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Research article

The rachitic tooth: The use of radiographs as a screening technique

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ABSTRACT

This study investigates morphological changes in pulp chambers of living and archaeological individuals with past vitamin D deficiency. Living individuals (n = 29), four with detailed medical and dental records and three groups of archaeological individuals (n = 25) were radiographed; selected individuals were further evaluated histologically for the presence of incremental interglobular dentin (IIGD), indicative of deficiency (28 living; 17 archaeological). Measurements of pulp horns/chambers from radiographs were conducted to quantify morphological observations. One group had clear skeletal evidence of rickets from St. Matthew, Quebec (n = 1) and St. Jacques, France (n = 4); a second group had slight skeletal indicators from Bastion des Ursulines, Quebec (n = 6); and a third group lacked both skeletal and radiological evidence of deficiency from St. Antoine (n = 6) and Pointe-aux-Trembles (n = 4). Results showed archaeological individuals with clear and slight skeletal evidence of past deficiency displayed constricted or chair shaped pulp horns. Living individuals with deficiency exhibited similar pulp chamber morphology. Radiographic pulp horn/chamber measurements corroborated morphological findings and significant differences were found in pulp horn/chamber measurements between those with and without deficiency. Results suggest that radiograph assessment of teeth can be used as a screening technique to elucidate patterns of deficiency and select individuals for microCT or histological assessment.

1. Introduction

Vitamin D deficiency has emerged as a significant public health problem in many communities (Holick, 2006). Identifying the number of individuals who may have experienced vitamin D deficiency has significant potential to further our understanding of the range of factors that may have compromised the health of people in the past (Brickley et al., 2017, 2014). Identification of individuals who may have experienced past deficiency based on skeletal changes is difficult as few adults retain clear skeletal changes associated with rickets (Hess, 1930). Disturbances in metabolism are reflected in the microstructure of developing tooth dentin (Arana-Chavez and Massa, 2004; Foster et al., 2014) and one consequence of vitamin D deficiency is the presence of mineralisation defects in teeth. Recently it has been demonstrated that conditions that disrupt vitamin D, calcium, and phosphate pathways cause systemic mineralisation defects in teeth known as incremental interglobular dentin (IIGD) (D'Ortenzio et al., 2016), which is observed as clear bands of bubble-like spaces that follow incremental lines within the dentin matrix (Noyes and Thomas, 1921).

Clinical studies have demonstrated that vitamin D deficiency from nutritional and genetic causes produce morphological changes in dental structures that can be detected radiologically (e.g., Seow and Latham, 1986; McDonnell et al., 1997). We hypothesised that abnormal mineralisation related to vitamin D deficiency in childhood can be detected radographically in both living and archaeological individuals through quantifiable pulp chamber changes in permanent molars. Teeth from known living controls and three groups of archaeological individuals were radiographed to evaluate if there were measurable differences in the pulp horns and pulp chambers in those with deficiency; histological analysis confirmed the presence or absence of incremental interglobular dentin (IIGD). Radiographic imaging provides a useful screening tool, enabling an inexpensive non-destructive means of identifying individuals who experienced past episodes of deficiency. We focused on permanent dentition as dentin in these teeth provides a record from early childhood to young adulthood (dentin is present in the 1st permanent molar before birth and the 3rd permanent molar

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ends at ~18 years), permitting the development of a chronological profile of deficiency throughout an individual's early life.

1.1. Background

Dental pulp consists of connective tissue derived from mesenchyme (embryonic connective tissue) cells and is localised within the pulp chamber and root canals of the tooth (Avery, 2002:190). The pulp contains cells that provide nutritive, sensory, and defensive functions and permits preservation of vitality of the tooth. Dental pulp is divided into two components: 1) the odontoblasts, which are the cells responsible for the production and maintenance of predentin and dentin, and 2) blood vessels and nerves. Deciduous teeth are fast forming, as many secretory odontoblasts are active at one time (Dean, 2016); permanent teeth are slower to form as fewer odontoblasts are simultaneously active. Due to their greater size and slower rate of formation, it takes approximately 3–6 years for the completion of dentin in the crowns of each permanent tooth, during which time endocrine and nutritional factors can influence enamel and dentin formation (Schour and Massler, 1940; Hillson, 2002:123). Similar to the failure of osteoid mineralisation in bone (Foster et al., 2014), systemic factors, such as conditions causing rickets, can also influence the internal environment of the tooth leading to an absence of secondary dentin formation, producing distinguishable morphological changes to the pulp chamber.

Clinical studies have demonstrated that conditions that disrupt vitamin D, calcium, and phosphate pathways lead to the development of a number of abnormalities of the dentition and surrounding structures and these are listed in Table 1. Many of the dental abnormalities have a number of possible causes and so for this study pulp horn morphology was selected for investigation because changes in pulp horns are only caused by conditions linked to mineralisation defects (D'Ortenzio et al., 2016; Table 1).

2. Materials

Teeth from living individuals were collected to act as controls (n = 29). Four individuals provided medical histories and bitewing dental radiographs, (three supplied blood serum 25(OH)D levels), of those, three were diagnosed with previous vitamin D deficiency (KT1–KT3), and one had no previous deficiency (TT1). KT3 was diagnosed with osteopenia. Permanent molars from twenty-five other living individuals were collected for radiographic and histological analysis to provide a comparative sample (1st molars: n = 8; 2nd molars: n = 7; 3rd molars: n = 10) (HIREB ethics approval 2246). These individuals (n = 25) had no pre-existing medical condition, however they may have experienced asymptomatic nutritional vitamin D deficiency, therefore histological analysis was undertaken to check for IIGD. Table 2 presents all individuals in the study sample.

Radiological and/or histological data were also evaluated from three different groups of archaeological skeletons classified as 'slight' to 'marked' for deformities associated with previous vitamin D deficiency. Teeth with carious lesions and/or severe attrition were avoided where possible. Based on information in Table 1, when excluding individuals with carious lesions, there is a higher chance that individuals with previous episodes of deficiency may also be excluded. The three archeological groups consisted of individuals of European ancestry, therefore the criterion described in Brickley et al. (2010) were used. The first archaeological group from Saint Matthew, Quebec (n = 1) and Saint Jacques, France (n = 4) had severe skeletal deformity from healed rickets and histological evidence of at least one systemic episode of IIGD. Radiographs were conducted on the permanent molars of these individuals whose skeletal deformities were severe.

The site of Bastion des Ursulines, Quebec City, curated at the Canadian Museum of History, Ottawa, was chosen as the second group, because some individuals buried at this site and will have spent their childhoods in conditions that were conducive to the development of rickets. Cases of rickets have been reported from Euro-Quebecois cemeteries (e.g., Larocque, 1999; Morland and Ribot, 2010), and findings by D'Ortenzio et al. (2016) showed that some individuals in these communities experienced multiple episodes of deficiency. Macroscopic examination of bowing defects was conducted on 32 adults. Ten were chosen for further radiographic analysis of the dentition, six with skeletal indicators of a deficiency and four with none observed (Table 2).

To evaluate how many individuals, might have had deficiency and recovered, but may be missed when radiographic techniques are used, a third group was selected to investigate individuals who lacked clear skeletal and dental radiographic indicators of vitamin D deficiency. These individuals from Saint Antoine (n = 6), and Pointe-aux-Trembles (n = 4), Montreal were buried in Euro-Quebecois cemeteries (Table 2). Individuals were first screened for an absence of skeletal deformity indicating healed rickets, 10 were identified, and permanent molars were radiographed to confirm that no morphological changes were present in the pulp chambers. To establish how many cases of deficiency were missed using radiographs, histological analysis was conducted on this set of individuals.

3. Methods

3.1. Radiographs to observe pulp horn and pulp chamber morphology

To observe pulp horn and pulp chamber morphology, radiographic images were taken on living and archaeological permanent molars (n = 50) and bitewing radiographs supplied by dentists were analysed for four additional living individuals (Table 2). Four locations using two different types of radiological equipment were used for archaeological individuals (see Table 1 in Supplemental Data A for locations and specifications). Single permanent molars and/or mandibles and maxillae containing molars were placed on the imaging plate at a distance from the source ranging from 20 cm to 150 cm, depending on the radiograph set-up. Clinical radiographs were obtained in a standardised orientation (buccolingual direction) that clearly showed the pulp chambers. To ensure consistency and facilitate comparison with clinical cases, archaeological dentition was positioned as perpendicular to the X-ray beam as possible in order to obtain the intersection of the X-ray beam in a buccolingual orientation (Worth, 1963:3). The number of pulses (exposure time) was selected (~6–29 pulses). Images were then converted to TIFF files and saved to a database.

3.2. Blind test

To establish if other researchers could use the radiograph instructions produced for identification of individuals with a previous episode of vitamin D deficiency a blind test was conducted using radiographs of archaeological individuals with slight to marked skeletal indicators of vitamin D deficiency and presence or absence of IIGD was confirmed by histological analysis (Saint Matthew, Saint Jacques, Bastion des Ursulines). See Supplementary Data B for further information. Known clinical images were also used. Twelve radiographs of permanent molars from individuals with and without deficiency were examined by six participants. Participants were instructed to examine the pulp chamber, paying close attention to pulp horns that were normal (evenly matched); uneven (referred to as chair shaped); or constricted (narrow), that could indicate deficiency (see Fig. 1a–c). Participants answered yes: clear evidence of uneven or constricted pulp horns; no: appears normal and evenly matched; possible yes: could have morphological changes, but not definitive; or undecided: not sure. See Supplementary Data B, Table 3 for detailed instructions, results, and examples from the blind test.

To aid in determining potential ages of deficiency reflected by changes to the morphology of the pulp chamber, Table 3 displays the approximate timing of pulp chamber initiation in the three types of permanent molar. For pulp horn shape changes to arise, deficiency has

Table 1
Dental abnormalities of vitamin D deficiency observed radiographically.

Dental abnormality	Conditions that disrupt vitamin D, phosphate, calcium pathways	Source	Other causes of dental abnormality	Source
Enlarged pulp chambers*	Vitamin D-resistant hypophosphatemic rickets	Seow (1984); Seow and Latham (1986)	Odontogenesis imperfecta (rare hereditary disease)	Cahuana et al. (2005)
	Nutritional rickets	McDonnell et al. (1997); Davit-Béal et al. (2014)		
High, constricted (narrow) pulp horns extending into the dentin-enamel junction	Vitamin D-resistant hypophosphatemic rickets; Vitamin D-dependent rickets	Harris and Sullivan (1960); Hernández and Laguna (2013)	–	
	Nutritional rickets	McDonnell et al. (1997); Galhotra et al. (2015)		
Short roots	Vitamin D–dependent rickets	Zambrano et al. (2003); Souza et al. (2010, 2013)	Dentin dysplasia (rare genetic disease)	Arana-Chavez and Massa (2004)
Poorly defined lamina dura	Vitamin D-resistant hypophosphatemic rickets; Vitamin D–dependent rickets; Hyperparathyroidism	Zambrano et al. (2003); Pereira et al. (2004)	Dental abscess; Giant cell granuloma	Chapman et al. (2013)
	Nutritional rickets	McDonnell et al. (1997)	Osteoporosis; Old age	Worth (1963:181)
Apical radiolucency (transparency)	Vitamin D–dependent rickets	Zambrano et al. (2003)	Cysts; Benign and Malignant lesions; Infection	Razavi et al. (2013)
Dental abscesses	Vitamin D-resistant hypophosphatemic rickets	Chaussain-Miller et al. (2007); Douyere et al. (2009); Beltes and Zachou (2012)	Infection	Robertson and Smith (2009)
	Nutritional rickets	McDonnell et al. (1997); Davit-Béal et al. (2014)		
Radiolucency region near the dentin-enamel junction	Vitamin D-resistant hypophosphatemic rickets	Seow and Latham (1986); Pereira et al. (2004)	Pre-eruptive intracoronal resorption (lesion in the crown dentin)	Ari (2014)
Enamel hypoplasia	Vitamin D–resistant rickets	Seow and Latham (1986); Tumen et al. (2009)	Hereditary conditions; Trauma; Systemic metabolic stress; for full review see Goodman and Rose (1990)	Armelagos et al. (2009); Anthonappa and King (2015)
	Nutritional rickets	Davit-Béal et al. (2014); Galhotra et al. (2015)		
Normal but thin enamel	Vitamin D-resistant hypophosphatemic rickets	Hernández and Laguna (2013)	Dentinogenesis Imperfecta	Arana-Chavez and Massa (2004)
	Nutritional rickets	McDonnell et al. (1997); Davit-Béal et al. (2014)		
Increased incidence of carious lesions	Vitamin D-resistant hypophosphatemic rickets	Pereira et al. (2004); Chaussain-Miller et al. (2007)	Diet; Poor oral hygiene, for full review see Selwitz et al. (2007)	Hillson (2002:269–284)
	Nutritional rickets	Davit-Béal et al. (2014); Galhotra et al. (2015)		
Missing or unerupted teeth (agenesis)	Vitamin D-resistant hypophosphatemic rickets	Pereira et al. (2004); Rathore et al. (2013)	Idiopathic Hypoparathyroidism (rare decrease in blood calcium); Cysts; Osteomyelitis	Worth (1963:184–197)
	Nutritional rickets	Galhotra et al. (2015)		

*Notes: enlarged pulp chambers are different from taurodontism, which is an apical elongation of the pulp chamber that results in shortened roots associated with Neanderthal dentition, amelogenesis imperfecta, and Down's syndrome (Tulensalo et al., 1989). –No other known cause.

Table 2
Description of individuals in study sample.

Geographical Location	ID	Age (Years)	Serum Level (nmol/L)	Past Deficiency	Molars Radiographed	Molar used Histological Analysis
North America (Living individuals)	KT1	18	31	✓	U & L 1st, 2nd, 3rd	RM^3
	TT1	19	127	×	U & L 1st, 2nd, 3rd	RM_3
	KT2	42	–	✓	U & L 1st, 2nd, 3rd	×
	KT3	46	21	✓	U & L 1st, 2nd, 3rd	Rm_1
	M12	89	–	×	LM^1	LM^1
	M13	58	–	×	LM^1	LM^1
	M16	42	–	×	RM_1	RM_1
	M17	57	–	×	LM^1	LM^1
	M18	58	–	×	RM^1	RM^1
	M19	21	–	×	LM^1	LM^1
	M111	48	–	×	RM^1	RM^1
	M22	64	–	×	LM_2	LM_2
	M24	37	–	×	RM_2	RM_2
	M25	56	–	×	LM_2	LM_2
	M27	32	–	×	LM^2	LM^2
	M28	27	–	×	RM_2	RM_2
	M29	20	–	×	RM_2	RM_2
	M32	18	–	×	RM_3	RM_3
	M33	21	–	×	LM_3	LM_3
	M34	26	–	×	RM_3	RM_3
	M35	26	–	×	LM_3	LM_3
	M36	25	–	×	LM^3	LM^3
	M37	32	–	×	LM^3	LM^3
	M38	18	–	×	RM^3	RM^3
	M39	27	–	×	RM^3	RM^3
	M310	30	–	×	LM_3	LM_3
	M11	64	–	✓	LM_1	LM_1
	M210	42	–	✓	LM_2	LM_2
	M31	24	–	✓	LM^3	LM^3
Bastion des Ursulines, Quebec (1746–1747)	19G37-M02	18–21	–	×	U & L 1st, 2nd, 3rd	×
	19G37-E03	20–24	–	✓	U & L 1st, 2nd, 3rd	RM^1
	19G37-M01	25–29	–	✓	U & L 1st, 2nd, 3rd	×
	19G37-M03	18–22	–	✓	L 1st, 2nd, 3rd	RI^1
	39G6-B2	N/A	–	✓	U & L 1st, 2nd, 3rd	×
	19G37-F05	28–34	–	×	RM^1	×
	19G37-N01	18–21	–	✓	L 1st, 2nd, 3rd	×
	19G41-D01	45–54	–	✓	U & L 1st, 2nd, 3rd	×
	19G35-H01	20–24	–	×	RM^1	×
	19G37-F04	20–24	–	×	U & L 1st, 2nd, 3rd	×
Saint. Matthew, Quebec (1771–1860)	15A-S36	~23	–	✓	RM^2, RM^3	RM^1, RM^2, RM^3
Saint Antoine, Quebec (1799–1854)	STA 18K 55	50+	–	×	LM^1	LM^1
	STA 25C 55	17–25	–	×	L 1st, 2nd, 3rd	RM^1
	STA 25A 53	20–34	–	×	RM_1, RM_2, LM_1, LM_2	LM_1
	STA 22A511	17–25	–	×	RM_2, RM_3, LM_3	RM_2
	STA 25C520	16–25	–	×	RM_1, RM_2, RM_3	RM_3
	STA 25C518	35–49	–	✓	RM_1, LM_1	RM_1
Pointe-aux-Trembles, Quebec (1709–1843)	PAT 7A9513	16–25	–	×	U & L 1st, 2nd, 3rd	RM^1
	PAT 7A9546	35–49	–	×	LM_1	LM_1
	PAT 7A11 560	25–34	–	×	RM_3, LM_3	RM_3
	PAT 7A11 561	19–26	–	×	RM^2, LM^2	LM^2
Saint Jacques, France (A.D. 1225–1798)	SJ 384	40+	–	✓	U & L 1st, 2nd, 3rd	RM_1, RM_2
	SJ 562	40–58	–	✓	U & L 1st, 2nd, 3rd	LM_2, RC^1
	SJ 892	40–58	–	✓	U & L 1st, 2nd, 3rd	LM_3, RC_1
	SJ 970	45–56	–	✓	U & L 1st, 2nd, 3rd	LM^1, RM^3

Notes: U = maxillary; L = mandibular.
Age for living individuals was the age that the radiograph was obtained. Age at death for archaeological individuals was estimated using auricular surface (Lovejoy et al., 1985), pubic symphysis (Brooks and Suchey, 1990), and cementochronology (Naji et al., 2016). – Information not available.

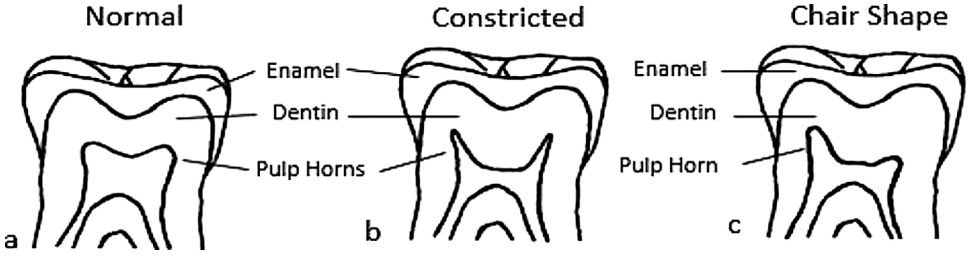

Fig. 1. (a–c) Diagram of different pulp chamber shapes in a generic permanent molar, a) normal: evenly matched pulp horns; b) constricted: high, narrow pulp horns; c) chair shape: pulp horns are uneven resembling a chair. Can be used for all three molars, recognising that the 3rd molar is more variable.

Table 3
Approximate age of pulp chamber initiation in permanent molars.

Tooth type (mandibular)	Pulp chamber initiation	
1st Permanent Molars	Male 1.5-2 years	Female 1.4-2 years
2nd Permanent Molars	Male 5–6.5 years	Female 4.5–6.5 years
3rd Permanent Molars	Male 10–12.5 years	Female 9.5–12 years

Note: Approximate age of initiation for pulp chambers as described in Moorrees et al. (1963:1492). Stage Crc (crown complete). Average age of mandibular molars is given.

to occur during the process of pulp formation. Third molars will show considerably more variation in size, contour, and number of roots than 1st and 2nd molars (Ahmed, 2012). Root formation was excluded, as this feature does not influence pulp chamber initiation. Evaluation of dental formation allows us to determine the approximate age when pulp changes arose and subsequently provides key information as to the age at which a deficiency occurred.

3.3. Pulp horn and pulp chamber measurements taken from radiographs for living and archaeological individuals

To quantify pulp horn and pulp chamber morphology, radiographs from both living and archaeological individuals (Table 2) were taken and measurements of pulp horns and pulp chambers were conducted blind to ensure unbiased calculations. Radiographs were imported into the image editing software program ImageJ to perform linear measurements. The scale from the radiograph images was used to calibrate the images imported into ImageJ. Using the Straight-Line Tool in ImageJ, the scale for each image was calibrated to the radiograph scale, where 1 mm equaled ~7 pixels. Pulp horn height (PH1-h and PH2-h), width (PH1-w and PH2-w), and pulp chamber height (PC-h) were measured using the Straight-Line Tool where measurements were automatically derived for pulp horns and pulp chambers (Fig. 2). Ratios were calculated for pulp horn heights (PH-h Ratio) to observe any differences between those with and without vitamin D deficiency (see Supplementary C for all measurements). The ratios were calculated to reduce the effect of external tooth size and the type of tooth used as pulp horns are proportional relative to the whole tooth. Kvaal et al.'s (1995) study did not find significant differences in measurements between teeth from the left or right side of the maxilla or mandible, therefore, teeth from both sides were used in this study. Student's *t*-tests (two-tailed) were conducted in a Microsoft Excel program that compared the five measurements taken from the pulp horns and pulp chambers of living and archaeological individuals with vitamin D deficiency to those without. Student's *t*-tests were also conducted to determine any significant differences in measurements between tooth types and between teeth on the left versus right side of the maxillae and mandible. To assess whether a dental restoration affected the shape of the pulp chamber, measurements were compared on KT3's (diagnosed with deficiency, Table 2) right (dental restoration) and left (no dental restoration) mandibular 1st molars. Bitewing dental radiographs were also obtained for KT3 for a 7-year period to determine if measurements of pulp chambers changed over time due to secondary or tertiary dentin formation.

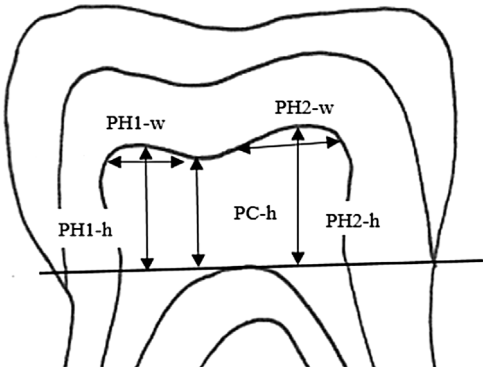

Fig. 2. Diagram of measurements taken on a generic permanent molar. PH1-h and PH2-h are pulp horn heights; PH1-w and PH2-w are pulp horn widths; PC-H is pulp chamber height (modified from Zilberman and Smith, 2001).

3.4. Histological analysis

Archaeological individuals who exhibited a number of dental and skeletal features associated with vitamin D deficiency were selected for histological analysis (n = 17). Living controls (n = 28), with and without deficiency, were also evaluated histologically to confirm the presence or absence of incremental interglobular dentin (IIGD). Histological analysis was undertaken to directly observe pulp chamber changes in individuals with deficiency, to determine presence/absence and severity of IIGD, and to estimate the age at which deficiency occurred (Table 2). Age of deficiency, based on location of IIGD in dentin, was determined using Moorreess' et al. (1963) developmental standards, and severity of deficiency was graded according to the scoring system described in D'Ortenzio et al. (2016). For example, for Grade 1, the amount of IIGD is less than 25% relative to the surrounding normal dentin, with small interglobular spaces, indicating a mild mineralisation defect. Grade 3 is the most severe with IIGD covering over 75% of the region of interest, accompanied by large spaces that follow the incremental lines in the dentin (D'Ortenzio et al., 2016).

Teeth were sectioned to expose the pulp chamber from the buccal side of the tooth to be consistent with the direction of the X-ray beam using a precision diamond wafering saw (Buehler IsoMet 1000) (Saunders et al., 2007). The samples were lapped and polished to remove saw marks with a Buehler MiniMet grinder-polisher and lapped using 400, 600, 1200 grit paper and a texmet pad with 3 μm diamond polish, followed by 1 μm diamond polish on a microcloth pad. The polished samples were ultrasonicated for 5 min in distilled water and glued to a microscope slide that adhered using a UV activated adhesive. For microscopic analysis, the thin-sections were imaged using a Nikon DsR:1 camera attached to an Olympus BX51 digital microscope, (40×–100× magnification).

4. Results

4.1. Radiographs observing morphology of pulp horns and pulp chambers for known living controls

Permanent molars of living indiviudals (n = 25) were radiographed (Table 2) to determine pulp horn and pulp chamber shape in those potentially lacking deficiency. Of these, three individuals (M11, M210, M31) displayed chair shaped pulp chambers, indicative of vitamin D deficiency and further histological analysis confirmed the presence of IIGD (see histological results below). Using Table 3, we approximated the ages of deficiency, based on location of IIGD, which ranged for each individual; M11 (LM_1) was 1.4–2 years; M210 (LM_2) was 4.5–6.5 years; and M31 (LM^3) was 9.5–12.5 years. Note that deficiency could have occurred earlier in M210 and M31 as we only had their 2nd and 3rd molars available for histological analysis.

We also examined bitewing radiographs of three living individuals with clinically diagnosed vitamin D deficiency. One diagnosed with rickets at age 4 (KT2), and two with asymptomatic deficiency diagnosed using serum 25(OH)D results (Table 2). All three individuals showed changes to the pulp horns, with the mandibular 1st molar showing the most marked change suggesting deficiency at ~1.8–2.6 years (Figs. 3 a–b , 4 a). In KT2, poor image quality due to overexposure prevented a definitive statement being made on the 2nd molar. The second mandibular molar for both KT1 and KT3 showed slight changes indicating deficiency continued. A right deciduous mandibular first molar from KT3 showed there was no deficiency from 14.5 to 17 weeks in utero to

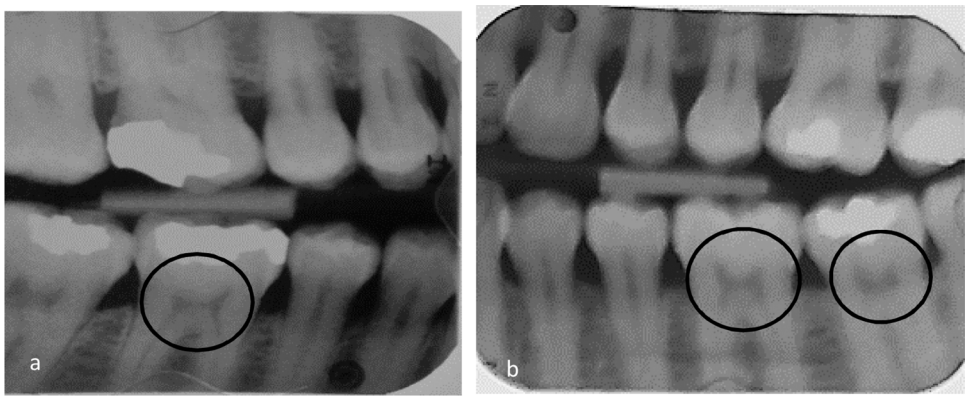

Fig. 3. a) Radiograph of KT3 (low in vitamin D), exhibits chair shaped pulp chamber in permanent mandibular right 1st molar (black circle); **b**) chair shape exhibited in permanent mandibular left 1st and 2nd molars (black circles).

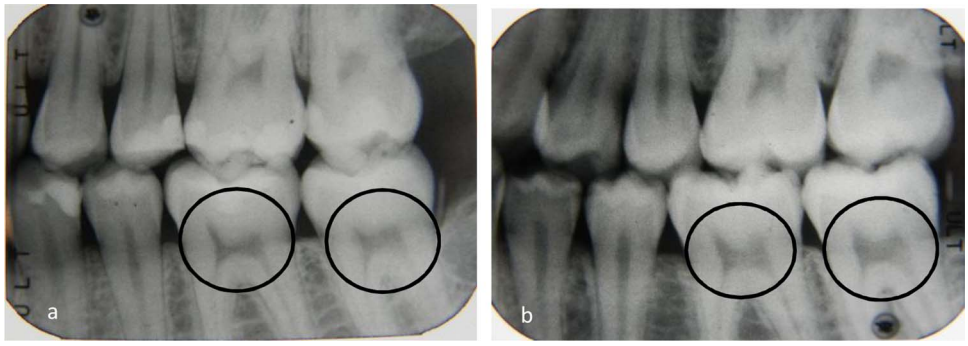

Fig. 4. a) Radiograph of KT1 (low in vitamin D) showing chair shaped, constricted pulp horns in the left 1st mandibualr molar and a chair shaped 2nd mandibular molar (black circles); **b**) radiograph of TT1 (normal vitamin D level) showing regular, even pulp horns in the left mandibualar 1st and 2nd molars (black circles).

5.5 months old (Hillson 2002), however bitewing radiographs showed chair-shaped 1st mandibular molars (Fig. 3a–b). Histological analysis on KT1's extracted 3rd molar, which begins to form at approximately 7–10 years (Hillson, 2002:123), confirmed the radiographic results (Fig. 4a); the presence of Grade 2 IIGD under the crown and the mesiobuccal cusp, which is the first cusp to form in the maxillary 3rd molar and could overlap with the formation of the pulp chamber in the second molar (Reid and Dean, 2006). Bitewing radiographs of individual TT1 (Fig. 4b), who lacked deficiency, did not display constricted or chair shaped pulp horns in any molars; the pulp horns were even and regular. Histological analysis of the 3rd molar confirmed the absence of IIGD (Grade 0) from ages ~7–19 years.

4.2. Radiographs observing morphology of pulp horns and pulp chambers for archaeological individuals

Table 5 presents pulp horn shape viewed histologically and radiographically, severity of IIGD grades, pulp horn/chamber measurements, and approximate timing of deficiency for the subsampled living and archaeological individuals (for a full description of all individuals, including tooth type analysed, see Supplemental Data A, Table 2). Radiographic images of molars from the first archaeological group (Saint Matthew, Saint Jacques), had marked skeletal evidence of vitamin D deficiency and were on the severe end of a deficiency spectrum. The maxillary 1st permanent molar of individual 15A-S36, from Saint Matthew, Quebec, revealed prominent constricted pulp horns. The Saint Jacques dentition showed that all individuals (n = 4) exhibited chair shape, constricted pulp horns in all maxillary and mandibular molars available, with pulp horn extensions towards the occlusal aspect of permanent teeth (Fig. 5a–d).

Radiographs of group two (Bastion des Ursulines, n = 10) consisted of cases with slight deformity of long bones revealed the presence of observable chair shaped pulp horns in five out of six individuals with bowing deformities. The exception was 19G37-M03 who had skeletal indicators of deficiency, but dental radiographs did not display constricted or chair shaped pulp horns. In the individuals where skeletal bowing was absent (n = 4), pulp horns appeared evenly matched, without constrictions.

4.3. Results of radiological and histological examination to determine how many vitamin D deficient cases were missed

Group three (Saint Antoine, Pointe-aux-Trembles) consisted of archaeological individuals who displayed no macroscopic skeletal indicators of past deficiency or characteristic pulp horn shape changes observed on radiograph. However, these individuals were from communities in which vitamin D deficiency was known to be present. Histological analysis was conducted to determine how many of these individuals had clear bands of IIGD suggestive of vitamin D deficiency (Table 2, Supplemental Data A). One individual (STA 25C 518) had chair shaped pulp horns in their right mandibular 1st molar observed radiographically also had Grade 1+ IIGD. Histological analysis showed that the other individuals (7/9) had no IIGD (as anticipated); however, the remaining two had Grade 1 IIGD (minimal interglobular spaces). One individual from Saint Antoine, Quebec (STA 18K 55) had only one molar available for analysis. The radiograph angle was not exactly perpendicular to the X-ray beam, which made it difficult to discern pulp horn shape.

Of the living individuals (n = 25) used as controls, 3 displayed chair shaped pulp horns radiographically and histological analysis showed that M11 had Grade 1.5–2 IIGD, whereas M210 and M31 exhibited Grade 2 IIGD, indicating that these individuals experienced moderate past vitamin D deficiency. The remaining 22 individuals did not display chair shape pulp horns and scored a Grade 0 for IIGD, indicative of an absence of deficiency (Fig. 6a–d).

4.4. Results of blind test

The blind test revealed an accuracy of 75% among participants for selecting the correct answer (yes: misshapen pulp horns detected; or no: none detected). Results obtained for each case varied, ranging from 17% correct (case 3) to 100% correct (cases 2, 4, 6, 11, 12). A review of

Table 4
Summary of mean values for measurements taken on permanent molars observed on radiograph.

Measurement (mm)	N, Mean, SD	Modern: no deficiency	Modern: with deficiency	Archaeological: no deficiency	Archaeological: with deficiency
PH1-h	N	14	34	44	62
	Mean	2.9	4.4*	2.6	2.7
	SD	0.8	1.9	0.9	1
	Range	1.6 – 4.9	2.5–8.6	1.4 – 4.7	1.2–4.3
PH2-h	Mean	2.6	2.7	2.5	1.6*
	SD	0.9	1.3	0.9	0.8
	Range	1.4 – 3.7	0.9 – 4.9	1.2–4.9	0.5 – 3.5
PC-h	Mean	1.8	2.1	2	1.2
	SD	0.7	1.5	0.9	0.7
	Range	0.9 – 3.0	0.8 – 6.1	0.8 – 3.9	0.3 – 2.9
PH1-w	Mean	1.2	0.7*	1.1	0.5*
	SD	0.2	0.2	0.2	0.2
	Range	1 – 1.7	0.4 – 1.1	0.9 – 1.5	0.2 – 1.2
PH2-w	Mean	1.3	0.6*	1.1	0.5*
	SD	0.3	0.2	0.2	0.3
	Range	1 – 1.9	0.3 – 0.9	1 – 1.4	0.1 – 1
PH-h Ratio		1.1:1	1.8:1	1.1:1	1.8:1

*Note: N is the number of molars measured on radiograph. Ph1-h = pulp horn 1 height; Ph2-h = pulp horn 2 height; PC-h = pulp chamber height; PH1-w = pulp horn 1 width; Ph2-w = pulp horn 2 width; PH-h Ratio = ratio between pulp horn heights.
*P value < 0.01.

the radiological images demonstrated that positive results were clearly linked to the quality and proper angulation of the radiograph observed. Evaluating the data indicated that differences in individual results were due to the positioning of the teeth on the radiograph plate. All cases that received 100% correct scores involved just one or two teeth with good magnification and positioning (see Table 3, Supplementary Data B). Positioning of teeth at a right angle to the X-ray beam is critical for good results, but once that has been accomplished, observation of pulp chambers can be completed with relative ease. The results show that with properly aligned radiographs, pulp chamber abnormalities, indicative of deficiency, were consistently identified by observers.

4.5. Pulp horn and pulp chamber measurements taken from radiographs for living and archaeological individuals

Table 4 presents the mean values for pulp horn/chamber measurements taken on living and archaeological individuals (n = 154) (for all measurements see Supplementary Data C). Table 5 displays measurements for selected individuals. There were no significant differences between measurements of teeth from archaeological individuals versus living individuals in both those with deficiency or those without. Measurements on pulp horn widths showed that pulp horns that were < 1 mm were associated with those with vitamin D deficiency, whereas those with widths > 1 mm, were without deficiency. Students *t*-tests determined that differences between pulp horn widths on living and archaeological individuals with deficiency versus those without were highly significant (living individuals p = 0.014; archaeological individuals p = 0.018). Pulp horn heights were compared and differences were also highly significant between living and archaeological individuals with and those without deficiency (p = 0.007, p = 0.015, respectively). There were no significant differences in measurements of teeth from the left or right side of the maxilla or mandible (p = 0.11–0.96) or between different tooth types (p = 0.06–0.67) for all individuals tested. Ratios conducted to compare pulp horn heights for all individuals found that those without deficiency had an approximate 1:1 ratio, whereas those with deficiency had close to a 2:1 ratio (Table 4). Pulp chamber heights ranged from 0.8-6.1 mm with archaeological individuals with deficiency having the narrowest pulp chamber height (0.3–2.9 mm) (Table 4).

Evaluation of pulp chamber measurements of teeth with dental restorations versus those without to assess possible pulp chamber shape change in KT3 showed that although there were slight differences between LM_1 (no restoration) and RM_1 (restoration) widths (e.g., PH2-w: 0.3 mm for RM_1; 0.5 mm for LM_1), these differences were not significant and the pulp horn height ratios (differences in height between both pulp horns) were identical (1.64:1) in both molars. More work is required to determine the full effect of dental restoration on pulp chamber measurements. Changes in the amount of secondary and/or tertiary dentin were not detectable over the 7-year time period in KT3's tooth with the dental restoration. The right mandibular 1st molar (dental restoration) was re-measured for each year and results showed that pulp horn/chamber measurements were almost identical over the seven-year period with fluctuations of only ± 0.01–0.03 mm for all measurements taken (Supplemental Data D).

5. Discussion

Radiographic imaging was specifically chosen for this preliminary study, as this technology is widely available, is non-destructive, and produced consistent results using a variety of different settings/exposure times, with the caveat that the positioning of the teeth on the radiograph plate is correct. The archaeological individuals in the first group (Saint Matthew, Saint Jacques), who displayed marked skeletal evidence of past rickets all (5/5) exhibited the formation of constricted and chair shaped pulp horns. These individuals with marked evidence of past rickets revealed extensions of the pulp horns into the occlusal edges of permanent molars (Fig. 5a–d), and all had a Grade 1+ to 3 IIGD severity score for deficiency. Fig. 4b displays a radiograph of pulp chambers for an individual known to have healthy 25(OH)D levels (TT1); this individual exhibited evenly matched pulp horns. Among individuals who had experienced severe rickets, indicated by marked skeletal deformity, the pulp horns were less similar in length (chair shaped) and/or constricted.

Histological results in both living and archeological individuals appear to support the concept that a certain severity in the threshold of deficiency is required before morphological changes develop in the pulp chamber. The data suggests that the level of disruption to the mineralisation of a tooth that occurs with Grade 1 IIGD is insufficient to affect the shape of the pulp chamber during development. For example, an individual from Point-aux-Trembles (PAT 7A9 513), had no changes to their pulp chambers when viewed radiologically, but had Grade 1 IIGD in their 1st molar that formed between ~1.5 and 2–3 years. Conversely, two individuals from Saint Jacques (SJ 384, SJ 970), also had episodes of deficiency starting at ~1.5 years, but both showed clear radiological dental features even though their episodes of deficiency were shorter. Their severity score was Grade 3 (Supplementary

Table 5
Summary of radiographic measurements and histological morphological data for selected individuals.

IIGD severity score	Identifier	Age (years)	Past deficiency*	Histological pulp horn/chamber shape	Radiological pulp chamber measurements (mm) of permanent molar	Approximate age of episode (yrs.) (Moorrees et al., 1963)
Grade 3	15A-S36	~23	✓	RM^2: constricted	RM^2: PH1-w: 0.61; PH2-w: 0.51; PH-h Ratio = 1.75:1	2 episodes between 1.5-2
	SJ 970	45–56	✓	LM^1: constricted; chair shape	LM^1: PH1-w: 0.27; PH2-w: 0.44; PH-h Ratio = 1.92:1	1.5
	KT1	18	✓	RM^3: moderately constricted: chair shape	RM^1: PH1-w: 0.61; PH2-w: 0.8; PH-h Ratio = 2.85:1	10.5
Grade 2	SJ 562	40–58	✓	LM^1: severely constricted	LM^1: PH1-w: 0.12; PH2-w: 0.14; PH-h Ratio = 1.38:1	1.5 – 2
Grade 1 +	19G37-M03	N/A	✓	N/A: single root (RI^1)	**RM_1: normal and even; multiple carious lesions obscured view	5.5–6.5
	STA 25C 518	35–49	✓	RM_1: moderately constricted; chair shape	RM_1: PH1-w: 0.71; PH2-w: 0.81; PH-h Ratio = 1.59:1	1.5 – 3
Grade 1	PAT 7A9 513	16–25	✓	RM^1: normal and even	RM^1: PH1-w: 1.06; PH2-w: 0.97; PH-h Ratio = 1.04:1	1.5 – 2
Grade 0	TT1	19	×	RM_3: normal and even	RM_2: PH1-w: 1.11; PH2-w: 1.17; PH-h Ratio = 1.02:1	–
	KT3	44	✓	– Rm_1 (deciduous tooth)	**RM_1: PH1-w: 0.54; PH2-w: 0.3; PH-h Ratio = 1.64:1	–
	STA 25A 53	20–34	×	LM^1: normal and even	LM^1: PH1-w:1.01; PH2-w: 1.02; PH-h Ratio = 1.01:1	–

Notes: Ages as set out in note for Table 3. *Deficiency diagnosed by blood serum levels (living individuals), skeletal changes, and presence/absence of IIGD in archaeological individuals. Full histological and radiological data available in Supplementary Data Table 2. Details on positioning covered in Supplementary Data B. **Observed RM_1 on radiograph as this forms near the same time as RI^1 and roots of Rm_1 (Moorrees et al., 1963).
PH1-w = pulp horn 1 width; PH2 = pulp horn 2 width; PH-h Ratio = ratio between pulp horn height.

Data A, Table 2). This indicates that it is not only the timing of deficiency, but the severity of deficiency that causes pulp chamber shape changes. The data indicates that an IIGD severity score of approximately a Grade 2 and above must be reached before alterations in permanent molar pulp chambers are observed (Supplemental Data A, Table 2). Grade 2 severity consists of interglobular spaces that are moderately large and more numerous than Grade 1 (D'Ortenzio et al., 2016).

Clinical cases of vitamin D deficiency reviewed in Roberts and Brickley (in press) indicate that the development of skeletal features is strongly linked to rates of skeletal growth. Once growth stops, as demonstrated by our data from living individuals and cases reviewed by Roberts and Brickley (in press), serum levels likely need to be much lower before skeletal changes develop that would allow paleopathologists to diagnose vitamin D deficiency. Dentin from the right deciduous mandibular first molar from KT3 (blood serum level 21 nmol/L) (Table 2), showed there was no deficiency from 14.5 to 17 weeks in utero to 5.5 months old (Hillson 2002), therefore deficiency developed at a later age. It is likely that osteopenia in KT3 is associated with the serum 25(OH)D result, and deficiency may have been present for a while. It is generally accepted that serum 25(OH)D > 50mnol/L are required for skeletal health and there are links to low bone mineral density (BMD) (Peterlik, 2012; Gallagher 2013). Dental changes to pulp chambers have appeared in individuals with blood serum levels of 31 nmol/L (e.g., KT1), suggesting that a threshold for severity of deficiency needs to be met before pulp chamber alteration occurs.

Although severe and long-standing vitamin D deficiency can produce clear skeletal changes, similar to those observed in group one (Saint Matthew, Saint Jacques), many individuals who experience deficiency will not be identified from paleopathological investigation of skeletal changes, particularly individuals who survive to adulthood (Hess, 1930; Brickley et al., 2010). To determine if radiograph analysis of pulp chamber shape could be used to identify individuals who had experienced rickets during childhood using a collection where skeletal changes (i.e., bowing of leg bones) were not marked, the second group of individuals from Bastion des Ursulines, Quebec was used. For individuals who exhibited bowing deformities (n = 6), dental radiographs revealed constricted, and/or chair shaped pulp horns in permanent molars in five out of six cases. However, one individual (19G37-M03), had skeletal changes, but did not have a positive dental radiological result for the three mandibular molars. Due to extensive carious lesion damage on the molars, an incisor was used for histological analysis. Evidence of Grade 1+ IIGD was observed histologically in a maxillary incisor (see Table 5). Timing of deficiency in this individual occurred later than the period in which the pulp chamber was forming in the permanent molars. The IIGD observed in the incisor that was formed at ~5.5–6.5 years, whereas pulp chamber formation is initiated in the 1st molar at an earlier age (~1.5–2 years).

It is not conclusive that the negative controls did not experience vitamin D deficiency, as they may have had slight deficiency but lacked skeletal and radiographic indicators. This appears to be the case with two individuals from the third group of individuals (Saint Antoine, Pointe-aux-Trembles) who had no skeletal indicators of deficiency and lacked morphological changes to the pulp horns on radiograph. Histological analysis revealed Grade 1 IIGD (mild), suggesting that some individuals who experienced deficiency that caused very slight mineralisation defects may not develop morphological changes in the pulp chamber and would be missed in radiological screening.

Examination of dental radiographs were useful to evaluate if there were measurable differences in the pulp horns and pulp chambers of those with or without past vitamin D deficiency. We demonstrated that measurements on maxillary and mandibular 1st, 2nd, and 3rd molars were not difficult to perform and significant differences were found in pulp horn widths and for the ratios between pulp horn heights in those with deficiency versus those without (Table 4) (Supplemental Data C). Measurements on pulp horn widths showed that pulp horns labelled

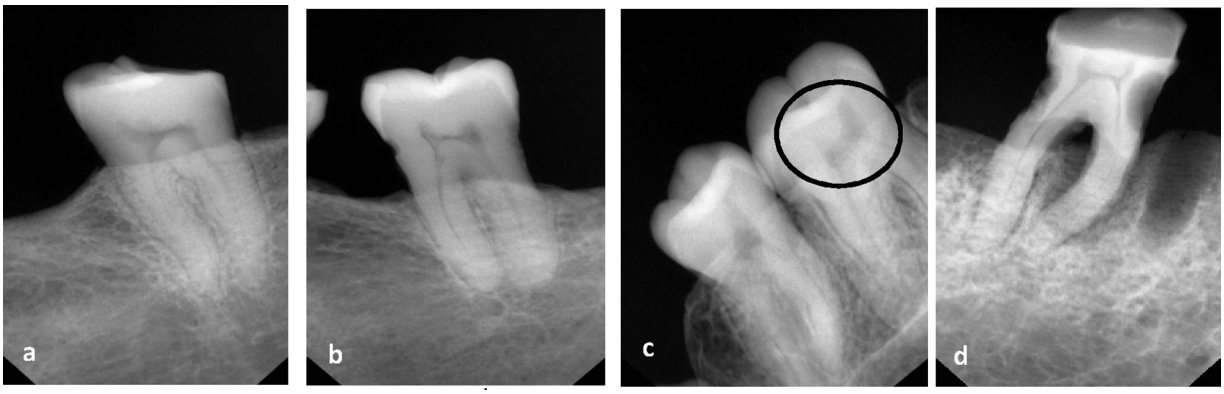

Fig. 5. **a**) SJ384, right mandibular 2nd molar exhibiting chair shape; **b**) SJ562, right mandibular 2nd molar exhibits constriction and a slight chair shape; **c**) SJ892, left mandibular 2nd and 3rd molars, 2nd molar exhibiting chair shape (black circle); **d**) SJ970, right mandibular 1st molar with carious lesion and occlusal wear exhibits constricted pulp horns.

morphologically as 'constricted' were < 1 mm and were associated with those with vitamin D deficiency, whereas those labelled 'normal' were > 1 mm, were found on those without deficiency. Those without deficiency had an approximate 1:1 ratio for pulp horn height, whereas those with deficiency had close to a 2:1 ratio. This signifies that the 'chair shape' observed on radiograph in those with deficiency was validated by the measurements, where one pulp horn was shorter relative to the other. As anticipated, measurements of pulp horn widths and heights taken on living individuals with deficiency were similar to archaeological individuals with deficiency and vise versa, further validating the trends found (Table 4). This was also evidenced in the living individuals (n = 25) used as controls, where 3 individuals displayed chair shaped pulp horns radiographically and histologically they had Grade 1.5-2 IIGD (M11, M210, M31), indicative of moderate asymptomatic vitamin D deficiency (Fig. 6a–d). Pulp chamber widths showed that all 3 individuals had constricted pulp horns (< 1 mm) and the ratio between pulp horn heights ranged from 1.5 to 1.8:1, which is higher than the 1.2:1 ratio found in the living individuals without deficiency.

Teeth with dental restoration were avoided where possible, as this may influence the shape of the pulp horns by producing reparative (tertiary) dentin adjacent to the irritated zone of a tooth (Stanley et al., 1966; Kraus et al., 1969). One individual (KT3) had radiographs that showed a dental restoration in the right mandibular 1st molar (Fig. 3a), but lacked a dental restoration in the left mandibular 1st molar (Fig. 3b), suggesting that the chair shape observed could have been the result of the restoration. Measurements found that differences between the two were not significant indicating that the dental restoration did not affect the pulp chamber shape in KT3 through subsequent growth of tertiary dentin. This individual likely received prompt dental care before the carious lesion was able to progress to the dentin layer. Similarly, radiographs of KT3 were taken in progression over a seven-year period and pulp chambers were measured to determine potential growth of secondary and/or tertiary dentin. Results showed that pulp horn/chamber measurements were indistinguishable over the seven-year period with fluctuations of only ± 0.01-0.03 mm for all measurements taken (Supplemental Data D), suggesting that in KT3, there was little to no secondary/tertiary dentin growth, as we would have observed a measurable reduction in the pulp chamber.

Radiological examination of pulp chambers can aid in determining the timing of deficiency in individuals who have pulp changes in their 1st molars (Table 3), however continual dentin deposition reduces the size of the pulp chamber with age, due to recurrent secretion of dentinal matrix by odontoblasts (Solheim, 1992; Kvaal et al., 1995; Goldberg, 2014). This may affect our ability to observe pulp horn shape in very old individuals, as the chamber condenses in size over time altering the original pulp horn shape. Examination of KT2 and KT3's pulp chamber shape (living adults diagnosed with evidence of deficiency), showed pulp chamber changes in permanent molars observed radiographically at age 42 and 46, years. M12 was an 89-year-old living individual without deficiency who exhibited normal even pulp horns and a measurable pulp chamber in the left maxillary 1st molar demonstrating that radiographic examination of pulp chamber shape could still be useful for middle to old-aged adults. However, further work is required to determine how long such features might remain consistently visible for very old-aged adults because the pulp chamber shape can be obliterated with age, making it difficult to observe radiographically.

The exact mechanisms for morphological changes in the pulp chamber are not fully understood, but are likely linked to severity of deficiency. As the pulp chamber and dentin are part of the same functional unit, the pulpodentinal complex (Dean, 2016), we hypothesize that in cases of vitamin D deficiency, there are periods of non-mineralisation in dentin affecting the shape of the pulpal margin during tooth development. Absence of dentin mineralisation could result in a less homogeneous or uneven circumpulpal zone directly affecting pulp horn shape. Another explanation relates to the growth of calcospherites (calcium salts) in dentin. In normal dentin, the calcospherites continue to grow uniformly, in all directions during tooth formation until contact is made with other calcospherites, forming a homogeneous matrix (Shellis, 1983; Dean, 2016). A lack of dentin mineralisation leads to modification of the calcospherite shape and size and calcospherites close to the pulp chamber that could alter the normal formation of pulp

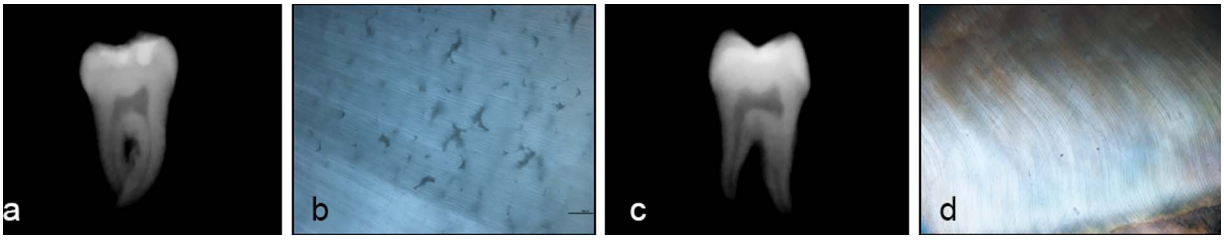

Fig. 6. **a**) M210 radiograph showing chair shape pulp horns in LM_1; **b**) M210 exhibits Grade 2 IIGD (crescent shaped spaces), indicative of moderate deficiency; **c**) M13 radiograph showing even pulp horns in LM^1; **d**) M13 exhibits Grade 0 IIGD (no spaces), indicating absence of deficiency. Histology 100× magnification.

horns in individuals with deficiency. Pulp chamber changes linked to vitamin D, calcium, and phosphate imbalance could potentially initiate an immune response from the dental pulp. Studies have shown that odontoblasts initiate an immune and/or inflammatory response to injury, microbial infiltration, and systemic disease (Yu and Abbott, 2007). Absence of mineralisation could disrupt the odontoblast layer surrounding the pulp chamber and initiate chemotactic (cell movement) signals that cause intercellular spaces to become filled with fluid and proteins, similar to that of an inflammatory response (Kraus et al., 1969:184; Avery, 2002:206). The fluid-filled intercellular spaces are unable to communicate due to a decrease in permeability of pulpal and dentin cells, thus preventing further formation of the pulp horns (Avery, 2002:208).

Although tooth formation differs from bone formation, tooth mineralisation is susceptible to similar failures as bone during vitamin D deficient episodes (Vital et al., 2012; Foster et al., 2014). We propose that loss of vitamin D signaling directly affects dental cell functions that stop the mineralisation process and subsequently affect the shape of the pulp horns. As the pulp chamber and pulp horns form the bulk of the tooth, incomplete mineralisation potentially compromises pulp horn shape. Our preliminary work suggests that the deficiency needs to occur during the initiation of the pulp chamber formation, but if this happens, then it is severity of deficiency that results in pulp chamber changes. Additional work using clinical radiographs and associated medical data will further understanding of the pathogenesis of pulp tissue to obtain detailed insight into the formation of dentin and pulp chambers in individuals with vitamin D deficiency. Further studies are needed to explore questions such as whether there is a differential sensitivity to deficiency in different tooth types as changes appear most marked in the 1st molar and this could be partly linked to speed of development. Preliminary results from our investigation suggest that radiograph assessment could be used as a screening method to elucidate patterns of deficiency in communities and aid in the selection of individuals for further histological or microCT assessment.

6. Conclusions

The results of this study indicate that individuals with measurable morphological changes to their pulp chamber observed radiographically will have experienced a condition that causes mineralisation defects. To the best of our knowledge, only conditions linked to vitamin D, calcium, or phosphate imbalance cause these types of pulp chamber changes linked to abnormal mineralisation (see D'Ortenzio et al., 2016). Pulp shape abnormalities could be the first stage of evidence for a previously undiagnosed vitamin D deficiency. Our study indicates that severity of a deficient episode needs to be approximately Grade 2 or greater to exhibit marked pulp chamber changes, as less severe deficiency will not influence the formation of the pulp chamber. The use of permanent molars makes it possible to investigate a wide range of ages (~1.5–12.5 years) that cover critical periods of development of past individuals, enabling a nuanced understanding of patterns of deficiency in the past. Radiographs permit measurements to be taken to corroborate morphological observations and provide a non-destructive aid in the diagnosis of individuals with vitamin D deficiency, particularly where skeletal changes are very subtle. The techniques outlined in this study also have the potential to provide a useful tool to aid in the selection of individuals for microCT or histological assessment. Importantly, the technique can be used on both modern and archaeological individuals, thus addressing the call made by Wright and Yoder (2003) for stronger interaction between modern health research and paleopathology. Radiograph screening of teeth enables better interpretations of health data from past societies and provide a time depth to inform current health debates on vitamin D deficiency in present communities.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpp.2017.10.001.

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