, 2020; B) Age of IGD based on tables from AlQahtani *et al.*, 2010.

7.2.3 Dental Calculus

Based on the osteobiographies, the general population exhibits mainly absent to slight levels of dental calculus (56% individuals, N=23/41) similar to the *aqlla* (44% of individuals, N=12/27). The level of dental calculus is slightly higher at the *Huaca* Burial Platform with a few individuals exhibiting mild (15% of individuals, N=4/27) and heavy levels of dental calculus (7% of individuals, N=2/27; Figure 7.8). A chi-square revealed no significant difference between groups (χ^2 = 10.88, p-value = 0.054, Cramer's V = 0.58). Thus, while there is no significant difference in the levels of calculus in Farfán, the individuals from the *Huaca* Burial Platform have a wider range of calculus levels which is supported by the moderately high effect size. No significant association was found between the level of calculus and age (χ^2 = 38.88, p-value = 0.299, Cramer's V = 0.34). However, an association was found between calculus and sex (χ^2 = 34.69, p-value = 0.003, Cramer's V = 0.41).

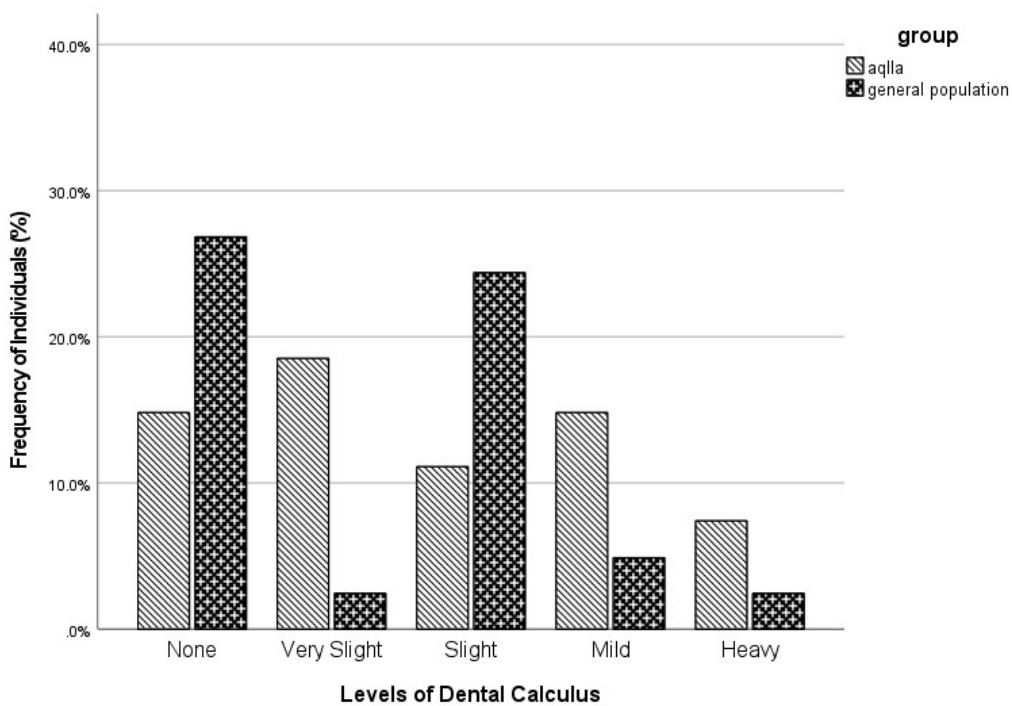

Figure 7.8. Distribution of dental calculus in the entire sample in (N=43) Farfán.

7.2.4 Dental Health and Contextual Variables in Farfán

Association between sex or age with dental health have been found based on chi-square tests for AMTL, wear level, LEH and only for sex in IGD and calculus. The association is likely influenced by the profiles of the samples including mostly females (29%, N=20/68) and subadults (57%, N=39) limiting the data available for males and older individuals. In consequent, it must be noted, that this bias in Farfán's population negatively affects the power of the statistical analysis. Thus, while the profiles of the Farfán's individuals seems to have an influence on the dental pathological lesions, the control of these variables did not change the main conclusion obtained. Table 7.7 below considering only the females and subadults in Farfán shows that the conclusion regarding dental health between the *aqlla* and the general population are not affected by the population structure, except for LEH. Chi-square test for the presence of LEH found no significant differences between groups when only females and subadults are considered ($\chi^2 = 2.53$, p-value = 0.112, Cramer's $V = 0.24$ vs. $\chi^2 = 6.03$, p-value = 0.049, Cramer's $V = 0.30$) which supports the hypothesis that a few individuals are affecting the comparison. Considering the effect sizes are similar between both tests, the results tend toward the same conclusion that the *aqllakuna* exhibit more LEH than the general population.

Table 7.7. Statistical test results for dental pathological lesions in a subset of females and subadults in Farfán.

Dental Pathological Lesions		N	χ^2	p-value	Cramer's *V*	t	p-value	Cohen's *d*
Abscesses	Presence	44	0.442	0.517	0.10	-	-	-
	#	44	-	-	-	1.41	0.167	0.42
AMTL	Presence	43	0.14	0.907	0.02	-	-	-
	#	43	-	-	-	-1.36	0.181	0.42
Caries	Presence	43	1.12	0.289	0.16	-	-	-
	#	43	-	-	-	0.07	0.948	0.02
Wear	Level	43	10.34	0.066	0.49	-	-	-
LEH	Presence	44	2.53	0.112	0.24	-	-	-
	#	43	-	-	-	-0.14	0.989	0.00
IGD	Presence	27	4.47	0.215	0.41	-	-	-
	#	13	-	-	-	-1.36	0.202	0.82
Calculus	Presence	38	3.27	0.70	0.29	-	-	-

The isotopic data collected on 23 individuals from the entire sample of primary burials and available in White *et al.* (2020) (see Appendix C for details) have been compiled to highlight trends between diet, geographic origin, and dental health. The dental characteristics focus on wear level, carious lesions, and number of episodes of LEH and IGD. The *aqlla* had a diet rich in maize ($\delta^{13}C$: -10.92 ± 0.81, mean ± sd, N = 17) and protein ($\delta^{15}N$: 11.32 ± 1.45, mean ± sd, N=17). Oxygen isotopes suggest that *aqlla* individuals were from various locations across Peru including highlands, non-local coast, and local coast (White *et al.*, 2020). The individuals from the general population did not include a sample as large as the one from the *Huaca* Burial Platform with six individuals with stable carbon ($\delta^{13}C$: -10.77 ± 0.90, mean ± sd) and nitrogen ($\delta^{15}N$: 10.33 ± 1.59, mean ± sd) isotopes and nine individuals with oxygen isotopes. Based on oxygen isotopic data, most of the individuals from the general population lived in the Jequetepeque Valley but were originally from other regions, especially in Compound VI Cemetery.

As seen in Table 7.8, no clear correlation can be established between diet and the total number of each pathological lesion. A negative correlation was calculated between the geographic origin and the total number of caries ($\delta^{18}O$ incisor: $R = -0.72$, p-value = 0.004; $\delta^{18}O$ bone: $R = -0.44$, p-value = 0.043). A significant correlation was also found between $\delta^{18}O$ from incisor and molar ($R = 0.98$, p-value = 0.003) which supports the association between the origin of the individuals and where they grew up. However, the weak correlation between the oxygen isotopes in incisors and bones ($R = 0.16$, p-value = 0.637) is most likely associated with the small sample size of incisors and the broader isotopic variation in teeth compared to bone samples. The only statistically significant correlations between the dental characteristics observed quantitatively are between the number of LEH episodes and caries ($R = 0.39$, p-value = 0.008) and the number of LEH and IGD episodes ($R = 0.44$, p-value = 0.003).

Table 7.8. Pearson correlation for quantitative variables considered in Farfán.

Correlations

		abscess	AMTL	caries	# LEH	# IGD	Stature	$\delta^{13}C$	$\delta^{15}N$	$\delta^{18}OI$	$\delta^{18}OM$
AMTL	Pearson Correlation	.197									
	Sig. (2-tailed)	.167									
	N	51									
caries	Pearson Correlation	.202	.001								
	Sig. (2-tailed)	.155	.996								
	N	51	51								
# LEH	Pearson Correlation	.187	.268	**.389****							
	Sig. (2-tailed)	.214	.075	**.008**							
	N	46	45	45							
# IGD	Pearson Correlation	-.108	-.251	.036	**.435**						
	Sig. (2-tailed)	.713	.386	.903	**.003**						
	N	14	14	14	14						
Stature	Pearson Correlation	.145	.123	-.110	.085	.527					
	Sig. (2-tailed)	.436	.501	.549	.679	.078					
	N	31	32	32	26	12					
$\delta^{13}C$	Pearson Correlation	.319	-.316	.113	.130	.025	-.055				
	Sig. (2-tailed)	.197	.201	.655	.633	.954	.859				
	N	18	18	18	16	8	13				
$\delta^{15}N$	Pearson Correlation	-.016	-.085	.061	-.210	-.492	.249	.199			
	Sig. (2-tailed)	.944	.713	.794	.389	.216	.352	.401			
	N	21	21	21	19	8	16	20			
$\delta^{18}OI$	Pearson Correlation	-.060	-.636*	**-.720****	-.140	-.053	.150	-.001	-.160		
	Sig. (2-tailed)	.839	.014	**.004**	.664	.920	.659	.998	.620		
	N	14	14	14	12	6	11	10	12		
$\delta^{18}OM$	Pearson Correlation	-.015	-.252	-.260	.104	.007	-.339	-.282	-.143	**.981****	
	Sig. (2-tailed)	.967	.483	.468	.776	.989	.373	.401	.675	**.003**	
	N	10	10	10	10	6	9	11	11	5	
$\delta^{18}OB$	Pearson Correlation	-.133	.104	**-.435***	-.146	-.246	.412	.051	.260	.160	-.550
	Sig. (2-tailed)	.554	.644	**.043**	.539	.524	.100	.857	.298	.637	.079
	N	22	22	22	20	9	17	15	18	11	11

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

OI refers to the oxygen isotope obtained from incisors, OM is obtained from molars, and OB is obtained from bone samples.

Statures extracted from the osteobiographies are compiled in Appendix H.

Correlation between #LEH and #IGD is based on the data obtained from micro-CT in 45 teeth.

7.2.5 *Aqlla* on the North Coast: Farfán vs. Túcume

This section presents the results of the comparison between the *aqllakuna* found at Farfán based on the osteobiographies and the *Huaca* Larga at Túcume, the other known *aqlla* site on the North Coast of Peru. A comparison between sites regarding the socio-demographic profiles is followed by a comprehensive summary of their dental health. Appendix E includes the details of the dental pathological lesions recorded at Túcume.

In both sites, the *aqlla* cemeteries contains more females than males (Farfán, N=1 male; Túcume, N=3 males) which is expected as males were not *aqllakuna*. These four males were not considered for the comparison between the *aqlla* sites. The age distribution at the *Huaca* Larga in Túcume indicates that the majority of the females were young adults (53%, YA=10) and the others were divided between subadults (26%, JUV=1, AD=4) and middle-aged adults (21%, MA=4). The *Huaca* Burial Platform population contains more old adults (19%, OA=5) and subadults (62%, CH=3, JUV=7, AD=6) than Túcume (Table 7.8). Based on Pearson's chi-square tests, the age (χ^2 = 19.28, p-value = 0.004, Cramer' V = 0.66) and sex (χ^2 = 10.64, p-value = 0.005, Cramer's V = 0.49) distribution between sites differ significantly due to the presence of undetermined sex in subadults and the larger range of ages at Farfán compared to Túcume (Table 7.9).

Table 7.9. Age and sex distribution in the *aqllawasi* in Farfán (N= 26) and Túcume (N=19).

		Age Distribution (% of individuals)								
Site	**Sex**	NEO	IN	CH	JUV	AD	YA	MA	OA	Total
Huaca Burial Platform, Farfán	NA	0	0	11.5	15.4	3.8	0	0	0	30.8
Huaca Burial Platform, Farfán	F	0	3.8	0	0	19.2	7.7	7.7	19.2	57.7
Huaca Burial Platform, Farfán	F?	0		0	11.1	0	0	0	0	11.5
Huaca Larga, Túcume	NA	0	0	0	0	0	0	0	0	0
Huaca Larga, Túcume	F	0	0	0	5.3	21.1	52.6	21.1	0	100.0
Huaca Larga, Túcume	F?	0	0	0	0	0	0	0	0	0

Data from the *Huaca* Burial Platform obtained from the osteobiographies compiled by Nelson for Farfan's data (Nelson, pers. comm. 2019); data from Túcume obtained from Toyne (2002). Age represents neonatal (NEO), infant (IN), child (CH), juvenile (JUV), adolescent (AD), young adult (YA), middle-aged adult (MA), old adult (OA).

The individuals at the *Huaca* Larga site have a lower level of occlusal wear (none= 82%; very slight=18%) while the *Huaca* Burial Platform has a higher level and broader range with 28% of individuals with a very slight level, 20% with moderate, and 12% with a heavy level of occlusal wear (Table 7.10). A chi-square test reveals a significant difference between sites ($\chi^2 = 30.72$, p-value < 0.001, Cramer's $V = 0.83$).

Table 7.10 Distribution of the level of occlusal wear recorded in the *aqllawasi* from Farfán and Túcume.

	Level of Occlusal Wear					
Site	None	Slight	Slight-Moderate	Moderate	Moderate-heavy	Heavy
Huaca Burial Platform, Farfán	1	7	2	1	5	5
Huaca Larga, Túcume	15	4	0	0	0	0

Data for the *Huaca* Burial Platform obtained from the osteobiographies compiled by Nelson for Farfán (Nelson, pers. comm. 2019); data from Túcume obtained from Toyne (2002).

The individuals from the *Huaca* Burial Platform exhibit slightly more AMTL, abscesses, and LEH than the individuals from the *Huaca* Larga. In contrast, the number of carious lesions is slightly higher at Túcume. However, t-tests reveal no significant difference between the *aqlla* from Farfán and the individuals from Túcume (p-value > 0.05) regarding the number of teeth lost antemortem, the number of abscesses, the number of carious lesions, and the number of LEH (Table 7.11).

Table 7.11. Comparison of the quantitative dental health characteristics recorded through the osteobiographies in Farfán (N=26) and Túcume (N=19).

	***Huaca* Burial Platform, Farfán**		***Huaca* Larga, Túcume**		**t-test**		
Pathological Lesions	Mean	SD	Mean	SD	t	p-value	*d*
# AMTL	0.77	1.57	0.32	0.67	-1.177	0.246	0.37
# Abscesses	0.96	1.58	0.32	0.58	-1.760	0.086	0.55
# caries	2.05	2.06	2.32	2.69	0.364	0.718	0.11
# LEH	1.81	1.63	2.78	1.90	1.695	0.095	0.55

Mean and standard deviation (sd) calculated obtained from the osteobiographies compiled by Nelson for Farfan's data (Nelson, pers. comm. 2019); data from Túcume obtained from Toyne (2002).

As seen in Table 7.12, the prevalence of dental lesions in both *aqlla* varies between 21% and 83% of females in Túcume and between 36% and 81% of *aqllakuna* in Farfán. With the exception of the prevalence of calculus, chi-square tests reveal no significant difference between sites (p-value > 0.05).

Table 7.12. Prevalence of dental health characteristics in the *aqlla* of Farfán (N=26) and Túcume (N=19).

Dental pathological lesions	*Huaca* Burial Platform, Farfán	*Huaca* Larga, Túcume	χ^2	p-value	Cramer's *V*
Abscess	36%	26%	0.475	0.491	0.11
AMTL	36%	21%	1.154	0.283	0.17
Caries	68%	58%	0.465	0.495	0.11
Calculus	77%	42%	4.359	0.037	0.35
LEH	81%	83%	0.037	0.847	0.03

Data for the *Huaca* Burial Platform obtained from the osteobiographies compiled by Nelson for Farfán (Nelson, pers. comm. 2019); data from Túcume obtained from Toyne (2002).

Fifty-eight percent of individuals from Túcume have no dental calculus, while 26% have slight and 11% have moderate levels of calculus. The *aqlla* from Farfán have similar results: 15% of individuals with no calculus, 31% with very slight and slight, and 12% with moderate (Table 7.13). A chi-square test does not reveal a significant difference between sites (χ^2 = 12.71, p-value = 0.013, Cramer's V = 0.53).

Table 7.13. Distribution of the level of calculus recorded in the *aqllawasi* from Farfán (N=26) and Túcume (N=19).

	Level of Dental Calculus				
Site	None	Very Slight	Slight	Moderate	Heavy
Huaca Burial Platform, Farfán	4	5	3	4	2
Huaca Larga, Túcume	11	0	7	3	1

Data for the *Huaca* Burial Platform obtained from the osteobiographies compiled by Nelson for Farfán (Nelson, pers. comm. 2019); data from Túcume obtained from Toyne (2002).

7.3 Methodological Comparisons

This section focuses on the comparison of the three methods (macroscopy, micro-CT, and histology) performed on the focused sample of 45 teeth from 32 individuals to address hypothesis #4 (see p.7). All the observations were made by the author. The data obtained from macroscopy and micro-CT were compiled on the 45 teeth while the data for the comparison between histology and micro-CT was collected on a sub-sample of 14 teeth from 14 individuals. The histological protocol was done on 15 teeth, but one thin-section (LLM3 from E T7) was unsuccessful. This section covers intra-observer variation and the comparison of methods for each dental characteristic including caries, occlusal wear, LEH, IGD, dental calculus, hypercementosis, and other dental anomalies.

Correlative tomography was used to register the histological images to the micro-CT volumes of six teeth to highlight the difference in resolution between methods (Figure 7.9). Appendix M describes the results further of this protocol. The combination of both imaging techniques allowed a more accurate comparison between the thin-section and the corresponding micro-CT slice for the dental characteristics. For the eight other teeth with histological images, the correspondence with micro-CT slice was approximated based on visible features.

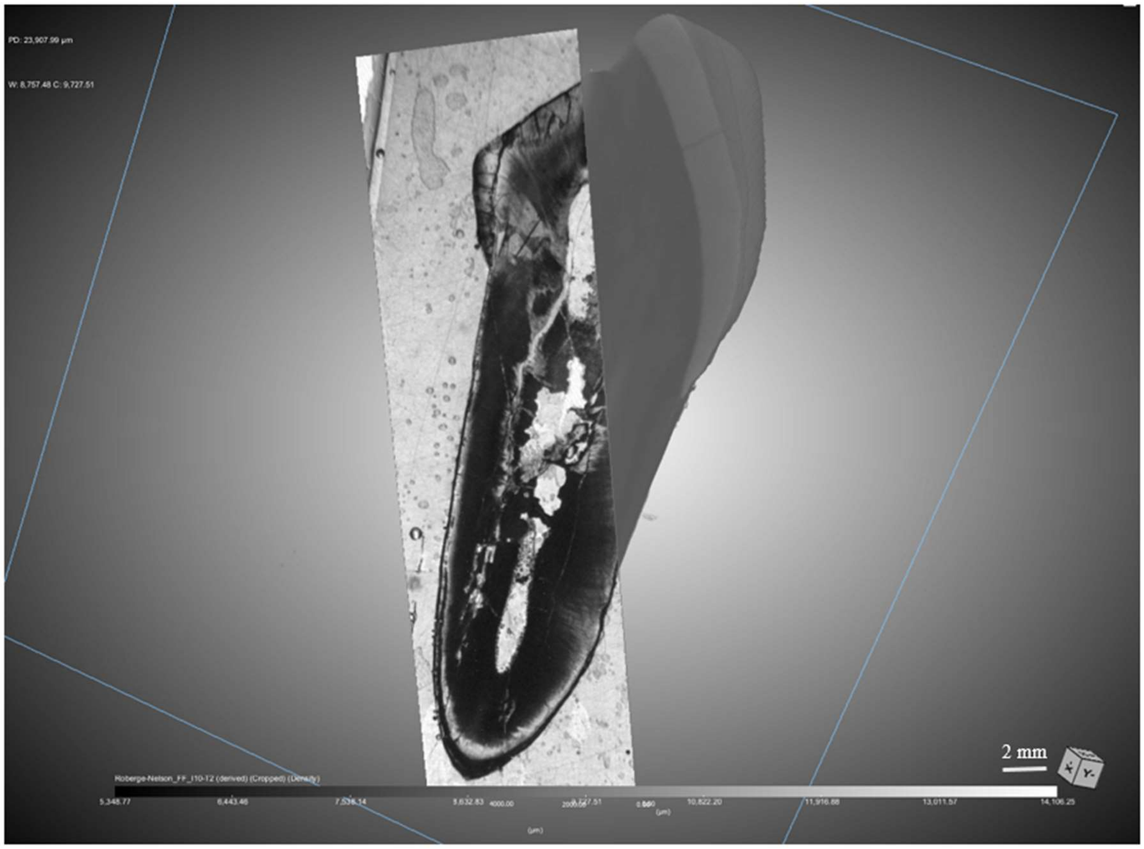

Figure 7.9. Image of correlative tomography done on the upper right first incisor of individual I10 T2 showing one half of the mesio-distal surface with histology, and one half with micro-CT. Voxel size of 12.5 µm. The gray scale indicates changes in the density of the material.

7.3.1 Intra-Observer Variation

To evaluate intra-observer variation, a random subsample of 11 teeth was re-examined macroscopically two weeks after the first assessment. The two sets of observations were compared using the weighted kappa coefficient. The value was between 0.81 and 1 depending on the pathological lesion being observed with more variation for the presence of LEH due to the ranking system (“absent”, “possible”, “probable”, “present”) (Table 7.14). Similarly, observations were recorded twice, two months apart, on all the teeth with the micro-CT. The weighted kappa coefficient obtained varied between 0.73 and 1 for the different pathological lesions which more variation in the recording of calculus. Thus, the observations are considered reliable (Landis & Koch, 1977). The second observations done with micro-CT were considered for the comparative analysis since they reflect the experience in recording pathological lesions with this technique.

Table 7.14. Kappa coefficient to evaluate intra-observer variation using macroscopy (N=11) and micro-CT (N=45).

Dental Characteristics	Kappa Macroscopy	Kappa Micro-CT
# Caries	0.96	0.92
Wear Levels	0.94	0.92
Presence of LEH	0.81	0.94
# LEH	1.00	0.87
Presence of IGD	-	0.90
Dental Calculus Levels	1.00	0.73
Hypercementosis	1.00	0.92

7.3.2 Macroscopy, Micro-CT, and Histology

7.3.2.1 Carious Lesions

Macroscopy vs. Micro-CT

A total of 13 carious lesions on nine teeth were noted with macroscopy. The observations from micro-CT revealed more caries with a total of 16 on ten teeth which also included "possible caries". This classification was used when a lesion only partially fulfilled the list of criteria established in Table 6.5. The ability to see the internal structure of the teeth allowed for the measurement of the depth of the lesion and documented the demineralisation of dental tissue underneath the surface due to the infection. This is especially visible on the ULM3 from individual J25 T8 (Figure 7.10). With macroscopy, 2 caries were recorded on this tooth, but 4 were noted with micro-CT as smaller caries, considered as pits on macroscopy, fulfilled the criteria for caries identification on micro-CT. Despite these differences in the total number of caries, a paired t-test performed on the two datasets did not find a significant difference ($t = 0.44$, p-value = 0.661, Cohen's $d = -0.09$).

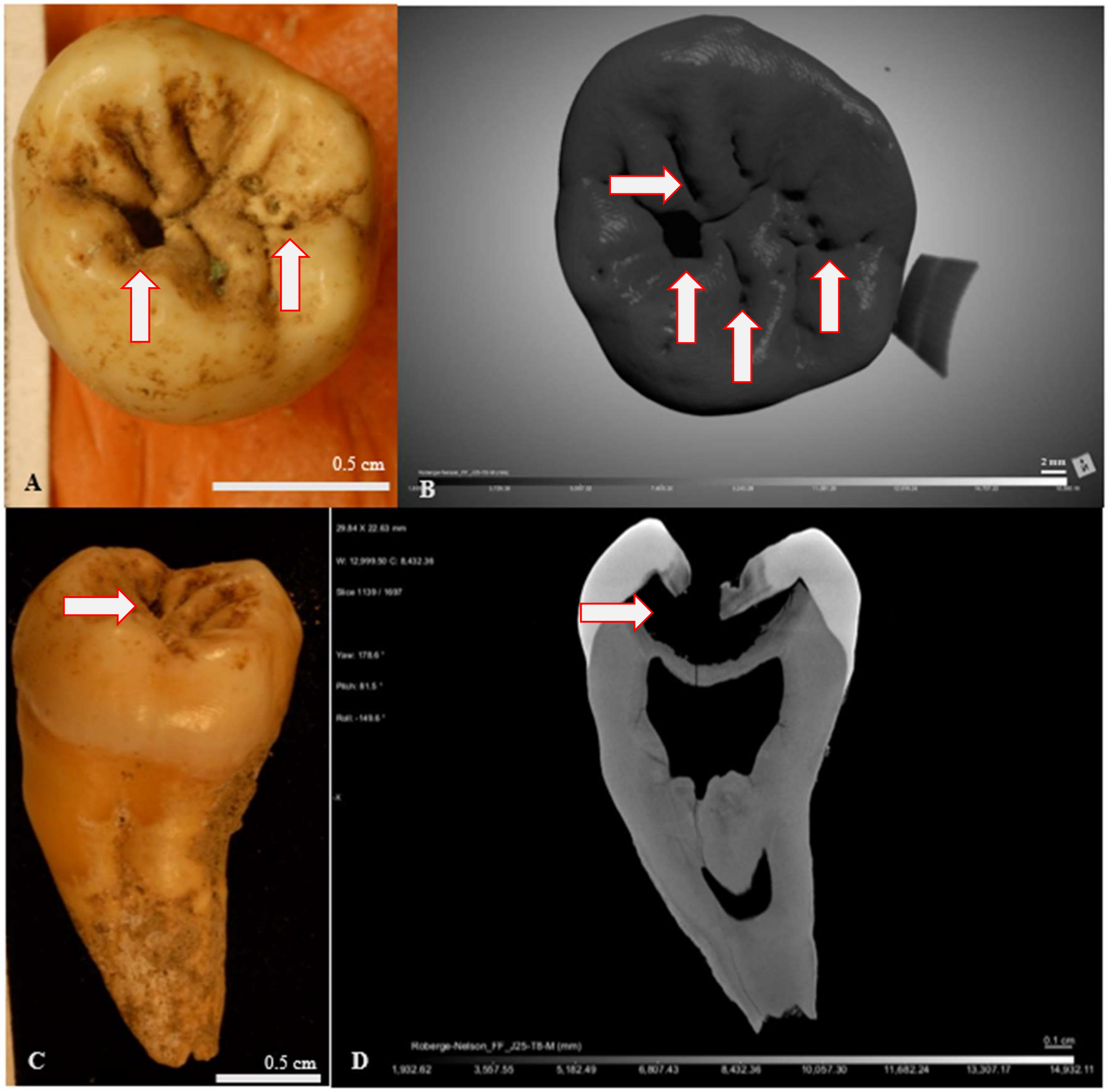

Figure 7.10. Carious lesions in ULM3 from individual J25 T8. A) occlusal surface macroscopically, the arrows identified the two carious lesions. B) Occlusal surface from micro-CT volume, voxel size at 12.5 µm. C) Lingual surface macroscopically. D) Mesio-distal cut done on the micro-CT volume to highlight destruction of tissue.

<u>Histology vs. Micro-CT</u>

One caries and one "possible" caries were clearly identified on both the thin-section and the corresponding micro-CT slice in the URM3 of individual N T1a (Figure 7.11). However, by considering the whole tooth, a total of three caries were recorded with micro-CT instead of the two recorded with histology. This difference, not significant based on a t-test (t = 1.12, p-value = 0.237, Cohen's d = 0.46) is due to the analysis of the 3D volumes with micro-CT which allows the analysis of the whole tooth while histology only captures one specific slice of the tooth.

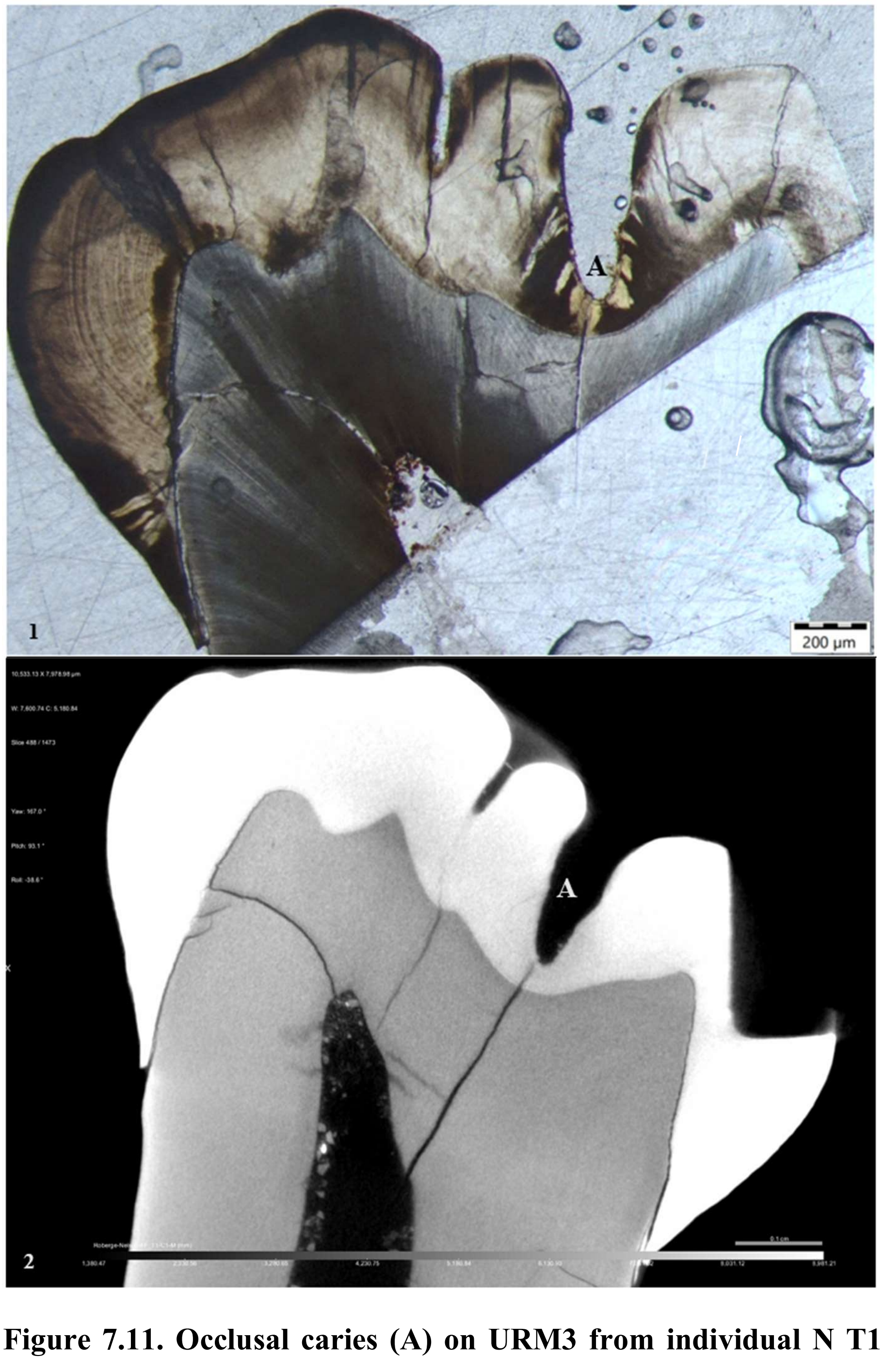

Figure 7.11. Occlusal caries (A) on URM3 from individual N T1a. 1) Thin-section observed at a resolution of 160X under a light microscope; 2) corresponding view on the micro-CT volume, voxel size at 12.5 um.

7.3.2.2 Occlusal Wear

Macroscopy vs. Micro-CT

The level of occlusal wear was similarly recorded between macroscopy and micro-CT as seen in Table 7.15. The slight variation found was not statistically significant based on a chi-square test (χ^2 = 0.46, p-value = 0.977, Cramer's V = 0.73). An example of a "moderate" level of occlusal wear can be found in Figure 7.12.

Table 7.15. Distribution of occlusal wear level using macroscopy and micro-CT (N=43).

	Wear Level					
Methods	None	Slight	Slight-Moderate	Moderate	Moderate-Advanced	Advanced
Macroscopy	10	11	8	9	5	0
Micro-CT	10	9	10	9	5	0

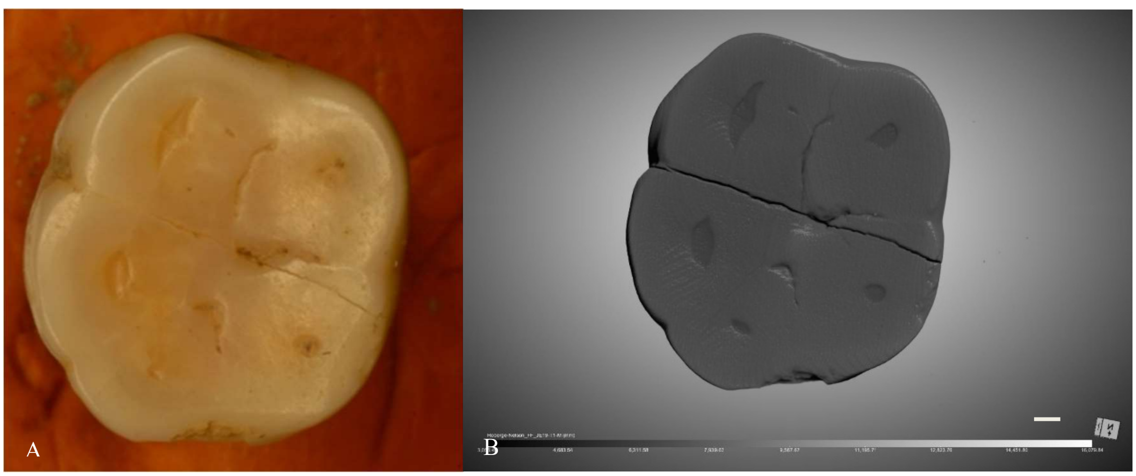

Figure 7.12. LLM1 from individual JE19 T1 presenting a "moderate" level of occlusal wear. A) Macroscopy; B) Micro-CT view, voxel size at 12.5 µm.

Histology vs. Micro-CT

Although the difference was not statistically significant based on a chi-square test (χ^2 = 5.95, p-value = 0.311, Cramer's V = 0.46), the comparison between histology and micro-CT revealed more variable results as seen in Table 7.16. This is principally due to the inability to assess the occlusal surface with histological data.

Table 7.16. Distribution of occlusal wear level using histology and micro-CT (N=13).

	Wear Level					
Methods	None	Slight	Slight-Moderate	Moderate	Moderate-Advanced	Advanced
Histology	3	3	4	1	2	0
Micro-CT	1	4	3	5	0	0

7.3.2.3 Linear Enamel Hypoplasia

Macroscopy vs. Micro-CT

Linear Enamel hypoplasia (LEH) was detected slightly more often with micro-CT compared with macroscopy. Macroscopic observations identified seven teeth with clear LEH, four with "probable" LEH, and one with "possible" LEH. Micro-CT, in comparison, identified eight teeth with clear LEH, seven teeth with "probable" LEH, and one tooth with "possible" LEH. However, a chi-square test for the presence of LEH did not highlight a significant difference between the two methods (χ^2 = 1.06, p-value = 0.787, Cramer's V = 0.11).

The total number of episodes of non-specific stress in these 45 teeth showed a few differences in the detection of LEH (Figure 7.13). A total of 23 defects were observed macroscopically in 15 teeth for an average of 0.51 ± 0.87 (mean ± sd) defects per tooth. The average was 0.58 ± 0.99 (mean ± sd) defects per tooth with micro-CT for a total of 26 defects in 16 teeth. The number of enamel defects detected macroscopically vs by micro-CT is not statistically different based on a paired t-test (t = 0.34, p-value = 0.735, Cohen's d = 0.07).

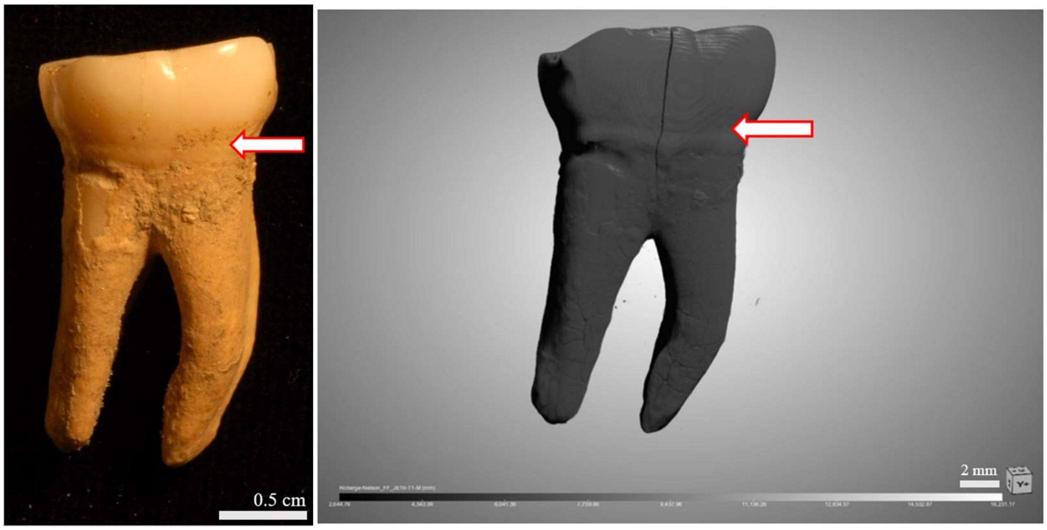

Figure 7.13. LEH (arrows) on the LLM1 from individual JE19 T1. A) macroscopic view; B) 3D view from micro-CT, voxel size at 12.5 µm.

Histology vs. Micro-CT

Among the 14 teeth for the comparison of micro-CT and histology, seven had evidence of LEH with micro-CT (4 present and 3 probable) compared to eight teeth observed via histology (4 present, 3 probable, 1 possible). The difference between methods is not significant for the presence of LEH (χ^2 =1.08, p-value = 0.783, Cramer's *V* = 0.20).

The total number of LEH defects was 13 with histology and 17 with micro-CT showing a slightly higher detection with micro-CT. On average, the histological analysis documented 0.93 ± 1.00 (mean ± sd) defects per tooth while with micro-CT the average was 1.21 ± 1.48 (mean ± sd) defects per tooth. A t-test found no significant difference in the number of LEH detected (t = 0.60, p-value = 0.554, Cohen's *d* = 0.23).

7.3.2.4 Interglobular Dentin

Macroscopy vs. Micro-CT

Since it is necessary to access the internal structure of the tooth to assess IGD, this characteristic was not observed macroscopically.

Histology vs. Micro-CT

IGD was present in all 14 teeth evaluated through micro-CT and histology, however, while the defect was identified as clearly present in all the histological slices, it was characterised as “possible” and “probable” in respectively two and three teeth with micro-CT. This slight difference leads to significant difference according to a chi-square test ($\chi^2 = 6.09$, p-value = 0.048, Cramer’s *V* = 0.47). However, the moderate effect size suggests this difference is not as pronounced. It is most likely due to the categories (“possible”, “probable”, “present”) which are not acknowledged by the statistical test.

The number of episodes of IGD detected by histology vs. micro-CT is significantly different (t = -3.89, p-value = 0.001, Cohen’ *d* = -1.47) with histology revealing more defects. A total of 96 episodes were noted with histology for an average of 6.9 ± 2.1 (mean ± SD) episodes per tooth, whereas 51 episodes for an average of 3.6 ± 2.2 (mean ± SD) per tooth, were noted on the corresponding micro-CT slice. The “possible” IGD lines are the faintest and were often not systematically visible on micro-CT scans. The “slab” function on micro-CT showed similar results (see Appendix L). Figure 7.14 shows the difference in visibility between histology and micro-CT.

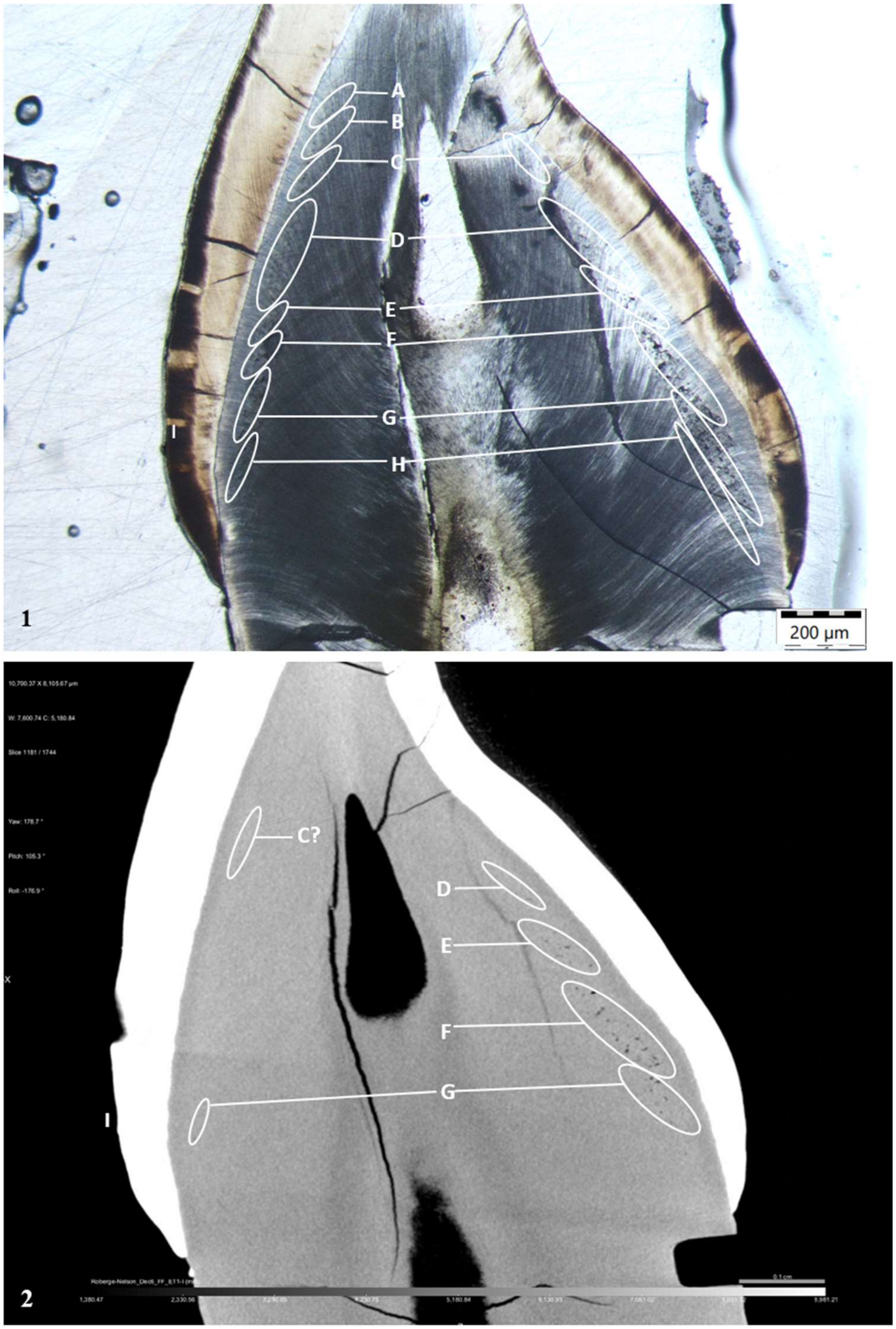

Figure 7.14. Identification of IGD and LEH in the crown of URI1 from individual E T1 from the *Huaca* Burial Platform. 1) Mesio-distal slice obtained with histological analysis seen under a light microscope at 125X; 2) 2D mesio-distal slice with micro-CT corresponding to thin-section, voxel size of 12.5 µm. A, B are possible IGD

(faint, unilateral, discontinuous, not on micro-CT); C are probable IGD (visible-faint, bilateral, discontinuous, on micro-CT); D are probable IGD, possibly 2 lines on the lingual side (visible-faint, bilateral, discontinuous, on micro-CT); E, F, G are distinct IGD (visible, bilateral, discontinuous, on micro-CT); H is probable IGD (faint, bilateral, discontinuous, not on micro-CT); I possible LEH.

7.3.2.5 Comparison with Higher Resolution Micro-CT

Among the 14 teeth processed for histological analysis, eight teeth were scanned focusing on their crown prior to the cut with a greater resolution (voxel size at 5.5 µm or 6.75 µm). The resolution does not affect the visibility of carious lesions or LEH. However, a slight difference can be seen for IGD. The greater resolution revealed slightly more IGD episodes than the micro-CT slices at 12.5 µm. In total, 40 episodes of IGD were noted on the micro-CT slices with a voxel size of 5.5 µm or 6.75 µm compared to 38 episodes on micro-CT volumes with a voxel size of 12.5 µm. Yet, even in the higher resolution micro-CT scans, histology still revealed significantly more episodes than micro-CT volumes ($t = 3.92$, p-value = 0.006).

7.3.2.6 Dental Calculus

<u>Macroscopy vs. Micro-CT</u>

The presence of dental calculus was identified in six teeth from the focused sample using micro-CT. It was present on only four teeth macroscopically including URM3 from C T11B seen in Figure 7.15. This comparison revealed the possibility of detecting slight levels of calculus with micro-CT that is not always easily identifiable macroscopically. A chi-square test shows no significant difference between methods ($\chi^2 = 0.45$, p-value = 0.502, Cramer's $V = 0.07$).

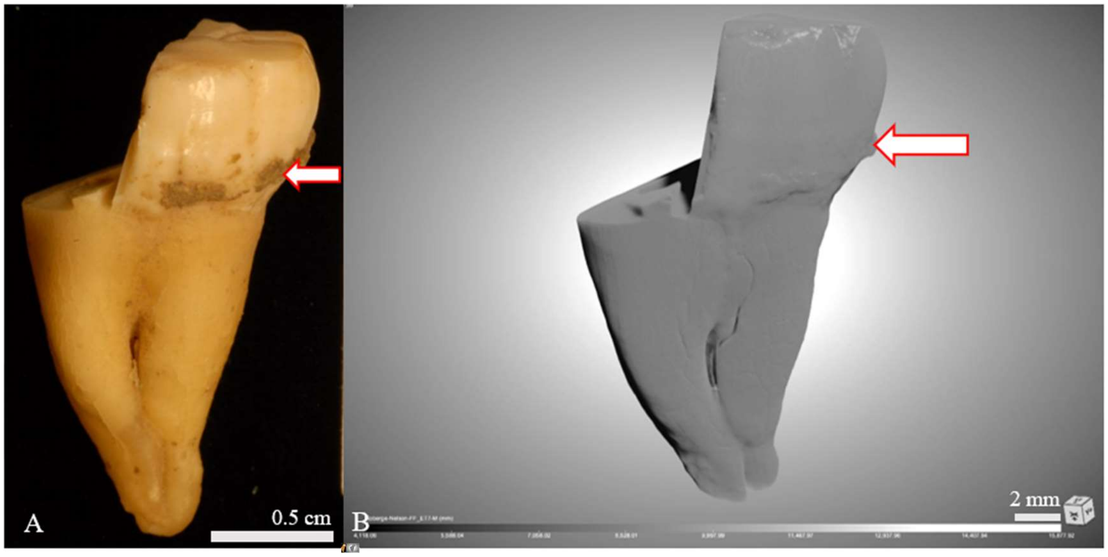

Figure 7.15. Slight dental calculus (arrows) on URM3 from individual E T7. A) View macroscopically; B) 3D view from micro-CT.

<u>Histology vs. Micro-CT</u>

Dental calculus is present in one of the 14 teeth cut for histological analysis (LLI1 from E T7 as seen in Figure 7.16). The calcified deposit is present in two teeth from the micro-CT data (LLI1 from E T7, and ULP4 from CH 1b). However, while this difference is not statistically significant based on a chi-square-test ($\chi^2 = 0.37$, p-value = 0.541, Cramer's $V = 0.12$).

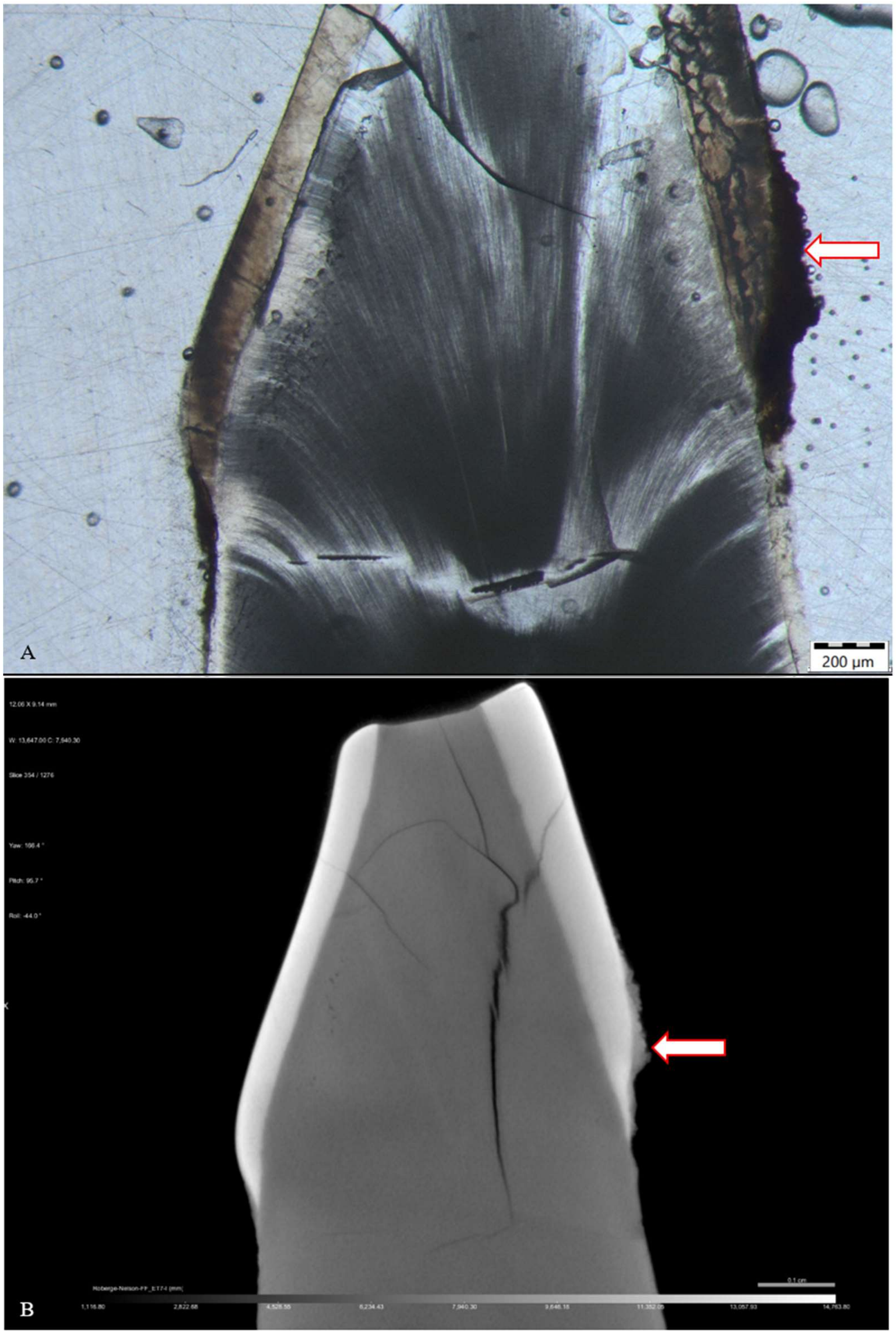

Figure 7.16. Slight dental calculus (arrows) on LLI1 from individual E T7. A) View from thin-section under a light microscope at 160X; B) view of corresponding micro-CT slice on the bottom with a voxel size of 12.5 µm.

7.3.2.7 Hypercementosis

Macroscopy vs. Micro-CT

Hypercementosis was observed on four teeth in the focused sample using macroscopy, including the URM3 from C T11A (Figure 7.17). In comparison, with micro-CT, since it is possible to observe the thickness of the cementum, slight hypercementosis could be observed in three additional teeth for a total of seven teeth. This difference is not statistically significant based on a chi-square test ($\chi^2 = 0.93$, p-value = 0.334, Cramer's V = 0.10).

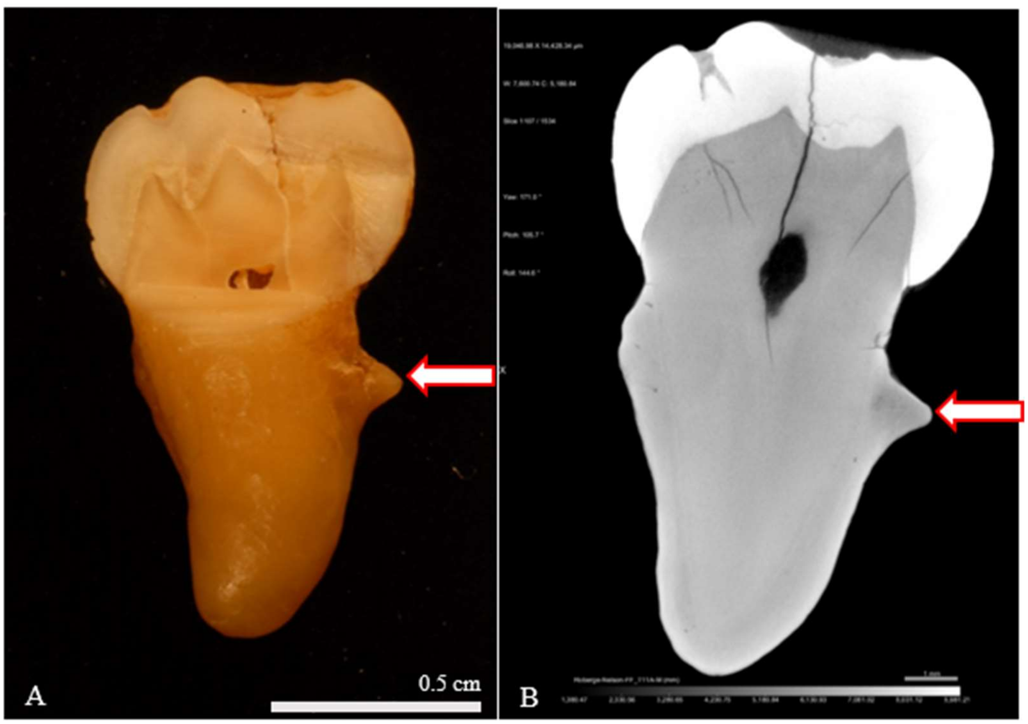

Figure 7.17. Hypercementosis (arrows) in URM3 from individual C T11A. A) As seen from macroscopy; B) Seen on grayscale with micro-CT, voxel size 12.5 µm.

Histology vs. Micro-CT

Slight hypercementosis was seen on the URC of individual J14 T2 on the thin section obtained with histology (Figure 7.18). With micro-CT, it was recorded in two teeth from the sub-sample. The observations between methods do not differ statistically significantly according to a chi-square test ($\chi^2 = 0.37$, p-value = 0.541, Cramer's $V = 0.12$).

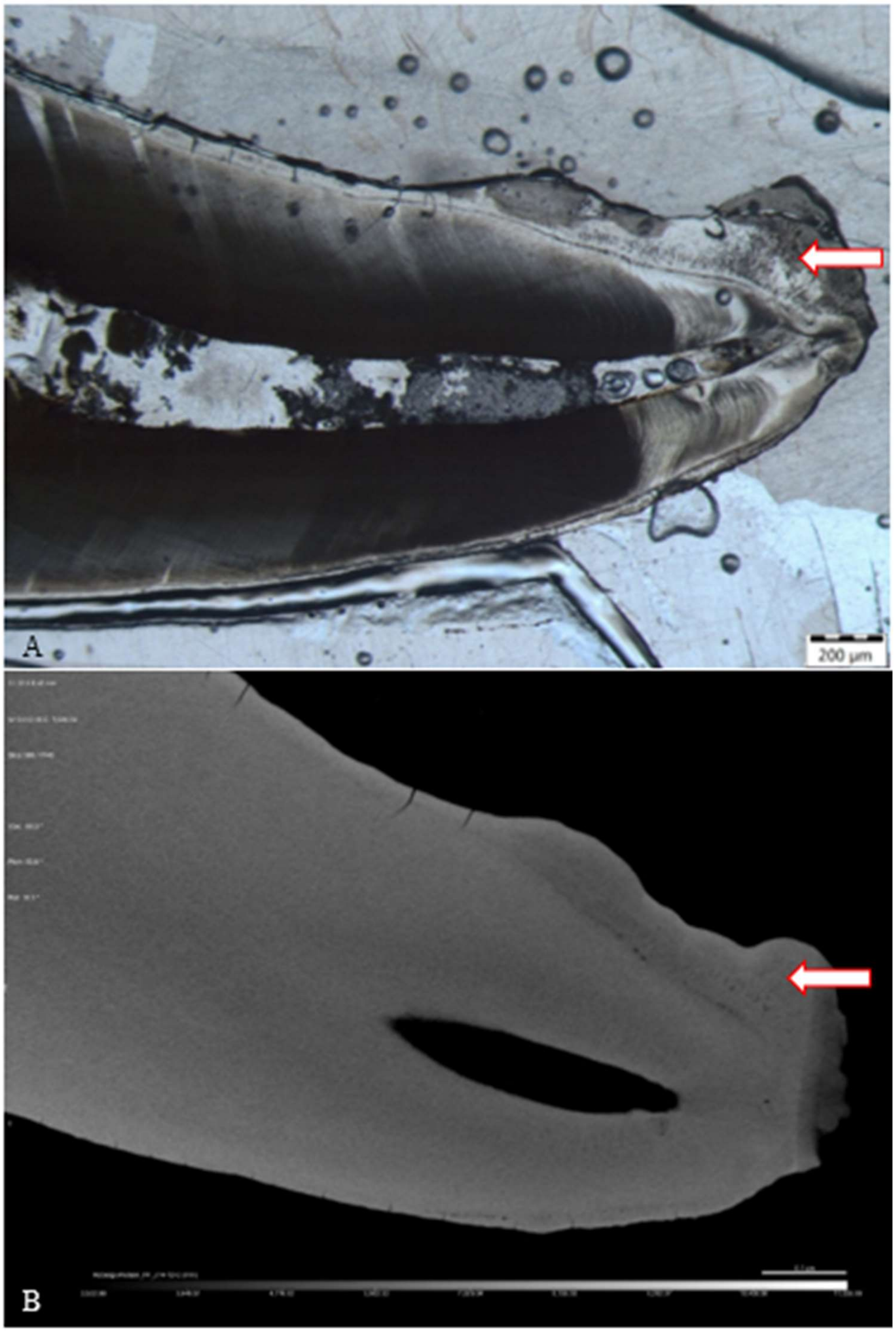

Figure 7.18. Hypercementosis (arrows) in URC from individual J14 T2. A) Thin section observed with a light microscope at 125X; B) corresponding micro-CT slice, voxel size at 12.5 µm.

7.3.2.8 Additional Defects

In addition to the dental pathological lesions just presented, some supplementary characteristics were noted in the focused sample with micro-CT data, that are not visible macroscopically. Possible pulp stones were observed in six teeth (3 from Cemetery J, 1 from Compound VI Cemetery, and two from the *Huaca* Burial Platform) (Figure 7.19). As well, possible enamel pearls were present in six teeth (2 from Cemetery J and 4 from the *Huaca* Burial Platform (Figure 7.20). Pulp stones and enamel pearls can be identified on micro-CT based on the different densities of dental tissues; pulp stones have a similar density as dentin, while enamel pearls have the density of enamel. These defects were not observed in the subsample for histological analysis.

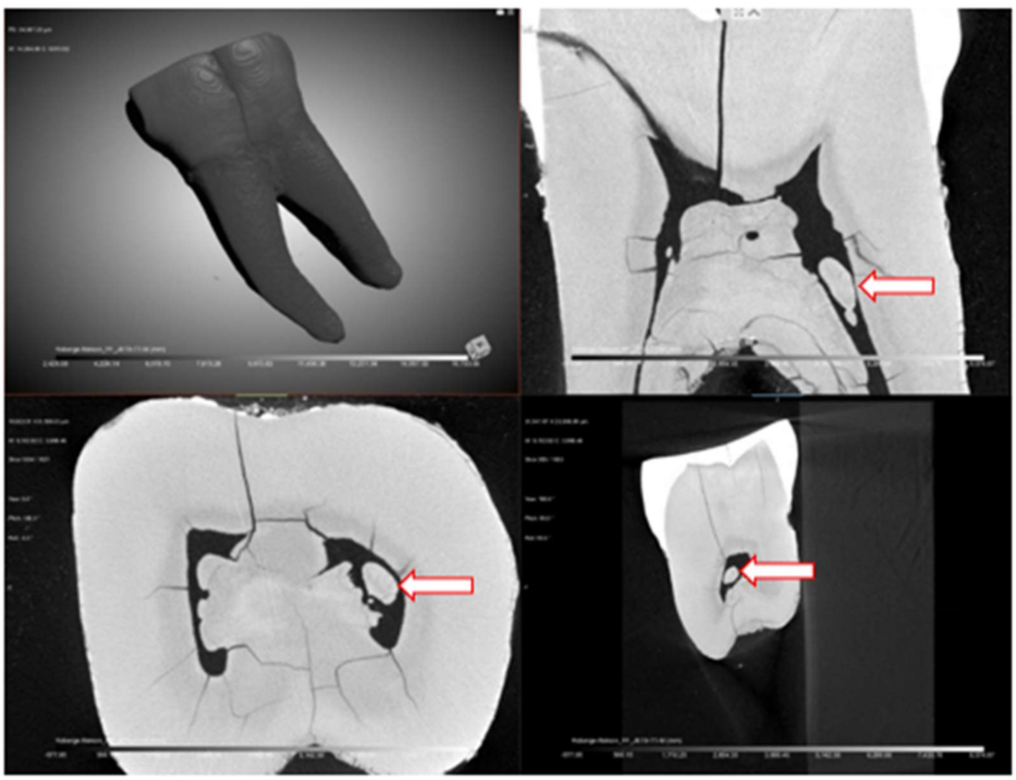

Figure 7.19. View from the four views in Dragonfly of pulp stone (arrows) found in LLM1 from individual JE19 T1 as seen from the occlusal cut (bottom left), bucco-lingual cut (upper right), and medio-distal cut (lower right).

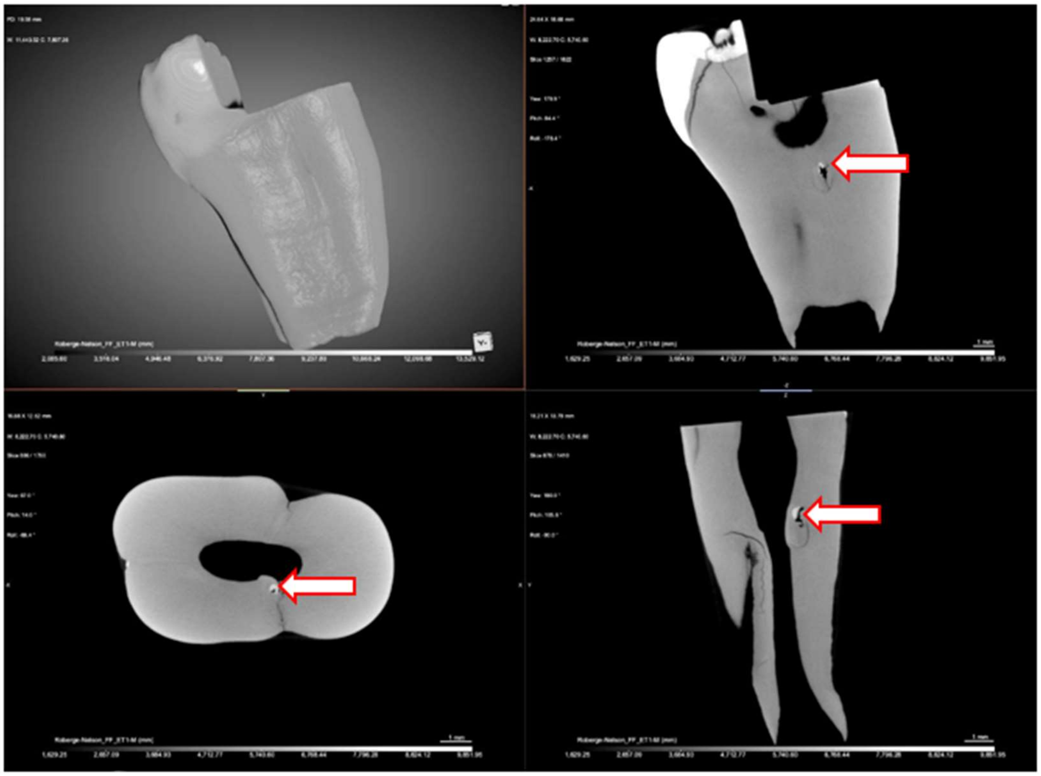

Figure 7.20. View from the four views in Dragonfly of a possible enamel pearl (arrow) found in URM3 from individual E T1 as seen from the occlusal cut (bottom left), medio-distal cut (upper right), and bucco-lingual cut (lower right).

7.3.2.9 Summary

<u>Macroscopy vs. Micro-CT</u>

In brief, for the comparison between macroscopy and micro-CT, the difference in detection was not statistically significant for any of the externally visible pathological lesions. However, as seen in Figure 7.21, the comparison between macroscopy and micro-CT volumes of 45 teeth reveals higher but non-significant detection rates of calculus, hypercementosis, presence and number of LEH, and carious lesions with micro-CT. The wear levels were similar between the methods. Some characteristics including IGD, pulp stones, and internal enamel pearls could not be seen with macroscopy.

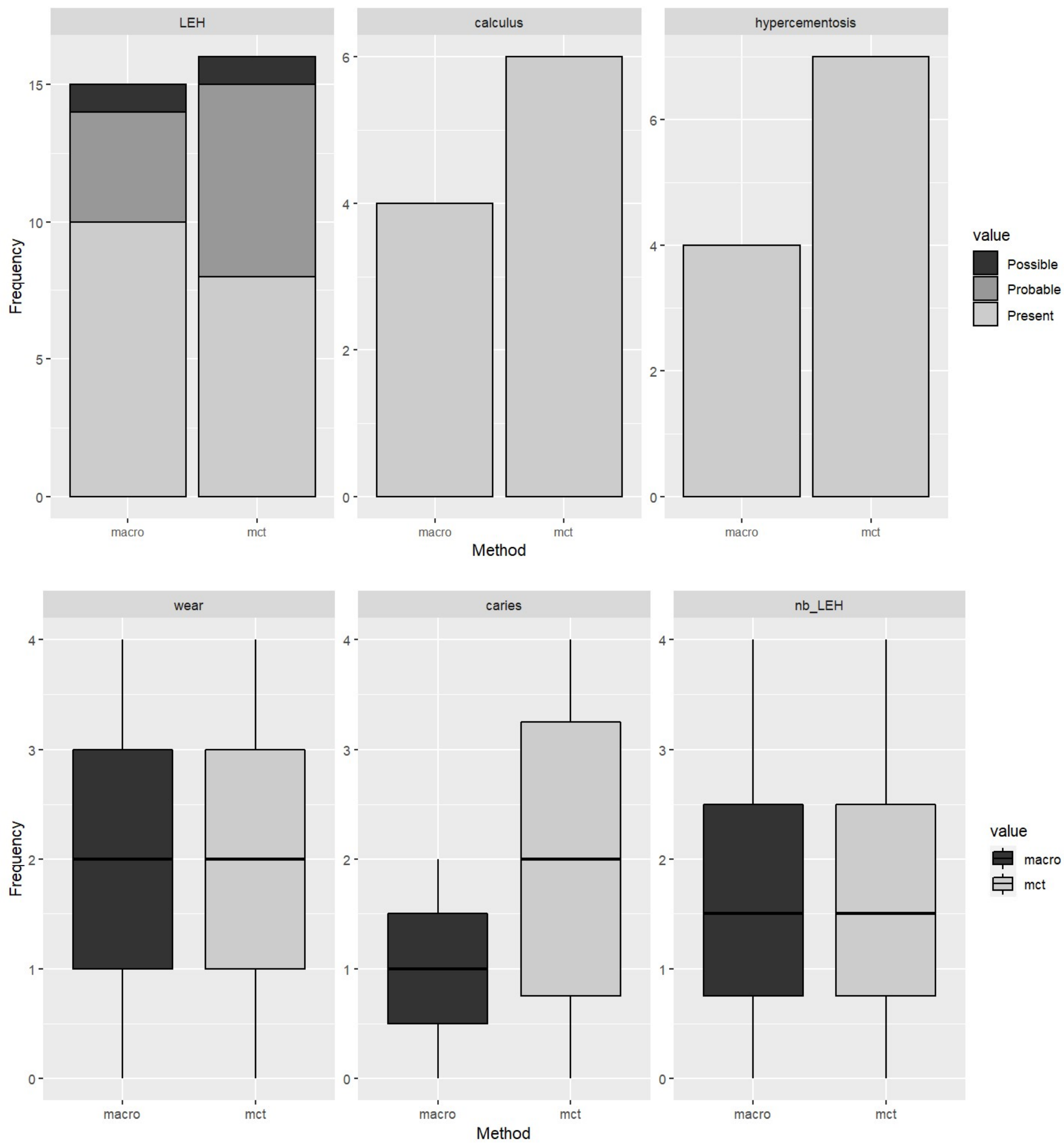

Figure 7.21. Summary of the detection rate of dental pathological lesions in 45 teeth using macroscopy and micro-CT.

Histology vs. Micro-CT

The comparison between histological analysis and corresponding micro-CT slices also presented no significant difference in the detection of pathological lesions except regarding the number of episodes of IGD. Figure 7.22 illustrates a higher wear level and more IGD episodes, but fewer carious lesions, fewer episodes of LEH, and less hypercementosis and calculus detected with histology compared to micro-CT. The presence of IGD were similar between these methods. Pulp stones and enamel pearls were not present in the thin-sections and therefore could not be compared.

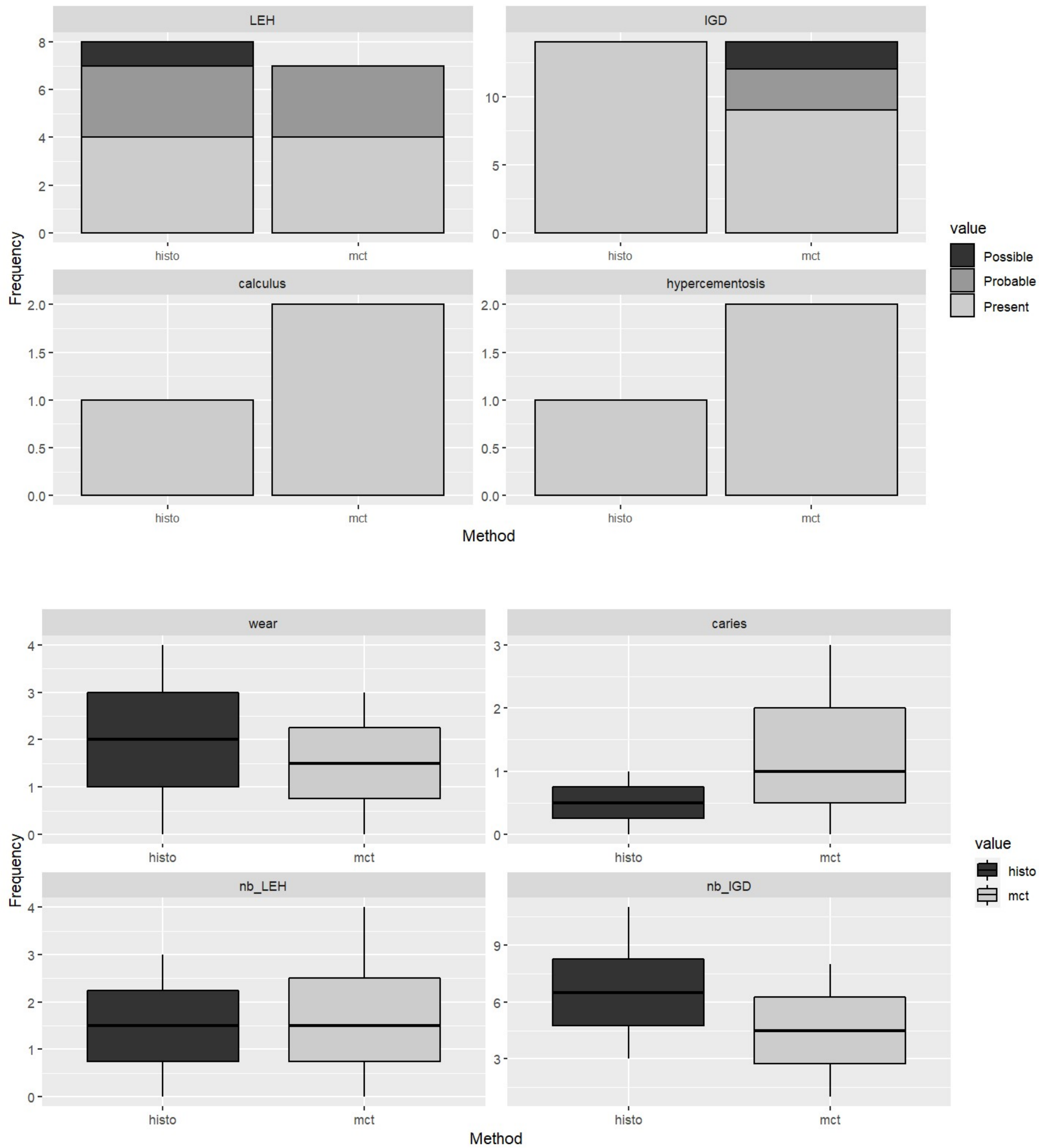

Figure 7.22. Summary of the detection rate of dental pathological lesions in 14 teeth using histology and micro-CT.

Chapter 8 - Discussion

This thesis investigates the identity of the *aqllakuna* excavated at Farfán through their teeth using a multi-modal approach. It aims to evaluate if the dental health of the *aqlla* from the *Huaca* Burial Platform is representative of the portrait of these females depicted in the Spanish Chronicles. The individuals excavated in the four other cemeteries at Farfán were used as a comparative sample to represent the "general population" on site. A comparative methodological approach is used to foster discussions about the use of destructive vs. non-destructive analysis.

The Spanish Chronicles represent a unique source of information to interpret observed patterns in bioarchaeological evidence. However, the Chronicles offer differing and undoubtedly biased accounts to characterise the Incas and the *aqlla* institution. Details differ from one source to another but overall, the ethnohistorical sources describe the *aqllakuna* as highly valued young females selected before puberty based on their beauty, rank, and/or skills to cook, weave fine cloth, brew *chicha*, and practice rituals to honour deities hidden behind the walls of their *aqllawasi* (Acosta, 2011 [1590]; Cieza de León, 2010 [1553]; Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Garcilaso de la Vega 2010 [1609]; Polo de Ondegardo, 1873 [1572]). These females were dedicated to religious and economic purposes for the state until they reached approximately fourteen years of age. Then, they could be given (or taken) to marry elite males, become concubines, serve as another Inca wife, be sacrificed or become *mamakuna* to train new *aqllakuna* (Cobo, 1979[1653]; Sarmiento, 1967[1572]). Based on this description, this chapter will discuss the following data expectations about the *aqlla* by focusing on evidence of dental health in Farfán:

1) As these females supposedly brewed *chicha* regularly, which involves mastication of maize to start the fermentation process, they should have a higher frequency of carious lesions and higher wear than the general population who experienced a regular diet and occasional chicha brewing.

2) As these females were supposedly venerated and considered as part of the elite, they may have had access to better food and cultural conditions resulting in generally good health, so they should have a lower prevalence of physiological stress lesions than the general population.

3) As these females were supposedly drawn mostly from local elite families, which would have shared similar lifeways, they should have a large degree of uniformity in their dental health when compared to the general population in Farfán.

Finally, the research goal regarding the comparative methodology will be discussed to evaluate if:

4) Micro-CT reveals as many features as traditional histological methods without destroying teeth and thus can be used as an alternative, or complement, micro-analytical method.

8.1 The *Huaca* Burial Platform as an *Aqlla*

The demographic profile of the *aqlla* cemetery supports the expected bias toward females. This cemetery contains eight individuals of unknown sex (all subadults), 18 females, and one male (CH 1a) ranging in age from infancy (1.0-2.9 years of age) to old adulthood (> 40 years of age). The single male has characteristics reminiscent of the *Dos Cabezas* giants thought to be *eunuchs* (Donnan, 2001), including tall stature (165.7 cm) and osteoporosis. While not an *aqlla,* the male was included among the individuals from the *Huaca* Burial Platform for the analyses between Farfán's cemeteries.

The females excavated at the *Huaca* burial Platform are mostly young individuals (63% of subadults) which agrees with the *aqllakuna* being inducted into the institution at a young age. However, the age range presents more variability than expected with some individuals much younger than the 8 to 14 years of age claimed in the ethnohistorical documents. The cemetery includes one infant (1.0-2.9 years; N T1c), three children (3.0-5.9 years; CH 1e/ f/ d), seven juveniles (6.0-11.9 years; CH 1g, C T2D/ E/ F , E T6/ T9/ T10), six adolescents (12.0-19.9 years; C T11C, C T2B/ C, E T1/ T4, N T1a), two young adults (20.0-29.0 years; CH 1b, C T2A), two middle adults (30.0-39.0 years; CH 1c, E

T5), and five old adults (> 40 years; C T11A/ B/ D, E T7, N T1b). It is interesting to note that the infant and children from the North Tomb 1 and the Looted Chamber 1 are located around the platform whereas the East Tomb, Center Tomb 11, and Center Tomb 2 are in the center of the platform (Figure 8.1). The position of the infant and children around the platform might indicate an association with the *aqllawasi* without being identified yet as *aqlla.* Given their young age, they may have been orphans given to the *aqllawasi* for potential future service (Guamán Poma de Ayala, 2006 [1615/1616]), or their burial around the platform may have been a form of offering (Mackey & Nelson, 2020). This would also explain the presence of the infant which does not align with the expected virginity. Older individuals associated with the *aqllawasi* who may have been buried in and around the *Huaca* burial Platform include guards (Betanzos, 1996 [1576]) and servants (Garcilaso de la Vega, 2010 [1609]; Guamán Poma de Ayala, 2009 [1615/1616]), The single male in the sample, and some of the middle and older-adult females (if not *mamakunas*), may have held these latter roles. With these possibilities in mind, the youngest *aqllakuna* may well have been the juveniles, and the oldest may have been the adolescents, with a few young adult females remaining in the *aqllawasi.* This scenario is broadly concordant with the ethnohistorical sources.

Numerous textiles, ceramics, and weaving materials were buried with the females of the *Huaca* Burial Platform (Appendix D) supporting the high-status activities associated with the *aqlla* institution. Personal grave goods were interred with all females, but weaving implements were only found with two adults (E T5, E T7) and an adolescent (E T4). Individual E T7 had high-status grave goods, which combined with her age (> 60 years), suggested she was probably a *mamakuna* (Mackey & Nelson, 2020).

The overall health of the females was variable. Diverse spinal conditions including degeneration of the spine, scoliosis, and spondylolysis were reported in seven individuals (N T1a/ b; E T1/ T7; C T11A/ B/ C). Additional health issues were recorded including one individual with evidence of blood-borne infection (N T1c), one individual with avascular necrosis of the femoral head or a slipped femoral epiphysis (E T5), three juveniles with scurvy (E T6; E T9; E T10), one individual with tuberculosis (N T1b), and one with bowed fibula (C T11C) that might be associated with rickets, dysplasia, or a congenital defect (Mackey & Nelson, 2020).

The oxygen isotope values suggest that these individuals were from various regions as suggested by Cieza de León (2010 [1553]). Among the *Huaca* Burial Platform, four females had spent time in the highlands (C T11A/ D; E T5/ T6), two females were from non-local coasts (C T11B; E T10), and nine individuals were from the local coast (C T11C; E T1/ T4/ T7/ T9; N T1a/ b/ c; CH 1a) (Mackey & Nelson, 2020). It is worth noting that, at the *Huaca* Burial Platform, the *mamakuna* E T7, the individuals from North Tomb 1 and the male from Chamber 1 were all local to the Jequetepeque region suggesting that high status individuals were drawn from the local area. Thus, geographic origins might have had an impact on the role of the individuals associated with the institution.

The carbon and nitrogen isotope values were also quite variable within this cemetery. Among the individuals with isotopic data, the diet was high in maize and marine protein for seven of them (C T2B/ C; N T1a/ b; E T1/ T4/ T7), including the *mamakuna* (E T7) who has the highest nitrogen isotope value suggesting high protein intake. A diet rich in protein, but low in maize was found in the male (CH 1a). The individuals from the East Tomb and the Center Tomb 11, located in the middle of the platform, exhibit a lower level of protein intake than the individuals from the Center Tomb 2, North Tomb 1, and looted Chamber 1 interred around the platform. The average isotopic signatures for carbon and nitrogen were recorded in E T10 while E T9 presents a low protein-intake profile but an average $\delta^{13}C$ value. E T6 has a low maize intake and the lowest protein intake on site. In brief, in the East Tomb, the *mamakuna* presents values supporting a richer diet, followed by the adolescents while the juveniles, affected by scurvy, had a diet lower in maize and protein than the other *aqllakuna* on site. This parallel between age and diet might be the reflection of the social structure within the *aqllawasi*.

Preliminary bioarchaeological evidence from the *Huaca* Burial Platform suggests this cemetery was associated with an *aqlla*. The females from the *Huaca* Burial Platform exhibit more variability than all the other mortuary sites at Farfán in terms of health, geographic origin, and diet, than all the other mortuary samples at Farfán, which is non-concordant with the description of the *aqlla* in the Spanish Chronicles (Mackey & Nelson, 2020). The *Huaca* Burial Platform contains the largest number of individuals of any of the cemeteries at Farfán, so offers a sample size upon which to better reconstruct the lifeways of the *aqlla*. The detailed analysis of the *aqlla*'s dental health adds another line of evidence to their demystification.

8.2 Hypothesis #1 – Cariogenicity

Maize was considered a life-supporting crop in the Andes (Rostworowski, 1975). It was also associated with power and status (Hastorf, 2006). Therefore, higher status individuals and elites, believed to have access to a preferential diet, would have a diet richer in maize than lower status individuals (Costin & Earle, 1989). A diet rich in carbohydrates such as maize is correlated with an increase in carious lesions (Powell, 1985). The harsh and coarse texture of maize combined with the use of mano and metate grinding stones likely introducing rock chips to the food which contributes to attrition in teeth (Hillson, 1996).

The Spanish Chronicles describe the *aqlla* as being part of an elite group brewing *chicha* for ceremonies as one of their main responsibilities (Betanzos, 1996 [1576]; Cobo, 1979 [1653]; De Las Casas, 1892 [1552]; Garcilaso de la Vega, 2010 [1609]; Guamán Poma de Ayala, 2009 [1615/1616]; Pizarro, 1921 [1571]; Polo de Ondegardo, 1873 [1572]). *Tinajas* (large jars) and *crisoles* (small jars) to ferment and drink *chicha* (Hayashida, 2008) were excavated in Farfán in association with the Compound VI Cemetery and the *Huaca* Burial Platform suggesting that the beverage was produced and consumed at these sites. *Chicha* could also have been produced somewhere else and transported to the site (Mackey & Nelson, 2020). An activity such as brewing *chicha* where the maize kernels need to be chewed before being spit in a jar for fermentation, should lead to a higher prevalence of caries and an increased rate of attrition in teeth (Murphy, 2004).

The archaeological evidence from the *Huaca* Burial Platform agrees with a high consumption of maize by the *aqlla* as supported by the grave goods and the stable carbon isotope values. The combination of diet and activity associated with maize, led to the expectation to find a higher prevalence of carious lesions and a more advanced wear level in the *aqlla* compared to the general population in Farfán.

8.2.1 Carious Lesions

The data from the osteobiographies revealed a prevalence of 68% of individuals from the *aqlla* cemetery had at least one caries which is very similar to the prevalence found in Farfán's general population (64% of individuals). The caries prevalence was similar in the *aqllakuna* from Túcume (58%% individuals) (Toyne, 2002).

The average number of carious lesions per individual was slightly lower at the *Huaca* Burial Platform (1.96 ± 2.06) than the other cemeteries combined (Cemetery J, Cemetery I, Mound G Cemetery, and Compound VI Cemetery) (2.00 ± 2.30), but the difference is not statistically significant. These values are a bit lower than the average number of carious lesions per individual in the *aqllakuna* from Túcume (2.32 ± 2.69) (Toyne, 2002). The general population average is influenced by four non-*aqlla* individuals with a high number of carious lesions (J25 T8 with 7 caries, J26 T3 with 6 caries, JE19 T1 with 6 caries, and I T3 with 5 caries).

Among the *aqlla* cemetery, the highest number of carious lesions was recorded in N T1a (6 lesions), E T5 (6 lesions), E T7 (5 lesions), and C T2D (5 lesions). While these individuals consumed maize as evidenced by their $\delta^{13}C$ values (see Appendix C), the isotope results do not suggest they had a diet especially rich in maize. These four individuals are also not markedly older than the individuals from the general population (include respectively an adolescent, mid-adult, old adult, and juvenile). No correlation was found regarding the number of carious lesions, isotopic data, or age. Thus, these results suggest that brewing *chicha* from maize was not associated with higher caries rate in the *aqllakuna* from Farfán.

Many recipes and methods for *chicha* production exist. Household production likely used a technique that involved chewing the maize kernels to ferment with saliva. However, this method was not convenient to produce the 9 to 15 litres of *chicha* that has been estimated to be consumed per person at special events (Jennings, 2005). It is also possible to brew *chicha* using sprouted maize (malting) which is then dried, ground, mixed with water, cooked and fermented (Cutler & Cardenas, 1947; Moore & Moore, 1989). The second technique might have been preferred in the *aqlla* institution to produce the large quantity of chicha needed for festivals and ceremonies (Gagnon *et al.*, 2015). Thus, the *aqllakuna* might not have brewed chicha using their mouth, as much as first thought, which would explain why there is no statistically significant difference in cariogenicity and occlusal wear between the *Huaca* Burial Platform and the general population.

Other factors such as hormonal changes, frequency of consumption, and tooth morphology, can influence the development of carious lesions. Frequent oestrogen fluctuation in females, especially during puberty and pregnancy, triggers physiological changes which decrease immune competence (Lukacs, 2008). Thus, females are, in theory, more susceptible to carious lesions than males as seen in Maranga (Boza Cuadros, 2010), Morrópe (Klaus & Tam, 2010), Rinconada Alta (Lacerte, 2019; Salter-Pedersen, 2011), Pasamayo (Okumura, 2014), Chachapoyas (Tran, 2016), and Machu Picchu (Turner, 2015). However, a higher caries frequency in females than males is not constant in the Andes, as demonstrated by findings at the archaeological sites of Morrópe (Klaus, 2008), Los Pinos (Pezo-Lanfranco & Eggers, 2010), and Puruchuco-Huaquerones (Murphy & Boza, 2012; Williams & Murphy, 2013). Pubertal estrogen changes amongst the *aqllakuna* could have been a factor contributing to caries frequency, however, the *aqllakuna* were supposedly virgins, and thus they should not have gone through the hormonal changes associated with pregnancy. This factor could be part of the reason why the *aqlla* lacked the hypothesised higher caries rate.

8.2.2 Tooth Loss

Tooth loss occurred more frequently in the general population than in the *aqlla* cemetery (AMTL: 45% vs. 39%; PMTL: 48% vs. 48%). Moreover, the *aqllakuna* exhibit a significantly lower amount of antemortem and postmortem tooth loss than the other cemeteries combined (AMTL: 0.78 ± 1.53 vs. 2.48 ± 4.14, $t = -2.04$, p-value = 0.048; PMTL: 1.96 ± 2.98 vs. 3.21 ± 5.63, $t = -1.03$, p-value = 0.309 teeth per individual, mean ± sd). An association was found between age and the prevalence of AMTL in Farfán ($\chi^2 = 23.97$, p-value = 0.001, Cramer's $V = 0.75$) with more tooth loss in older individuals.

AMTL is often caused by pathological lesions such as caries and infection (Hillson, 1996). Thus, the general population most likely had a higher prevalence of caries, leading to AMTL, than was detected in the remains. This hypothesis is supported by tooth type where more molars were loss antemortem than anterior dentition in the individuals from Farfán (67% of molars vs. 20% of premolars vs. 13% of canines and incisors). Tooth morphology influences its susceptibility to carious lesions (Hillson, 2001). The numerous crests and large occlusal surface of molars make them more susceptible to carious lesions (Buikstra & Ubelaker, 1994). The absence of these teeth, and the ones lost postmortem, reduce the number of teeth available to recorded pathological lesions. Thus, the prevalence and frequency of dental pathological lesions was likely higher than documented at Farfán.

8.2.3 Occlusal Wear Level

The wear level is different between the two Farfán groups, although the difference does not reach statistical significance at a 0.05 level. At the *Huaca* Burial Platform 29.6% of the individuals have an absent or slight wear level while 40.7% have a moderate-heavy or heavy wear level. In the general population, 52% have an absent to slight wear level while only 22.5% of individuals have moderate-heavy or heavy level of occlusal wear. This difference reflects the demographic profiles of the groups as older individuals have more advanced wear ($\chi^2 = 61.85$, p-value = 0.003, Cramer's $V = 0.54$) and the *Huaca* Burial Platform contains a slightly higher proportion of older individuals compared to the other cemeteries (18.5% vs 12.5% of old adults). Thus, it is not surprising to find a slightly more advanced wear level in the *aqlla* cemetery.

Wear is also more pronounced in individuals with a coarse diet (Buikstra & Ubelaker, 1994) and thus, the more advanced wear of the *Huaca* Burial Platform individuals could also be the result of a diet high in coarsely ground maize. If more advanced wear was a direct consequence of the frequent *chicha* brewing, it would be observed in the younger *aqllakuna* compared to the individuals of the same age in the general population. A moderate-heavy wear level was recorded in the juvenile E T9 and advanced wear was found in two other juveniles (C T2D/ E) from the *Huaca* Burial Platform. Most of the *aqlla* subadults including infant to adolescent exhibit a slight wear level (58.3%, N=12).

The higher wear level in these three *aqlla* juveniles might be a consequence of the lower density of deciduous tooth enamel being more susceptible to abrasive food like maize or *chicha* brewing compared to permanent dentition.

8.2.4 Abscesses

Carious lesions and advanced wear increase the risk of pulp infections which can be seen via dental abscess. Therefore, abscesses are often considered regarding their association with caries (e.g. Murphy, 2004; Lowman *et al.*, 2019). Adults with numerous carious lesions often present more than one abscess (Murphy, 2004). In Farfán, the *aqllakuna* had a statistically similar number of abscesses per individual compared to the general population (0.96 ± 1.58 vs. 1.24 ± 2.96, respectively, t = -0.42, p-value = 0.679). The average of the general population is influenced by two individuals from Cemetery J who had an especially high number of abscesses (J14 T1 with 7 and J25 T6 with 14 abscesses).

8.2.5 Summary Cariogenic Lesions – Hypothesis #1

The dental pathology data for Farfán do not support expectations for hypothesis #1 stating that these females should have a higher frequency of carious lesions and higher wear as they supposedly brewed *chicha* regularly compared to the general population, who experienced a regular diet and occasional chicha brewing. In other words, the results do not show that the *aqllakuna* had a higher caries rate or more advanced tooth wear, which could lead to more AMTL and abscesses, than the general population in Farfán.

In fact, there was a slight trend towards a lower level of carious lesions, AMTL, and abscesses in the *aqlla*. The effect size is a more informative measurement to quantify the association between variables and contribute to the interpretation of the data. Here, the low effect sizes calculated (Cohen's *d* caries = 0.02; AMTL = 0.52; abscess = 0.12) align with the not statistically significant result found at 0.05 level. Thus, even with the small sample size more subject to Type I error, the effect sizes support that the difference in cariogenicity between cemeteries might be caused more by individual differences within Farfán population than the presence of the seemingly distinct social group of *aqlla.* It is worth mentioning that, even the general population leaned toward high-status individuals as seen at the Compound VI Cemetery (containing governors from the Chimú royal lineage) and Cemetery J (containing individuals affiliated with administrative duties), which might have influenced the outcomes observed (Mackey & Nelson, 2020).

While these results cannot confirm fewer pathological lesions associated with carious lesions at the *Huaca* Burial Platform, similar numbers were recorded at Túcume. Thus, the difference might be representative of a lower level of carious lesions found in *aqlla* institutions despite their diet rich in maize (as indicated by stable carbon isotopes). In these circumstances, the low number of carious lesions might be the result of drinking a lot of *chicha.* The beverage has lower cariogenicity compared to other maize preparations due to the fermentation process, where the maize sugars are consumed by yeast leaving a small amount for oral bacteria. The presence of ethanol following fermentation could also influence carious lesions due its antimicrobial properties (Gagnon & Juengst, 2019; Luoma, 1972). *Chicha* contains approximately 1-3% ethanol following fermentation (Vargas-Yana *et al.*, 2020). It would be interesting to look at alcohol levels in hair from Farfán as done by Cartmell *et al.* (2005) to evaluate if the levels of ethanol where high enough to prevent caries. Moreover, a study in a modern population found a difference in oral biofilm pH between sweetened liquid and baked goods, where liquids caused a sudden drop in pH but the pH returned to normal faster than with baked goods (Kashket *et al.*, 1994). The shorter amount of time with a variable oral pH, combined with the viscosity of a liquid decreasing the adhesion of food particles (Brudevold *et al.*, 1988; Caldwell, 1970), suggest that drinking a lot of *chicha* could result in fewer carious lesions. Additional analyses such as other dietary isotopes analysis or examining dental calculus for food particles (see Gagnon & Juengst, 2019) may better establish the extent of *chicha* consumption in the *aqlla*.

8.3 Hypothesis #2 – Physiological Stress

Physiological disbalance occurring due to malnutrition and infectious disease can lead to abnormalities in dental tissues during development. Thus, by evaluating developmental defects in enamel such as linear enamel hypoplasia (LEH) and in dentin such as interglobular dentin (IGD), it is possible to get insight into the childhood of individuals. Based on the assumption that the females from the *Huaca* Burial Platform were drawn from elite families, they might have had access to better food and cultural practices which should have led to a lower prevalence of physiological stress lesions than the rest of the population prior to their arrival in the *aqllawasi*. Elite individuals are often associated with preferential access to resources (Cucina & Tiesler, 2003; Costin & Earle, 1989; Dulanto, 2008) and thus should have fewer episodes of malnutrition, and possibly also childhood illness, as a better diet leads to a more robust immune system (e.g. Shimada *et al.*, 2004). Moreover, since the *aqlla* supposedly joined the secluded institution before puberty, it might be possible to observe lifestyle changes after they entered the *aqllawasi*. For example, their sequestered status might have led to less sunlight exposure than the regular population, which might have been seen as a higher prevalence of vitamin D deficiency via IGD lesions in the dentin.

8.3.1 Linear Enamel Hypoplasia

Linear enamel hypoplasia (LEH) was present in 63% of individuals from the *Huaca* Burial Platform compared to 35% in the other cemeteries combined. The *aqlla* have a significantly higher prevalence of LEH than the common population ($\chi^2 = 6.03$, p-value = 0.049). In fact, the difference might be more pronounced than observed in the current comparison since the author recorded more LEH episodes than were noted in the osteobiographies. Two of the cemeteries in the general population exhibit high prevalences of LEH as well. Cemetery I and Cemetery J exhibit respectively 60% and 47% of individuals with at least one enamel defect, values that are closer to the *aqlla*.

The difference between groups might be due to a higher proportion of younger individuals (24% of individuals between 0-2.9 years of age) in the general population compared to the *aqllakuna* (4% of individual between 0-2.9 years of age). Because most LEH episodes formed between 3 to 5 years of age, the neonates (0-0.9 years of age) and infants (1.0-2.9 years of age) would have fewer LEH as they died before LEHs could form later in life. Thus, while statistical evidence supports a higher prevalence of LEH in the *aqllakuna* before their induction into the *aqlla*, difference in the demographic distribution of the groups must be taken into account when interpretating the data.

While the *aqlla* have a higher prevalence of LEH, they exhibit slightly fewer LEH defects per individual compared to the general population. The general population has an average of 1.96 ± 2.47 (mean ± sd) LEH episodes per individual whereas the *Huaca* Burial Platform individuals have an average of 1.81 ± 1.63 (mean ± sd) LEH episodes per individual. More than 5 different LEH episodes were recorded in only two individuals from the *Huaca* Burial Platform (6 episodes in C T2A; 5 in N T1a) but were found in six individuals from the other cemeteries (8 episodes in J25 T9; 6 episodes in J25 T1, I10 T2; and 5 episodes in J26 T1b, JE19 T1, I3 T1a). These results suggest that per individual, the *aqlla* may have experienced slightly, but not statistically significantly ($t = -2.01$, p-value = 0.810), fewer episodes of childhood illness or malnutrition than the general population.

8.3.1.1 LEH & Other Variables

No correlation was found between the number of episodes of LEH and adult stature ($R = 0.09$, p-value = 0.679). The only two individuals considered as "short", had more than five LEH episodes (C T2A stature = 142.0 cm; N T1a stature = 141.0 cm). Yet, individuals of average and above average stature height also often had multiple LEH episodes. Thus, the physiological stress indicated by LEH episodes, seemingly did not affect the overall growth of the individual.

Pearson's correlation tests found non-significant associations between the number of LEH and geographic location during childhood ($\delta^{18}O$ incisors: $R = -0.14$, p-value = 0.664; $\delta^{18}O$ molar: $R = 0.10$, p-value = 0.776) and the number of LEH and diet ($\delta^{15}N$: $R = -0.21$, p-value = 0.389; $\delta^{13}C$: $R = 0.13$, p-value = 0.633) in Farfán individuals. Thus, contrary to expectation, the individuals with diets lower in maize or protein did not develop more episodes of LEH.

8.3.2 Interglobular Dentin (IGD)

Interglobular dentin was detected in the majority of the 32 individuals from the Farfán population (75% of the population) included in the focused sample (including distinct and possible cases). This includes 92% of individuals from the *Huaca* Burial Platform (N=11 out of 12 individuals) and 65% of individuals from the general population (N=13 out of

20 individuals). While the *aqllakuna* exhibit a higher prevalence than the general population, the difference is not statistically significant ($\chi^2 = 2.06$; p-value = 0.151). The effect size measured indicates a moderate association between the cemetery and the presence of IGD in individuals (Cramer's $V = 0.25$) in other words being in the *aqlla* cemetery increases the probability of having IGD by 25%.

8.3.2.1 Vitamin D Deficiency

Interglobular dentin is commonly associated with vitamin D deficiency. Vitamin D deficiency can be caused by a lack of sunlight exposure, dietary deficiency, or, rarely, genetic variations in vitamin D synthesis or activation (Brickley *et al.*, 2020). Risk factors including increased age, darker skin pigmentation, and hormonal variation, such as occurs during pregnancy, lactation, and menopause; these can all negatively affect the acquisition of vitamin D in the body (Brickley *et al.*, 2020). Sunlight exposure is influenced by season, altitude, latitude, cultural norms, clothing, and environment (Lockau *et al.*, 2019). Regarding the Farfán population, these individuals should have had ample exposure to sunlight to prevent vitamin D deficiency as the site is located near the Equator (7°17'52.2"S 79°28'22.9"W) and the clothing pictured in the Spanish Chronicles showed there should have been sufficient skin exposure. An association between vitamin D level and altitude has been reported in Beer *et al.* (2020) with a 4% increase in prevalence with every 100 m above sea level. However, the site of Farfán is only 50-meters above sea level, so its altitude should not have been a factor in vitamin D deficiency. Unfortunately, no data can reveal the average of sunshine hours during the Late Horizon period. Cloud coverage data between 1980 and 2016 in San Pedro de Lloc, at approximately 15 km from Farfán, showed a cloudier sky between October and April with an average of "overcast" or "mostly cloudy" varying between 28% to 75% during the year (Weather Spark, 2022). If the cloud coverage was similar during the Inca Empire, the cloud coverage might have led to a higher susceptibility to vitamin D deficiency during summer months (especially December to February).

Some of the highest rates of vitamin D deficiency are found in modern communities living near the Equator (see Spiro & Buttriss, 2014; Creo *et al.*, 2017). Only a few reports have been published on vitamin D deficiency in South America. Relying on 25OHD levels in serum, these studies often focus on a specific part of the population, such as the elderly or women undergoing hormonal changes. The serum 25OHD levels measured in elderly from across Argentina show a gradient between 2% to 14% of vitamin D deficiency, and 50% to 73% of vitamin D insufficiency, with lower rates in people living closer to the equator (Oliveri *et al.*, 2004). In Chile, 60% of postmenopausal women had vitamin D deficiency (González *et al.*, 2007) while 46% of a Guatemalan elderly population were vitamin D deficient (Sud *et al.*, 2010). In Columbia, a 7% vitamin D deficiency, and a 24% insufficiency, were recorded in pregnant women while toddlers had 43% insufficiency (Beer *et al.*, 2020). These data are similar to the results from in Peru where clinical studies have reported a prevalence of vitamin D deficiency between 46% (Kramer *et al.*, 2021) and 57% in adults (Wingfield *et al.*, 2014), while 51% of adults had insufficient 25OHD levels (Kramer *et al.*, 2021). Another study on children found a deficiency in 47% of individuals in Lima vs. 7% of individuals in Tumbes (Checkley *et al.*, 2015). The main explanations for these deficiencies were older age (Oliveri *et al.*, 2004; Sud *et al.*, 2010), urbanisation (Beer *et al.*, 2020; Checkley *et al.*, 2014; Kramer *et al.*, 2021; Oliveri *et al.*, 2004; Wingfield *et al.*, 2014), and hormonal variations (Beer *et al.*, 2010; Gonzalez *et al.*, 2007). The modern Peruvian prevalences are lower than the one found in Farfán population. Part of this difference may be explained by the use of different measures or markers of vitamin D levels. Modern clinical studies do not assess teeth for IGD as a marker of vitamin D deficiency because they have easier and more definitive and reliable tests. Bioarchaeologist cannot use these tests as they require body fluids. At this time the threshold of 25OHD serum values that are associated with IGD development is unknown. IGD could develop before 25OHD serum levels indicate vitamin D insufficiency or deficiency or may not develop until 25 OHD levels are extremely low.

The IGD defects observed in Farfán occurred between 0 (incisors) and 13 (third molars) years of age. Thus, older age and hormonal variation are not factors that increased the susceptibility of the individual to have vitamin D deficiency. Maternal deficiency can be transmitted to the infant (Barrett & McElduff, 2010) which might partly explain the IGD that developed in neonates. Thus, hormonal variation and maternal deficiency cannot explain the high prevalence of IGD in Farfán.

In archaeology, vitamin D deficiency can be readily identified from bone deformities such as rickets and osteomalacia. Rickets and osteomalacia (pathological lesions characteristic of vitamin D deficiency) are rare in Peruvian archaeological samples (Foote, 1927; Weiss, 2014). However, IGD can be formed in cases not severe enough to cause bone deformities such as long bone deformation (D'Ortensio *et al.*, 2018). Evidence of bowed fibula was recorded in three individuals in Farfán including two *aqllakuna* and one individual from Compound VI Cemetery (C T11C, E T10, and I T2). Only E T10 had a tooth to analyse for IGD, which revealed eight separate episodes of IGD supporting the interpretation of their bowed fibula as due to vitamin D deficiency. However, this individual was diagnosed with scurvy (vitamin C deficiency) in which bowed long bones can also occur (Huss-Ashmore *et al.*, 1982). Thus, vitamin D deficiency might not be the only reason for the development of bowed long bones in Farfán.

Interglobular dentin has also been recorded in association with the consumption of certain foods low in calcium, like maize (Mellanby, 1928). Some modern studies suggested that rickets can develop due to low calcium (see Mays & Brickley, 2022 for a review). IGD was not assessed in these studies, but the defect was likely present considering the bone demineralisation present which relies on similar calcification mechanisms between the skeletal tissues. However, the impact of the calcium deficiency can be reversed with enough vitamin D. Thus, even though maize was important in the Inca diet, IGD should not have formed if vitamin D was sufficient.

Other conditions have been associated with IGD including rare hereditary conditions affecting vitamin D homeostasis such as hypophosphatemia (Murayama *et al.*, 2000) and fluorosis (Fejerskov *et al.*, 1979; Kuijpers *et al.*, 1996; Schour & Smith, 1934). Hypophosphatemia is a rare genetic disorder where low phosphate levels occur due to a genetic mutation controlling phosphate metabolism. The imbalance in phosphate leads to calcium imbalance and rickets (Sharma *et al.*, 2018). Hereditary conditions are rare and generally associated with many health issues in addition to defect in calcification of the skeleton. Considering the lack of treatment in the past for such conditions, the individuals would likely not have survived (Brickley *et al.*, 2020). The rarity of the genetic conditions, the little rickets/ osteomalacia prevalence on site, and the non-continuity of the age range for IGD occurrence (no evidence of defect in individuals older than 13 years of age) suggest that hereditary conditions are an unlikely explanation for IGD development in Farfán.

8.3.2.2 Fluorosis

Fluorosis occurs with high consumption of fluorine leading to toxic effects in the body. Fluorine, found in groundwater, substitutes for hydrogen to form fluorapatite instead of hydroxyapatite, which increases bone susceptibility to fracture. Fluorine also stimulates the activity of osteoblasts (Ortner, 2003).

The first visible effect of excess fluorine is dental fluorosis characterised by brown staining of the enamel and hypomineralisation of dentin leading to IGD defects. The severity of the defects is positively correlated with fluoride levels in the water and time of exposure (Goldberg, 2016). Eventually, the toxicity of fluoride can induce periosteal hyperostosis mostly located at the insertions of tendons and ligaments and in more severe cases, fusion of vertebrae (Ortner, 2003). These skeletal changes occur mostly in older individuals (> 50 years of age) (Littleton, 1999). Enamel hypoplasia and pulp stone have also been reported after chronic fluoride toxicity (Brown & Hardisty, 1990). One individual from Farfán has a hypocalcification in their incisor possibly due to fluorosis (C T2F). Unfortunately, the teeth from this individual were not available for the focused sample. Many of the Farfán teeth have brown staining, and while this could just be due to postmortem alteration, chronic fluorosis cannot be ruled out as the cause. Still, no other individuals from Farfán have been suggested to have fluorosis (Mackey & Nelson, 2020).

Fluorosis is rarely reported in archaeological and modern populations in the Andes. A modern study in Arequipa (South of Peru) found 75% of children (N=292) between 11 to 14 years of age had dental fluorosis (Calderon *et al.*, 2021) while, in Lima, 23% of children (N=26) between 4 to 6 years of age consumed more than the recommended limit of fluorine through water fluoridation (Rodrigues *et al.,* 2009). This limit has been established at 0.05-0.07 mg fluoride/ kg body weight/ day to avoid detrimental effects from fluorine (Burt, 1991).

The water consumed by Farfán residents came mainly from the Jequetepeque River. One ecological report has been recently published on the fluoride level in the Jequetepeque River (Chávez & Ortiz, 2019) reporting a concentration between 0.10 to 0.24 mg/l which is lower than the established standard of 1 mg/l. However, the data were collected in the Cajamarca region on the other side of the Represa de Gallito Ciego, which is quite far from Farfán, so the results may not be representative of the fluoride levels on site. Therefore, the water from the Jequetepeque river probably did not represent a health risk for Farfán population, but future research should determine fluoride levels closer to the site. Systemic ICPMS analysis could be used to better understand fluoride levels. Moreover, since fluorosis in skeletal samples cannot be directly predicted from water levels of fluoride in the past (Littleton, 1999), further analysis will be necessary to confirm that fluoride was an uncommon health risk at Farfán. Such work has begun with element mapping of the teeth using BSE-SEM which can detect element concentrations down to about 0.5 wt.%. The analysis did not detect fluorine in the thin-sections and thus, if present, the concentrations of fluorine were lower in the teeth than the detection rate (see Appendix G). Overall, current data suggest fluorosis was absent or uncommon in Farfán, so it is an unlikely explanation for the high prevalence of IGD in the sample.

8.3.2.3 Age of Defects

The age of occurrence of each episode of IGD was calculated for the teeth processed for histological analysis (N=14). All episodes occurred before 13 years of age with the majority occurring between 1 to 5 years of age, similar the age distribution of LEH. LEH age-of-occurrence corresponds to the age of an episode of IGD in four teeth from C T11B, E T10, J25 T8, and I10 T2, with a total of six LEH-IGD episodes occurring between 1.5 to 4.0 years of age. The other thin-sections did not have LEH so co-occurrence regarding age of formation with IGD could not be assessed however, a significant correlation was found between the number of episodes (R = 0.44, p-value = 0.003). The paired LEH-IGD episodes might be associated with the same event during childhood which could help narrow the possible explanations for the childhood illnesses or stress events causing LEH in Farfán. To the knowledge of the author, this co-occurrence has been found in only two other studies, one in dogs where LEH and IGD co-occurred because of a dietary deficiency in vitamin D (Mellanby, 1928; Mellanby, 1934) and one from an archaeological population from Montreal from the 19th century (Bigué, 2021). Bigué (2021) hypothesised that more severe episodes of vitamin D deficiency might induce LEH, but a larger sample size would be necessary to address this hypothesis. No correlation was found between evidence of rickets/ osteomalacia, and IGD formation in the Montreal sample.

The meaning of LEH-IGD co-occurrence will need a larger sample size to be further explored. It is important to note that no significant correlation was found between the number of LEH and IGD in any of the Farfán groups. Teeth included more episodes of IGD (average of 6.11 ± 2.07 episodes per individual in the *aqlla*) than LEH (1.81 ±1.63 episodes per individual). Moreover, IGD episodes were slightly more frequent than LEH episodes. IGD occurred multiple times per year (approximately every 4-6 months) whereas the LEH episodes occurred approximately every 6-12 months. This overlap between the age of occurrence of both physiological defects might be a coincidence or caused by common cyclic stressors.

The Incas had an annual calendar similar to the Georgian calendar used nowadays with 12 months and 4 seasons based on the Sun's position (autumn = 93.15 days, winter = 93.40 days, spring = 89.46 days, summer = 89.23 days) (Zuidema, 1986). Festivities such as *Quapac Raymi* during the December Solstice (a festival where young noble males were initiated into manhood and received their ear plugs) and *Inti Raymi* for the winter solstice were important annual events Other festivities occurred on an irregular period following the moon and sun cycles (Zuidema, 1986). These festivals might have contributed to the pattern of IGD occurrence throughout the year. For example, wearing specific clothing or painting for certain festivals could limit temporarily skin exposure to sunlight on a semi-regular basis. Festivals may have involved consuming imported food and drink and these could have contained a lot of fluoride. These specific examples are not directly mentioned in the ethnohistorical documents but are offered here as ideas to stimulate thought about the periodicity of IGD episodes.

8.3.2.4 IGD & Other Variables

The expression of IGD did not vary with social status. Multiple IGD episodes were found in E T4 (N=4 episodes), E T7 (N=6 episodes), and N T1b, who were identified as particularly high-status based on their grave goods (Mackey & Nelson, 2020). Among, the young *aqllakuna* possibly affected by scurvy, E T10 had eight episodes of IGD while IGD presence could not be confirmed in E T6 (probable on micro-CT). The presence of IGD is also relatively uniform across cemeteries as it is present in 92% of individual from the *Huaca* Burial Platform, 66% of individuals from Cemetery I (N=3), 78% from Cemetery J (N=9), 43% from Compound VI Cemetery (N=7), and 100% from Mound G Cemetery (N=1). The prevalence is highly influenced by the sample size as seen at the Mound G Cemetery where only one tooth from one individual was available for analysis. The other cemeteries have small sample sizes too (N=3, 7, 9, and 12) so the prevalences may not be representative of all of the individuals originally buried on this site even if the demographic profiles of the focused sample are representative of the primary burials.

As was the case with LEH, there was no correlation between the number of episodes of IGD and isotopic value. Thus, geographic origin did not influence the development of IGD ($\delta^{18}O$ incisor: R= -0.05, p-value=0.920; $\delta^{18}O$ molar: R = 0.01, p-value = 0.989; $\delta^{18}O$ bone: R = -0.25, p-value = 0.524) nor their diet ($\delta^{13}C$: R = 0.03, p-value = 0.954; $\delta^{15}N$: R = 0.25, p-value = 0.352).

Considering that IGD is associated with poor mineralisation in dentin, a structure similar to bone, it might have caused a delay in individual's growth. Four individuals from Farfán (all *aqllakuna*) showed delayed post-cranial fusion compared to their dental age (E T6, E T9, C T2C, and C T2B). Only one of these individuals was available for the focused sample (E T6). IGD was detected in this *aqlla* (only through micro-CT) but at a comparatively minor level. This individual did not have LEH episodes, while the others had one (C T2B/ C) and three (E T9) episodes. Thus, potentially delayed epiphyseal fusion does not appear to be associated with a high number of IGD or LEH episodes.

8.3.3 Summary for Physiological Stress – Hypothesis #2

Hypothesis #2 relies on the assumption that as these females were supposedly venerated and considered as part of the elite, they may have had access to better food and cultural conditions resulting in generally good health. This preferential access to resources might be observable through a lower prevalence of physiological stress lesions in the *aqllakuna* compared to the general population.

The average LEH at Farfán is lower than other archaeological sites during the Late Horizon, such as Rinconada Alta (Lacerte, 2019) and Túcume (Toyne, 2002). Toyne (2002) described the sample from Túcume as having a low number of stress episodes that might reflect the high status of the sample. Thus, the individuals buried at Farfán might have experienced better environmental conditions than other populations in the Andes. Indeed, individuals from Farfán had a prevalence of LEH similar to the ones encountered in Puruchuco-Haquerones (Murphy, 2004). A possible explanation for the low LEH number might be the location of Farfán at the crossroads of the Inca road system, which would have facilitated the exchange of resources and migration between areas.

The unexpected high prevalence of LEH and IGD in Farfán does not seem to be explained by geographic origin, diet, or status (no statistically significant correlation with isotope value, stature, and the number of IGD or LEH). The correlation found between the number of IGD and LEH suggests an association between the two defects in Farfán. Thus, the development of dental defects associated with physiological stress is likely due to environmental factors or habits that occurred in all individuals in Farfán or a combination of factors.

The high prevalence and frequency of LEH and IGD in Farfán in all cemeteries with a prevalence higher in the *aqlla* does not agree with the expectations from the Spanish Chronicles. This observation suggests that the *aqllakuna* did not have a better access to resources or cultural practices during their childhood. On the contrary, they experience more episodes of childhood illness, malnutrition, or vitamin D deficiency during their first years of life especially prior to 5 years of age than the general population at Farfán.

Therefore, based on these results, hypothesis #2 supposing less physiological stress in the *aqllakuna* is rejected.

8.4 Hypothesis #3 – Uniformity Within the *Aqlla* Institution

The information in the ethnohistorical documents claims the *aqllakuna* were venerated and shared similar lifeways. This suggests they should have a large degree of uniformity in their health and lifeways when compared to the general population of Farfán. Yet, in contrast to the expectations of uniformity, Mackey and Nelson (2020) reported a greater variability among the *Huaca* Burial Platform individuals in terms of cranial deformation, geographic origin (30% born outside the Jequetepeque valley), diet, age, stature, and general health than the other cemeteries on site. Their dental health, on the other hand, upheld the expected uniformity as the *aqllakuna* show more similarity regarding each dental characteristic compared to the general population. However, when compared to the sample from Túcume, the *aqllakuna* from Farfán have more variation in dental characteristics, except for carious lesions and LEH. Table 8.1 shows a summary of the dental health characteristics of these three groups in which one can assess sample uniformity based on standard deviation values.

Table 8.1. Summary of the dental health characteristics recorded through the osteobiographies in Farfán and Túcume.

	Aqlla, Farfán		General Population, Farfán		*Aqlla*, Túcume	
Pathological Lesions	Mean	SD	Mean	SD	Mean	SD
Wear Level	2.81	1.78	2.03	1.82	0.21	0.42
AMTL	0.77	1.57	2.48	4.14	0.32	0.67
Abscesses	0.96	1.58	1.24	2.96	0.32	0.58
Carious Lesions	2.05	2.06	2.00	2.30	2.32	2.69
LEH	1.81	1.63	1.96	2.47	2.78	1.90
Dental Calculus	1.17	0.95	1.28	1.34	0.63	0.90

Mean and standard deviation (sd) calculated from the osteobiographies compiled by Nelson for Farfán's data (Nelson, pers. comm. 2019); data from Túcume obtained from Toyne (2002).

Thus, there is more dental health variability in the general population compared to the *aqlla* cemetery from Farfán. However, it is important to acknowledge that the greater variation encountered in the general population might simply be caused by differences between the four cemeteries. Differences regarding age-at-death distribution, spinal degeneration, trauma, and stable carbon and nitrogen isotopes are not significant between the four cemeteries (Mackey & Nelson, 2020) but some variation is noted. For example, Cemetery J includes more subadults and has a high rate of dental pathological lesions, Cemetery I has the highest rate of dental pathological lesions, the highest stature, the highest prevalence of LEH, but the lowest prevalence of spinal pathology, Mound G shows overall good dental health and few LEH, but it also has the highest prevalence of individuals with infectious disease. Compound VI Cemetery, which is the only cemetery with more males than females, has the lowest average stature, lowest infectious disease prevalence, few LEH, and the highest oxygen isotope value suggesting that these individuals spent time in another coastal region (Mackey & Nelson, 2021) probably to the north (Nelson, pers. comm., 2021). Since the general population include four distinct cemeteries with their own characteristics, it is not surprising that they exhibit greater variability than the *aqlla* and the higher uniformity of the *Huaca* Burial Platform could just be a function of greater variation in other cemeteries rather than being caused by similar *aqlla* lifeways.

Farfán was an administrative and ceremonial site and thus included mostly individuals considered of moderate to high status such as *aqlla* and administrators. Thus, presumably, the individuals needed a reasonable status to be buried at Farfán minimising the number of low-status burials. Thus, it is possible that the homogeneity within populations might be associated to the bias of individuals buried at the site. This uniformity might also be the results of a heterarchical government structure where access to resources is approximately equal between individuals instead of a hierarchical structure as found under the Inca polity (Tran, 2016). Cueller (2013) also noted that unequal access to resources was not as pronounced in the Andes before the arrival of the Spaniards. However, other authors have reported variation in oral health associated with social status due to differential diet in pre-Hispanic Andean populations (Murphy, 2004; Shimada *et al.*, 2004; Bader, 2012; Klaus and Tam, 2010).

In comparison, the females from Túcume exhibit less variation, except for carious lesions and LEH, but they also have a significant variation in their age distribution compared to the *aqllakuna* from Farfán (χ^2 = 19.28, p-value = 0.004). Indeed, the majority of the females at Túcume were young adults (53%) while the *Huaca* Burial Platform had more subadults including children, juveniles, and adolescents (62%). No old adults were found at the *Huaca* Larga (Túcume) while it represents 19% of the individuals from the *Huaca* Burial Platform.

Despite, the statistically significant correlation calculated between the geographic origins of the individuals ($\delta^{18}O$ incisor) and the number of carious lesions (R = -0.72, p-value = 0.004), the geographic origin cannot explain the difference in the variation of dental pathological lesions. Isotopic analysis on the *aqllakuna* from Túcume suggest these females were also from various geographic origin (Hewitt, 2013). Thus, the greater variation in Farfán might be the result of a greater variation in age distribution or environmental conditions compared to the individuals from Túcume.

8.4.1 Summary on Uniformity – Hypothesis #3

Hypothesis #3 stipulates that since these females were supposedly drawn mostly from local elite families, which would have shared similar lifeways, they should have a large degree of uniformity in their dental health when compared to the general population in Farfán. The results show that the *aqllakuna* from Farfán present more uniformity in dental health than the general population on site, but a wider age range, poorer health, and more variability in their dental health, except for carious lesions and LEH, than the *aqlla* from Túcume.

The overall uniformity within Farfán suggested similar lifeways in the *aqllakuna* and the general population on site. Indeed, even if slightly more uniformity was found among the *aqllakuna* compared to the general population, the difference is not pronounced enough to claim more uniformity within the female institution. For example, the comparison with other sites in the Andes such as Rinconada Alta where 2.40 ± 1.23 LEH episodes (Lacerte, 2019) or Túcume 2.78 ± 1.90 vs. 1.81 ± 1.63 LEH episodes at the *Huaca* Burial Platform and 1.96 ± 2.47 LEH episodes in the general population suggest less episodes of childhood stress in Farfán, but not necessarily more uniformity. The homogeneity between the *aqlla* and the general population at Farfán might have been influenced by the bias in the individuals buried at the site which seemingly focused on moderate to high status individuals based on their grave goods and dental health. Moreover, the greater variation observed in the general population is likely influenced by the variation between the four cemeteries as each of them has their unique characteristics.

The greater variation in the *aqllakuna* from Farfán compared to the ones from Túcume might be due to the age distribution. Indeed, a wider age distribution is found among the *aqllakuna* from Farfán compared to the *aqllakuna* from Túcume. This age distribution including more subadults at Farfán could partly explain that less carious lesions were found in these females compared to the ones at Túcume.

8.5 Hypothesis #4 - Methodological Comparison

The use of a multi-method approach gave another perspective to the detection of dental pathological lesions in bioarchaeological material. Micro-CT allows the detection of internal structures of teeth without destroying the sample. The scanner can detect lesions such as interglobular dentin that would not be observable only through macroscopy. Histological analysis can be performed to assess internal tissues, but it is destructive.

The development of medical equipment such as radiography and computed tomography (CT) imaging to examine archaeological material without damaging it has been revolutionary. Scientists can exploit non-invasive and non-destructive techniques to see beyond structures and wrapping to detect hidden abnormalities in bones and teeth (Cox, 2015). For example, Bulbul *et al.* (2017) use CT images to detect the prevalence of carious lesions, dental developmental anomalies, periapical lesions, and periodontal disease in a living population. Nowadays, studies tend to use multiple techniques to complement their examination (e.g. Seiler *et al.*, 2013), but few have looked into the accuracy of imaging to assess lesions compared to macroscopic examination. With the increasing concerns about destructive analysis, it is important to find reliable alternatives such as non-invasive micro-CT. Thus, micro-CT should be as accurate as “traditional” methods to detect dental pathological lesions. The visibility of each dental pathological lesion is discussed in the following sections, including carious lesions, occlusal wear level, LEH, IGD, dental calculus, hypercementosis, and additional dental defects observed in Farfán.

8.5.1 Carious Lesions

Significantly more carious lesions were recorded by the author compared to the amount reported in the osteobiographies. This difference is mostly due to the recording of smaller lesions for the methodological comparison. Indeed, in the focused sample, micro-CT revealed 17 caries while macroscopic analysis only identified 13 lesions. The difference, however, was not statistically significant ($t = 0.44$, p-value = 0.661). The higher detection rate with micro-CT is mostly due to the ability to assess the depth and shape of a potential carious lesions and to detect demineralisation patches in the enamel and/or dentin that were not visible from the tooth's surface. As seen in another archaeological study, carious lesions can be hidden behind calculus, and therefore teeth considered "healthy" in appearance might reveal another story (Özkan *et al.*, 2015). Micro-CT is considered the most reliable method to investigate caries in archaeological teeth when they are covered by dirt, or calculus to prevent underestimation (Tomczyk *et al.*, 2014).

The ability to assess demineralisation, lesion depth and morphology, and see the internal tooth structures to evaluate if dentin is affected offer considerable advantages to differentiate normal pits and crenulations from carious lesions with micro-CT. Especially on molars, smaller carious lesions were not identified with macroscopy due to their location in the groove of a molar and their small size. Although, this enhanced visibility of all pits and grooves can make it difficult to differentiate the boundaries of normal pits vs. early-stage caries.

In clinical studies, micro-CT has been considered reliable to detect early-stage caries in human teeth. While clinical studies have established micro-CT as a reliable alternative to detect carious lesions in human teeth (Arnaud *et al.*, 2017; Boca *et al.*, 2017; Kamburoglu *et al.*, 2011; Kawato *et al.*, 2009; Mitropoulos *et al.*, 2010; Rovaris *et al.*, 2018, 2019; Soviero *et al.*, 2012), they do not consider the poor level of preservation sometimes encountered in archaeological samples. Diagenetic changes can mimic or hide carious lesions (Hillson, 1996).

The difficulty of differentiating biogenetic lesions from diagenetic ones can explain part of the variation in the recording of carious lesions. It supports the need to clearly establish the characteristics to identify carious lesions in micro-CT, as well as the best resolution settings, since no standards have yet been developed, in opposition to the macroscopic observation of caries (Buikstra & Ubelaker, 1994). In terms of resolution settings, a tendency to overestimate the presence and size of caries was mentioned by Nascimento *et al.* (2018) depending on contrast adjustment. Low brightness and high contrast settings could lead to 'over-detection'. These settings can also affect the coloration when using lookup tables to analyse volumes and the visibility of demineralisation based on the transition between light grey and darker grey (as done by Boca, 2017). It is hoped the Farfán caries rate determined by micro-CT was not affected by these problems, but the possibility cannot be ruled out. Researchers should clearly establish their criteria and always note image settings when publishing micro-CT caries research (as done with this research) to increase the reproducibility of methods and accuracy of results.

For histology, demineralisation, irregular edges, and depth/ severity of carious lesions can be observable, but the thin-section must be located exactly where the carious lesion is located. This rarely occurs. It is not possible to assess the total number of carious lesions visibility with histology, since only a small section of each tooth will be examined under the microscope. Moreover, the grinding process might destroy smaller carious lesions and cause additional damage to the sample, thus limiting the detection of caries. The comparison of the thin-sections with their corresponding micro-CT slices revealed the same number of caries identified (N=2). Since micro-CT allowed the examination of multiple consecutive tooth sections, useful to identify an infected site, and has a good inter-observer reproducibility (Mitropoulos *et al.*, 2010) it is considered a more reliable alternative.

8.5.2 Occlusal Wear Level

Macroscopy and micro-CT can both rely on Buikstra and Ubelaker's (1994) standard to record occlusal wear which makes the methods more comparable. The differences observed were mainly associated with intra-observer variation (kappa = 0.92).

Macroscopic wear level could be assessed all at once allowing more consistency within the evaluation while micro-CT had to be assessed one tooth at the time, and therefore the analysis of the 45 teeth had to be spread on multiple days. The Buikstra and Ubelaker's (1994) dental wear standard relies on the visibility of dentin. In the Farfán sample, dentin was not often visible even on the teeth with more advanced wear even when the occlusal surface was completely flat. Micro-CT allows the distinction between enamel and dentin based on density. It is also possible to look at the thickness of the enamel which is helpful to assess wear level. As micro-CT scans can be seen in 3D, differences in thickness and angle can be seen more accurately than with macroscopic observation, making micro-CT as, if not more, reliable as the "classical" method to assess occlusal wear.

With histology, the wear level cannot be assessed using Buikstra and Ubelaker's (1994) standard due to the 2D perception of the volume. Wear can be assessed from thin-sections when using the categorical wear scale from this thesis (i.e., none, slight, slight-moderate, moderate, moderate-advance, advanced), which is more resistant to variation between methods. While the difference in occlusal wear level reported with the two methods is not statistically significant different due to sample size ($\chi^2 = 5.95$, p-value = 0.311), the ability of micro-CT to consider the whole occlusal surface makes it more reliable than histology to evaluate occlusal wear level.

8.5.3 Linear Enamel Hypoplasia

More enamel defects were detected with micro-CT than with macroscopy, in terms of the number of teeth with LEH and the total number of episodes. A total of 26 defects in 16 teeth was detected with micro-CT as opposed to 23 defects in 15 teeth from macroscopy. This slight difference was not statistically different (t = 0.34, p-value = 0.735). This difference can be explained by the high resolution of the micro-CT allowing the observation of faint or thin defects in enamel, such as accentuated striae of Retzius. However, it is important to recognize normal fine lines in enamel, such as perikyamata and (normal) striae of Retzius, and not erroneously count these as enamel lesions. Dirt and dental calculus can also hide some LEH (Buikstra & Ubelaker, 1994) that would become visible on micro-CT.

Histological methods detected 13 LEH in seven teeth while micro-CT detected 17 LEH in 8 teeth (t = 0.60, p-value = 0.554). Again, micro-CT had a slightly higher detection rate, but difference was not significant. Yet, other factors make histology generally poor method for LEH detection. Diagenetic change can alter the ability to see the LEH defects accurately especially when teeth are not well preserved (e.g. J25 T8). The destructive process of the histological method can destroy the defects. Moreover, LEH is not uniform across the tooth, it can vary in depth and height from the CEJ. Since histology only represents a specific slice of the sample, and cannot show all sides, these factors can interfere with the detection of an enamel defect.

While there is a difference in detection of LEH between methods, they all are capable of accurately identifying the defects, at least in well-preserved teeth. Micro-CT can be used to combine the advantage of a microscopic 3D view, with information derived from the macroscopic and histological observations.

8.5.4 Interglobular Dentin

Interglobular dentin requires the observation of the internal structure of the tooth. Therefore, IGD cannot be assessed through macroscopic observation and thus, was not reported in the osteobiographies. Commonly it relies on histological methods and light microscopy, but histology is a destructive method leading to the loss of the sample. D'Ortensio *et al.* (2016), Colombo *et al.* (2019), and Veselka *et al.* (2019) have shown the possibility to detect IGD through micro-CT. Their results suggest detection is possible with both techniques, but some information is lost with micro-CT. In this thesis, using optimised micro-CT parameters, similar results were found. Micro-CT was reliable to evaluate the presence of IGD (χ^2 = 6.09, p-value = 0.048, Cramer's V = 0.47) but significantly fewer episodes of IGD were detected with micro-CT than with histology (t =-3.89, p-value= 0.001). A total of 96 IGD episodes was noted with histology for an average of 6.9 ± 2.1 episodes per tooth whereas 51 episodes for an average of 3.6 ± 2.2 episodes per tooth were noted with micro-CT.

IGD episodes detected in Farfán were usually Grade 1 (< 25%) or Grade 2 (25-50%) based on the scale from D'Ortensio *et al.* (2016). D'Ortensio *et al.* (2018) suggested that

Grade 1 IGD might be developmental interglobular dentin (DIGD) occurring during tooth development. DIGD is a natural defect in dentin calcification similar to IGD, but it does not follow incremental lines and it looks like a "small sparse granular patch of spaces that can have a slightly elongated or wave-like appearance" (D'Ortensio *et al.*, 2018:105). DIGD tends to be localised near the cemento-enamel junction or the mantle dentin (D'Ortensio *et al.*, 2018). The shape of the patches could not be differentiated in the lower category of IGD defects (identified as possible defects) in micro-CT due to the resolution limitations, nor could incremental lines be identified. However, in most cases, the patches were linearly located within the primary dentin and all the episodes observed under light microscopy were identified as IGD based on shape, location, and amount affected, as done in D'Ortensio *et al.* (2018), suggesting that even the possible IGD defects observed in micro-CT volumes correspond to IGD episodes instead of DIGD. Moreover, considering that DIGD are not always visible on histology, they will likely not be visible with the lower resolution of the micro-CT scans.

Dentin is composed of 20% of organic matrix making it more susceptible to diagenetic defects than enamel (Hillson, 1996). In previous research, vitamin D deficiency has been evaluated in teeth without wear or dental caries as these two processes often initiate reparative dentin formation (tertiary dentin) (Brickley 2020). Other normal physiological processes can occur in dentin such as "marbling" which has a dappled appearance and can disrupt dentin mineralisation (Colombo *et al.*, 2019). However, with the lower resolution of micro-CT compared to histology, the loss of details can limit our ability to differentiate "normal" disruptions in mineralisation from IGD episodes. To minimise this, D'Ortensio *et al.* (2018) suggest using bilaterality and the orientation of the episode along incremental lines as main characteristics to consider. These were established for analysis through histology. Since bilaterality within the tooth is not systematically present on micro-CT, the category system used for this research (absent, possible, probable, distinct) allowed considering potential IGD defects even if they did not possess all the usual criteria.

Micro-CT also allows the observation of the defect in a 3D volume, an acknowledgement not discussed in current literature due to this being a novel technique. Previous articles focus on the comparison between the information retrieved between histology and the approximately corresponding micro-CT slices. As seen with our current analysis, IGD is a defect that exist in a 3-dimensional plane. It is not uniformly distributed as concentric circle within the tooth. For example, in the Farfán sample, IGD defects were more frequent on the lingual side of the teeth. The exploration of the 3D view revealed the presence of episodes of IGD that were present in the thin-section but not visible in the corresponding slice on micro-CT. This 3D perspective of IGD can contribute to a better understanding of its aetiology and development of the defect (BSE-SEM in Appendix G also contributes to this discussion). There is no standard yet to approximate the age of defects in 3D volumes obtained from micro-CT. Ages of occurrence have only been established in a 2D model developed from thin-sections (Brickley *et al.* 2020). The limitation in the visibility of the incremental lines, and the visibility of episodes makes it difficult to associate the age with the defects in micro-CT. Thus, the number of episodes of IGD and their age of occurrence was not assessed in the micro-CT scans, limiting the comparison between methods. The development of an accurate way to determine the number of separate episodes, and to then estimate their age of occurrence needs to be established for micro-CT to become a more reliable alternative tool for IGD research.

In brief, in agreement with Colombo *et al.* (2019) and Veselka *et al.* (2019), micro-CT can detect the presence of IGD in individuals, but it does not provide as much detail as histological analysis. Episodes that were characterised as “faint” on histology cannot be detected with micro-CT and episodes that are bilateral in histology might appear unilateral within the tooth in micro-CT. Micro-CT can detect the presence of IGD, but it cannot systematically represent all the different episodes of IGD in dentin. The limitation in the visibility of the incremental lines, and the visibility of episodes, make it difficult to associate age with the defects in micro-CT. Thus, we suggest using micro-CT as a screening device to identify which teeth have the defects and should be destroyed for further analysis to establish the age of occurrence of each IGD episode if necessary. This would limit the destruction of irreplaceable archaeological teeth.

8.5.5 Dental Calculus

Dental calculus could be evaluated by the three different methods and in the osteobiographies. Dental calculus was reported in the ostobiographies in 16 individuals but was only found in three of the 45 teeth analysed in the focused sample. However, dental calculus was reported, like wear level, as a global dental indicator instead of tooth specific in the osteobiographies while this characteristic was reported on each tooth in the focused sample. Moreover, dental calculus was not systematically reported in the osteobiographies in opposition to the focused sample. Calculus was only recorded macroscopically in four teeth from the focused sample while it was slightly more detected by micro-CT with six teeth presenting evidence of calcified plaque ($\chi^2 = 0.45$, p-value = 0.502). This difference results mainly from the ability to detect changes in density and composition on micro-CT not always visible macroscopically especially since the level of calculus was usually slight.

Among the 14 teeth cut for histology, dental calculus was present in one (LLI1 from E T7) and was present in two of these 14 teeth with the micro-CT (LLI1 from E T7 and ULP4 from CH 1b). Thus, there is a difference in the detection between methods ($\chi^2 = 0.37$, p-value = 0.541). The analysis of thin-sections is limited by what can be seen in the 2D view. With micro-CT, the distinction between dirt and very slight dental calculus could not be easily made (no difference in colour or texture). This might lead to an overestimation of teeth with dental calculus in the sample.

8.5.6 Hypercementosis

Micro-CT detected more teeth with hypercementosis (N=8) than with macroscopy (N=4). While the difference was not statistically significant, micro-CT was considered more reliable to assess hypercementosis than macroscopic observations. It can be more clearly identified in micro-CT as it allows the measurement of the thickness of cement. Fewer teeth were identified in osteobiographies because hypercementosis was evaluated in the socket where the root was usually not visible. Moreover, with micro-CT, hypercementosis could be detected in teeth such as an M3 from C T11A which did not a priori appear affected by hypercementosis from macroscopic observations.

8.5.7 Other Dental Features

Other unexpected dental characteristics were encountered in Farfán which are not often reported in the Andean literature (e.g. Thomas *et al.*, 2019), but can reveal additional information on past lifeways and thus have been reported as additional comments during analysis. Among these were discolouration, enamel pearls, and pulp stones. Change in enamel coloration associated with fluorosis, or hypocalcification, can only be observed through macroscopy since differences in color are not visible on micro-CT unless there is a variation in density. Regarding hypocalcification, it was not encountered macroscopically and therefore it is not known if the variation in enamel density is sufficient to be observed on micro-CT. The three other dental defects were better assessed through micro-CT due to the 3D perspective and the approximation of density.

The ability to approximate density was also useful to confirm the presence of enamel pearls. Enamel pearls can be seen macroscopically and were recorded in the osteobiographies. The enamel pearls recorded in the focused sample could not be seen macroscopically due to their location near a root groove or within the dentin. While archaeological studies usually report enamel pearls on the outer surface of the tooth, enamel pearls can also be found in the dentin (Ortner, 2003). Micro-CT allows the recording of this characteristic and the possibility to confirm that the density is the same as enamel through the attenuation coefficient (e.g. Anderson *et al.*, 1996). This feature was not observed in the histological sample since the tooth selected with this feature (M3 from E T7) was lost during preparation of the sample. The loss of this sample highlights the importance of alternative and non-destructive method for analysis.

Pulp stone detection requires the observation of the internal structure of the tooth and therefore cannot be assessed macroscopically. To be observed with histology, one will need to cut precisely at the location of the stone to report its presence. Thus, micro-CT, which could determine exactly where to cut the tooth is the most advantageous method to record pulp stones. Similar than enamel pearls, the 3D perspective, and the possibility to compare the density of the structure, aids in the diagnosis of pulp stone. The exact factors promoting the formation of pulp stones are unclear, but some factors seem to contribute to their occurrence including a diet high in fat and protein. Although, the $\delta^{15}N$ values in individuals with pulp stones did not suggest any specific pattern, as it was present in one individual with a high protein diet (E T7) and in another with average protein intake in the population (C T11B). Factors such as diet (Nicklisch *et al.*, 2021), dental trauma, periodontal disease (Fatemi *et al.*, 2012), systemic diseases including dentinogenesis imperfecta, dentin dysplasia, cardiac disease (Nayak *et al.*, 2010), kidney disease (Yeluri *et al.*, 2015), Marfan syndrome (Bauss *et al.*, 2008), and Van der Woude syndrome (Kantaputra *et al.*, 2002) are thought to contribute to the formation of pulp stones.

8.5.8 Correlative Tomography

The combination of images taken at different resolutions is not new, as it has long been used for "scout and zoom" strategies for finding regions of interest (ROIs) at low resolution for interrogation at higher resolution with another imaging modality (Burnett *et al.*, 2014; Caplan *et al.*, 2011). However, the extension of the concept into the 3rd dimension, and the increasingly sophisticated image processing and registration techniques that have recently become available, have led to a great expansion in the field in the last decade. The use of correlative tomography, or the registration of a 2D image into a 3D volume, is an interesting method to ensure the comparison between histology and micro-CT and locate the thin-section accurately. It allows combining some of the advantages regarding the visibility of the micro-CT and histology methods including the level of detail obtained from histology and the visibility of the surrounding dental tissues with micro-CT.

8.5.9 Summary Methodological Comparison – Hypothesis #4

No statistically significant differences were found between methods in the dental pathological lesions recorded here between macroscopy and micro-CT. Internal characteristics such as IGD, pulp stones, and enamel pearls could not be recorded macroscopically, but were accessible with micro-CT representing a great advantage of the method. This ability to cut through the teeth contributed to the higher, although not statistically significant, detection rates of calculus, hypercementosis, presence and number of LEH, and carious lesions were observed with micro-CT.

Similarly, the recording of dental pathological lesions did not detect a significant difference between micro-CT and histology except for the number of IGD episodes. Micro-CT can detect the presence of IGD in teeth, but the resolution obtained here did not provide enough details to observe as many IGD episodes as in the thin-sections. Even if micro-CT does not provide as much details as traditional histological analysis, the advantage of the 3D analysis compared to the thin-section is useful to have a more accurate number of lesions recorded regarding caries, wear, LEH, calculus, hypercementosis, enamel pearls, and pulp stones.

Thus, the results obtained on carious lesions, wear, LEH, IGD, dental calculus, and hypercementosis support hypothesis #4 to use micro-CT as a non-destructive alternative method to record or complement dental analysis.

8.6. Limitations of the Project

Some limitations were encountered with the sample influencing the results obtained, including the sample size, the use of secondary data, time limitations, and the lack of standards for micro-CT scans. The teeth brought back to Western were not selected with this analysis in mind, some teeth mostly from the *Huaca* Burial Platform had already been sectioned which could have destroyed some lesions. This highlights the importance of using non-destructive methods as much as possible to preserve archaeological samples for future research.

The postmortem modifications encountered by these individuals and the small sample size are the two main limitations faced within the sample. Small sample sizes ($N < 30$) highly influence the statistical power of analysis. Observations for each pathological lesion were not available for every individual. Thus, while the two groups compared include 27 individuals from the *Huaca* Burial Platform and the 41 individuals from the general population, the analytical sample includes a smaller comparative sample. This small sample size increases the risk for Type I error and thus slightly different conclusion could have been obtained with greater sample size. To limit this, the effect sizes and raw data were considered to highlight trends instead of simply relying on p-values.

The majority of the data on the whole population were secondary data and since dental health was not the focus of analysis differences were observed between the author's data and the osteobiographies. The latter were prioritised for evaluating the lifeways between the *aqlla* and the general population in Farfán as a single tooth cannot be representative of the whole dental health of an individual. The recording of more pathological lesions on the analytical sample due to the use of multiple methods suggests that more lesions would have been recorded in all individuals as well which might have led to greater differences between the *aqlla* and the general population.

The use of the biocultural approach is also limited to the information available. It is not possible to report all the variables involved in the biocultural model and thus we cannot be sure of the explanations surrounding the distinction between the *aqllakuna* and the general population (as seen in Hodder, 2014). Dental health is used as a proxy for the different lifeways and conditions surrounding the individuals from Farfán but cannot assess all the components involved. For example, it is not possible to know if the IGD defects observed are associated solely with vitamin D deficiency and the practices leading to the deficiency or if it is associated with fluorosis without additional analyses.

There is a lot of variability in the recording of dental pathological lesions. Most research follows Buikstra and Ubelaker's (1994) standards which rely on broad characteristics observed with the naked eye. Thus, unfortunately, it cannot be directly translated to analysing micro-CT images. One of the most challenging issues was the diagnosis of carious lesions. For Ubelaker and Buikstra (1994), the main feature to identify carious lesions is the coloration of the lesion however, this characteristic cannot be observed on micro-CT volumes. Instead, we can observe the shape, edges, depth, and demineralisation processes all characteristics mentioned mostly in the clinical literature where they do not encounter preservation issues in the dental material. Indeed, although Farfán is one of the largest samples on the North Coast of Peru, it has also been damaged by looting, rain, and run-off from the adjacent mountain leaving poorly preserved material.

The material from Farfán was poorly preserved especially in Compound VI Cemetery affecting the recording of macroscopic lesions, the quality of the resolution and the appearance of lesions in micro-CT. The diagenetic damages cause additional defects in dentin that can mimic pathological lesions (e.g. caries and IGD). The poor preservation, also reported in Feilen (2004), had an impact on the quality of histological images as a lower density in dental tissue is ground away faster than higher density. Some teeth such as the incisor from J25 T8 were so poorly preserved that they would be destroyed by the vibration of the saw.

The analysis of micro-CT was simplified and shortened due to time constraints, but the use of other filters and tools in Dragonfly such as segmentation might reveal a more standardised way to diagnose pathological lesions and a more reliable way to record physiological stress lesions such as LEH and IGD more accurately.

Chapter 9 - Conclusion

The *aqlla* institution created during the Inca Empire (1470-1532 AD) has been a subject of fascination in Andean archaeology. The first written evidence of the institution was compiled into the Spanish Chronicles by Spaniards and *mestizos* following the Spanish Conquest. They offered many descriptions regarding Inca culture and institutions including the *aqlla*, a female institution reduced to a convent or a group similar to the vestal virgins. The chroniclers described the *aqllakuna* as females selected around 8 to 10 years of age based on their status or beauty to be sequestered into an *aqllawasi* to cook, weave, and brew *chicha* for economic and religious purposes. The Spanish Chronicles are invaluable documents to better understand the Inca Empire. However, the authors offered a biased perspective on a culture they considered as having "depraved custom" (Cobo, 1979[1653]:30), "false religion" (Garcilaso de la Vega, 2010 [1609]: 336), and "people with such mundane, vile, and cowardly spirits" (Cobo, 1979 [1653]: 32). Furthermore, they never interacted directly with the female institution explaining some of the inconsistencies between authors and building the mystery around these underrepresented females.

Archaeological evidence needs to be used to assess the portrait of the *aqlla* in the Spanish Chronicles, but few archaeological sites have been identified as *aqlla*. So far, only two *aqlla* sites have been excavated on the North Coast of Peru: Farfán and Túcume. Previous analyses on both sites suggest discrepancies between the archaeological evidence and the written description. In Túcume, the *aqllakuna* presented a wider age range, poorer health, more trauma, and more variability than expected from the assumptions based on the Spanish Chronicles (Toyne, 2002). Evidence from Farfán also suggests that the *aqllakuna* presented more variability in terms of origin, age, status, and health than expected (Mackey & Nelson, 2020). By focusing on the unique perspective of dental health, this thesis offered a new approach to pursue the investigation into the *aqlla*'s lifeways of the *aqllakuna* while incorporating a strong methodological component.

This project is the first to investigate the *aqlla* through their dental health. The results obtained adds to the mystery surrounding these females. More specifically, this thesis addressed four hypotheses:

1) As these females supposedly brewed *chicha* regularly, which involves mastication of maize to start the fermentation process, they should have a higher frequency of carious lesions and higher wear than the general population who experienced a regular diet and occasional chicha brewing.

2) As these females were supposedly venerated and considered as part of the elite, they may have had access to better food and cultural conditions resulting in generally good health, so they should have a lower prevalence of physiological stress lesions than the general population.

3) As these females were supposedly venerated and shared similar lifeways, they should have a large degree of uniformity in their dental health when compared to the general population.

4) Micro-CT is an accurate alternative to detect, or complement, dental pathological lesions compared to macroscopy and histology.

A brief overview of the findings of this thesis are presented below.

9.1 Lifeways in the *Aqllawasi*

To address the hypotheses, dental material dating from the Late Horizon (1470-1532 A.D.) from the site of Farfán was analysed with a focus on individuals from the *Huaca* Burial Platform which was identified as an *aqlla* cemetery. The comparison with teeth from four other local cemeteries (Cemetery I, Cemetery J, Mound G Cemetery, and Compound VI Cemetery) suggests that the *aqllakuna* were not different than the general population in Farfán.

1) The *aqllakuna* were exposed to similar cariogenicity as they express similar wear levels, number of caries, number of AMTL, and abscesses as the general population.

2) The *aqllakuna* did not experience less physiological stress during childhood, evaluated through LEH and IGD, than other individuals in Farfán. On the contrary, these females exhibit a significantly higher prevalence of LEH than the general population.

3) Based on their dental health, the *aqllakuna* exhibit slightly more uniformity than the other cemeteries in Farfán. However, they present more variability in their dental health compared to the females from Túcume.

Better dental health was not associated with a specific cemetery, social status, diet, or geographic origin in Farfán. The comparisons between the different groups are generally not statistically significant, but some trends can be observed including a slightly more advanced wear level, less carious lesions, less AMTL, more IGD, and more dental calculus in the *aqllakuna* compared to the general population. Therefore, the dental health observed in Farfán contradicts the assumption from the Spanish Chronicles. The dental health at the *Huaca* Burial Platform suggests for less cariogenicity than the general population despite the brewing of *chicha*. Thus, either brewing was not a major component of their lives, or they used alternative method to chewing corn to initiate fermentation. Moreover, the *aqllakuna* did not represent a more advantageous and homogenous subset of the population. In fact, more physiological stresses during childhood, and more variability were detected in the *aqlla* compared to the general population with no association with their geographic origin. Thus, these females likely came from various origins and social status while the high variability within the institution suggests a hierarchy within the *aqllawasi* not thoroughly documented.

Thus, the dental health in Farfán revealed more complexity within the *aqlla* institution than described in the ethnohistorical documents. Additional considerations regarding the environment and the social structure of the *aqlla* should be taken into account to understand their lifeways.

It is important to note that the individuals excavated on archaeological sites represent a biased sample of the lager population as they are limited to the individuals who were buried on site. It does not include all death within the population. Thus, it is possible that the individuals buried at Farfán were associated mainly with administrative and ceremonial duties and thus, shared more similar lifeways with the *aqllakuna* than populations including more commoners. This could explain why the difference was not as pronounced between the *aqllakuna* and the general skeletal population. The sample size available in Farfán is also quite small which led to lower statistical power for analysis. Individuals presenting more lesions (e.g. 14 abscesses in an individual from Cemetery J) have a higher influence on the results. Considering the context, these analyses relied on the individual as the unit of analysis to have an overview of their dental health instead of a specific tooth. The osteobiographies were therefore prioritised to investigate the lifeways at Farfán, but detailed dental analysis recorded here more pathological lesions than the osteobiographies provided by Dr Nelson for the same teeth suggesting more dental lesions in each individual than previously reported.

This research highlights how much of the *aqlla* remains unknown. I believe, the apparent structure within the *aqllawasi* should be investigated in greater depth to better understand who these females where because the bioarchaeological evidence does not support the writings of the Spanish Chronicles. That fact highlights the importance of not solely relying on ethnohistorical writings to understand past institutions and the importance to consider the bias and context surrounding those texts.

The high prevalence of IGD, highly consistent with vitamin D deficiency, has not been previously documented in Andean archaeology. The presence of IGD is a completely novel and unexpected information on an population that venerated the Sun. It opens new questioning regarding the Inca and the *aqlla* as they should have had access to enough resources including ample sunlight to avoid vitamin D deficiency. Vitamin D is important for many physiological processes including influences on the immune system and cardiovascular health (Brickley *et al.*, 2020). The ability to diagnose cases of deficiency not severe enough to exhibit evidence of rickets without destroying samples by using micro-CT enhances our ability to shed light on health and disease in past communities. Moreover, the superposition of LEH and IGD with most defects occurring prior to 5 years of age suggests the presence of underlying elements during the first years of life in the Andes increasing the susceptibility to physiological stress. The biocultural approach relying on biology, socio-cultural, and environmental factors is necessary to investigate this association more deeply in the future.

9.2 Methodological Comparison

The greater resolution in micro-CT compares with other imaging method such as clinical CT scan can reveal many details unavailable with other imaging techniques without destroying the sample. In agreement with other comparative studies (e.g. Colombo *et al.*, 2019; Veselka *et al.*, 2019), micro-CT can detect dental pathological lesions in a population. The detailed dental analysis using macroscopy, micro-CT, and histology on a subsample from Farfán including 45 teeth from 32 individuals revealed more variation in the recording of dental pathological lesions between methods than first expected. The results highlight advantages and disadvantages on the accuracy of micro-CT over “classical” methods. Additional observations with a larger and less damage sample will be necessary to “confirm” the accuracy of micro-CT, the current results agree in this direction. This research has shown some advantages from micro-CT over classical histology including the assessment of a 3D volume, the ability to assess multiple lesions, and the non-destructive aspect. However, classical histology remains superior in term of resolution.

With this research, the quantitative results support the hypothesis that micro-CT can be a reliable alternative method to assess dental health compared to macroscopic or histological observations. Indeed, there were no statistically significant differences regarding the recording of pathological lesions between macroscopy and micro-CT. In addition, while the results were not statistically significant based on the threshold of 5%, the qualitative assessment shows that micro-CT is more reliable than macroscopy and histology to assess hypercementosis, enamel pearls, pulp stones, and to evaluate the overall level of preservation of teeth. This method allows the assessment of wear level similarly to macroscopic observations; however, it can lead to a slight overdetection of LEH and caries mostly due to the lack of standards to records these two lesions with alternative methods.

In comparison with destructive histological analysis, micro-CT represents a good alternative to preserve unique archaeological material and assess internal structure even when the material is poorly preserved such as encountered with the sample from Farfán. The poor preservation state of some teeth limited the ability to perform histological analysis, an issue that can be overcome with micro-CT. The ability to achieve high resolution with micro-CT can also allow the detection of small defects like IGD, although significantly less episodes were detected with micro-CT compared to histology. While it is not yet possible to report as many details as histology, micro-CT could be used as a screening tool to validate the presence and location of defects of interest in teeth. Moreover, micro-CT can be used to assess weaknesses in the structure of the tooth that could affect the ability to perform histological analysis.

The main limitations for the comparison between methods were associated with the poor preservation level of some teeth and the expertise required for each method. Indeed, despite the high resolution of images obtained, one requires certain expertise to be comfortable with Dragonfly and distinguish diagenetic damage from pathological presentation. In many cases diagenetic damage affected the visibility and diagnosis of pathological features on micro-CT scans and histology. While micro-CT is a good alternative to avoid additional damage to the sample and observe them, it can be difficult to differentiate between biogenetic and diagenetic patterns. This issue led to a slight overdetection of dental pathological lesions with micro-CT compared to “classical” methods. This conclusion supports the importance of considering and reporting preservation levels when it comes to dental health analysis. To improve the reliability of micro-CT it would also be important to establish standards to assess dental lesions in archaeological samples to include the influence of diagenetic changes which are not considered in clinical settings more familiar with the use of imaging methods to explore dental pathological lesions in livings subjects. Taphonomic changes and lesions are represented and evolved differently in remains. The increasing transdisciplinarity between fields will surely continue to promote the use of imaging techniques in archaeological research.

In recent years, a few researchers have used micro-CT to investigate damages in burnt bone (Ellingham & Sandholzer, 2020), following temporal impact of decomposition in the soil on bones (Tomanik *et al.*, 2017), or bone bioerosion (Booth *et al.*, 2016). No studies have looked into the impact in teeth or the alteration of diagenetic changes in the presence of pre-existing pathological lesions such as IGD, wear level, and caries affecting the dentin, which is less dense than enamel and thus more susceptible to diagenetic alterations. Understanding the impact of diagenetic change is important to properly diagnose pathological features and to assess the integrity of the sample prior to performing destructive analysis to maximize the preservation of the sample.

9.3 Future Directions

To enhance the impact and the precision of results, there is an increasing development of quantitative tools in dental anthropology. For example, Lagan & Ehrlich (2021) have developed software to calculate occlusal wear levels based on photos (MolWear). Other studies have published quantitative methods to estimate the percentage of original crown height (McFarlane *et al.*, 2021), to quantify the severity of IGD (Snoddy *et al.*, 2020), or to distinguish caries based on attenuation coefficient in dental tissues (Hoffmann *et al.*, 2021). For these quantitative assessments, histological methods were used to access the internal structure of the tooth and obtain accurate measurements. Micro-CT could be an interesting alternative to contribute to the development of quantitative methods. Numerous tools are available for quantitative analysis in Dragonfly such as segmentation to take 3D measurements of changes in tissue density due to lesions which might overcome the limitation of the lower resolution with micro-CT compared to histology. Micro-CT has already been considered reliable for the measurement of LEH (e.g., Marchewka *et al.*, 2014b) and pulp/tooth ratio through segmentation (Selig *et al.*, 2021). It would be interesting to use segmentation for other dental pathological lesions such as wear and IGD. The new research on ”super-resolution”, a deep learning models used in software to increase resolutions in scans (see Isaac & Kulkarni, 2015), could expand the applicability of micro-CT for the detection of small defects like IDG. Thus, micro-CT opens up possibilities to standardise recording, increase sample size, and promote data sharing as done with the IMPACT database (Nelson & Wade, 2015) while limiting the destruction of samples.

Finally, the comparison with the individuals from Túcume found similar dental health in that *aqlla* as the one in Farfán despite the slight difference in age distribution. It will be interesting to compare with other *aqlla* in the Andes such as the one in Cuzco (Eaton, 1916), Huanuco Pampa (Morris & Thompson, 1985), Pachacamac (Uhle, 1903), or the one at Tomebamba in Equator (Idrovo Urigüen, 2000) to see if the *aqlla* lifeways where local or universal across the Inca Empire.

9.4 Conclusion

This thesis increases knowledge on the mysterious female institution of the *aqlla*, and while it could not completely demystify the lifeways of these "Chosen Women", it demonstrates the necessity to not solely rely on historical documents to understand the past. According to the dental analysis on Farfán's population, despite their portrait in the Spanish Chronicles, the *aqllakuna* were not different than the general population on site. Their dental health at Farfán was slightly poorer than in the sample found at Túcume, but the *aqllakuna* from Farfán also presented more variation in terms of age, overall health, and isotopic values than what is known from Túcume. The recording of IGD, a new palaeopathological defect so far unreported in Andean populations, gives a new insight into the living conditions under the Inca Empire. Each method of analysis has advantages and disadvantages that should be taken into consideration when analysing dental health in past populations. The use of advanced imaging tools such as micro-CT and software (e.g. Dragonfly) offer a lot of potential for the analysis of unique bioarchaeological material without destroying it.

In this exciting period for the development of new and more powerful imaging technologies, the research with micro-CT is meant to increase. Research such as this thesis contributes to the rise of non-destructive method to explore the past without altering samples. Having the ability to obtain details such as the extent of wear, or detection of defects in dentin open new areas of expertise that were not available for large sample sizes prior to the development of this non-destructive method. Micro-CT can shed light on the dental health of understudied population for which little sample is available, such as the *aqlla*, without destroying them. The unexpectedly high prevalence of IGD in Farfán is a good example of the power and potential of micro-CT to obtain new information and challenge our knowledge of the past. The rise of new quantitative tools such as segmentation or “super-resolution” offer exciting possibilities to reinforce the precision and reliability of micro-CT to assess dental pathological lesions such as IGD.

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Appendix A. Glossary

Apupanaca: Governor of a province (around ten thousands of individuals), selected and take care of the *aqlla* institution.

Aqlla / *aclla*: refer to ‘Chosen Women’; girl selected around 8-10 years of age to weave, spin, cook, and brew chicha in the confinement of their religious institution.

Aqllakuna/ acllacuna: combination of the word *aqlla* meaning ‘Chosen Women’ and *kuna* which indicate the plural form of a noun or a member of a class (Betanzos, 1996 [1576]).

Aqllawasi: House of the ‘Chosen Women’.

Ayllu: family unit, related group from a common ancestor who shared land and resources (Kolata, 2013).

Camay: a creative power understood by the Incas to bring life to and animate all material things (Kolata, 2013).

Çancu: bread consumed during Inca ceremonies (Garcilaso de la Vega, 2010 [1609]).

Chicha: beer usually made of fermented maize, an important element for ceremonies in Precolumbian cultures.

Coya/ *qoya*: Queen, first wife of the Inca (Kolata, 2013).

Crisoles: small jars, often unfired, used to drink *chicha*.

Curaca/ Kuraka: a community political leader; a “chief” (Kolata, 2013).

Huaca (*Waka’s*): a sacred object strongly infused with *camay*. It could be a shrine, a fixed point in the landscape, or any unusual or extraordinary object, place, or person. (Kolata, 2013).

Khipu (*quipu*): system of knotted, multicolored cords used for recording figures and narrative information (Kolata, 2013).

Mamakuna / *mamacona*: Older *aqlla* remaining virgin and cloistered in charge of ceremonies and training of the new *aqllakuna*.

Mestizo: Spanish and Andean mix ancestry.

Mitima/ mitmaq (pl. *mitimaes/ mimaqkuna*): a conquered population relocated to other provinces of the empire for political, defensive, or economic reasons (Kolata, 2013).

Tambos/ *tampus*: stations distributed regularly along the roads for supplies and temporary lodging.

Tawantinsuyu: meaning "the four quarters together"; the Incas' names for their empire (Kolata, 2013).

Tinajas: large jars used for *chicha* production.

Yana (pl.*yanakuna*/ *yanacona*): a "personal retainer" attached to a noble households. These individuals owed allegiance to their households rather than to their natal communities and were exempt from the tributary obligations associated with their community of origin (Kolata, 2013).

Appendix B. – Example with Individual C T11D of Dental Information Available In the Osteobiographies Filled In By Dr Nelson.

Stress Indicator Summary: (See also:)

Dental Enamel Hypoplasia: (teeth involved, pits or lines, distance from cemento-enamel junction)

mand rt canine 3.6 left canine 3.6. 3.8

max rt I' - 3.3 left canine 2.2

2.5 3.2

Porotic Hyperostosis: (includes cribra orbitalia - location, healed/unhealed, severity - light, moderate, severe)

NO

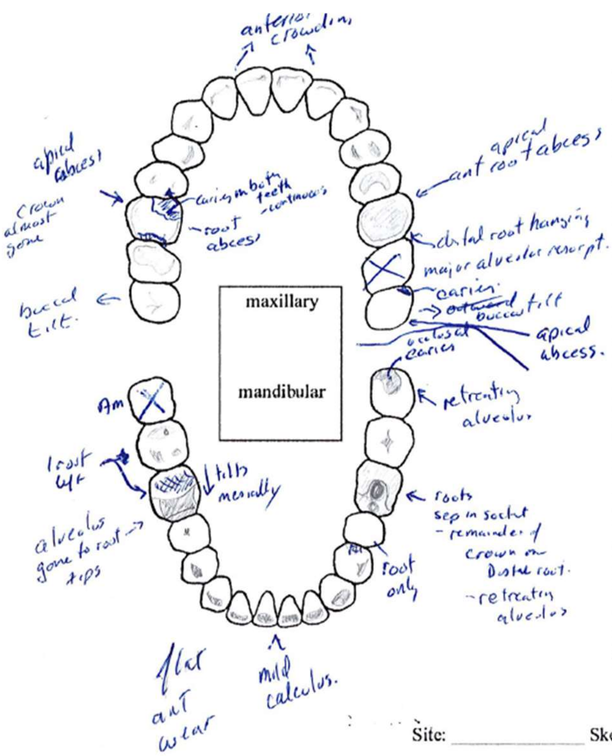
Harris Lines: (number, thickness, degree of opacity, location, % of diaphysis crossed)

2.5.3

3.3.6

3.5.4

Site: Skeleton #: page 2 of 9



Dental Pathology

Comments:

Key: CO = caries on occlusal surface
CI = interproximal caries
CC = cervical caries K = calculus
AR = alveolar resorption
SUP = supragingival
SUB = subgingival
AB = abscess H = hypoplasia
- shade for wear pattern

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Appendix C. Isotopic Data in Farfán.

Table C.1. Isotope values of teeth and bones for primary burials with data in Farfán after Table 1 in White *et al.* (2020).

ID	Age	Tooth 1	δ^{18} Op	CI	Tooth 2	δ^{18} Op	CI	element	δ^{18} Op	CI	δ^{13} C	δ^{15}N	Yield collagen	C/N	Comments on diet	Comments on geographic residence
G T4	MA	NA	17.5	-	-	-	-	bone	16.7	-	- 12.3	10.4	-	3.4	Lowest level of maize; Typical level of protein	Grew up in another coastal region, lived locally but spent some time in the last ten year on another coast
G T11	OA	NA	16.6	-	-	-	-	bone	16.4	-	- 10.2	12.1	-	3.3	High level of maize; High level of protein	Grew up and lived locally
I3 T1a	MA	-	-	-	-	-	-	bone	16.8	-	- 10.8	10.9	9.77	-	Typical level of maize; Typical level of protein, probably of marine origin	-
I10 T2	MA	-	-	-	M3	16.4	-	-	-	-	-	-	-	-	-	Grew up locally
I10 T3	CH	-	-	-	M1	17.9	-	bone	17.0	-	-	-	0.77	-	-	Grew up in another coastal region, recent move to Jequetepeque valley
J14 T2	YA	-	-	-	-	-	-	bone	16.4	-	-	-	0.54	-	-	-
J25 T6	MA	I2	17.5	-	-	-	-	bone	16.9	-	-9.6	11.5	3.0	3.2	High level of maize; High level of protein, probably of marine origin	Grew up in another coastal region, moved to Jequetepeque valley around 9 years of age
J25 T9	YA	I1	17.2	-	-	-	-	bone	-	-	-10.5	8.3	5.1	3.1	Typical level of maize; Low level of protein	Grew up in another coastal region, lived locally
C T11A	OA	I1	14.3	2.6	M3	14.1	-	bone	15.5	2.8	-11.8	10.0	-	3.1	Typical level of maize; Typical level of protein, probably of marine origin	Grew up in the highlands, recent move to Jequetepeque valley
C T11B	OA	I1	18.3	2.7	M3	-	2.6	bone	15.5	2.8	-11.0	10.9	-	3.1	Typical level of maize; Typical level of protein, probably of marine origin	Grew up in another coastal region, lived locally
C T11C	AD	I2*	-	-	M3	16.0	2.8	bone	15.9	2.8	-11.1	11.0	-	3.1	Typical level of maize; Typical level of protein, probably of marine origin	Grew up and lived locally

ID	Age	Tooth 1	δ^{18} Op	CI	Tooth 2	δ^{18} Op	CI	element	δ^{18} Op	CI	δ^{13} C	δ^{15}N	Yield collagen	C/N	Comments on diet	Comments on geographic residence
C T11D	OA	I2*	-	-	M2	19.7	2.8	bone?	11.4	2.7	-11.1	10.6	-	3.1	Typical level of maize; Typical level of protein, probably of marine origin	Grew up in another coastal region, moved to the highlands (bone), then moved to the Jequetepeque valley
C T2A	YA	-	-	-	-	-	-	bone?	-	-	-	-	0.7	-	-	-
C T2B	AD	-	-	-	-	-	-	bone	-	-	-10.1	12.0	-	3.2	High level of maize; High level of protein, probably of marine origin	-
C T2C	AD	-	-	-	-	-	-	bone	-	-	-10.1	13.6	-	1.9	High level of maize; High level of protein, probably of marine origin	-
C T2D	JUV	-	-	-	-	-	-	bone	-	-	-	-	0.4	-	-	-
C T2E	CH	-	-	-	-	-	-	bone	-	-	-	-	0.9	-	-	-
C T2F	JUV	-	-	-	-	-	-	bone	-	-	-	-	0.6	-	-	-
CH 1a	MA	PM1	16.9	3.2	-	-	-	tooth	-	-	-12.0	13.3	-	-	Low level of maize; High level of protein, probably of marine origin	Grew up locally
E T1	AD	I1	15.9	2.6	M3	15.0	2.6	bone	15.7	2.8	-11.5	10.5	-	3.2	High level of maize; High level of protein	Grew up and lived locally
E T4	AD	I1	17.2	-	M3	17.0	2.6	bone	15.5	2.8	-11.2	10.5	-	3.1	High level of maize; High level of protein	Grew up and lived locally
E T5	MA	I1	14.5	2.6	M3	13.4	3.0	bone	15.5	2.7	-10.5	10.4	-	3.1	Typical level of maize; Typical level of protein	Grew up in the highlands, recent move to the Jequetepeque valley
E T6	AD	I1	19.1	2.7	M3	18.4	2.8	bone	15.2	2.8	-12.2	8.2	-	3.1	Low level of maize; Low level of protein	Grew up in another coastal region, moved to the Jequetepqeue Valley between 6 to 12 years of age
E T7	OA	I2*	-	-	M3	16.3	2.6	bone	15.1	2.8	-9.7	12.3	-	3.1	High level of maize; High level of protein	Grew up and Lived locally
E T9	JUV	-	-	-	-	-	-	bone	16.3	2.8	-10.5	10.2	-	3.1	Typical level of maize; Low level of protein	Lived locally
E T10	JUV	I1	17.5	2.6	-	-	-	bone	15.3	2.7	-10.4	10.6	-	3.1	Typical level of maize; Typical level of protein	Origin from another coastal region, moved to the Jequetepeque valley between 4 to 6 years of age

ID	Age	Tooth 1	δ^{18} Op	CI	Tooth 2	δ^{18} Op	CI	element	δ^{18} Op	CI	δ^{13} C	δ^{15}N	Yield collagen	C/N	Comments on diet	Comments on geographic residence
N T1a	AD	I1*	-	-	M3	16.1	3.0	bone	16.7	2.7	-10.9	12.4	-	3.3	High level of maize; High level of protein, probably of marine origin	Grew up and lived locally
N T1b	OA	-	-	-	M3	15.1	2.8	bone	15.3	2.7	-9.6	12.7	-	3.2	High level of maize; High level of protein, probably of marine origin	Grew up and lived locally
N T1c	NEO	Frags of incisor*	-	-	M1	16.9	3.2	bone	16.9	2.8	-12.0	13.3	-	-	Low level of maize; High level of protein	Origin from the Jequetepeque valley
I T1	IN	-	-	-	-	-	-	bone	19.5	2.8	-	-	-	-	-	Origin from another coastal region
I T3	AD	-	-	-	-	-	-	bone	-	-	-	-	0.5	-	-	-
I T4	IN	-	-	-	-	-	-	bone	-	-	-	-	0.5	-	-	-
II T1	OA	-	-	-	-	-	-	bone	20.0	2.8	-	-	-	-	-	Lived in another coastal region
II T2	AD	-	-	-	-	-	-	bone	-	-	-	-	0.6	-	-	-
II T3	OA	-	-	-	-	-	-	bone	-	-	-	-	0.6	-	-	-
II T4	MA	-	-	-	-	-	-	bone	-	-	-	-	0.4	-	-	-
III T1	OA	-	-	-	-	-	-	bone	-	-	-	-	0.6	-	-	-
III T2	MA	-	-	-	-	-	-	bone	19.0	2.8	-	-	0.6	-	-	Lived in another coastal region
III T3	NEO	-	-	-	-	-	-	bone	-	-	-10.7	8.4	-	-	Typical level of maize; Low level of protein, might had inadequate breast-feeding; possibly fed with a supplemental mush of maize and other foods	-

Age: NEO (neonate) between 0 and 0.9 year, IN (infant) between 1 and 2.9 years, CH (child) between 3 and 5.9 years, JUV (juvenile) between 6 and 11.9 years, AD (adolescent) between 12 and 19.9 years, YA (young adult) between 20 and 29 years, MA (mid adult) between 30 and 39 years, OA (old adult) more than 40 years.
* Insufficient enamel for analysis.
Gray: individuals included in the focused sample.
CI: Cristallinity index, indicator of diagenetic damages

Carbon and nitrogen isotope analysis was done on collagen while the oxygen isotope analysis was done on the bioapatite phosphate component of rib bone samples and enamel. A subsample of 39 individuals among the primary burials was analysed for isotope values using the facilities of the Laboratory for Stable Isotope Science at The University of Western Ontario, London, Ontario, Canada (White *et al.*, 2020). Details regarding the methodology and isotopic analyses can be found in White *et al.* (2020).For the Farfán sample, the C/N ratios have an average of 3.1, the bone crystallinity index (CI) ranges from 2.5 to 3.1 for an average of 2.8, and the tooth CI ranges from 2.5 to 3.1 with an average of 2.7. This value suggests that "they had not undergone sufficient diagenesis to affect the primary isotopic signature" (Mackey & Nelson, 2020: 362). Among the sample, 13 individuals from the *Huaca* Burial Platform and Compound VI Cemetery had low collagen yields (i.e., below 1.0 ‰) and therefore were not analysed isotopically. Five teeth had insufficient enamel for analysis.

The isotopic values of Farfán individuals suggest a diet rich in maize and marine resources (average $\delta^{13}C_{col}$= -10.8 ± 0.8‰, range -12.3 to -9.6‰, N=25) and variable in protein (average $\delta^{15}N_{col}$ = +10.9 ± 1.4‰, range +8.2 to +13.6‰, N=25). The high δ15N average is most likely due to a combination of the arid environmental conditions, high tropic level marine resources, and/or the use of organic fertilisers (White *et al.*, 2020).

The overall $\delta^{18}O_p$ value in enamel (average= +16.5±1.5‰, range +13.4 to +19.7‰, N=30), which reflects where a person lived during childhood during the formation of the relevant tooth, include ten individuals (30% of the sample) having values outside the local range suggesting non-local origin. These individuals migrated from the highlands ($\delta^{18}O_p$ < +15‰, N=2) and non-local coast most likely from the northern region (Nelson, pers. comm., 2021) ($\delta^{18}O_p$ > +17‰, N=11) to the Jequetepeque Valley when they were young. The overall $\delta^{18}O_p$ in bone, which reflects approximately the last ten years before death, falls within the Jequetepeque Valley value (average = +16.2 ± 1.6‰, range +11.4 to +20.0‰, N=29) with only four individuals outside the local range. These individuals most likely came to the valley within the last decade prior to their death, so their $\delta^{18}O_p$ in their teeth still reflects their previous location(s).

Appendix D. Grave Goods in Farfán.

Table D.1. Grave goods and offerings excavated with the internments in Farfán.

Skeleton Number	Cemetery	Sex	Age	Orientation	Personal Grave Goods	Communal Offerings
G T3a	G	F	MA	N	5 ceramic vessels (1 blackware stirrup spout vessel, 1 blackware spout and handle vessel, 3 redware *ollas*), shroud, copper beads, folded copper pieces, 2 spindle whorls	-
G T3b	G	U	NEO	E	-	-
G T4	G	F	MA	N	seed, 9 ceramic vessels (4 *ollas*, 3 jars, 1 jar and handle vessel, 1 stirrup spout vessel), llama bones (llama, 1-12 months old; llama, foetus; llama, 3.4-4 years old; disarticulated elements of llama, indeterminate age)	-
G T5	G	U	IN	S-E	seeds of lucuma fruit	-
G T6	G	U	CH	S-E	-	-
G T11	G	F	OA	S-E	20 gourds, *Nectandra* seed necklace, 27 ceramic vessels (15 cooking *ollas*, 4 plates, 2 bowls, 6 jars), cinnabar, textiles, copper discs, 6 copper rings, folded copper pieces, 1 silver bead, 4 llama feet, guinea pig bones, spindle whorls, 6 weaving swords, weaving basket, 2 copper needles	-
G T12	G	U	NEO	E	3 ceramic vessels (2 *ollas*, 1 jar), folded copper pieces	-
I10 T1	I	U	CH	N-W	5 ceramic vessels	-
I10 T2	I	F	MA	W	4 ceramic vessels, folded copper pieces, llama bones	-
I10 T3	I	U	CH	S	spindle whorls, llama bones (llama, 6-11 months old; llama, 12-14 years old), guinea pig bones	-
I3 T1a	I	M	MA	W	2 gourd bowls, 5 ceramic vessels (domestic wares), whole Spondylus shell	-
I3 T1b	I	U	IN	S	-	-
J14 T1	J	M	OA	S	gourds, 3 ceramic vessels (2 cooking *ollas* and 1 stirrup spout), camelid fiber and cotton textile, copper needles	-
J14 T2	J	F	YA	S	1 ceramic vessel	-
J14 T3	J	U	IN	E	4 ceramic vessels	-
J25 T1	J	M	YA	N	seed, maize, 1 ceramic vessel	-
J25 T2	J	U	NEO	E	folded copper pieces	-

Skeleton Number	Cemetery	Sex	Age	Orientation	Personal Grave Goods	Communal Offerings
J25 T3	J	U	CH	W	maize, fruit, 4 ceramic vessels	-
J25 T4	J	U	IN	E	maize, 1 ceramic vessel, shell beads	-
J25 T5	J	U	IN	E	1 ceramic vessel, shell beads	-
J25 T6	J	M	MA	N	3 gourd bowls, 3 ceramic vessels, folded copper pieces, shell beads	-
J25 T7	J	F	MA	N	gourd, 5 ceramic vessels, shell beads, whole *Spondylus* shell, needles	-
J25 T8	J	F	YA	S	2 ceramic vessels, shell beads, whole Spondylus shell, chalk	-
J25 T9	J	F	YA	N	2 ceramic vessels, weaving basket	-
J26 T1a	J	U	CH	W	3 ceramic vessels	-
J26 T1b	J	U	JUV	W	folded copper pieces, shell beads	-
J26 T2	J	U	NEO	E	1 ceramic vessel	-
J26 T3	J	U	JUV	W	gourd, 4 ceramic vessels, folded copper pieces, 2 copper tweezers, whole Spondylus shell	-
JE19 T1	J	F	OA	-	shreds of fine pottery, feathers (possibly flamingo)	-
CH 1a	HBP	M	MA	-	-	15 ceramic vessels, fragments of metal and copper discs, camelid and bird bones
CH 1b	HBP	F	YA	-	-	
CH 1c	HBP	F	MA	-	-	
CH 1d	HBP	U	CH	-	-	
CH 1e	HBP	U	CH	-	-	
CH 1f	HBP	U	CH	-	-	
CH 1g	HBP	U	JUV	-	-	
C T11A	HBP	F	OA	E	*Nectandra* seed necklace, textile bag, rings, whole Spondylus shell, weaving swords, weaving basket with wooden spindles, copper spindle whorls, and needles	136 ceramic vessels (18 plates, 13 jar), chicha poured over the textile bundles (17 small rolled bundles recovered)
C T11B	HBP	F	OA	N	*Nectandra* seed necklace, textile bag, decorated copper rings, whole Spondylus shell, weaving basket with wooden spindles, copper spindle whorls, and needles	
C T11C	HBP	F	AD	W	3 ceramic vessels, *Nectandra* seed necklace, textile bag, decorated copper ring, whole Spondylus shell	
C T11D	HBP	F	OA	S	gourd, *Nectandra* seed necklace, a small textile bag, decorated copper rings, guinea pig bones, whole Spondylus shell, weaving swords	

Skeleton Number	Cemetery	Sex	Age	Orientation	Personal Grave Goods	Communal Offerings
C T2A	HBP	F	YA	N	copper rings, shell beads	30 ceramic vessels (17 jars, 7 stirrup spout vessels), 10 Spondylus shells including one with dried cochineal insects (red pigment to dye textiles), 2 gourds, a copper needle, two plates with food remains
C T2B	HBP	F	AD	N	copper rings, shell beads	
C T2C	HBP	U	AD	N	copper rings, shell beads, cinnabar	
C T2D	HBP	U	JUV	N	copper rings, shell beads, cinnabar	
C T2E	HBP	U	JUV	N	copper rings, shell beads, cinnabar	
C T2F	HBP	U	JUV	N-E	copper rings, shell beads, cinnabar	
E T1	HBP	F	AD	N	2 ceramic vessels	32 ceramic vessels (15 jars, 2 jars with handles, 8 stirrup spout vessels, 6 complete plates, plate fragments), large textile bundles
E T4	HBP	F	AD	E	3 gourd bowls, 19 ceramic vessels, 10 weaving swords, 1 wooden figurine (might represent *Zaramama*, mother of maize)	
E T5	HBP	F	MA	N	1 ceramic vessel (black stirrup spout vessel), cloth with remains of beans (*Phaseolus vulgaris),* thread, and a spindle whorl	
E T6	HBP	F?	JUV	N	1 ceramic vessel, 1 gourd bowl with unidentified organic remains	
E T7	HBP	F	OA	S	4 ceramic vessels (2 miniature jar and handle, 1 olla, 1 blackware jar), cotton and camelid fiber textile bag (tapestry fragment in the bag), other textile, 1 ear spool, discs, copper beads, silver beads, silver object, copper *tumi*, spindle whorl, 4 needles including 1 bone needle held in a case made of deerskin	
E T9	HBP	F?	JUV	S	1 ceramic vessel, llama bones	
E T10	HBP	F?	JUV	S	-	
N T1a	HBP	F	AD	W	2 copper rings, silver-plated copper rattle, gourds, silver plates, llama bones	Llama, 3.5 years old, llama, 1-12 months old, llama, foetus guinea pig (*Cavia porcellus*), silver-plated objects, 2 human offerings, 32 ceramic vessels, 1 communal shroud
N T1b	HBP	F	OA	N	cinnabar, 6 copper rings, silver object, silver plated copper rattle, silver-plated copper bowl, copper *tumi* knife, 36 shell beads, necklace-remnants of thread and quartz, gourd fragments, whole Spondylus shell	
N T1c	HBP	F	IN	W	-	
I T1	VI	U	CH	E	shell beads	-
I T2	VI	U	IN	E	3 ceramic vessels	-
I T3	VI	M	AD	E	4 ceramic vessels, copper discs, over 60 copper beads, 30 shell beads, part of a necklace	-

Skeleton Number	Cemetery	Sex	Age	Orientation	Personal Grave Goods	Communal Offerings
I T4	VI	U	CH	S-W	4 ceramic vessels, feline remains	-
II T1	VI	F	MA	E	1 ceramic vessel	-
II T2	VI	F	OA	E	6 ceramic vessels, 3 copper rings, several necklaces with copper and shell beads, spindle whorls	-
II T3	VI	M	OA	E	3 ceramic vessels (2 redware *ollas*, 1 blackware jar), shroud, folded copper pieces, llama bones, a copper spindle whorl	-
II T4	VI	M	MA	E	1 ceramic vessel	-
III T1	VI	F	OA	E	4 ceramic vessels	-
III T2	VI	M	MA	E	3 ceramic vessels (blackware monkey jar and 2 redware *ollas*), copper beads, copper tweezers, shell beads, a shell ornament with a green stone inlay	-
III T3	VI	U	NEO	E	1 ceramic vessel	-

Information compiled from chapter 3A and table on p.77-78 for the *Huaca* Burial Platform (HBC); chapter 4A and table on p.163 for Cemetery J (J); chapter 5A and table on p.200 for Cemetery I (I); chapter 6A and table on p. 226 for Mound G (G), and chapter 7A and table on p.251 for Compound VI Cemetery (VI) in Mackey and Nelson (2020).
Sex: F= Female, F?= probable female, M= Male, U= Undefined
Age: NEO (neonate) between 0 and 0.9 year, IN (infant) between 1 and 2.9 years, CH (child) between 3 and 5.9 years, JUV (juvenile) between 6 and 11.9 years, AD (adolescent) between 12 and 19.9 years, YA (young adult) between 20 and 29 years, MA (mid adult) between 30 and 39 years, OA (old adult) more than 40 years.

Appendix E. Dental Data from Túcume.

Table E.1. Profiles and Dental Health Data of Individuals from *Huaca Larga,* Túcume (N=22) based on Toyne (2002).

ID	Sex	Age	# Teeth	# AMTL	# Carious Lesions	Level Calculus	# Abscesses	# Stress Episodes (LEH)	Age of Enamel Defects	Wear Level	*Freq. Abscesses (%)	Other Comments
R3E1	F	YA	31	0	3	2	2	1	3.5-4.0	0	6.5	-
R3E2	F	YA	31	0	1	0	0	3	2.0-2.5; 3.0-3.5; 5.0-5.5	0	0.0	-
R3E3	F	YA	31	0	0	0	0	0	NA	0	0.0	Enamel defects present but not measured
R3E4	F	YA	28	0	2	0	0	1	4.5-5.0	0	0.0	-
R3E5	F	MA	31	0	4	0	0	3	3.5-4.0; 4.0-4.5; 5.0-5.5	0	0.0	-
R3E6	F	AD	31	0	0	0	0	2	2.0-2.5; 3.0-3.5	1	0.0	UXI2s peg teeth, brown staining
R3E7	F	YA	29	1	10	0	0	2	4.5-5.0; 5.5-6.0	0	0.0	-
R3E8	F	YA	28	0	0	0	0	0	NA	1	0.0	Brown staining
R3E9	F	AD	31	0	5	0	1	5	1.5-2.0; 2.5-3.0; 3.0-3.5; 3.5-4.0; 4.5-5.0;	0	3.2	-
R3E10	F	MA	25	2	0	1	1	0	NA	0	4.0	-
R3E11	F	YA	32	0	0	0	0	5	1.0-1.5; 2.5-3.0; 3.0-3.5; 3.5-4.0; 5.0-5.5	0	0.0	-
R3E12	F	AD	26	1	5	3	1	4	3.0-3.5; 3.5-4.0 (x2); 4.5-5.0	0	3.8	-
R3E13	F	MA	23	2	3	2	0	0	NA	1	0.0	UM3s absent

ID	Sex	Age	# Teeth	# AMTL	# Carious Lesions	Level Calculus	# Abscesses	# Stress Episodes (LEH)	Age of Enamel Defects	Wear Level	*Freq. Abscesses (%)	Other Comments
R3E14	F	JUV	27	0	0	0	0	2	4.5-5.0; 5.0-5.5	0	0.0	Brown staining
R3E15	F	YA	28	0	5	1	1	5	2.5-3.0; 3.0-3.5; 3.5-4.0 (x2); 4.5-5.0	1	3.6	-
R3E16	F	YA	30	0	3	1	0	4	3.5-4.0; 4.5-5.0 (x2); 5.0-5.5	0	0.0	-
R3E17	F	YA	30	0	3	1	0	4	2.5-3.0; 3.0-3.5; 3.5-4.0; 4.0-4.5	0	0.0	-
R3E18	F	MA	32	0	0	1	0	3	3.0-3.5; 3.5-4.0; 4.5-5.0	0	0.0	2 supernumerary residual canines
R3E19	F	AD	31	0	0	0	0	6	2.5-3.0; 3.0-3.5 (x2); 4.0-4.5 (x2); 5.0-5.5	0	0.0	-

Sex: F= Female;
Age: JUV (juvenile) between 6 and 11.9 years, AD (adolescent) between 12 and 19.9 years, YA (young adult) between 20 and 29 years, MA (mid adult) between 30 and 39 years, OA (old adult) more than 40 years;
Dental Calculus: 0=None, 1=slight, 2=mild, 3=heavy;
Age of enamel defects based on the formulae from Goodman & Rose (1990),
Wear Level: 0=None, 1= slight; Other Comments: UXI2= upper second incisors with unknown side. UM3s= upper third molars;
* calculated Freq. Abscesses= # Abscesses/ # Teeth* 100.

Appendix F. Clinical CT Scans.

Clinical CT scanners have a resolution of 40X lower than micro-CT in this case (500 μm vs 12.5 μm), but they are usually easier to access since they can be found in any hospital and thus, represent a more accessible alternative to destructive analysis than micro-CT. Thus, the 45 teeth in the focused sample were also scanned with the clinical CT scanner from St-Joseph's hospital in London, Ontario to compared with the data obtained from micro-CT scans.

All teeth were embedded in two floral foam blocks (6.6 X 8.6 X 19.8 cm) (Figure F.1-F.2) to be scanned with the Canon Aquilion One scanner at St. Joseph's Healthcare at a slice thickness of 0.5 mm, 80 KVp, and a tube current of 200 mA. Enough distance was left between teeth to minimize streak artefacts. They were scanned twice using two different reconstruction algorithms. The first reconstruction volume was scanned using Bone Hi-res algorithm while the second volume was with SEMAR algorithm (Bone sharp with SEMAR). SEMAR scans are designed to reduce the streak artifacts in the CT scans associated with metal and very dense substances, such as enamel (Funama *et al.*, 2015). As seen in Figures F.3 and F.4, the volumes are sharper with Bone Sharp with SEMAR when compared with Hi-res filter. However, the difference is not important enough to detect more pathological lesions. In both cases, as seen in Figures F.5 to F.10, the resolution does not allow the observations of as many details as micro-CT. With the colored lookup table, it is possible to observe some variations in the density of dental tissues, but it is difficult to differentiate between biogenic and diagenetic damages. The occlusal wear level can be approximated, although it is difficult to follow the standard from Buikstra and Ubelaker (1994). Only the more pronounced carious lesions can be observed such as the large carious lesions in ULM3 from E T5. LEH and IGD cannot be recorded in any teeth.

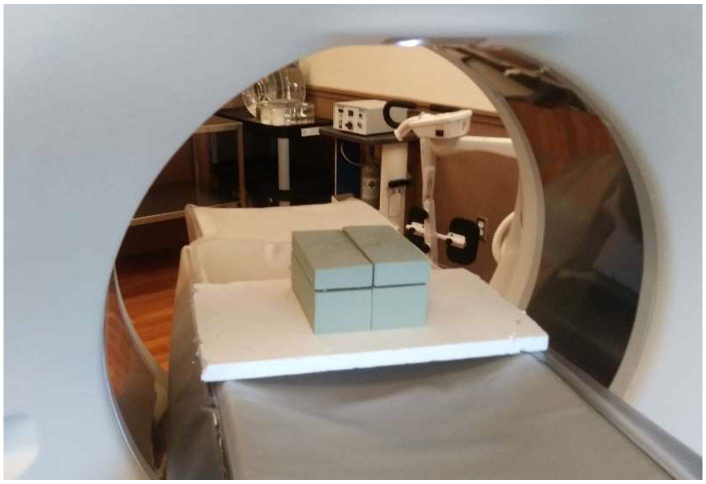

Figure F.1. Set-up of 45 teeth in florist foam inside the clinical CT scan at St-Joseph's hospital, London, Ontario. Picture taken by Émy Roberge.

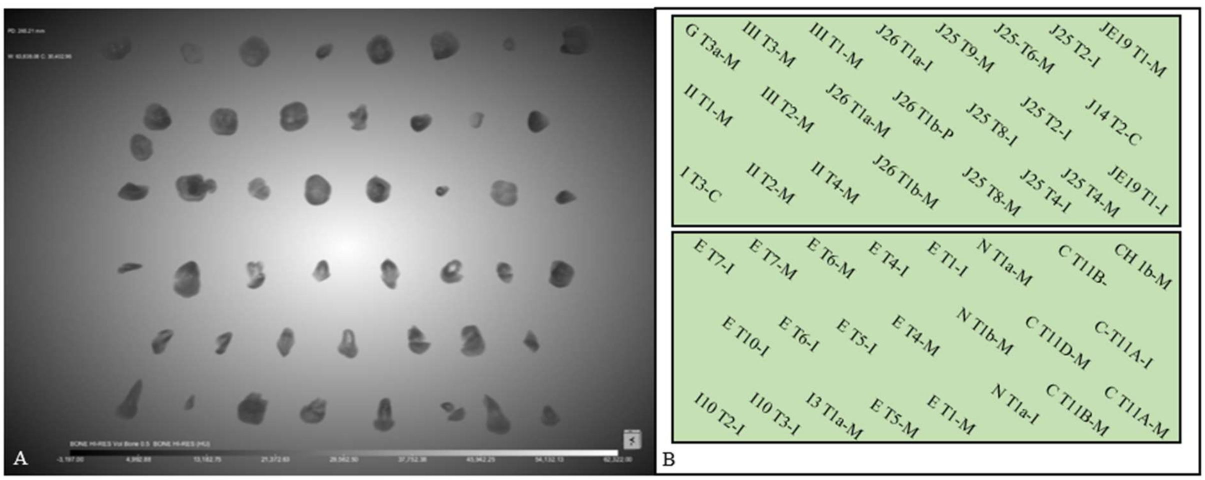

Figure F.2. Occlusal view of the 45 teeth obtained with the clinical CT scan, Bone Hi-res filter. A) volumes seen in the 3D view on Dragonfly 2020.2; B) Identification of teeth as embedded in the florist foam. The last letter refers to tooth type either incisor (I), canine (C), premolar (P), or molar (M).

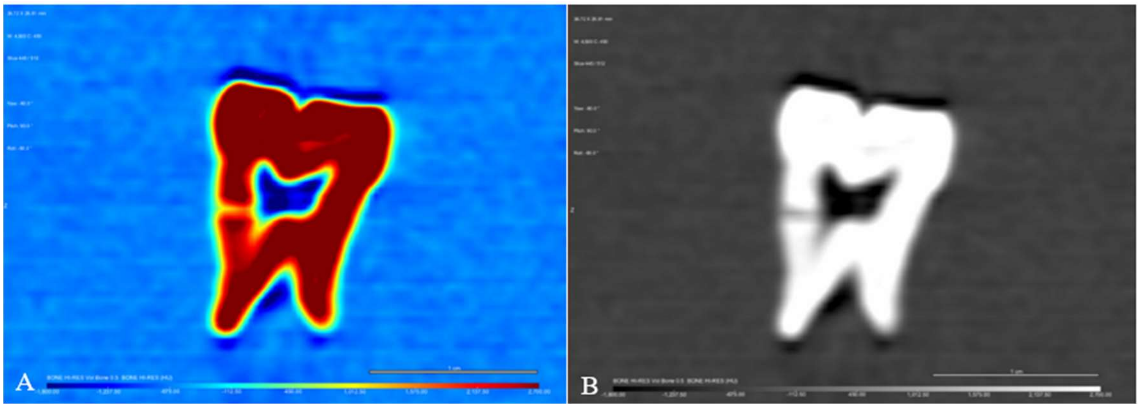

Figure F.3. Medio-distal view of ULM3 from E T5 obtained from the clinical CT scan bone Hi-res filter. A) seen through look up table "Jet". B) seen through grayscale.

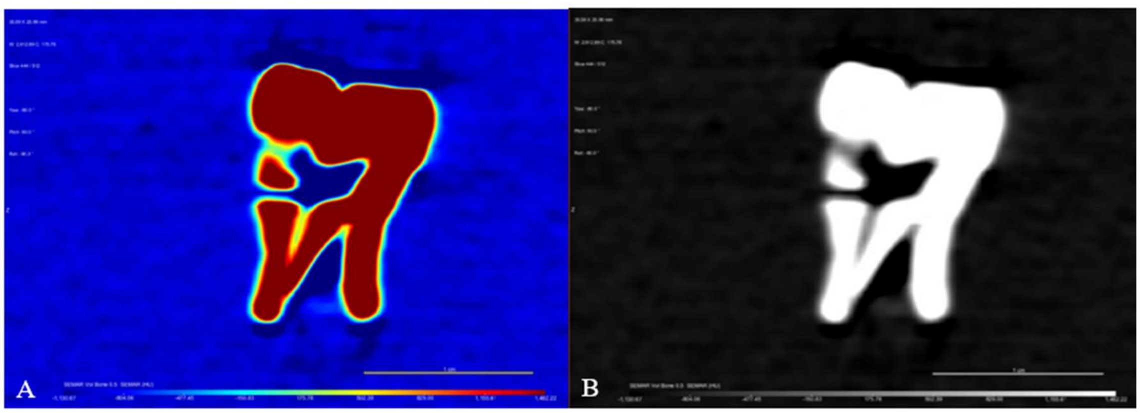

Figure F.4. Medio-distal view of ULM3 from E T5 obtained from the clinical CT scan Bone sharp with SEMAR filter. A) seen through look up table "Jet". B) seen through grayscale.

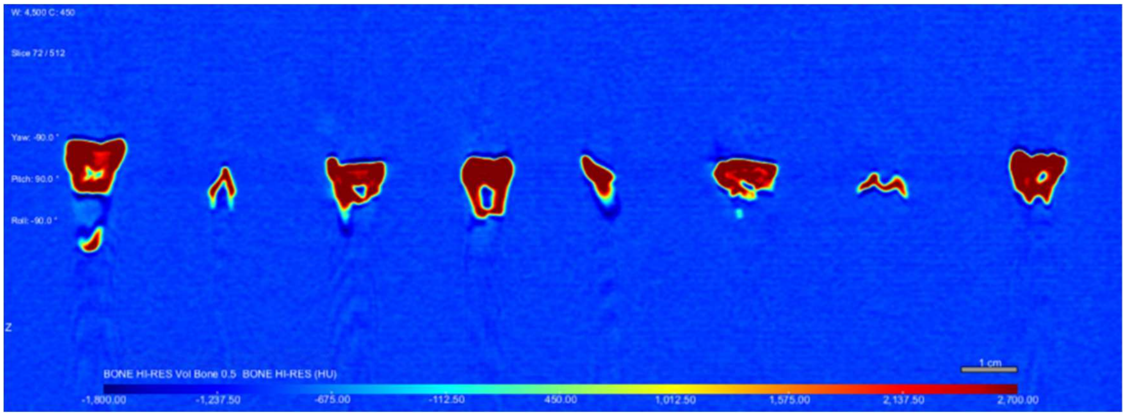

Figure F.5. Medio-distal view of the first row of teeth obtained from the clinical CT scan bone Hi-res filter, using the look up table "Jet". From left to right, the teeth represent LLM1 from JE19 T1, uri2 from J25 T2, LRM3 from J25 T6, LLM3 from J25 T9, ULI1 from J26 T1a, ULM2 from III T1, xxm1 from III T3, LLM1 from G T3a.

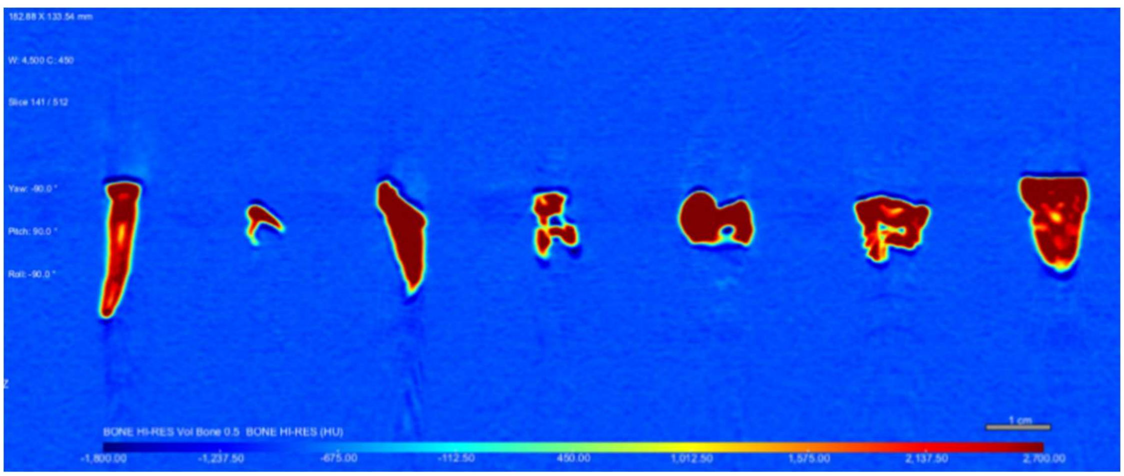

Figure F.6. Medio-distal view of the second row of teeth obtained from the clinical CT scan bone Hi-res filter, using the look up table "Jet". From left to right, the teeth represent URC from J14 T2, uxc from J25 T2, ULI1 from J25 T8, urm2 from J26 T1b, LLM1 from J26 T1a, LRM1 from III T2, LRM3 from II T1.

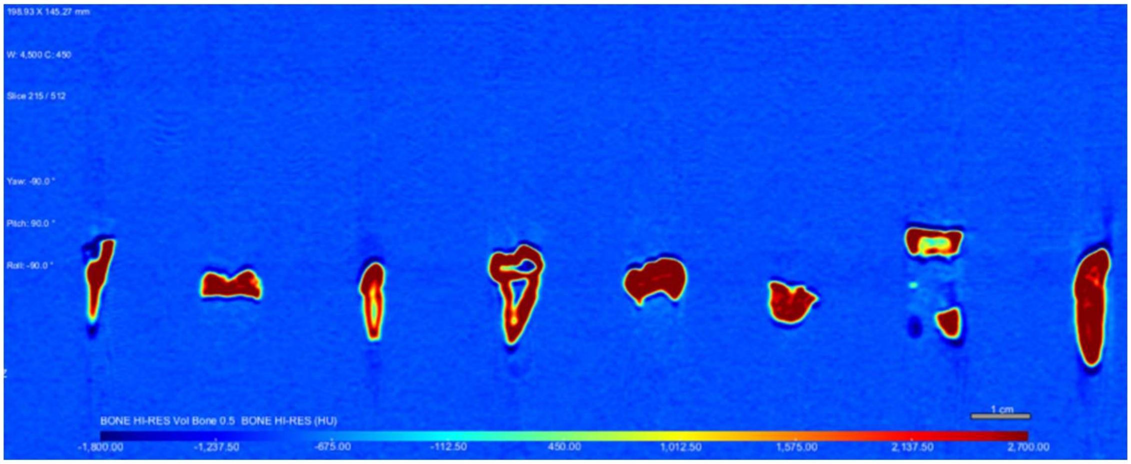

Figure F.7. Medio-distal view of the third row of teeth obtained from the clinical CT scan bone Hi-res filter, using the look up table "Jet". From left to right, the teeth represent LLI1 from JE19 T1, lli2 from J25 T4, ULM3 from J25 T8, URM1 from J26 T1b, LLM2 from II T4, LLMX from II T2, LLC from I T3.

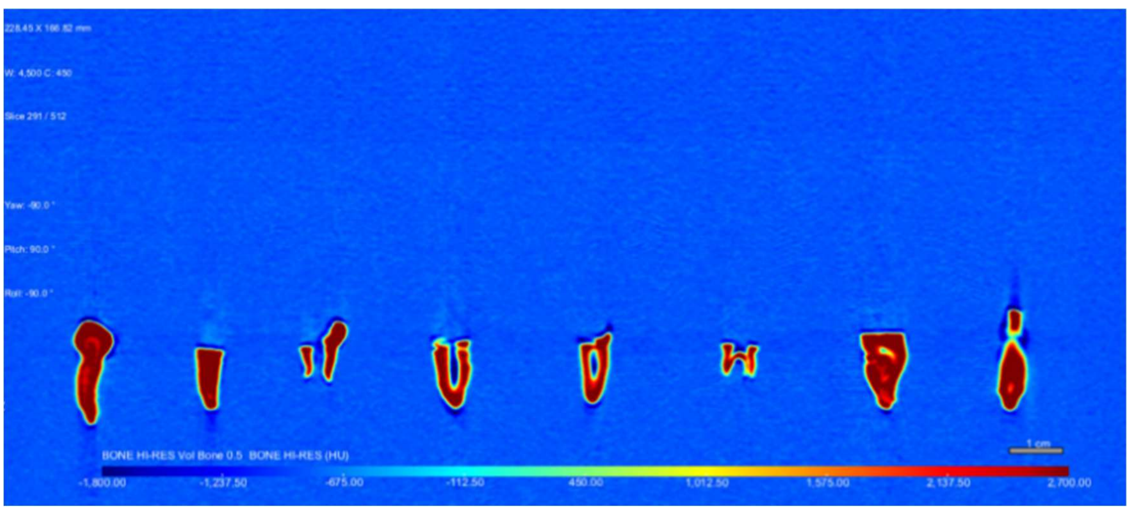

Figure F.8. Medio-distal view of the fourth row of teeth obtained from the clinical CT scan bone Hi-res filter, using the look up table "Jet". From left to right, the teeth represent ULP4 from CH 1b, URI2 from C T11B, URM3 from N T1a, URI1 from E T1, ULI1 from E T4, URM2 from E T6, LLM3 from E T7, LLI1 from E T7.

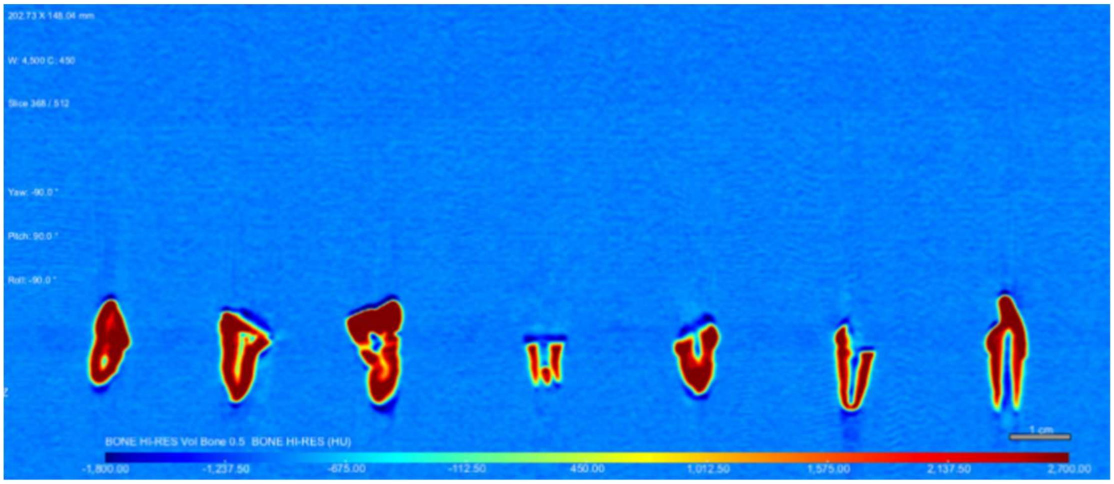

Figure F.9. Medio-distal view of the fifth row of teeth obtained from the clinical CT scan bone Hi-res filter, using the look up table "Jet". From left to right, the teeth represent URI1 from C T11A, LRM2 from C T11D, LRM3 from N T1b, ULM3 from E T4, ULI1 from E T5, URI1 from E T6, ULI1 from E T10.

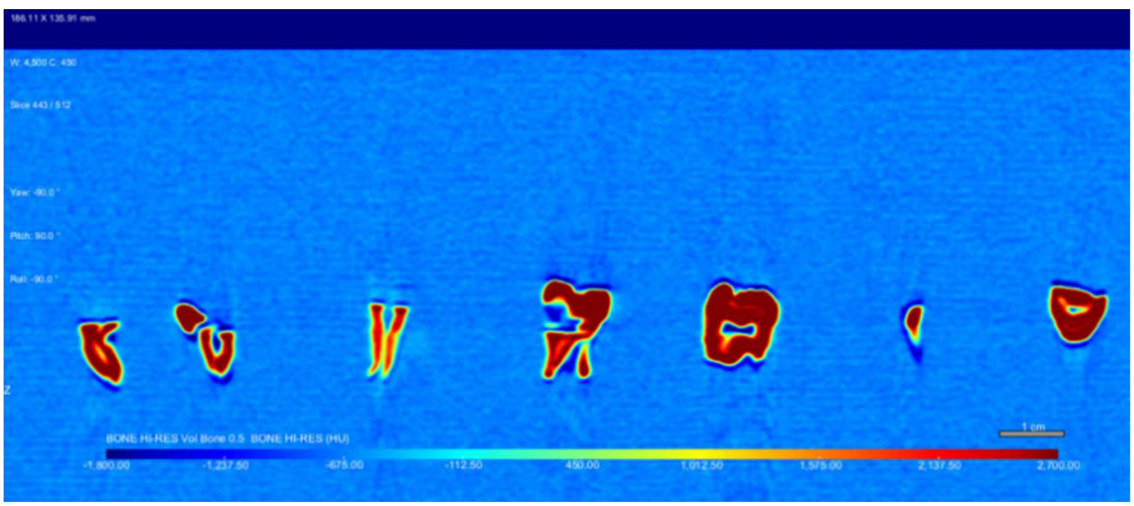

Figure F.10. Medio-distal view of the sixth row of teeth obtained from the clinical CT scan bone Hi-res filter, using the look up table "Jet". From left to right, the teeth represent URM3 from C T11A, URM3 from C T11B, ULI1 from N T1a, URM3 from E T1, ULM3 from E T5, LLM2 from I3 T1a, lli1 from I10 T3, URI1 from I10 T2.

Appendix G. BSE-SEM in 3 Teeth (CH 1b, E T1, and E T7) from the *Huaca* Burial Platform.

The comparison of histological analysis and micro-CT scanning to explore IGD has been done in a few recent articles (Colombo *et al.*, 2019; D'Ortenzio *et al.*, 2016; Veselka *et al.*, 2019). D'Ortenzio *et al.* (2016) extended the comparison between imaging methods by performing scanning electron microscopy (SEM) on their thin-sections. Their study suggests that SEM did not reveal additional information that could not be observed through histology. To add to the discussion, Backscattered Scanning Electron Microscopy (BSE-SEM) was performed on three thin-sections from Farfán. BSE-SEM relies on high-energy electrons to obtain high-resolution images that can distinguish small density differences and, with the proper settings, provide relative distribution of elements in the sample (Boyde & Jones, 1983).

Three thin-sections (ULP4 from CH 1b, URI1 from E T1, and LLI1 from E T7) were selected based on their quality (fewer artefacts and diagenetic damages) and visible IGD to perform BSE-SEM using the equipment at Surface Science Western, London, Ontario. The microscope (SU3900) was set at an accelerating voltage of 15 kV, a current of 60 W, a working distance of 10.6 mm, and a pressure of 50 Pa. The composition and homogeneity of the samples were analysed with EDS element mapping and element EDS point analysis. Thus, it was possible to evaluation the relative chemical composition of the sample in oxygen (O), calcium (Ca), carbon (C), phosphorus (P), nitrogen (N), magnesium (Mg), sodium (Na), chlorine (Cl), sulphur (S), and silicon (Si). The point analysis on enamel, dentin, and epoxy highlights the difference in composition in the different materials present on the thin-section. To confirm that the element map results were not due to beam artefact, carbon-coating of E T7 was done. Carbon was determined as the best coating option to avoid interaction with other components.

Results (Figures G.1 and G.2) do not add information regarding the detection of IGD similarly to D'Ortenzio *et al.* (2016), but that could contribute to discussions regarding the aetiology of the defect. One of the main observations was the difference regarding the depth of the defect highlighted by BSE-SEM even within the same episode of IGD. The development of the defect is not uniform and thus, it supports our previous observation regarding the loss of information associated with the imaging methods used. SEM, histology, or micro-CT analyse different thicknesses in the slices varying between 2-3 μm with SEM to 100 μm with histology affecting the accuracy of the comparison between methods since some defects cannot be detected at different thicknesses.

The element maps revealed, as expected, a high proportion of oxygen (O), phosphorus (P), and calcium (Ca) due to the hydroxyapatite ($Ca_{10}(PO_4)_6(OH)$) present in dental tissues. Approximately 97% of enamel and 70% of dentin are composed of the inorganic component (Hillson, 1996). Interestingly, while IGD is associated with a defect in the mineralisation of the calcospherite in dentin, the element mapping did not reveal variation in the composition surrounding the defect. Brighter colors in Figure G.3 indicates the presence of the component in the region of interest. The element composition is uniform around the IGD defect. A slight increase is visible near the defect in Cl, O, and C which is most likely from the epoxy. Indeed, the point analysis of the epoxy reveals a high relative proportion (75.9%) of C, 21.0% of O, and 1.0% of Cl (Figure G.4). Regarding the point analysis, as seen in Figure G.5 of E T7, a slight decrease in the relative proportion of Ca was seen in the defect passing from >30% in the surrounding area to 27% on the defect. However, Ca was present at 26.6% in the dentin surrounding the defect in E T1 (Figure G.6) suggesting that this variation might be a normal variation across the tooth. The coated sample showed a slightly more intense signal for Cl, Mg, and C, but the difference was not enough to require coating samples.

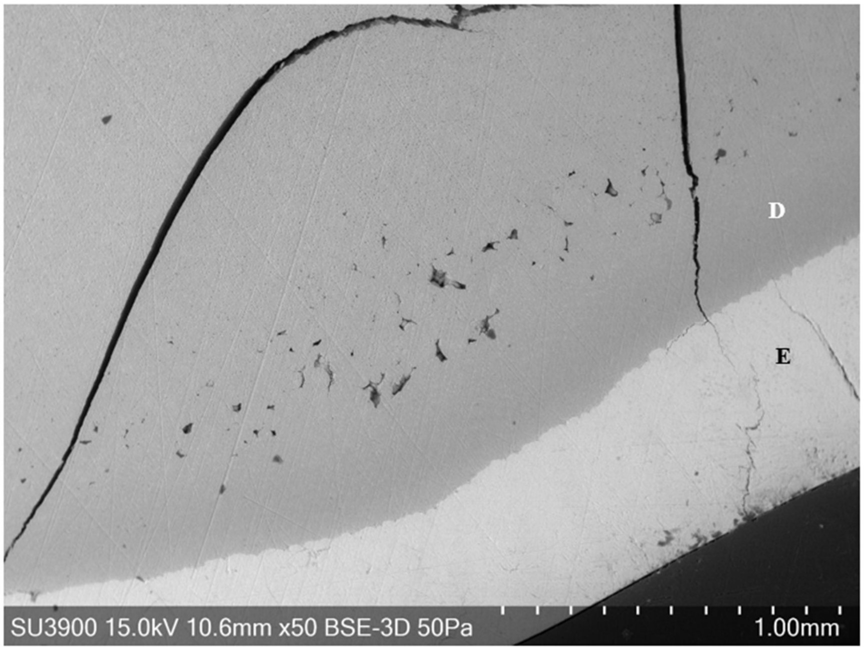

Figure G.1. View of a mineralization defect (IGD), occurring around 3.5 years of age, observed on the lingual side of a thin-section (mesio-distal cut) of the URI1 from individual E T1, magnification x50. E indicates enamel and D indicates dentin.

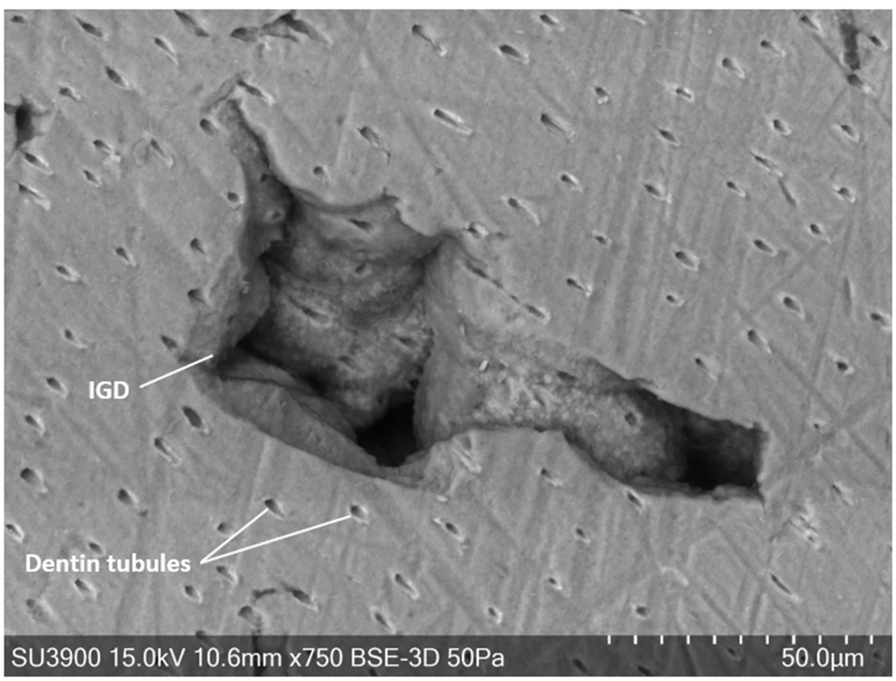

Figure G.2. View of a mineralization defect (IGD), occurring around 3.5 years of age, observed on the lingual side of a thin-section (mesio-distal cut) of the URI1 from individual E T1, magnification x750.

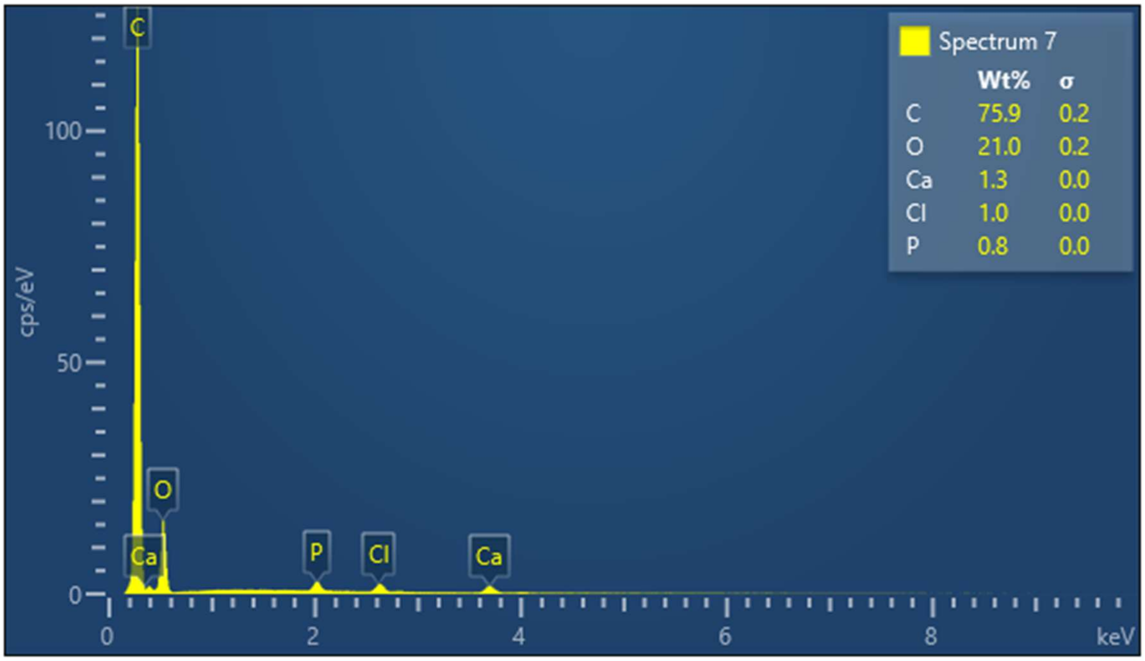

Figure G.3. Point analysis done through BSE-SEM of the epoxy surrounding the thin-section of LLI1 from E T7.

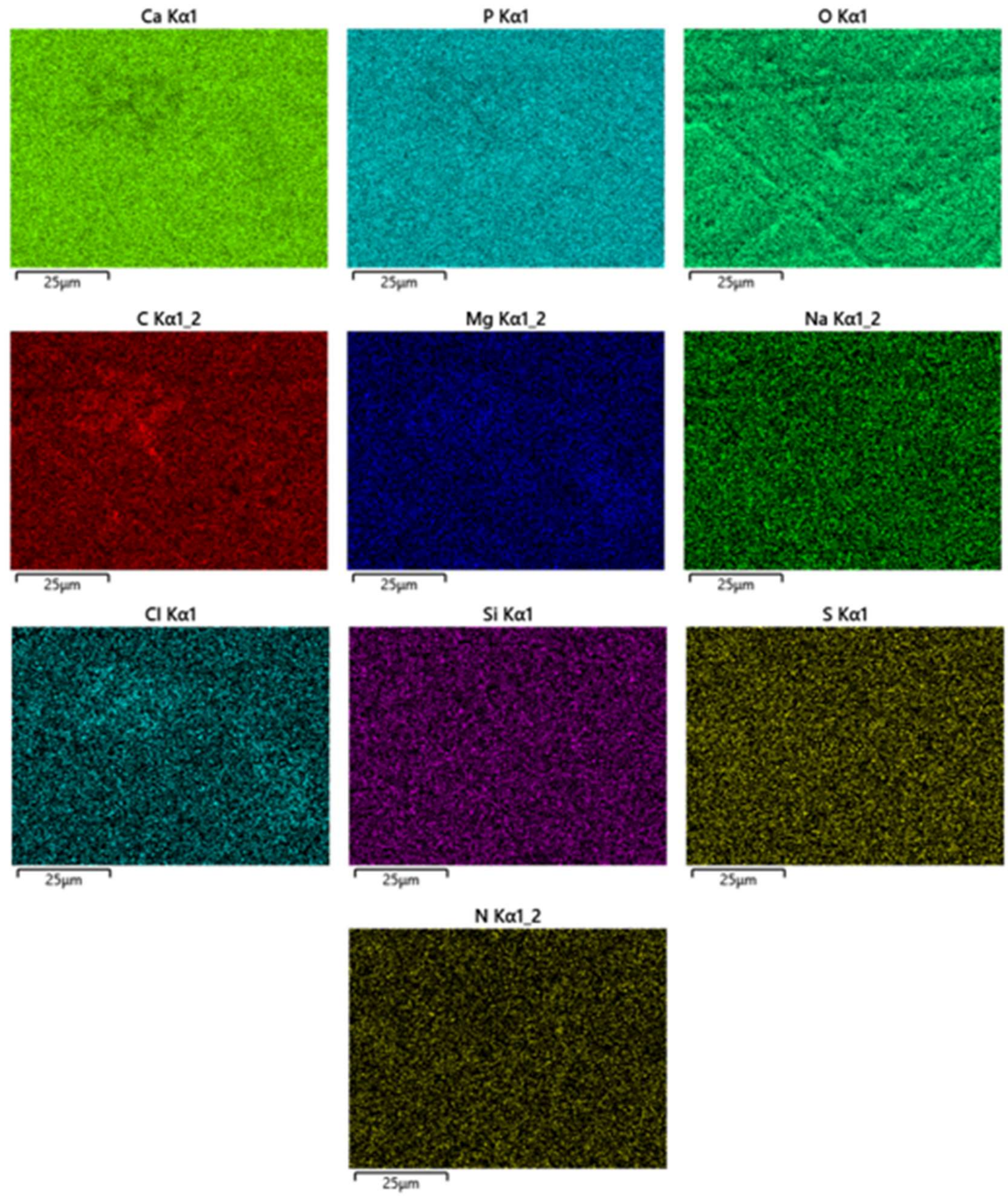

Figure G.4. Mapping element analysis done through BSE-SEM of a development defect (IGD) on the thin-section of the LLI1 from E T7 (should be viewed in color).

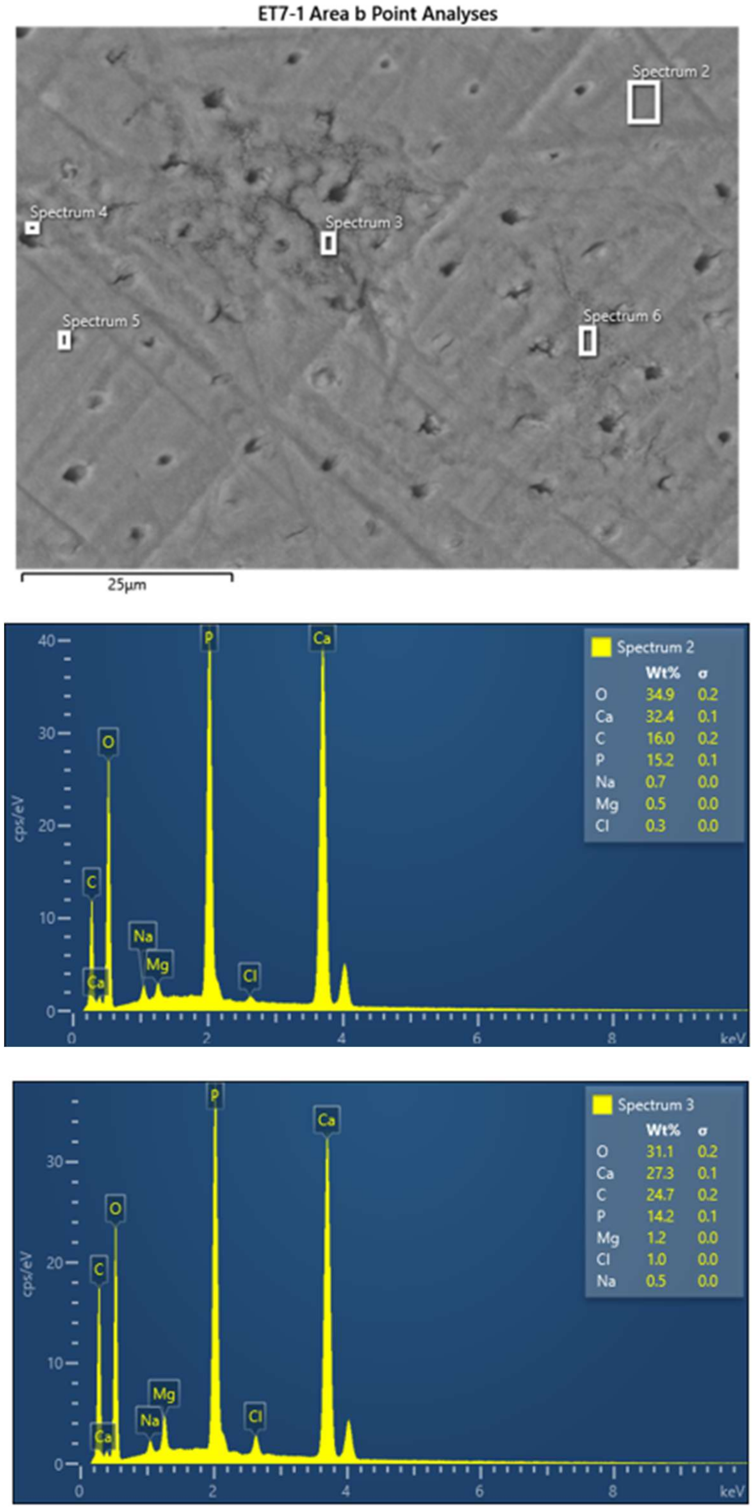

Figure G.5. Point analysis done through BSE-SEM of the developmental defect in dentin (IGD) in the thin-section of LLI1 from E T7.

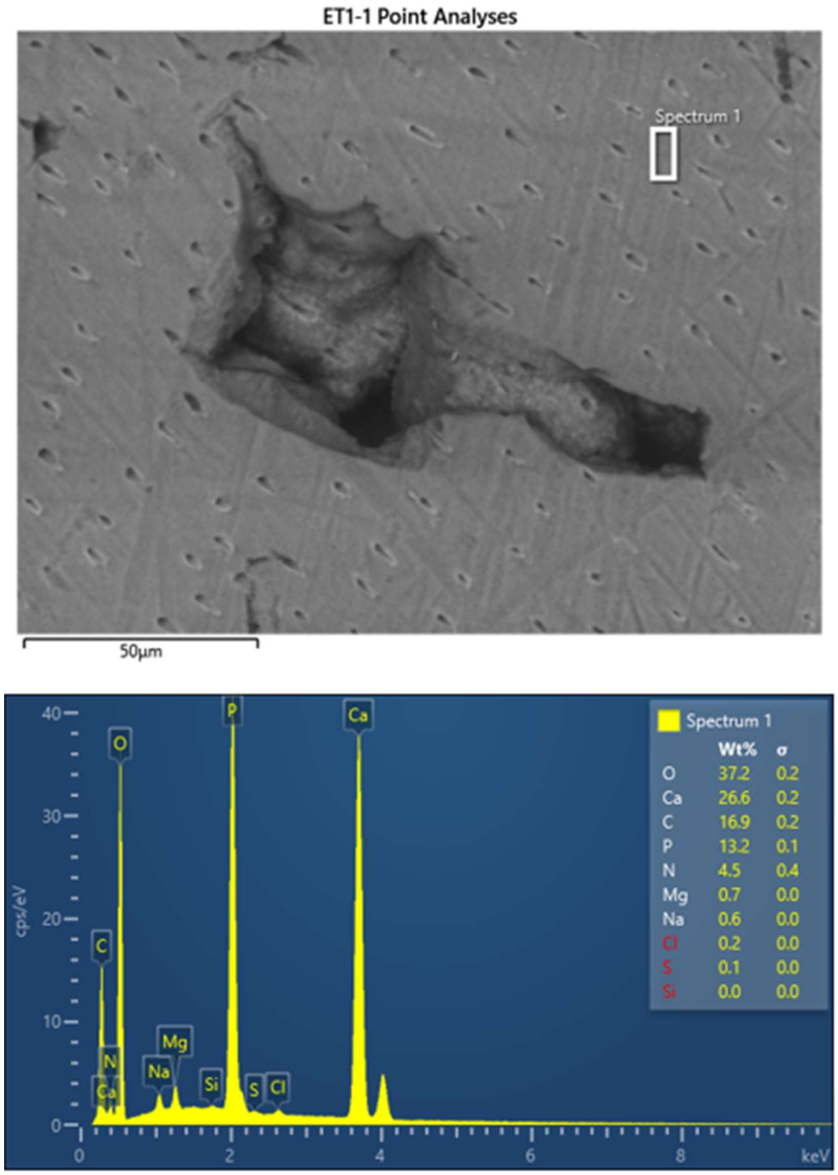

Figure G.6. Point analysis done through BSE-SEM of the dentin surrounding the developmental defect (IGD) in the thin-section of URI1 from E TI.

Appendix H. Osteobiographies in Farfán

Table H.1. Profiles, dental health, and other pathological lesions of primary burials individuals from Farfán (N=68 individuals) based on the osteobiographies from Dr Nelson (Nelson, pers. comm. 2020).

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	LEH	# LEH	Age LEH (years)	# Carious Lesions	Comments
G T3a	G	F	MA	-	29	3	0	2	1	Y	1	-	2	Enamel defects present; Lumbar osteophytes
G T3b	G	U	NEO	-	3	-	-	-	-	-	-	-	-	Endocranial periostitis; Femoral and humeral long bone shafts indicate possible meningitis
G T4	G	F	MA	152.8	27	0	0	1	2	Y	1	3.5-4.0	0	Mild crowding lower anterior; Chips on LRM3, LRM1, LRP4 , LRM2, ULM2, URM3, URM2, URM2 , URC , ULM1 , ULM2; M3 agenesis
G T5	G	U	IN	-	-	0	0	0	-	N	0	-	-	Cranium is fragmented; all teeth present
G T6	G	U	CH	-	20	-	-	-	-	N	0	-	-	Supernumerary lower left premolar
G T11	G	F	OA	145.9	30	2	1	4	2	N	0	-	1	Supernumerary tooth loss looking like a premolar at LLM1 ; Developmental dysplasia of the hip, cerebral palsy; Periodontal disease.
G T12	G	U	NEO	-	-	-	-	-	-	-	-	-	-	-
I10 T1	I	U	CH	-	10	0	0	0	NA	Y	3	0.5-1.0	5	LL/RM1 and URM1 forming in crypt; Slight alveolar bone loss on maxilla; urm1/2 and uxix loose; Upper left alveolar bone absent

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	LEH	# LEH	Age LEH (years)	# Carious Lesions	Comments
I10 T2	I	F	MA	149.9	25	5	2	1	1	Y	6	3.0-3.5; 3.5-4.0;	4	Permanent canine in empty socket XLI1 and XLI2; chip on URM1, buccal defect on LLP3
I10 T3	I	U	CH	-	16	0	0	1	0	N	0	-	0	All m1 in crypt, both lxm2 crown forming
I3 T1a	I	M	MA	157.4	17	13	5	5	1	Y	5	2.5-3.0; 3.0-3.5	2	LRM1 has root exposed; Hypercementosis on ULM2 and ULM3: Advanced or complete alveolar resorption on mandibular molars; Calculus obscures LEH on ULP4; Abscess unsure on right maxilla due to postmortem damages; Chip on ULC; No crown on LLP4
I3 T1b	I	U	IN	-	-	-	-	-	-	-	-	-	-	No teeth info
J14 T1	J	M	OA	155.5	22	8	7	5	1	-	-	-	2	Moderate alveolar loss on anterior regions but severe in posterior regions; Teeth too worn to distinguish if LEH; Chips on URM1, URP4, URP4, URI2, ULI2
J14 T2	J	F	YA	144.7	31	1	2	2	3	Y	1	4.0-4.5	5	Mild alveolar bone loss; Large hole ULM2; Chips on LRI2, URI2
J14 T3	J	U	IN	-	-	-	-	-	-	-	-	-	-	No teeth info
J25 T1	J	M	YA	158.3	5	1	0	-	-	Y	6	2.0-2.5; 3.0-3.5; 3.5-4.0; 4.5-5.0	4	Moderate alveolar bone loss on maxilla, slight on mandible; Left side destroyed; Chips on LLI1, LRM1, URM1; Fragment of URM1, URP4, URP3, URC, URI2, LRM3, LRM2, LRM1, LRP4, LRP3, LRC, LRI2, LRI1, LLI1; Schmorl's Nodes

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	EH	# Episodes EH	Age EH (years)	# Carious Lesions	Comments
J25 T2	J	U	NEO	-	12	0	0	0	0	N	0	-	0	Nothing observable, very fragmented
J25 T3	J	U	CH	-	20	-	0	1	0	N	0	-	0	Some anterior crowding; Destruction of the hip joint (osteomyelitis or bone infection)
J25 T4	J	U	IN	-	3	0	0	0	0	N	0	-	0	LRM1 and LLM1 forming; No maxilla; Infectious disease present
J25 T5	J	U	IN	-	9	0	0	-	1	N	0	-	1	Moderate to severe alveolar bone loss; Enamel pearls on ulm2; Periodontal disease
J25 T6	J	M	MA	158.1	32	0	14	4	1	-	-	-	3	Enamel very brittle; Alveolar resorption; Crowns destroyed on URM2, ULP4, and LRM1; Chips on LRM3, LLC, and LLM3; Probable infectious osteomyelitis of the spine
J25 T7	J	F	MA	151.8	19	13	0	4	1	Y	3	4.0-4.5; 4.5-5.0	3	Moderate alveolar resorption where teeth removed on maxilla and complete on mandibular; ULM2 has hypercementosis on roots; Antemortem chip on URI2, ULI1, ULI2
J25 T8	J	F	YA	144.6	32	0	1	2	0	Y	4	3.0-3.5; 3.5-4.0; 4.0-4.5;	7	Gap of 3.88 mm at alveolar margin; ULM3 tilted buccally and distally
J25 T9	J	F	YA	146.0	32	0	0	1	1	Y	8	1.5-2.0; 2.0-2.5; 2.5-3.0; 3.0-3.5; 4.0-4.5; 4.5-5.0	0	Buccal enamel defects (creamy white) on LRM2 and LLM2; Chips on LLM2, ULM1; Slight crowding on anterior teeth
J26 T1a	J	U	CH	-	0	0	0	0	0	N	0	-	0	-

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	EH	# Episodes EH	Age EH (years)	# Carious Lesions	Comments
J26 T1b	J	U	JUV	-	8	0	0	1	0	Y	5	1.5-2.0; 3.0-3.5; 3.5-4.0; 4.0-4.5; 4.5-5.0	2	Buccal defects on llm2, llm3, urm1; Infectious lesions on bone
J26 T2	J	U	NEO	-	-	-	-	-	-	-	-	-	-	No teeth info
J26 T3	J	U	JUV	-	14	0	1	0	1	Y	2	2.0-2.5; 2.5-3.0	6	Severe alveolar resorption at ulm1, ulm2 and urm2; M2s forming; lower left molars destroyed; Periodontal disease
JE19 T1	J	F	OA	149.4	28	3	3	2	1	Y	5	2.0-2.5; 2.5-3.0; 3.0-3.5; 3.5-4.0; 4.0-4.5	6	LRP3 to LRM3 are broken off; Irregular occlusal profile; Hypercementosis on M3s; Alveolar resorption at ULM3; Deep shovelling on ULI1; Active alveolar remodelling on maxilla
C T11A	HBP	F	OA	140.3	25	7	2	5	3	N	0	-	3	Crown and root mostly resorbed on URP4; Hole to root URM1; Crowns broken on ULP3 and URM1; Chips on ULC and ULM1; Alveolar bones completely resorbed on mandible; 2.5 cm gap between LRI1 and LLI1; Large defects on incisors looking like labial shovels; Extensive periodontal loss
C T11B	HBP	F	OA	140.8	32	0	3	4	1	N	0	-	0	Alignment error of URM3 and ULM1; Crown destroyed on URM1; Large labial defects on URI1 and ULI1; Unusual wear pattern on LXI1s associated with holding something with her front teeth; Several chips

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	EH	# Episodes EH	Age EH (years)	# Carious Lesions	Comments
C T11C	HBP	F	AD	146.8	32	0	1	1	-	Y	3	2.5-3.0; 3.0-3.5; 3.5-4.0	2	Slight shovels on URI1 and ULI1; M3s erupting; Prominent lingual cusps on URP3 and ULP3; Abscess at ULI2 consistent with an anatomical formation known as "dens en dente"; bowed fibula might indicate rickets
C T11D	HBP	F	OA	139.3	30	2	4	4	2	Y	3	3.0-3.5; 3.5-4.0; 4.0-4.5	4	Crowding upper anterior; Crown almost gone on URM1; Buccal tilt on URM3 and ULM3; Alveolus gone to root tips LRM1; Roots only LLP4 and LRM2; Retreating alveolus on LLM3 and LLM1; Distal root hanging on ULM1; Major alveolar resorption on ULM2; Periodontal disease; Alveolar resorption on LRM1
C T2A	HBP	F	YA	142.0	28	2	0	3	2	Y	4	2.5-3.0; 3.0-3.5; 3.5-4.0; 4.0-4.5	1	Upper M3's have enamel defects similar to pearls on the occlusal surfaces; Chip on ULM1; Roots fragment on LRM2 and LRM1 with crown gone and alveolar resorption; Lingual tilt on LRM3, LLM3; Buccal tilt on ULM3; Mild anterior crowding
C T2B	HBP	F	AD	-	27	0	0	1	0	Y	2	3.0-3.5; 3.5-4.0	0	Possible calcification in left canine; Slight crowding on anterior; Discoloration hypocalcification on almost all teeth; No evidence of periodontal disease

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	EH	# Episodes EH	Age EH (years)	# Carious Lesions	Comments
C T2C	HBP	U	AD	-	29	0	0	0	0	Y	1	4.0-4.5	0	EH on LLC could be a defect; Occlusal enamel defect on LLP4; M3's erupting; Chip on LRM1; Pit surrounded by enamel ring on URI2; Deep shovelling on upper anterior
C T2D	HBP	U	JUV	-	26	0	0	5	1	Y	3	3.0-3.5; 3.5-4.0; 4.0-4.5	5	Various vertical defects; M2s forming; Defect on URI1; Chip on ulm2
C T2E	HBP	U	JUV	-	14	0	0	5	0	Y	1	4.0-4.5	0	URI2 erupting
C T2F	HBP	U	JUV	-	8	0	0	2	-	Y	1	1.5-2.0	1	Fragmentary maxilla and mandible; Minor abrasion on all deciduous teeth present; Fluorosis-type hypocalcification in the incisor areas; Variety of small possible chips
CH 1a	HBP	M	MA	165.7	7	1	0	4	2	N	0	-	0	Only mandible available; Two enamel chips on LRM3 and LLM2; Red staining on occlusal surface; LLM3 missing agenesis; Alveolar resorption throughout the mandible; Osteoporosis
CH 1b	HBP	F	YA	151.1	22	1	0	2	2	Y	2	2.5-3.0; 3.0-3.5	2	Few enamel chips; Periodontal disease; Impacted LXM3s; LRI2 only has broken root; UXM3s missing from agenesis; Infected alveolar bone on maxilla; LRM3 tilted medially; Interproximal wear facet with LRM2; Double shovel on ULI1; Alveolar resorption throughout, severe on LLM3

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	EH	# Episodes EH	Age EH (years)	# Carious Lesions	Comments
CH 1c	HBP	F	MA	142.9	2	1	5	5	-	Y	1	3.5-4.0	0	No mandible; Teeth worn to root; Mild alveolar resorption on maxilla; Hypercementosis on ULC root; Maxilla broken post M2's; Periodontal disease
CH 1d	HBP	U	CH	-	-	-	-	-	-	-	-	-	-	-
CH 1e	HBP	U	CH	-	-	-	-	-	-	-	-	-	-	-
CH 1f	HBP	U	CH	-	-	-	-	-	-	-	-	-	-	-
CH 1g	HBP	U	JUV	-	4	0	0	-	-	-	-	-	0	Minor enamel abnormalities visible on the alveolar third of LRM1; Moderate abrasion on all deciduous teeth; Polishing spots on permanent first molars
E T1	HBP	F	AD	151.9	32	0	0	1	1	Y	2	3.0-3.5; 4.5-5.0	3	Lingual tilt on lower M3s; Mild crowding; Shovels on URI2, URI1, ULI1, ULI2; Chips on URP4, ULI2, ULP4, and ULM1; Vertical enamel defects on upper incisors; Porous bone around M3s (possibly infection); Severe abrasion on all teeth except 3Ms
E T4	HBP	F	AD	143.7	32	0	0	1	2	Y	1	2.5-3.0	3	Vertical labial enamel defects on upper anterior; No crowding; Tilt of URM3 and ULM3; Minor hypocalcification on some teeth; Extensive staining so not all teeth carefully examined; Slight periodontal infection

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	EH	# Episodes EH	Age EH (years)	# Carious Lesions	Comments
E T5	HBP	F	MA	149.7	31	1	4	4	1	Y	2	4.0-4.5; 4.5-5.0	6	Complete alveolar resorption on LLI1; Chips on LRM1, LLM1, URP3, ULP4, ULM1; Gapping hole due to breaks in ULM2 and URM1; Rotation of ULI1 and LLI2; 3.5 mm gap between UXI1s; Extensive periodontal disease around abscesses; No calcified deposits but plaque
E T6	HBP	F?	JUV	-	28	0	0	1	1	N	0	-	0	Little wear on m1s; M3s not erupted; Buccal defects on LRM2, LRM1, LLM1, and LLM2; Evidence of scurvy
E T7	HBP	F	OA	151.5	31	1	1	5	0	Y	3	3.0-3.5; 3.5-4.0; 4.0-4.5	5	Deep shoved hole on URI2 and ULI2; Chips on URM2, URI1, ULI2, ULM2, LLM3, and URM2; Mild crowding on lower anterior; Alveolus resorbed at URP3; Gaps of 6.5 mm between URP4 and URP3, 2.0 mm between URC and URI2, 3.0 mm between ULI2 and ULC, 3.0 mm between ULP3 and ULP4; Slight periodontal; URC protruded laterally and erupt above URM1, oriented buccal side up; ULC is part of tooth row but has erupted between ULP3 and ULP4
E T9	HBP	F?	JUV	-	23	2	2	4	3	Y	3	1.5-2.0; 2.5-3.0; 3.0-3.5	1	M2s all in crypts; Alveolus for ULP3 and ULP4 completely resorbed; lrc rotated; No crown on llm2; Active remodelling at lrm1 and lrm2; Evidence of scurvy

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	EH	# Episodes EH	Age EH (years)	# Carious Lesions	Comments
E T10	HBP	F?	JUV	-	23	0	0	1	-	N	0	-	2	Buccal defect on LRM1; All M2s in crypt; ULI2 rotated; Permanent premolar at alveolar margin for ULP3; Evidence of scurvy
N T1a	HBP	F	AD	141.0	30	0	0	1	1	Y	6	1.5-2.0; 2.0-2.5; 2.5-3.0; 3.0-3.5; 3.5-4.0; 5.0-5.5	6	No evidence of LXM3's; Tips nubbin on URC, ULC, and ULI2; Chips on URM2 and ULM1; Deep shovels on upper anterior
N T1b	HBP	F	OA	139.2	31	0	0	4	1	Y	1	3.5-4.0	1	Silver stains on left side; Red stains on anterior teeth; LLM3 impacted; Chips on LRM2, LRM1, LLP4, LLM2, URP4, and ULM2; ULI1 and ULP3 rotated; Tuberculosis lesions; Lesion consistent with temporomandibular joint syndrome
N T1c	HBP	F	IN	-	-	-	-	-	-	-	-	-	-	No info on teeth; Extensive woven subperiosteal bone probably associated a blood borne systemic infection
I T1	VI	U	CH	-	8	0	0	0	0	N	0	-	0	Abrasion on anterior teeth; lli1 fractured
I T2	VI	U	IN	-	-	-	-	-	-	-	-	-	-	No info on teeth; Bowed long bones (rickets?)
I T3	VI	M	AD	157.7	32	0	0	1	1	N	0	-	5	Possible defects on LLM2 and LRM2; Incisors not shovelled; Periodontal disease
I T4	VI	U	CH	-	-	-	-	-	-	-	-	-	-	No info on teeth
II T1	VI	F	MA	144.7	30	2	0	3	0	Y	1	4.5-5.0	0	Crowns missing at LRP2, LRI2, LRI1, LLI1, LLI2, URC, and URI1; Crowding on ULI1, ULI2, and ULC

ID	Cemetery	Sex	Age	Stature (cm)	# Teeth	# AMTL	# Abscesses	Wear Level	Level Calculus	EH	# Episo des EH	Age EH (years)	# Carious Lesions	Comments
II T2	VI	F	OA	142.6	-	-	-	4	0	-	-	-	-	Some teeth missing on the maxilla; No details available
II T3	VI	M	OA	157.7	27	0	0	5	-	N	0	-	0	Most teeth now represented only by roots
II T4	VI	M	MA	157.7	29	3	-	5	-	-	-	-	0	Very fragmented; X-ray shows multiple lesions that could be multiple myeloma (cancer affecting blood plasma cells) or metastatic carcinoma
III T1	VI	F	OA	146.0	19	13	0	4	0	-	-	-	0	Crown fragmentary; Impossible to distinguish EH due to preservation; Mandible well resorbed after tooth loss; Vertebral collapsed
III T2	VI	M	MA	155.7	21	5	0	1	-	-	-	-	0	Alveolar resorption where antemortem loss; Supernumerous tooth between LLP1 and LLP2; Crowding on LRC, LRI2, and LLI2; Vertebral collapsed
III T3	VI	U	NEO	-	-	-	-	-	-	-	-	-	-	No info on teeth; Skeleton fragmentary

Sex: F= Female, M= Male, U= Undetermined; Age: NEO (neonate) between 0 and 0.9 year, IN (infant) between 1 and 2.9 years, CH (child) between 3 and 5.9 years, JUV (juvenile) between 6 and 11.9 years, AD (adolescent) between 12 and 19.9 years, YA (young adult) between 20 and 29 years, MA (mid adult) between 30 and 39 years, OA (old adult) more than 40 years; Dental Calculus: 0=None, 1=slight, 2=mild, 3=heavy. Wear Level: 0=None, 1= Slight, 2= Slight-moderate, 3=Moderate, 4= Moderate-advanced, 5= Advanced; NA= no information/ not available. For teeth identifications: the first letter refers to the upper (U), lower (L), or unknown (X); the second letter refers to right (R), or left (L); the third letter indicates tooth type whether incisor (I), canine (C), premolar (P), or molar (M); the digit indicates which tooth specifically based thus for one side, in an adult, will have I1, I2, C, P3, P4, M1, M2, M3; lower case letter indicates deciduous teeth. For Isotopic Data: Y indicates that isotopic information is available for this individual while N is not present; Grey lines: part of the sample.

Appendix I. Data Recording Checklist for Detailed Dental Analysis Using Multiple Methods

ID: ______________________________

Tooth analysed: __________________

Preservation state: Incomplete Poor Fair Good

Method: Macroscopy Micro-CT Histology

Dental Measurements:

Mesiodistal diameter (max. width of the tooth): _________________________________

Buccolingual diameter: ____

Crown height (occlusal surface to CEJ): ______________________________________

Total height: ____

Root damage associated with an abscess: Present Absent

Comments:

____
____
____
____

Wear Level:

None Slight Slight-moderate Moderate Moderate-advanced Advanced

None: Unworn, no dentine exposure
Slight: Moderate blunting, point of dentine exposure
Slight-moderate: At least one large dentine exposure, thick enamel rim
Moderate: Large dentine area exposed (approximately 50% of occlusal surface), enamel rim thick and complete
Moderate-advanced: Greater dentine exposure, enamel visible on the edge
Advanced: full dentin exposure, no enamel left on the crown or very little, loss of the crown

<u>Caries</u>: Present Absent

Type:

1. Occlusal
2. Interproximal
3. Buccal
4. Lingual
5. Cervical
6. Root
7. Large
8. Noncarious pulp exposure

Size: ____

<u>Enamel Hypoplasia</u>: Present Absent

Type of defect (based on Buikstra & Ubelaker, 1994):

Number: ____

Distance from CEJ: ____

Other comments:

____
____
____
____

<u>Dental Calculus</u>: Present Absent

Other observations:

____
____
____
____

<u>Hypercementosis</u>: Present Absent

Other observations:

____
____
____
____

<u>Interglobular Dentine:</u> Present Absent

Grade: 0 1 2 3

Table I.1. Scoring system for interglobular dentine (IGD) after Table 3 in D'Ortenzio *et al.* (2016:157).

Grade	Interglobular space	Description	Defect in dentine mineralisation
0	No IG space	Dentine homogeneous/ No IGD	Absent
1	Minimal IG space	IG spaces present but small < 25% relative to surrounding dentine	Mild
2	Moderate IG space	IG spaces larger and more numerous 25-50% relative to surrounding dentine	Moderate
3	Large IG space	IG spaces large, very numerous with a clear scalloped or bubbled appearance > 75% relative to surrounding dentine	Severe

<u>Number of episodes:</u> ______________________________

<u>Additional Comments/ Observations:</u>

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Appendix J. Parameters for Micro-CT Scans.

The filament of the micro-CT was changed resulting in a change in brightness in the scans. Thus, to keep the same brightness throughout the focused sample, the parameters for the micro-CT were adjusted based on a water calibrator. All scans were calibrated with air at 0 and water at 1,000.

Table J.1. Parameters for micro-CT scans of teeth based on scanning date.

ID	Molar	Date Scanned	Incisor	Date Scanned	Other	Date Scanned
C T11A	URM3	22-11-2020	URI1	29-11-2020	-	-
C T11B	URM3	22-11-2020	URI2	29-11-2020	-	-
C T11D	LRM2	22-11-2020	-	-	-	-
CH 1b	-	-	-	-	ULP4	13-11-2020
E T1	URM3	13-11-2020	URI1	06-12-2020	-	-
E T4	ULM3	27-11-2020	ULI1	28-11-2020	-	-
E T5	ULM3	23-11-2020	ULI1	03-12-2020	-	-
E T6	URM2	23-11-2020	URI1	03-12-2020	-	-
E T7	LLM3	23-11-2020	LLI1	29-11-2020	-	-
E T10	-	-	ULI1	27-11-2020	-	-
N T1a	URM3	27-11-2020	ULI1	28-11-2020	-	-
N T1b	LRM3	13-11-2020	-	-	-	-
G T3a	LLM1	21-02-2021	-	-	-	-
I10 T2	-	-	URI1	13-12-2020	-	-
I10 T3	-	-	lli1	13-12-2020	-	-
I3 T1a	LLM2	13-12-2020	-	-	-	-
J14 T2	-	-	-	-	URC	12-12-2020
J25 T2	-	-	uri2	12-12-2020	-	-
J25 T4	LLM1	20-02-2021	lli2	11-12-2020	-	-
J25 T6	LRM3	06-12-2020	-	-	-	-
J25 T8	ULM3	10-12-2020	ULI1	11-12-2020	-	-
J25 T9	LLM3	06-12-2020	-	-	-	-
J26 T1a	LLM1	21-02-2021	ULI1	12-12-2020	-	-
J26 T1b	URM1	10-12-2020	-	-	urm2	21-02-2021
JE19 T1	LLM1	10-12-2020	LLI1	11-12-2020	-	-
I T3	-	-	-	-	LLC	27-02-2021
II T1	LRM3	27-02-2021	-	-	-	-
II T2	LLMX	28-02-2021	-	-	-	-
II T4	LLM2	21-02-2021	-	-	-	-
III T1	ULM2	28-02-2021	-	-	-	-
III T2	LRM1	27-02-2021	-	-	-	-
III T3	xxm1	28-02-2021	-	-	-	-

Teeth scanned between 13-11-2020 and 03-12-2020: 82 kV, 150 µA.
Teeth scanned after 06-12-2020: 82 kV, 148 µA, 0.5 mm Al filter.
For teeth identifications: the first letter refers to the upper (U), lower (L), or unknown (X); the second letter refers to right (R), or left (L); the third letter indicates tooth type whether incisor (I), canine (C), premolar (P), or molar (M); the digit indicates which tooth specifically based thus for one side, in an adult, will have I1, I2, C, P3, P4, M1, M2, M3; lower case letter indicates deciduous teeth.

Appendix K. Lookup Tables in CT Scans.

The human eye can recognise thousands of different colors, but can only recognise about 30 shades of gray (Tjahjadi & Bowen, 1989). Thus, generating pseudocolor with lookup tables (LUTS) in Dragonfly 2020.2 can highlight characteristics during analysis that would not be easily observed in gray scale.

LUTs are predefined color maps that substitute a color for a particular grayscale intensity value for all pixels (Russ, 2016). They highlight subtle changes in grayscale value to improve the quality and perception of images. Red, Blue, and Green (RBG), or "Jet", is the preferred setting here to visualise medical images and improve the quality and perception of images (Selvapriya & Raghu, 2018).

Gray scales for many micro-CTs are not calibrated while clinical and pre-clinical CT scanners are. The latter use the Hounsfield Unit (HU) scale, where the air is assigned a value of -1,000 and distilled water a value of 0. Values ranges between -100 and +3,000 HU for teeth in clinical scans as seen in Table K.1.

Table K.1. Attenuation coefficient and Hounsfield Unit (HU) for dental material.

Razi *et al.*, 2014	NewTom VG		Soredex		Planmeca		Somatom CT			
material	mean	sd	mean	sd	mean	sd	mean	sd		
Teeth	616	373	610	353	633	341	685	407		
Watanabe *et al.*, 2012	micro-CT									
Material	mean HU ST	sd	DC 30	sd	DC 60	sd	DC 90	sd	DC 120	sd
Enamel	2869	569	2384	474	2382	565	2319	475	2294	475
Genisa *et al.*, 2018	CBCT									
Material	mean	sd								
Enamel	3721,2	123,9								
Dentin	2222,6	39,8								
Villa & Lynnerup, 2012	Clinical CT									
Material	median HU bog bodies	med HU cold-dry mummies	med hot-dry mummies	med HU forensic cases	med HU archaeological bones					
Teeth	-102	1291	2086	1954	2003					

On industrial micro-CT scans, the air is assigned a value of 0 and distilled water a value of 1000 (Ramsay, 2011). Greyscale values are projected based on those two anchors. Individual pixels can have grayscale intensities that vary from 0 (non-dense, black) to 65,535 (very dense matter; bright white) depending on their attenuation coefficients (the ability of a substance to block x-ray) on micro-CT. For the present study, the averages of the attenuation coefficient for different materials were established in a sample of ten teeth from Farfán (Table K.2). Overall, the pixel values for enamel range between 8,500 and 11,000; between 4,500 and 7,500 for dentin; and between 6,000 and 8,500 for cementum.

Table K.2. Attenuation coefficient in a sample of ten teeth from Farfán with 82 kV, 148 µA, 0.5 Al filter, Mo target on micro-CT.

Material	Mean	Sd
Water	1014	155
Air	-35	181
Enamel	9989	140
Dentin	6181	158
Cementum	7066	459
Enamel Pearl	8244	255
Pulp Stone	6618	281

To compare the different datasets, the imaging conditions and window/level settings need to be the same. For this study, Dragonfly's jet LUT and a window range from 1,000 to 10,000 were applied to highlight changes in density such as demineralisation from carious lesions and interglobular dentin (IGD) (example in Figure K.1).

Figure K.1. Images obtained through Dragonfly 2020.2. A) and B) represent the lower left second molar (LLM2) of individual I3 T1a from Cemetery I. C) and D) represent upper right first incisor (URI1) of individual ET10 from the *Huaca* Burial Platform. A) and C) are the pseudocolor images of the corresponding gray scales images seen in B) and D) respectively.

Appendix L. Slabs in Dragonfly 2020.2 on Two Incisors from Individual C T11B and E T1.

One of the main limitations of the comparison between techniques concerned the thickness of the slices compared. With histology, the thickness obtained is usually between 60-100 µm (FitzGerald, 2006) while with micro-CT scans, the thickness of the slice observed is equivalent to the voxel size, which in this case is 12.5 µm. With SEM the thickness of the visible slice is only 2-3 µm (Barker, pers. comm. 2021). Therefore, the comparison between the different methods might be affected by this difference in thickness especially with a defect such as interglobular dentin which is not distributed uniformly across the tooth. To acknowledge this difference, it is possible to increase the thickness of the slices seen on Dragonfly 2020.2 by creating a “slab”. A “slab” represents a stack of micro-CT slices with individual pixel values determined as the average intensity of lines projected through the volume. This function was applied on the scans of two teeth randomly chosen among the ones analysed histologically (URI1 from E T1 and URI2 from C T11B) to create a slab of 100-µm-thick of the view corresponding to the mesio-distal cut done through histology. As seen in Figure L.1, seven IGD defects could be recorded on a “slab” from the URI1 of E T1 as opposed to six IGD defects in a single slice with micro-CT scans, voxel size of 12.5 µm. In the URI2 from C T11B, one IGD defect was visible compared to two defects on the single slice from micro-CT scans, voxel size 12.5 µm (Figure L.2). Thus, using the “slab” function on Dragonfly 2020.2 can slightly increase the accuracy of the comparison with the histological view regarding the number of defects. It allows better visibility of the IGD defects compared with histological images; however it did not change the number of episodes detected. Since it did not add new information to the comparison, this step was not done on the analyses.

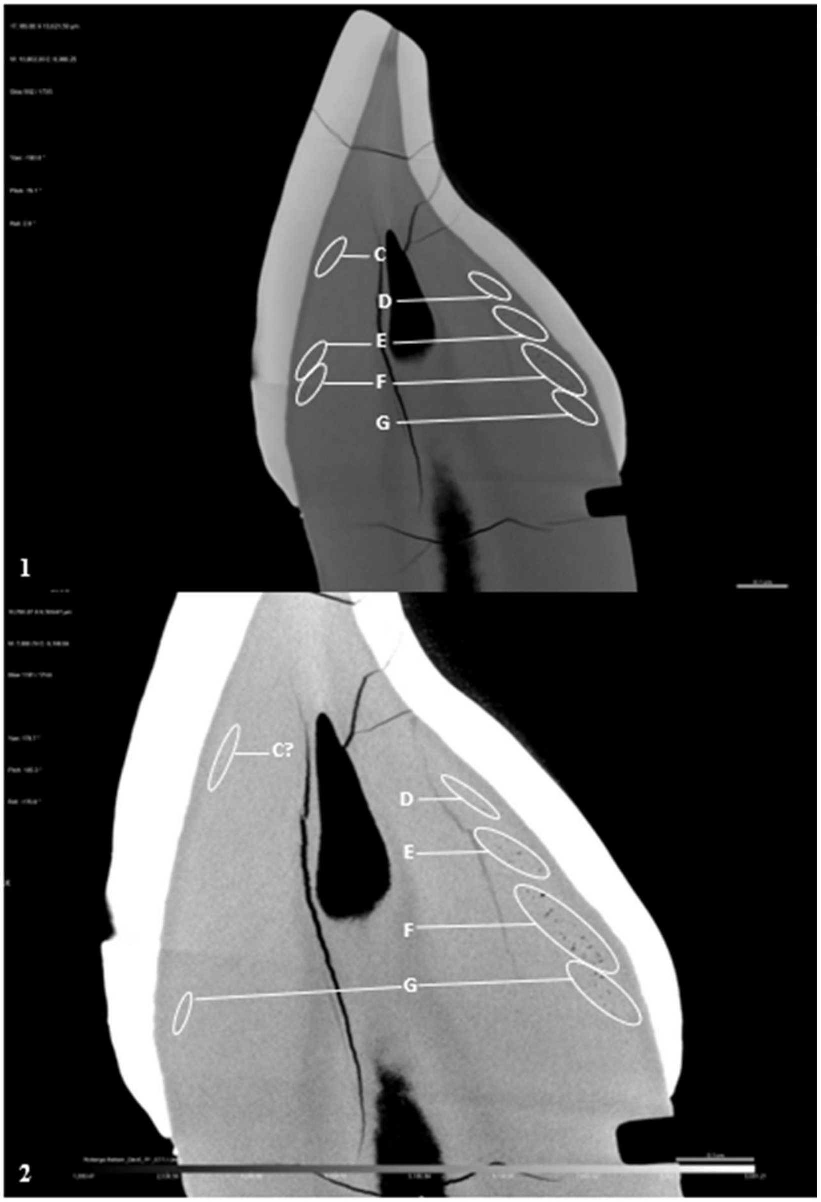

Figure L.1. Mesio-distal cut of the upper right first incisor from individual E T1, *Huaca* Burial Platform Cemetery. 1) 100-µm-thick slices (slab) obtained on Dragonfly 2020.2 after micro-CT scans, voxel size 12.5 µm. 2) image in micro-CT slice corresponding to thin-section, voxel size of 12.5 µm. The letters indicate different episodes of IGD.

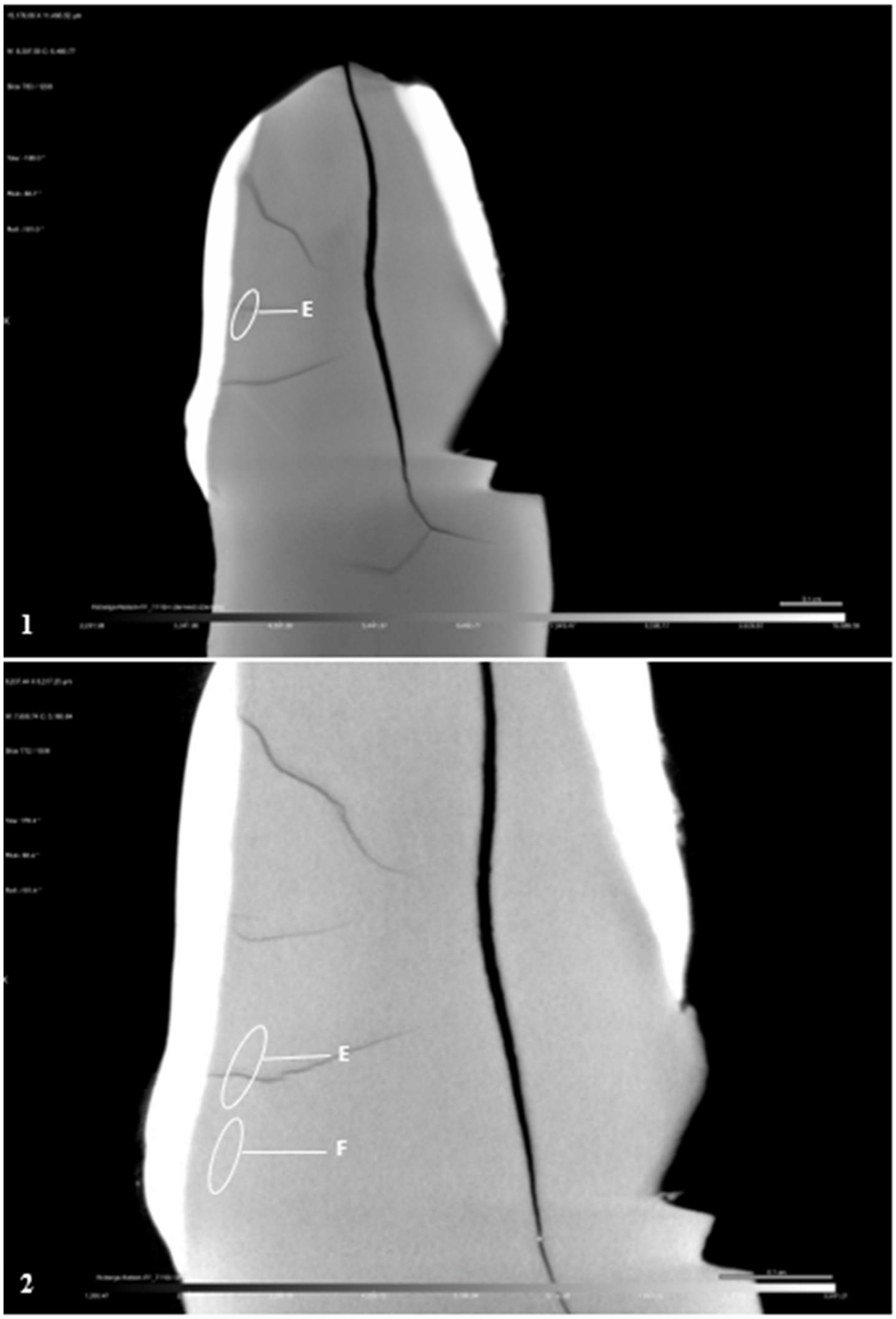

Figure L.2. Mesio-distal cut of the upper right second incisor from individual C T11B, *Huaca* Burial Platform Cemetery. 1) 100-µm-thick slice (slab) obtained on Dragonfly 2020.2 after micro-CT scans, voxel size 12.5 µm. 2) image in micro-CT slice corresponding to thin-section, voxel size of 12.5 µm. Letters indicate different episodes of IGD.

Appendix M. Correlative Tomography

Correlative tomography, also known as "multi-modal imaging", refers to the registration of 2D images such as histology onto 3D volumes created by tomography (CT, micro-CT, MRI) (Burnett *et al.*, 2014). Similar to correlative microscopy where the registration of images from two or more 2D imaging modalities or resolutions are merged into a single image, the objective is to take advantage of the properties of different imaging modalities such as colour, density, and resolution, to highlight specific features (Loussert Fonta & Humbel, 2015).

To contribute to current discussion of this novel approach joining 2D mapping with a 3D volume, correlative tomography was performed on six teeth from the analysed sample (URI1 from E T1, LLM1 from J26 T1a, URI1 from C T11A, URI1 from I10 T2, URI2 from C T11B, and LRM2 from C T11D). They were selected from the histological sample based on image quality and the amount of dental material left after sectioning. To register the histological images onto the CT volume, the remaining half of the six embedded teeth were rescanned with the micro-CT (82 kV, 148 µA, 1 frame per projection, with an 0.5 mm aluminium filter, molybdenum target, voxel size at 12.5 µm). The volumes obtained were registered with the full tooth scanned prior to histological analysis (Figure M.1) using "move" tool in Dragonfly 2020.2. This process allows the establishment of the angle of the thin-section to contribute to determination of the corresponding slice for correlative analyses. The location of the corresponding slice was approximated based on the measurements taken during the histological protocol and visual resemblance before registering the histological image onto the 3D volume using Dragonfly 2020.2 (Figures M.2 to M.4). Once the approximated location was found, a new dataset was derived from the current view to isolate the corresponding slice. The 2D image of the thin-section was downloaded in TIF format before being registered to the same position as the marked slices using the "Advanced Properties". The scale of the 2D image was adjusted as well as the position of the image using "move" tool. Finally, the 3D volume of the tooth was cropped one slice prior to the location of the thin-section so the 2D image could replace the corresponding slices.

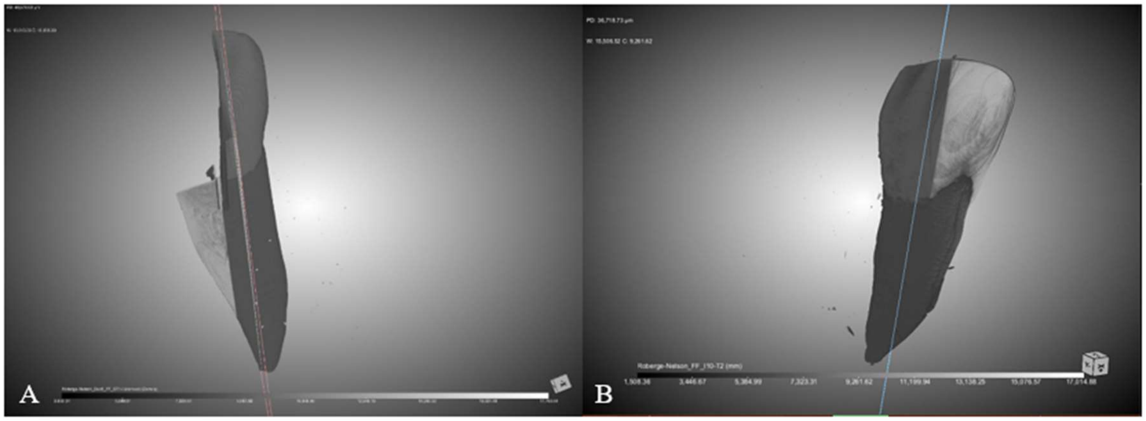

Figure M.1. Registration of the remaining embedded tooth volume onto the full tooth volume seen from the labial/buccal surface of A) URI1 from individual E T1; B) URI1 from individual I10 T2. White indicates the remaining embedded tooth volume while gray identify the full tooth volume. The perpendicular lines indicate the approximate location of 2D images of the thin-sections.

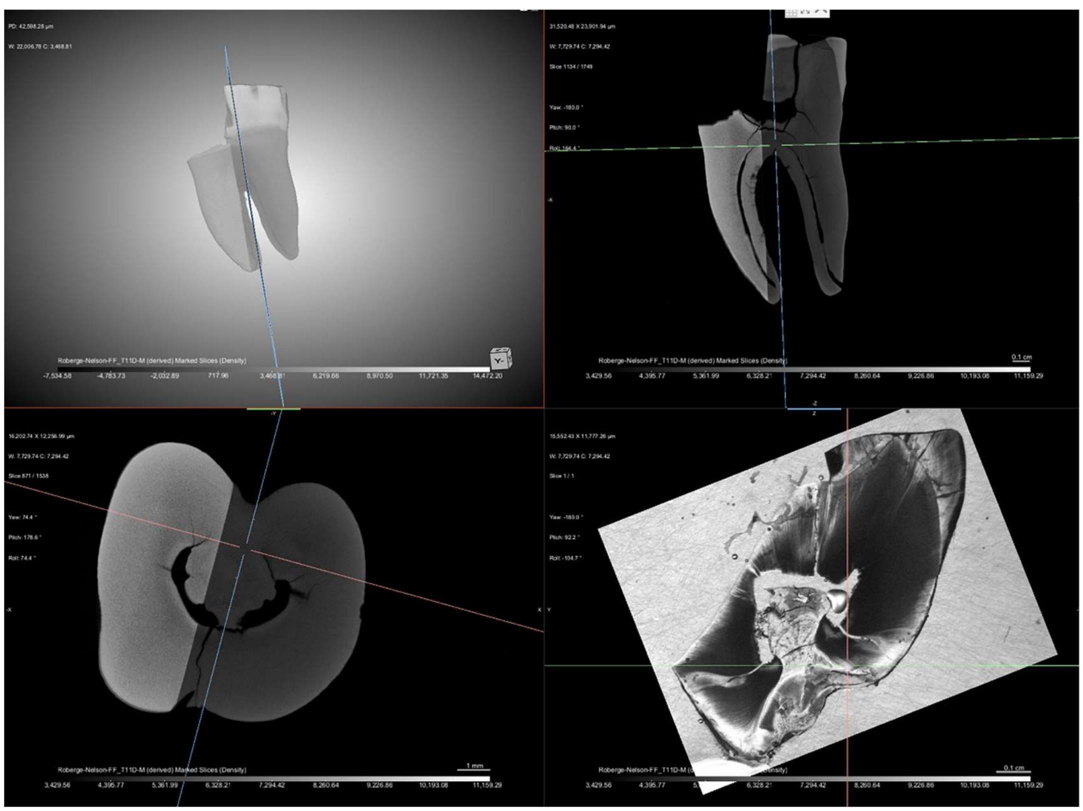

Figure M.2. Registration of the histological images to the micro-CT volumes of the LRM2 from individual C T11D at the corresponding location in Dragonfly 2020.2.

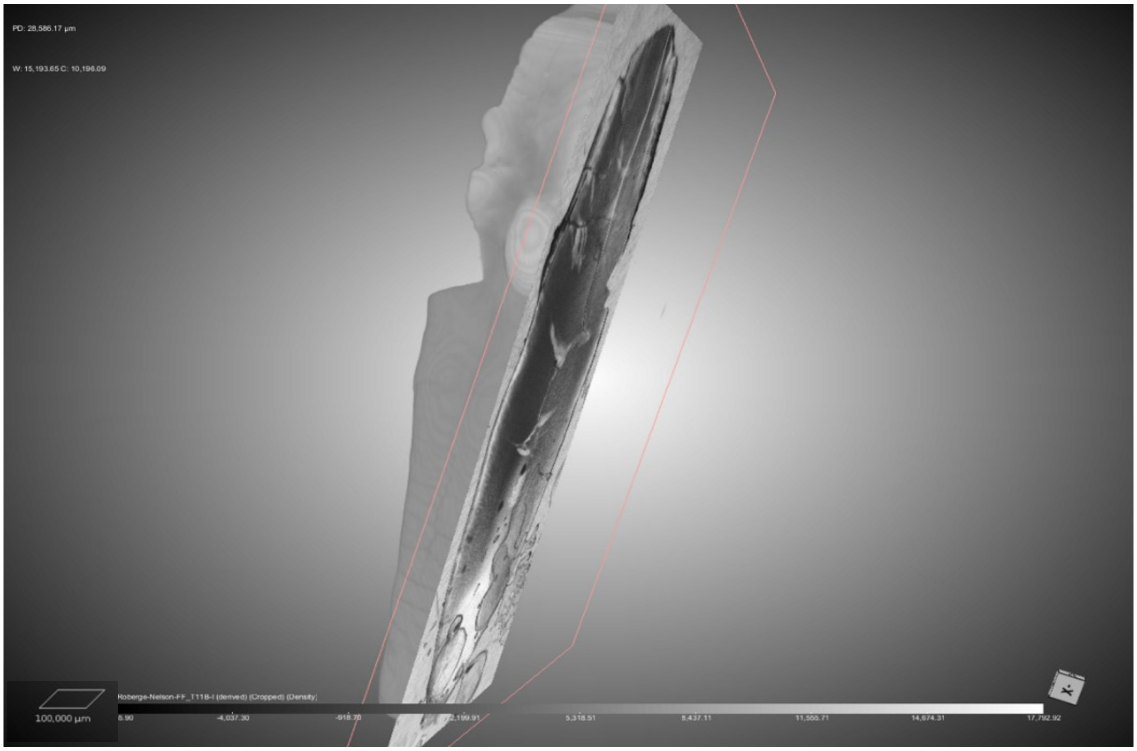

Figure M.3. Cut of the volume of the URI2 from individual C T11B at the location of the thin-section seen in the 3D view in Dragonfly 2020.2. View from the lingual side.

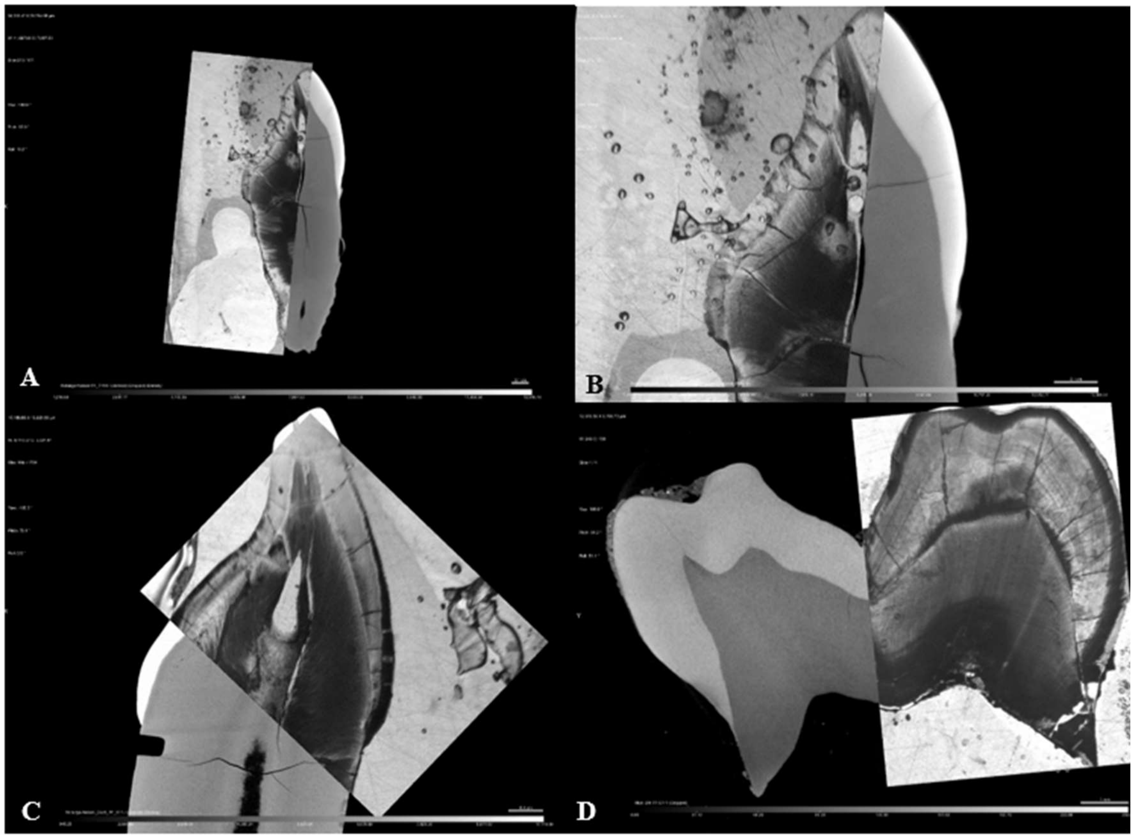

Figure M.4. Correlative tomography including visuals from the thin-section and views from the micro-CT at the corresponding slices. A) URI1 from individual C T11A; B) focus on crown of URI1 from individual C T11A; C) Crown from URI1 from individual E T1; D) LLM1 from individual J26 T1a.

Appendix N. Dental pathological lesions recorded through the detailed dental analysis

Table N.1. Compilation of the dental pathological lesions in the focused sample (N=45 teeth) recorded by the author using histology, macroscopy, and micro-CT.

ID	Tooth	Method	Wear Level	# Carious Lesions	Presence LEH	# LEH Episodes	Presence IGD	# IGD Episodes	Presence Dental Calculus	Presence Hypercementosis	Comments
CH 1b	ULP4	histology	1	0	0?	2	1	7	0	0	includes 1 IGD unilateral
		macroscopy	1	0	0?	2	NA	NA	1	0	-
		μCT	1	0	0?	1	1	2	1	0	slight calculus lingual
		μCT_5.5μm	1	0	0?	1	1	4	1	NA	2 larger perikymata lines, dentin defects near the root canal
E T1	URI1	histology	2	0	0	0	1	8	0	0	includes 2 unsure IGD
		macroscopy	1	0	0	0	NA	NA	0	0	-
		μCT	1	0	0	0	1	5	0	0	irregularities dentin-enamel junction
		μCT_6.75μm	1	0	0	0	1	NA	0	NA	-
	URM3	macroscopy	1	0	0	0	NA	NA	0	0	-
		μCT	1	0	1	1	1?	1	0	0	Random "patches" in dentin; potential occlusal caries (depth 1.4 mm, bowl-shaped, demineralisation), many cracks, 2 pits, enamel pearl
E T4	ULI1	macroscopy	NA	0	0	0	NA	NA	0	0	crown broken
		μCT	NA	0	0	0	0	0	1	0	crown broken
	ULM3	histology	0	0	1	2	1	4	0	0	Possible occlusal caries
		macroscopy	1	0	1	1	NA	NA	0	0	-
		μCT	1	1	1	2	0?	1	0	0	occlusal caries (cone-shaped, demineralisation, irregular edges,)

ID	Tooth	Method	Wear Level	# Carious Lesions	Presence LEH	# LEH Episodes	Presence IGD	# IGD Episodes	Presence Dental Calculus	Presence Hypercementosis	Comment
E T5	ULI1	macroscopy	2	0	0	0	NA	NA	0	0	-
		µCT	2	0	0	0	1	2	0	0	irregularities at the dentino-enamel junction buccal-lingual sides
	ULM3	macroscopy	3	2	0	0	NA	NA	0	0	occlusal and CEJ caries
		µCT	3	2	0	0	0	0	0	0	CEJ (demineralisation, 3.9 mm in diameters, irregular edges, bowl-shaped); occlusal? (irregular edges, cone shape, depth 0.6 mm)
E T6	URI1	macroscopy	0	0	0	0	NA	NA	0	0	brown staining
		µCT	0	0	0	0	1?	3	0	0	-
	URM2	macroscopy	NA	1	0	0	NA	NA	0	0	occlusal caries, brown staining
		µCT	NA	0	1?	0	1?	1	0	0	Possible occlusal caries (cone-shaped, smooth-edges, demineralisation, diameter 0.5 mm), one LEH?
E T7	LLI1	histology	4	0	0	0	1	6	1	0	includes 9 unsure IGD
		macroscopy	4	0	0	0	NA	NA	0	0	-
		µCT	3	0	0	0	1?	3	1	1	-
		µCT_5.5µm	3	0	0	0	1	3	1	NA	-
	LLM3	macroscopy	3	1	0	0	NA	NA	1	0	calculus buccal side
		µCT	3	1	0	0	1	1	1	0	occlusal caries (cone shape, irregular edges, 0.9 mm in diameter), pulp stone, enamel pearl
		µCT_6.75µm	NA	NA	0	0	1	1	1	0	bit of artefact on the buccal side since was cut to fit in the scanner view

ID	Tooth	Method	Wear Level	# Carious Lesions	Presence LEH	# LEH Episodes	Presence IGD	# IGD Episodes	Presence Dental Calculus	Presence Hypercementosis	Comment
E T10	URI1	histology	NA	0	1?	3	1	8	0	0	include 1 unsure IGD
		macroscopy	1	0	1	4	NA	NA	0	0	-
		µCT	0	0	1	4	1	4	0	0	Diagenetic crack in cross shape
		µCT_5.5µm	0	0	1	4	1	5	0	NA	-
C T11A	URI1	histology	2	0	0	0	1	6	1?	0	-
		macroscopy	2	0	0	0	NA	NA	0	0	-
		µCT	2	0	0	0	1?	3	0	0	-
	URM3	macroscopy	1	0	0	0	NA	NA	0	1	-
		µCT	1	0	0	0	1?	2	0	1	-
C T11B	URI2	histology	4	0	1	1	1	6	0	0	includes 7 unsure IGD
		macroscopy	1	0	1	2	NA	NA	0	0	crown with black substance on surface
		µCT	3	0	0	0	1?	2	0	0	-
	URM3	macroscopy	1	0	0	0	NA	NA	1	0	crown with black substance on surface
		µCT	2	0	1?	1	0	0	1	0	enamel pearl, pulp stone
C T11D	LRM2	histology	2	0	0	0	1	7	0	0	includes 2 unsure IGD
		macroscopy	3	2	0	0	NA	NA	0	0	occlusal caries
		µCT	3	0	0	0	1	1	0	0	wear facet distal side, enamel pearl, occlusal pits (irregular edges but straight shape and no demineralisation)
N T1a	ULI1	macroscopy	1	0	1	2	NA	NA	0	0	-
		µCT	1	0	0?	2	0?	2	0	0	very cracked, few “bubbles” defects in dentin but patches

ID	Tooth	Method	Wear Level	# Carious Lesions	Presence LEH	# LEH Episodes	Presence IGD	# IGD Episodes	Presence Dental Calculus	Presence Hypercementosis	Comment
N T1a	URM3	histology	1	1	0	0	1	3	0	0	one caries for sure the other probably not
		macroscopy	0	1	1?	1	NA	NA	0	0	-
		µCT	1	1	1	2	0?	2	0	0	2 occlusal caries (irregular, demineralisation, cone, >2mm depth but one might be pit 1,4 mm depth)
N T1b	LRM3	macroscopy	1	0	0	0	NA	NA	0	0	-
		µCT	1	0	0	0	0	0	0	0	enamel pearl
G T3a	LLM1	macroscopy	0	0	1	2	NA	NA	0	0	-
		µCT	0	1	1	2	1?	2	0	0	one LEH sure and possible 2; occlusal caries (Irregular edges, shape)
I10 T2	URI1	histology	3	0	1?	2	1	5	0	0	-
		macroscopy	3	0	1	2	NA	NA	0	0	-
		µCT	3	0	1	5	1	5	0	0	very cracked
		µCT_6.75µm	3	0	1	5	1	5	1	0	-
I10 T3	lli1	macroscopy	4	0	0	0	NA	NA	0	0	-
		µCT	4	0	0	0	0	0	0	0	diagenetic cracks in shape of cross through the tooth
I3 T1a	LLM2	macroscopy	2	1	0	0	NA	NA	0	1	occlusal caries
		µCT	1	1	0	0	1?	2	0	1	occlusal caries (irregular edges, cone-shaped, dentin affected, demineralisation, 1.7 mm in depth)
		µCT_6.75µm	1	1	N	0	1	3	1	1	-

ID	Tooth	Method	Wear Level	# Carious Lesions	Presence LEH	# LEH Episodes	Presence IGD	# IGD Episodes	Presence Dental Calculus	Presence Hypercementosis	Comment
J14 T2	URC	histology	1	0	1	2	1	8	0	1?	includes 8 unsure IGD
		macroscopy	3	0	0	0	NA	NA	0	0	-
		µCT	2	0	1?	2	1	7	0	1	IGD also in the root, LEH lines only on bucco-distal side
		µCT_5.5µm	2	0	1	2	1	5	0	NA	-
J25 T2	uri2	macroscopy	0	0	0	0	NA	NA	0	0	-
		µCT	0	0	0	0	0	0	0	0	tooth in development
J25 T4	lli2	macroscopy	1	0	0	0	NA	NA	0	0	-
		µCT	0	0	0	0	0	0	0	0	tooth in development, distinct cementum lines, diagenetic cross cracks
	LLM1	macroscopy	0	0	0	0	NA	NA	0	0	-
		µCT	0	0	0	0	1?	2	0	0	very cracked
J25 T6	LRM3	macroscopy	3	0	1?	1	NA	NA	1	0	-
		µCT	3	0	0	0	1?	2	1	0	very cracked, pattern in dentin
J25 T8	ULI1	histology	2	0	1?	1	1	9	0	0	-
		macroscopy	2	0	0	0	NA	NA	0	0	-
		µCT	2	0	1?	2	1	6	0	0	some dentin region more dense substance remaining toward apex maybe bone
		µCT_5.5µm	2	0	1	2	1	6	0	NA	-
	ULM3	macroscopy	0	2	1?	1	NA	NA	0	0	occlusal caries
		µCT	0	4	0	0	1?	1	0	0	enamel pearl, dentin destroyed under the crown, multiple occlusal pits, pulp stone, 4 occlusal caries (shapes larger at the bottom, irregular edges, enamel demineralisation)

ID	Tooth	Method	Wear Level	# Carious Lesions	Presence LEH	# LEH Episodes	Presence IGD	# IGD Episodes	Presence Dental Calculus	Presence Hypercementosis	Comment
J25 T9	LLM3	macroscopy	0	1	1	1	NA	NA	0	0	-
		µCT	0	1	1	1	1	2	0	0	enamel pearl; occlusal caries (shape, irregular edges, demineralisation dentin and enamel, 17 mm in depth), 3 potential caries (1.2 to 1.6 mm depth, slight enamel demineralisation) and numerous occlusal pits
J26 T1a	LLM1	histology	0	0	0	0	1	8	0	0	includes 2 potential IGD
		macroscopy	0	0	0	0	NA	NA	0	0	-
		µCT	0	3	1?	1	1	2	0	0	lingual caries (irregular edges, 1.5 mm in depth, shape, demineralisation); 2 occlusal caries (2.1 and 2.4 mm in depth, irregular edges, shape, demineralisation)
		µCT_6.75µm	0	3	0	0	1	2	0	NA	-
J26 T1a	ULI1	macroscopy	2	0	0	0	NA	NA	0	0	-
		µCT	2	0	0	0	0	0	0	0	lines along dentin tubules in the crown, apex rugous and curved toward buccal side,
J26 T1b	URM1	macroscopy	0	0	0	0	NA	NA	0	0	-
		µCT	4	2	0	0	0	0	1?	0	CEJ caries (reaches pulp, irregular edges, shape, demineralisation, 2.0 mm deep), occlusal caries (irregular edges, dentin demineralisation, shape, 1.5 mm deep)

ID	Tooth	Method	Wear Level	# Carious Lesions	Presence LEH	# LEH Episodes	Presence IGD	# IGD Episodes	Presence Dental Calculus	Presence Hypercementosis	Comment
J26 T1b	urm2	macroscopy	2	2	0	0	NA	NA	0	0	occlusal & interproximal caries, very slight buccal calculus
		μCT	2	2	0	0	0	0	NA	0	Pattern in dentin; occlusal caries (0.6 mm depth, bowl-shaped, irregular edges, dentin demineralisation); interproximal caries (irregular edges, bowl-shaped, dentin demineralisation, penetrates until root canal, 1.3 mm depth)
JE19 T1	LLI1	macroscopy	2	0	1	1	NA	NA	0	0	-
		μCT	2	0	1?	1	1?	2	0	0	Pulp stone
	LLM1	macroscopy	3	0	1	1	NA	NA	0	0	-
		μCT	3	0	1	1	1	3	0	1	enamel pearl, pulp stone
III T1	ULM2	macroscopy	4	0	0	0	NA	NA	0	1	-
		μCT	4	0	1?	0	1?	1	1	1	very cracked, many pits
III T2	LRM1	macroscopy	3	0	0	0	NA	NA	0	0	root fragmented
		μCT	3	0	0	0	1	2	0	0	root fragmented, very cracked, pulp stone
III T3	xxm1	macroscopy	0	0	0	0	NA	NA	0	0	-
		μCT	0	0	0	0	0	0	0	0	tooth in development
II T1	LRM3	macroscopy	4	0	1?	1	NA	NA	0	0	wear facet on mesial side, linguo-distal cusp chipped PM
		μCT	4	0	0	0	0	0	0	0	root fragmented, very cracked, incremental lines visible, enamel pearl, slight calculus, occlusal hole filled with dirt looks diagenetic

ID	Tooth	Method	Wear Level	# Carious Lesions	Presence LEH	# LEH Episodes	Presence IGD	# IGD Episodes	Presence Dental Calculus	Presence Hypercementosis	Comment
II T2	LLMX	macroscopy	4	0	0	0	NA	NA	0	0	root fragmented, mesial and distal wear facets
		µCT	4	1	0	0	0	0	0	1	1 pit, 1 occlusal caries (bowl-shape, irregular edges, reach dentin, enamel demineralisation, 1.8 mm in depth
II T4	LLM2	macroscopy	2	0	0	0	NA	NA	0	1	-
		µCT	2	0	0	0	0	0	0	1	poorly preserved, very cracked, dentin denser near pulp, 1 possible occlusal caries (smooth edges, dentin demineralisation, between 2 mm deep)
I T3	LLC	histology	0	0	0	0	1	11	0	0	includes 12 possible IGD (unilateral or really fainted especially on root)
		macroscopy	3	0	1	1	NA	NA	0	0	-
		µCT	3	0	N	0	1	8	0	0	-
		µCT_6.75µm	3	0	N	0	1	11	0	0	-

For teeth identifications: the first letter refers to the upper (U), lower (L), or unknown (X); the second letter refers to right (R), or left (L); the third letter indicates tooth type whether incisor (I), canine (C), premolar (P), or molar (M); the digit indicates which tooth specifically based thus for one side, in an adult, will have I1, I2, C, P3, P4, M1, M2, M3; lower case letter indicates deciduous teeth;
Wear Level: 0=None, 1= Slight, 2= Slight-moderate, 3=Moderate, 4= Moderate-advanced, 5= Advanced
Presence LEH/ IGD/ calculus/ hypercementosis: 0= Absent, 0? = Possible, 1?= Probable, 1= Present; NA= no information/ not available.
CEJ: cemento-enamel junction.

Curriculum Vitae

Name:	Émy Roberge
Post-secondary Education and Degrees:	Université de Sherbrooke Sherbrooke, Quebec, Canada 2015-2018 B.Sc.
	The University of Western Ontario London, Ontario, Canada 2019-2022 M.A.
Honours and Awards:	Faculty of Social Science Dean's Entrance Scholarship 2019
	Christine Nelson Graduate Award for Research in Archaeology and Bioarchaeology 2020
	Social Science and Humanities Research Council (SSHRC) Canada Graduate Scholarship 2020-2021
	Province of Ontario Graduate Scholarship (declined) 2020-2021
	Graduate Conference Travel Fund Award 2022
	Cockburn Student Prize Award – Best Student Poster Presentation 2022
Related Work Experience	Research Assistant Université de Sherbrooke 2019
	Teaching Assistant The University of Western Ontario 2019-2021

Publications:

Roberge, É., Nelson, A., & Waters-Rist, A. (2022, March 21-23). *High prevalence of interglobular dentin in an Inca population from Farfán (Peru) detected through*

correlative tomography. [Poster Presentation]. Palaeopathological Association (PPA) 49th Annual North American Meeting. Denver, Colorado, United States.

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