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MANDIBULAR INCISOR TEETH RADIOGRAPHY AND COMPUTED TOMOGRAPHY IN YOUNG HORSES

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SUMMARY

We have studied a total of seventeen anatomical pieces of horse incisors with ages between 1 and 4,5 years old. The anatomical pieces were divided in groups and identified as group I. 1-2.5 years: twelve anatomical pieces; group II. 3.5-4 years: one anatomical piece, and group III. 4-4.5 years: four anatomical pieces. From each incisor was evaluated: 1) the anatomical piece; 2) radiographs in the right lateral and intraoral views; 3) the computed tomography (CT) in the three planes of cut: axial, dorsal and sagittal, and 4) the three-dimensional reconstruction, highlighting the bone or the tooth, depending on the case. In group I., from 1 to 2.5 year with only deciduous incisor in the mouth, it was possible to see 4 different dental germs stages of the central permanent (301 and 401). Stage I: dental germs without content; stage II: with amorphous enamel content; stage III: with conical tooth formation, and stage IV: with very advanced phase of development. In group II., 2.5 years, the intraoral radiographs and CT show great internal development of 301 and 401 with a large pulp cavity and an infundibulum that occupies half of the occlusal surface of the tooth. In group III., 3.5-4 years, with corners (303 and 404) close to eruption, the radiographs and CT allow to observe the development level of each tooth, especially in the axial plane with clear differences between them. The joint use of radiographs and CT allows better understanding of the intraoral radiographs and makes a more attractive teaching of the age of the horse's mouth.

Key words: Incisors, Mandible, Radiology, CT scan, Horse.

INTRODUCTION

Actually, equine dentistry is one of the specialties with a great future in the equine industry worldwide. It's true that many veterinarians take care of horse's mouths performing routine procedures but very few are really specialized in this area. Since many years the Spanish Society of Odontology and Maxillofacial Surgery formed from the Faculty of Veterinary Medicine of Madrid, under the auspices of Prof. Dr. Fidel San Román, carries out a constant work of specialization in this area in small animals, horses and exotic animals. Currently, the elected president of the European Veterinary Dental Society is the Spanish Carla Manso with whom the Radiology Service has been working for several years.

The horse incisors pathologies are well known and described in numerous texts. The incisors radiology is very well described in terms of the pathologies present but a methodical description by age is lacking. The textbooks show many incisor x-rays in young horses but there are no methodical descriptions in the different age groups and less if they're young horses.

The use of computed tomography (CT) in the horse head is advancing all over the world. The incorporation of standing CT for the horse head is making the diffusion more and more quick in the equine referral clinics and research equine referral centers. There is really little interest in the incisors study by CT, possibly because the oral examination and the possibility of performing lateral and intraoral (occlusal) radiographs gives sufficient information by having little structures overlap. There are very interesting studies of basic research with microCT that is teaching important features in the incisors. However, its use will be increasing because there are dental malformations, tumors, fractures in young horses where the CT offers much better information than the simple inspection and radiographs realization. We aren't aware that there is a methodological radiological description by age and less if it's a description of the image characteristics produced by a CT, except some works already published in national and european congresses.

The CT produces a very high number of images that can be analyzed in three planes of cut: transverse or axial, sagittal and dorsal. In addition, the images can be reconstructed in three dimensions (what is known as volume rendering or VR) highlighting the bone or dental tissue, depending on the study region.

The joint use of programs that integrate radiographs and CT with videos in different cutting planes offers excellent teaching characteristics, not only for students but also for dentistry specialists, a line in which the Radiology Service has been working for several years.

Although there are many publications that help determine the age of the horse's mouth, specialists have a hard time determining the age in the period between 1 year (time of eruption of the corner permanent incisors) and 2.5 years (time of eruption of the central permanent incisors). In many occasions the specialists can't specify too much. We are convinced that a serial study with radiographs and CT help to better understand the dental development in this period of age.

In the Anatomy and Comparative Anatomy Pathology Departments of Cordoba Faculty of Veterinary Medicine there is an excellent piece collection of horse incisors (with incisive bone and mandible) thanks to the work of years of the Anatomy group members, especially the Prof. Dr. Andrés Diz. From this 52 pairs of incisors collection of all ages, we have chosen the youngest (17 cases) that we have photographed, radiographed and made CT, with the following **objectives**:

1. Describe the radiographic anatomy and CT of the horse's mandibular incisors with mixed dentition (permanent and deciduous incisors) between 1 and 4.5 years of age;
2. Understand the dental germs development in horses with mixed dentition, which can help determining the age more exactly, and
3. Through the joint use of X-rays and CT, perform and collaborate in a more attractive teaching of equine dentistry.

DENTAL ANATOMY

The mandible is an even bone, in the horse case the two mandibles quickly join forming a synostosis that make their separation impossible. They have a vertical portion or mandible branch, and a horizontal or mandible body (Barone, 1997; Clayton, 2005). In the body there are two portions: the rostral, that lodges the teeth and is called the incisive portion of the mandible, and the larger caudal portion serves as a support to the lower premolars and molars, and is the molar portion of the mandible. In the incisive portion, there is a dorsal surface, the lingual surface (that supports the apex of the tongue,) and a ventral or labial surface. The incisive portion of the mandible, has an alveolar arch that houses the six lower incisor teeth, and the molar portion of the body of the mandible, has an external surface or buccal surface, and an internal or lingual surface. This molar portion of the mandible, has its corresponding alveolar arch with its corresponding alveoli that houses the lower premolars and molars. The space between the alveolar arches, of the molar and incisive portions is a part without dental alveoli, which are called the bars. In males of a certain age, canines proliferate (Dixon and du Toit, 2011).

Incisor and canine teeth disorders can significantly affect prehension, function, comfort, horse aesthetics, and its location makes easier for owner and veterinarians to detect, examine, diagnose and treat incisor disorders (Earley and Rawlinson, 2013). Clinical history can include facial swelling, quidding, masticatory abnormalities, halitosis, resistance to biting, or no clinical signs (Earley and Rawlinson, 2013).

Adult horses have four types of teeth: incisors, canines, premolars and molars, in a rostrocaudal order (Barone, 1997). Each type of tooth has certain morphologic characteristics and specific functions; incisor teeth are specialized in prehension and cutting food (Dixon and du Toit, 2011).

The horse deciduous and permanent dental formula by Dixon and du Toit, 2011 is:

Deciduous teeth 2 (Di 3/3, Dc 0/0, Dm3/3): 24 teeth

Permanent teeth 2 (I3/3, C1/1, or 0/0, PM 3/3 or 4/4, M3/3): 36 or 44 teeth

Permanent dentition fully erupts prior to maturity. The teeth normally are long and hard enough to survive for the horse's life because they aren't subjected to prolonged and high levels of dietary abrasive forces that herbivore teeth must contend with. In contrast, long crowned teeth slowly erupt over most of the horse's life, at a rate of two/three millimeters per year, which is similar to the rate of attrition on the occlusal surface of the tooth, provided that the horse is on a fibrous diet. Concentrate diet, reduces the rate of occlusal wear and restricts the range of lateral chewing, thus dental overgrowth can occur (Dixon and du Toit, 2011).

Permanent incisors are formed from separate enamel organs that are derived from lingual extensions of the dental laminae of the deciduous teeth. Consequently, the deciduous incisors are normally displaced labially by the erupting permanent incisors. In all equine incisors, invaginations of enamel epithelium that will later become infundibula develop from the convex aspect of the "bell" into the papilla. The enamel organ in equine incisors is circular on transverse section. In the infundibula, cement deposition proceeds from cementoblasts, that are nourished by vasculature from the dental sac and via openings in the apical aspects of the infundibula (Fitzgibbons *et al.*, 2010).

At the rostral aspect of the incisors, the peripheral cement is usually worn away, thus exposing the shiny underlying enamel. The deciduous incisors often have little overlying cementum and thus appear whiter and shinier than their permanent successors. Incisor enamel is composed almost solely of equine type-2 enamel. Equine incisors are smaller and flatter than cheek teeth, have less support from adjacent teeth and undergo great mechanical stresses during prehension that could readily cause enamel cracks. Therefore, it's not surprising that they are largely composed of equine type-2 enamel

prisms. Equine incisors have just a single pulp and the infundibula is usually incompletely filled by cement (Dixon and du Toit, 2011).

The central deciduous incisors generally erupt during the first week of life, the middle emerge through the gums at four/six weeks and the corners erupt between six/nine months of life (Sisson *et al.*, 1953).

Deciduous incisors are whiter and contain wider and shallower infundibula than their permanent successors, which erupt on their lingual aspect. The deciduous and permanent teeth eruption can be used to estimate the horse's age up to five years old, with a reasonable degree of accuracy (Walmsley *et al.*, 1993).

The upper incisor teeth are in the incisive bone and the lower incisor in the rostral mandible, with the reserve crowns and apices converging towards each other. Incisor teeth are curved convexly on their labial aspect, and taper in uniformly from the occlusal surface toward the apex. With age, spaces eventually develop between equine permanent incisors, but these spaces development is delayed by the medial pressure of the corners on the remaining incisors. The fully developed incisor arcade in a young adult horse has an almost semicircular appearance, which gradually becomes shallower with age, due to alteration of teeth shape caused by progressive wear (Goody, 1983).

The occlusal angle of incisors also changes from almost vertical apposition in the young horse, to an increasing angle of incidence with age. Equine incisors also develop certain wear related macroscopic features that have been traditionally used for estimating age. The infundibulum present in all incisors is termed the "incisal cup". This funnel like enamel structure is oval in shape and *circa* one centimeter deep when the tooth first erupts. However, variations in its depth may cause the infundibulum to wear away more rapidly or slower than normal, and thus make aging difficult. The incisor infundibulum is usually incompletely filled with cement and consequently later becomes filled with food material and appears dark. When the infundibular cavity is worn away, it leaves behind a small ring of the remaining apical aspect of the infundibular enamel, located on the lingual aspect of the tooth, which is called the "enamel spot" (Walmsley *et al.*, 1993).

Due to the slower wear of enamel as compared to dentin, the enamel spot becomes elevated above the occlusal surface. The dental star represents exposure of secondary dentin on the occlusal surface of incisor teeth that was deposited in the former pulp cavity. It appears sequentially in the central, middle and corner incisors. This secondary dentin initially appears as a dark yellow, transverse line on the labial aspect of the infundibulum.

With further tooth wear, it gradually becomes oval in shape and moves toward the centre of the occlusal surface. Variations in incisor teeth appearance can also be due to individual and breed variation, differences in diets, environmental conditions, eruption times, mineralization rates, depth of enamel infundibulum, amount of infundibular cement and the presence of certain stereotypic behaviours, such as crib-biting and wind sucking (Eisenmenger, 1985).

The occlusal surface of individual incisors is elliptical in recently erupted incisors, but with wear, they successively become round, triangular, and then oval in shape. These changes are more apparent in the lower central, than in the middle, and corner incisors. (Richardson *et al.*, 1994, 1995)

TEETH SURFACES AND DIRECTIONS IN THE MOUTH

In this work, we use the terminology according to the American Veterinary Dental College (AVCD / 2009):

Vestibular, is the correct term referring to the tooth surface facing the vestibule or lips; buccal and labial, are acceptable alternatives.

Lingual, the surface of a mandibular or maxillary tooth facing the tongue, is the lingual surface. Palatal, can also be used when referring to the lingual surface of maxillary teeth.

Mesial and distal, terms applicable to tooth surfaces. The mesial surface of the first incisor is next to the median plane, on other teeth it's directed toward the first incisor. The distal surface is opposite from the mesial surface.

Rostral and caudal, are the positional and directional anatomical terms applicable to the head in a sagittal plane. Rostral, refers to a structure closer to, or a direction toward the most forward structure of the head. Caudal, refers to a structure closer to, or a direction toward the tail.

Deciduous dentition, period during which only deciduous teeth are present.

Mixed dentition, period during which both deciduous and permanent teeth are present.

Permanent dentition, period during which only permanent teeth are present.

NOMENCLATURE AND NUMERING TEETH

The incisors will be referred as right or left, maxillary or mandibular, first, second, or third, numbered from the midline (AVDC, 2009). The existence of conventional anatomical teeth names as well as various teeth numbering systems are recognized.

The correct teeth anatomical names are right or left; maxillary or mandibular; first, second, third or fourth; incise, canine, premolar, molar, as applicable, written out in full or abbreviated.

The **modified Triadan system** is presently considered to be the teeth numbering system of choice in veterinary dentistry (Floyd, 1991).

Both use of anatomical names and modified Triadan system, are acceptable for recording and storing veterinary dental information (AVDC, 2009).

The modified Triadan System of the dental nomenclature uses three digits to identify each tooth. The first digit refers to the quadrant, with 1 for upper right, 2 for upper left, 3 for lower left and 4 for lower right (Floyd, 1991).

The deciduous teeth are similarly identified using the prefix 5 to 8 for the four quadrants. Adult horses have twelve incisors in total, six in each arcade (Dixon and du Toit, 2011).

AGING

Equine incisors erupt lifelong, although their intrinsic growth ceases at the average age of seventeen (Muylle *et al.*, 1999).

The most appropriate teeth for aging horses are the lower incisors. When estimating a horse's age by its incisors, the eruption dates and the changes in appearance of the occlusal surfaces are the main criteria. Neither is wholly dependable but the first is the more reliable, although limited in application to younger animals. The second may be used throughout the life span but becomes increasingly inaccurate with age (Dyce *et al.*, 1996).

The deciduous incisors are smaller than the permanent ones. The surface of their crown is white and presents several small longitudinal ridges and grooves. The occlusal tables of deciduous incisors are oval in the mesiodistal direction (Muylle, 2011).

Permanent incisor teeth are larger and more rectangular than the deciduous incisors. Their crown surface is largely covered with cement and has a yellowish aspect.

The upper incisors, generally present two distinct longitudinal grooves on their labial surface, the lower incisors have only one clearly visible groove (Muylle, 2011).



Fig. 1 and 2 – Permanent incisors eruption in three-year old Lusitano horses.

The dental star, is a yellow/brown structure on the occlusal surface situated between the labial edge of the incisor and the infundibular cavity or “cup”. It consists of secondary and tertiary dentin that occludes the pulpal chamber when it risks being exposed by wear. In young animals, the dental star appears as a linear stripe because the occlusal end of the original pulp cavity is not conical but elongated in a mesiodistal direction. With age, dental stars become oval, and then round and move towards the center of the occlusal table. These progressive patterns reflect cross sections through the stuffed pulp cavity at various levels (Muylle, 2011). The pulp is located on the incisors labial aspect and varies in shape (oval) depending on its tooth position (Earley and Rawlinson, 2013)

The infundibulum, is an enamel infolding in the occlusal surface of the equine incisors. The superficial half of the infundibulum is empty or filled with food particles. This part is called the “cup”. The bottom of the infundibulum is filled with cement. When wear has brought this infundibular cement layer into the occlusal surface, the cup is filled in or has disappeared. The exposed cement core and the surrounding enamel ring are called the “mark”. The shape of the mark generally corresponds to the contour of the occlusal table of the incisor. In young horses, marks are oval in the mesiodistal direction. When wear progresses, marks become smaller and rounder and move lingually on the occlusal surface. With age, the cement of the infundibular bottom wears away and

eventually the remaining enamel spot disappears from the occlusal surface (Muylle, 2011).

Due to extensive wear, the sequential shapes of the occlusal tables represent the cross sections of the incisor teeth at various levels. The sequence ranges from oval in the mesiodistal direction, to trapezoid and triangular, and finally to oval in the labiolingual direction (Muylle, 2011).

When incisors are viewed in profile, the angle between the upper and lower incisors changes with age. In young horses, the upper and lower incisors are positioned in a straight line with each other. With age, the occlusal portions of the crowns wear off, and we look at different cross sections of the crown shape in profile. The angle between upper and lower incisors becomes, therefore, increasingly acute. The lower incisors are the first to obtain an oblique position followed at a later date by the upper incisor teeth (Muylle, 2011).

Many standard textbooks dealing with aging of horses suggest that the above-mentioned characteristics give an accurate indication of a horse's true age. However, some reports are inconsistent in their guidelines and show large discrepancies in the dental features described at specific ages. A possible explanation for the non-uniformity of existing guidelines, is the lack of evidence that any system was used to validate an author's recommendations for aging (Richardson *et al.*, 1994).

Most standard texts don't provide exact data concerning breed, sex and nutrition of the examined horses. However, anatomical, physiological, environmental, and behavioural differences between individuals ensure differences in rate of equine dental wear (Muylle, 2011).

In the Mini-Shetland pony, eruption of the middle and the corner deciduous incisors is retarded. The middle incisor, starts erupting at the age of four months, whereas the corner incisor breaks through the gums between twelve and eighteen months of age (Muylle, 2011).

The upper and lower permanent horse incisors erupt almost simultaneously. In some horses shedding begins with the maxillary, in others with the mandibular incisor teeth. Arabian horses shed their central, middle, and corner incisors at 2.5, 3.5 and 4.5 years of age, respectively. In Standardbreds and in Belgian draft horses, shedding generally occurs later, namely at nearly three, four and five years of age.

In Mini-Shetland ponies, eruption of the permanent incisors is still further delayed by two or three months (Muylle, 2011).

DENTAL IMAGING

Imaging is an extremely important diagnostic tool for evaluation of equine dentition, particularly for those parts of the teeth and associated structures that can't be evaluated during oral or endoscopic examinations. Radiography, is still the most widely used and accessible diagnostic technique for veterinarians in general practice and the adoption of digital radiography in many equine practices has undoubtedly resulted in improved image quality. Radiography, produces a two-dimensional image of a three-dimensional structure, and therefore, superimposition of the anatomically complex structures of the equine skull can present challenges to radiographic interpretation in some cases (Butler *et al.*, 2008). In recent years, the use of three-dimensional imaging techniques, particularly computed tomography (CT), has become increasingly widespread (Solano and Brawer, 2004). These techniques have led to significant improvement in our ability to accurately diagnose disorders of the equine dental structures and the anatomical regions that are closely associated with them, by their ability to produce high resolution images in multiple planes, and three-dimensional reconstructions of areas of interest (Barakzai 2011, Novales y Manso, 2014, Novales *et al.*, 2018).

RADIOGRAPHY

Low exposure, is required for intraoral incisor radiographs compared with that needed to image the cheek teeth. The plate should be placed between the incisors, as far caudally as possible, and held in position using long handled trimmer testers, with the plate held at a distance from the assistant. The x-ray beam is directed at 60–80° from the dorsal plane, depending on the conformation of the incisors, using a rostradorsal caudoventral oblique to image the maxillary incisors/canines, and a rostroventral caudodorsal oblique to image the mandibular incisors/canines. The beam should be centered on the centrals and collimation should include the rostral and lateral aspects of the lips. If necessary, the x-ray beam can also be angulated slightly from left to right to try and highlight the apices of incisors, at the edges of the incisor arcade without superimposition of adjacent teeth (Barakzai, 2011).

Although the superimposition of incisors of the right and left sides usually makes individual incisors impossible to distinguish, lateral incisors projections can be useful for identification and orientation of dysplastic or retained incisors, or for assessment of fractures of the premaxilla, or rostral mandible. Adding a slight 5–10° rostrocaudal angulation to a lateral radiograph centered on the canines, can provide left and right side separation, and allows individual reserve crowns and apices examination of these teeth (Barakzai, 2011).

NORMAL RADIOGRAPHIC ANATOMY

Deciduous incisors are more radiolucent, have shorter reserve crowns and roots, and a smaller cross sectional area than their permanent counterparts (Barakzai, 2011).

The reserve crowns and apices of the permanent incisors converge towards each other on an intraoral projection, and there may be partial superimposition of the reserve crowns and roots of the *Triadan* middle and corner incisors on a true dorsoventral radiograph. If the roots and reserve crowns of these teeth are to be examined in detail, a slight angulation of the x-ray beam can be used to prevent this superimposition. The incisor teeth gradually change their angle throughout life, the occlusal angles change from almost vertical in a young horse to an increasing angle of incidence, and the occlusal surface becomes more triangular in cross section with advancing age. In recently erupted incisors, the infundibula can be recognized on the obliquely projected occlusal surfaces and in those that have been longer in wear, traces of infundibular enamel and cement may be visible as thin, elliptical conical radiodense shadows (Gibbs, 2005).

The pulp cavities of the incisors should be evident as curvilinear radiolucent structures in the middle of these teeth (Barakzai, 2011).



Fig.3 (A, B and C) – Mandible Intraoral radiographs: note the triangular cross section of the occlusal surfaces of the central and middle teenage horse incisors in (C), comparatively to a yearling (A) and a three-year old horse (B). In (C), there is a vestigial dental remnant lying just caudal to 303 (Barazkzai, Equine Dentistry. 3rd ed.).

COMPUTED TOMOGRAPHY

Over the past decade, computed tomography (CT) has been increasingly used in equine examinations and is now available in many equine referral hospitals across the globe (Solano *et al.*, 2004).

CT is a valuable diagnostic tool that provides detailed cross-sectional images of tissues, providing good bone and soft tissue contrast, and eliminating the tissue superimposition problem (Heufelder *et al.*, 1994).

Equine head CT examination is indicated in cases where clinical and radiographic examinations are inconclusive (Novales *et al.*, 2013; Novales y Manso, 2014), when the exact location and extent of a lesion is needed for detailed therapy planning, such as for less invasive surgery, or radiation, or local chemotherapy, and also to accurately monitor cases following treatment (Annear *et al.*, 2008).

CT has proven to be very useful in fractures, dental disease, infection and neoplasia diagnosis of the equine head. Despite the fact that CT is increasingly used for equine dental pathology diagnosis, little information has been published to date on the appearance of equine dental tissues, in health and pathology. For equine dental imaging, a soft tissue algorithm is useful for soft tissue structures, followed by a reconstruction in a bone algorithm from the raw data, to allow detailed evaluation of dental and bony structures. The acquired sectional images can be reformatted in various two-dimensional planes or three-dimensional models (Novales *et al.* 2018). On transverse CT images of

normal teeth, the peripheral layer of cement is hypodense compared to adjacent enamel. The hyperdense zone of peripheral enamel extends from the erupted crown towards the apical region. Dentin, is less radiodense than enamel, and surrounds the hypodense pulp cavities (Puchalski, 2006).



Fig.4 – Standing horse head CT (A and B / Veterinary Computed Tomography 2011), and under general anaesthesia in dorsal recumbency (C / Equine Dentistry 2011).

MATERIAL AND METHODS

A study was made from a horse incisor pieces collection (with the incisive bone and mandible) of the Department of Anatomy and Pathology Anatomy of the Faculty of Veterinary Medicine of Cordoba, Spain. This collection consists of 52 pairs of incisors, of all ages. For this work we have only studied 17 anatomical pieces of young horses, whose numbers are not necessarily consecutive.

The horse breeds and sex are unknown, and all ages were determined by inspection by Carla Manso, diplomated by the European College of Veterinary Odontology. The ages were determined by previously published age guidelines (Richardson et al., 1994, 1995; Muyelle 2011). None of the anatomical pieces presented dental pathologies.

The anatomical pieces were named with the name “ANA” (Anatomy), numbered from 1 to 52, photographed on all their sides, and a radiological, and computed tomography (CT) study was performed.

Radiological studies were made with a fixed equipment of rotating anode, Odel brand, model C306-20. For the radiographs development, an indirect digital computerized

radiology (CR) equipment, Fujifilm brand, was used. Two projections were used per mandible, right lateral (65-70 kVp and 6 mAs) and intraoral (70 kVp and 2 mAs). The right lateral radiograph, was performed with the primary x-ray focus perpendicular to the sagittal plane of the head, and centered on the apex of the incisors (Henry *et al.*, 2016). For intraoral radiographs, the plates were placed inside the mouth as caudally as possible. The x-ray primary focus was inclined in rostroventral caudodorsal oblique, to image the mandibular incisors. The angle of inclination was about 60-80° on the ventral plane (Barakzai, 2011). The bisector angle technique (Baratt, 2011) was used to obtain a clearer image of the area.

CT studies were carried out with a high-speed dual equipment, General Electric brand, in helical mode with contiguous cuts of 0.6 mm and a pitch of 1,5. 120 kV and 30 mA were used, with bone algorithm, and matrix size of 512 x 512. The window width was 2,300 Hounsfield Units (UH) and the window level was 300 UH. The mandibular incisors, were scanned from the first incisor to the canines. Transverse cuts and reformatting were made in the ventral and sagittal planes, as well as three dimensional reconstructions.

Radiographs and CT images, were analyzed in DICOM, with Horos © program (free version for Apple 64 bit). Following the current indications of the AVCD (Barrat, 2019), the mandible intraoral radiographs were positioned with the teeth facing upwards and the left side to the right of the image. Until recently, each one put it as he wanted, even in books. The current trend is to put the maxillaries down and the mandibles up.

From each incisor was evaluated:

- 1) The anatomical piece;
- 2) Radiographs in the right lateral and intraoral views;
- 3) The CT in the three planes of court: axial, dorsal and sagittal, and
- 4) The three-dimensional reconstruction, highlighting the bone or the tooth, depending on the case.

Evaluation criteria:

1. The presence of all the incisors and their degree of eruption, depending on the estimated age. This included the existence of deciduous, permanent or both teeth simultaneously;

2. The appearance of dental germs, their location and degree of evolution, the degree of opening of the apex, the infundibulum (size, shape and present alterations), its enamel, the pulp canal (size, shape and alterations), dentin and the cement; and

3. Finally, the degree of development was evaluated in relation to the age and other incisors present (both deciduous and permanent).

RESULTS

We have studied a total of seventeen anatomical pieces of horse incisors, with ages between 1 and 4,5 years old. The anatomical pieces were divided in groups and identified as “ANA”:

Group I. 1-2.5 years: twelve anatomical pieces.

Group II. 2.5 years: one anatomical piece.

Group III. 3.5-4 years: four anatomical pieces.

Depending on the ages and the degree of development of the dental germs, the anatomical pieces studied could be divided into the following phases:

Group I. From 1 to 2.5 years. Only deciduous incisors in the mouth

In this age period, although in the inspection of the oral cavity only the deciduous incisors appear erupted: centrals (701, 801), middle (702, 802) and corners (703, 803), in the radiographs and CT already appears the dental germs of the central permanent (301 and 401). In intraoral radiograph, the deciduous incisors are small, rounded, with their mesiodistal oval occlusal surfaces and superficial infundibula. They have short reserve crowns, are small in cross section, contain abundant enamel and have short and wide

apices. At these ages the radiographs already show the dental germs of the central permanent incisors (301 and 401), that are shown as two radiolucent areas (in the radiographs) or hypodense (in the CT) in the lingual position to their deciduous (701 and 801). The comparative study of radiographs and CT allows to observe an identical development of both dental germs, distinguishing four different stages of development:

Stage 1. Dental germs of central permanents (301 and 401) without content (ANA-3; ANA-25). They are seen as two radiolucent (on radiographs) or hypodense (on CT) areas with rounded morphologies and no internal content (Fig.5 and 6);

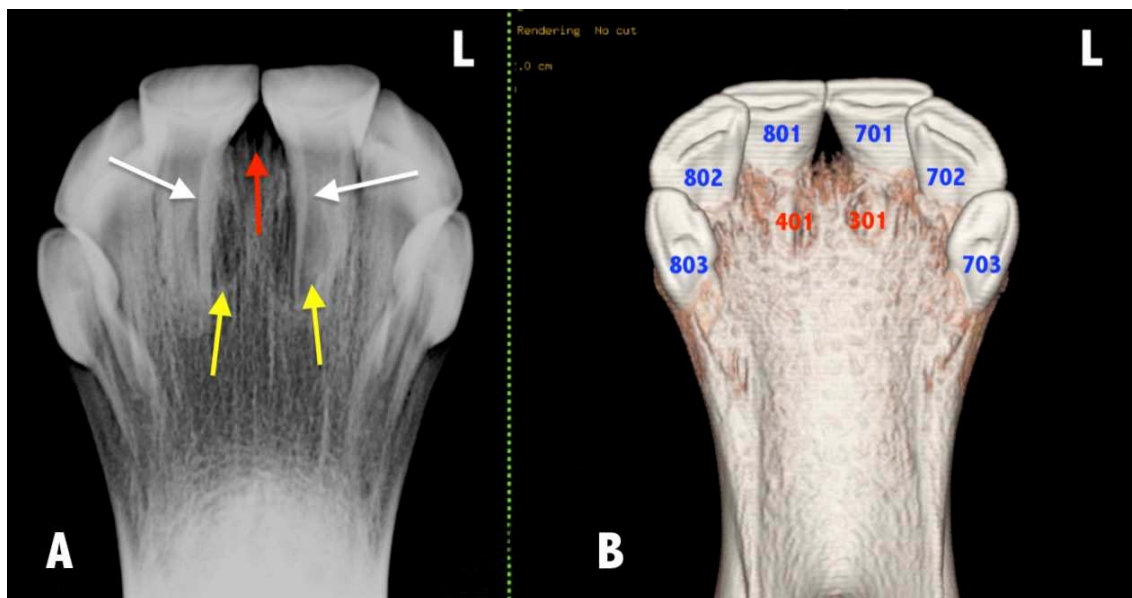


Fig.5 - Intraoral (occlusal) radiograph of the mandibular region (A) and the corresponding three-dimensional image (B). All the deciduous incisors are erupted. We can see the empty dental germs of the central permanent incisors (301 and 401) without their inner contents (yellow arrows). The mesial surfaces of the 701 and 801 reserve crowns aren't straight but open laterally (white arrows), which allows to clearly see the alveolar crest between these two teeth (red arrow). Left side (L). Estimated age between 1 and 2.5 years, closer to 1 year age (L: Left side).

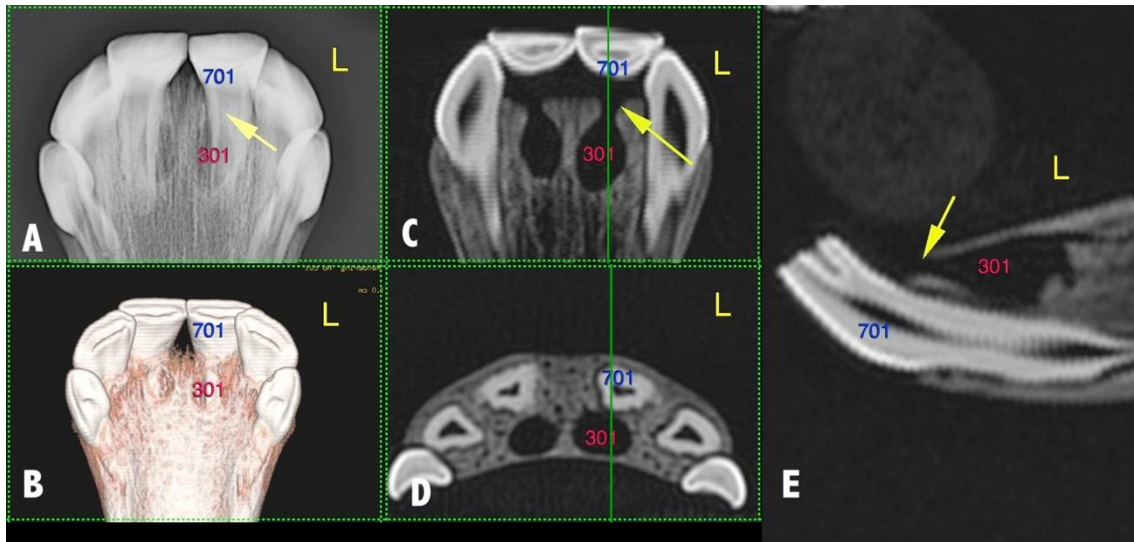


Fig.6 Intraoral radiograph (A), three-dimensional image (B) and cuts in the dorsal (C), axial (D) and sagittal (E) planes. The green line (in C and D) indicates the level at which the sagittal cut (E) was obtained. The 301 and 401 empty dental germs and their communication with the lingual surface of the mandible are appreciated. This communication can be seen well in the CT images but it's difficult to see in the intraoral radiographs because it's covered by the reserve crowns of the corresponding deciduous incisors. Estimated age between 1 and 2.5 years, closer to 1 year age (L: Left side).

Stage 2. Dental germs of central permanent (301 and 401) with amorphous enamel content (ANA-2; ANA-4). The germs elongate caudally and begin to show amorphous enamel, rather rounded, inside. In the CT, the dental germs don't exceed the corresponding deciduous (701 and 801), and the enamel is in contact with the lingual side (Fig.7);

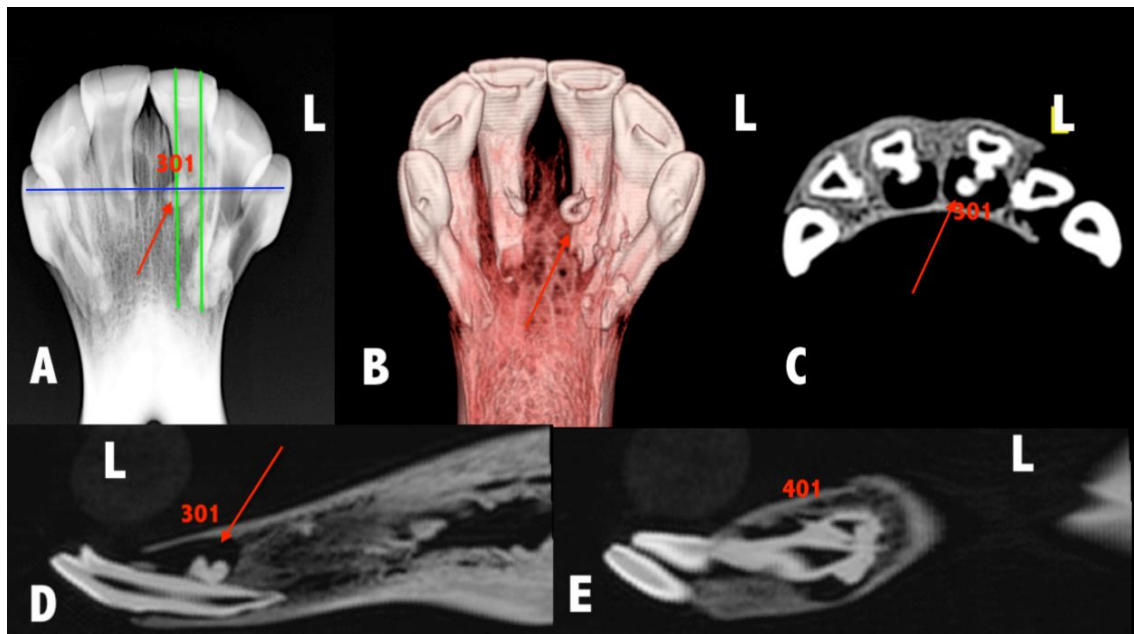


Fig.7 - Intraoral radiograph (A), three-dimensional image (B) and cuts in the axial (C) and sagittal planes (D and E). The blue line (in A) indicates the level at which the axial cut (C) was obtained. The green lines (in A) indicate the level at which the sagittal cuts were obtained (D and E). Inside the dental germs of 301 and 401 enamel with amorphous content inside it's appreciated. It's also forming an enamel rest in 401 (L: Left side).

Stage 3. Dental germs of central permanents (301 and 401) with conical tooth formation (ANA-1, ANA-19, ANA-27, ANA-30). The radiographs already show peripheral and infundibular enamel that begin to give a conical shape to the tooth. The CT shows layers of peripheral and infundibular enamel still in very thin concentric layers that already surpass the reserve crown of the corresponding deciduous (701 and 801) (Fig.8);

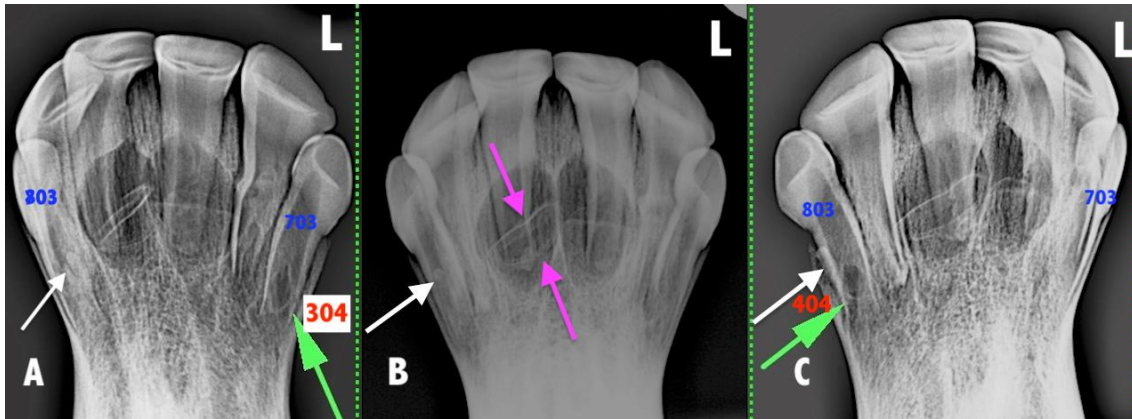


Fig.8 - Intraoral radiographs (A, B and C), with obliques A and C taken to highlight the lateral portions of the mandible. The 301 and 401 already show an advanced development of the dental germs interior with the beginning of the conical infundibulum formation (pink arrow). On the roots of the deciduous corners (703 and 803) the rudiments of the canines are superimposed. These teeth don't have deciduous precursors and, therefore, are numbered as permanent 304 and 404, although sometimes may be considered as 704 and 804.

Stage 4. Dental germs with central permanents (301 and 401) in very advanced phase of development (ANA, 17; ANA-20; ANA-31). Radiographs show the conical shape of the infundibulum and especially the dentin of the occlusal surface of the infundibulum. CT already shows in the sagittal plane that the apical portion of the peripheral enamel of the tooth and the infundibular enamel, reach the same level caudally. In the axial plane you can see a very advanced process of tooth formation with well developed layers of peripheral cement, peripheral enamel, pulp cavity, infundibular enamel and an infundibulum even without its inner contents. Only when the dental germs of 301 and 401 have lengthened and show an advanced evolutionary development, leave room for the appearance of the dental germs of the middle (302 and 402) still very early, as radiolucent areas (on radiographs) or hypodense (on CT) distal to the centrals and still without content (Fig.9 and 10).

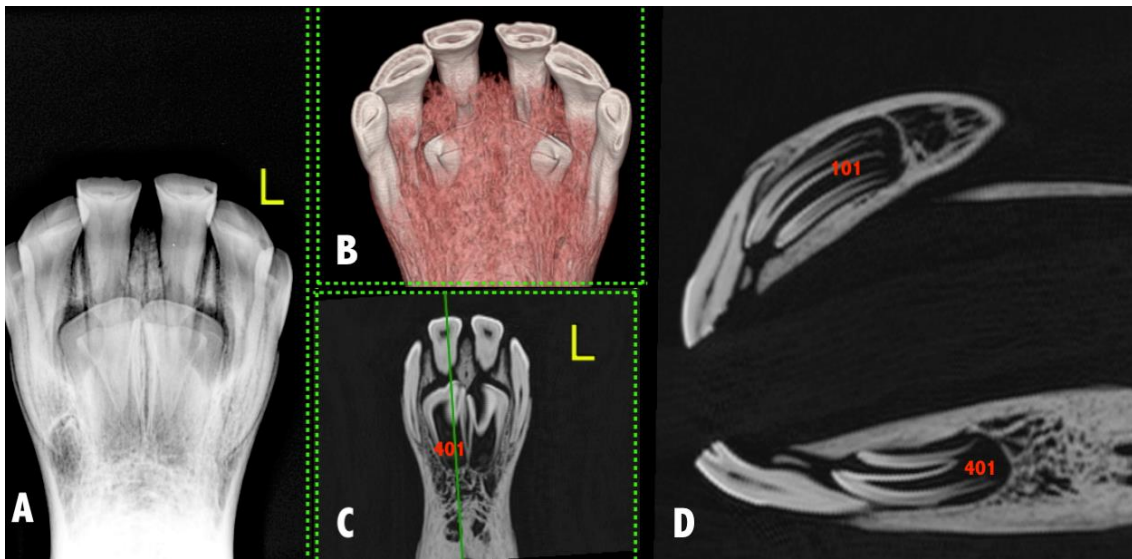


Fig.9 - Intraoral radiograph (A), three-dimensional image (B) and dorsal section (C). The green line in (C) indicates the level at which the sagittal section has been obtained in (D). It's shown as the centrals (301 and 401) are very developed. Although the maxilla hasn't been included in this work, a cut is made at 101 level to see that they show a lower development level in the mandibular incisor.

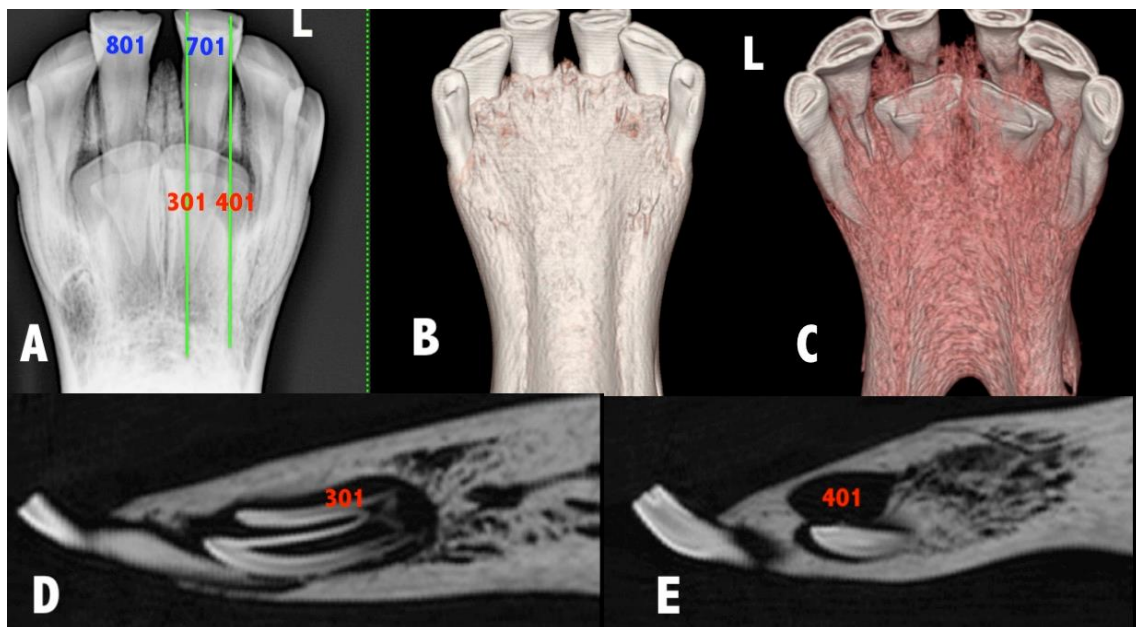


Fig.10 - Intraoral radiograph (A) and three-dimensional images highlighting bone (B) and dental tissue (C) where the degree of ossification of 301 and 401 is observed. The external surface of the bone observation (B) doesn't allow to see this detail. The green lines in (A) indicate the sagittal cuts obtained at 301 (D) and 401 (E) level. Once the permanent central incisors (301 and 401) show a great dental development, the dental germs of the middle permanents (401 and 402) begin to develop even without erupting.

Group II. 2.5 years. Centrals eruption (301 and 401) (ANA-18).

Although the oral examination only shows the eruption of the central incisors (301 and 401), the intraoral radiograph already shows the great internal development of the same, with a large pulp cavity and an infundibulum that already occupies half of the occlusal surface of the tooth. It's also possible to identify an advanced development of the middle incisors (302 and 402).

In this phase, the CT offers great detail about the relationship between deciduous and permanent incisors at the central and middle level (Fig.11 and 12).

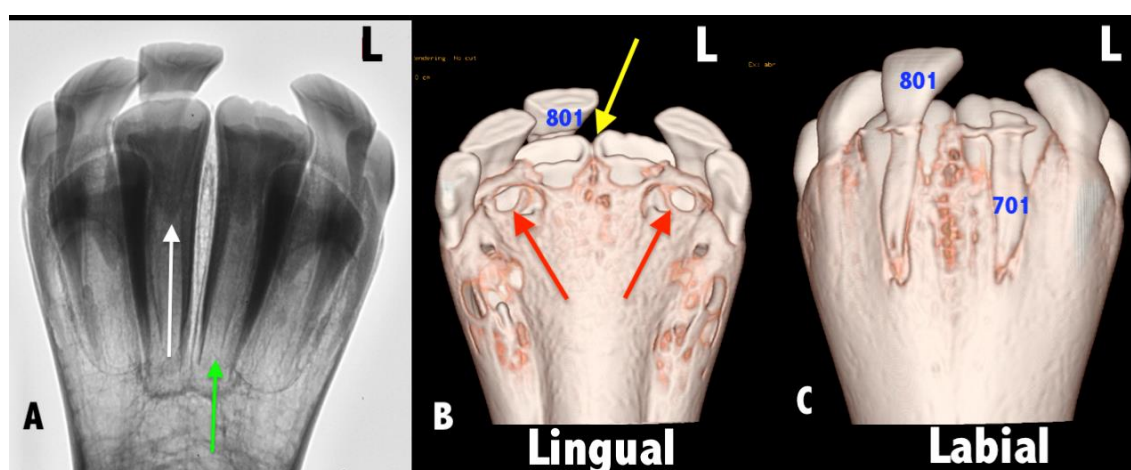


Fig.11 – Intraoral negative radiograph (A) and three-dimensional reconstruction by the lingual (B) and labial (C) surfaces. The radiograph offers a flat image of a three-dimensional structure. It's apparent how the permanent centrals (301 and 401) are starting to erupt and are expelling the corresponding deciduous centrals (701 and 801). The reconstruction shows how the degree of expulsion doesn't occur exactly symmetrically. From the occlusal surface, the exit holes of the dental germs of the middle permanents can be seen (302 and 402) (red arrows). From now on, it's difficult to see the alveolar crest (yellow arrow) on radiographs. In the permanent centrals (301 and 401) the infundibulum is well developed and occupies the occlusal half of the tooth. There aren't roots in the apical portion. Negative radiograph, shows a large, inconsistent, small trabeculum typical of a remodeling bone.

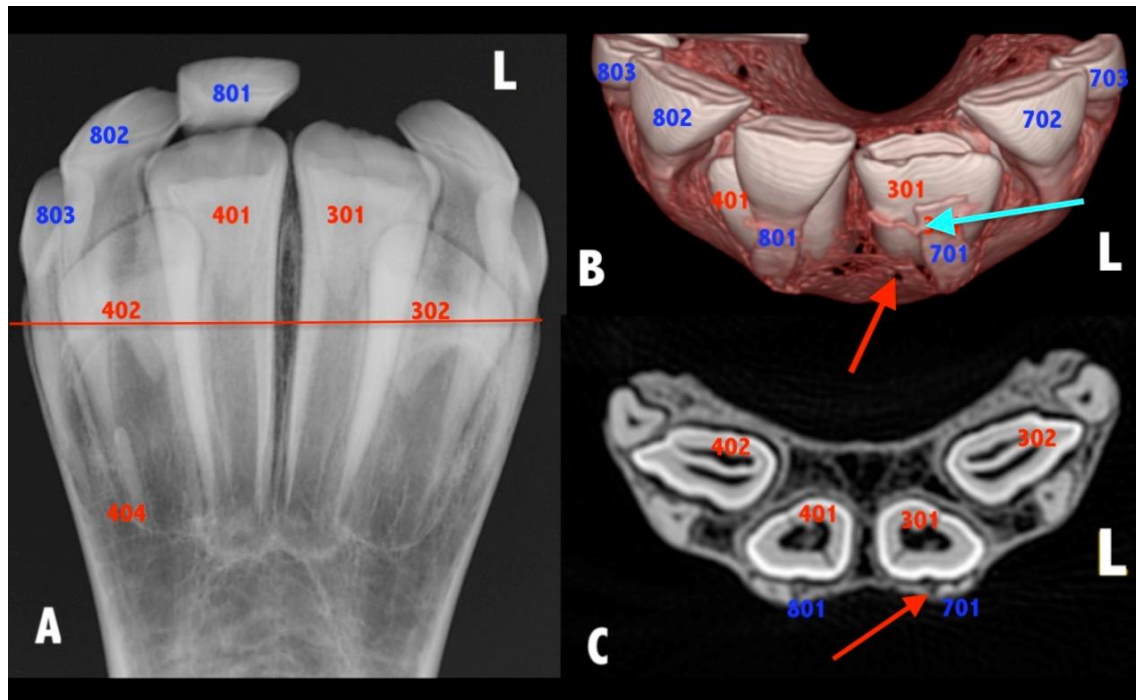


Fig.12 - Intraoral radiograph (A), three-dimensional image, on the labial surface of the mandible (B) and axial section (C), at the level indicated by the red line on the radiograph. The most important fact is that while the radiograph doesn't show 701, the central incisors (701 and 801) are close to the eruption and remains a remainder of 801 without being removed on it. However, the three-dimensional image still shows a remnant of 701 that hasn't yet been removed. We can also see a very rudimentary 401.

Group III. 3.5-4 years. Corners (303 and 403) and canines (304 and 404) eruption (ANA-14; ANA-15, ANA-24; ANA 28).

With the central incisors (301 and 401) quite developed, the middle (302 and 402) are expanding the amount of enamel on the occlusal side, extending its labial surface and expanding distally. This makes the amount of enamel in the reserve crown thicker on the distal surface than on the labial surface. The corners (303 and 403) close to the eruption are folding on their mesial sides and these changes in morphology are appreciable in intraoral radiography.

The CT allows observing, the level of development of each tooth, especially in the axial plane, that shows very clearly the differences between them. In this phase the canines, quite formed, are close to the eruption (Fig.13, 14 and 15).

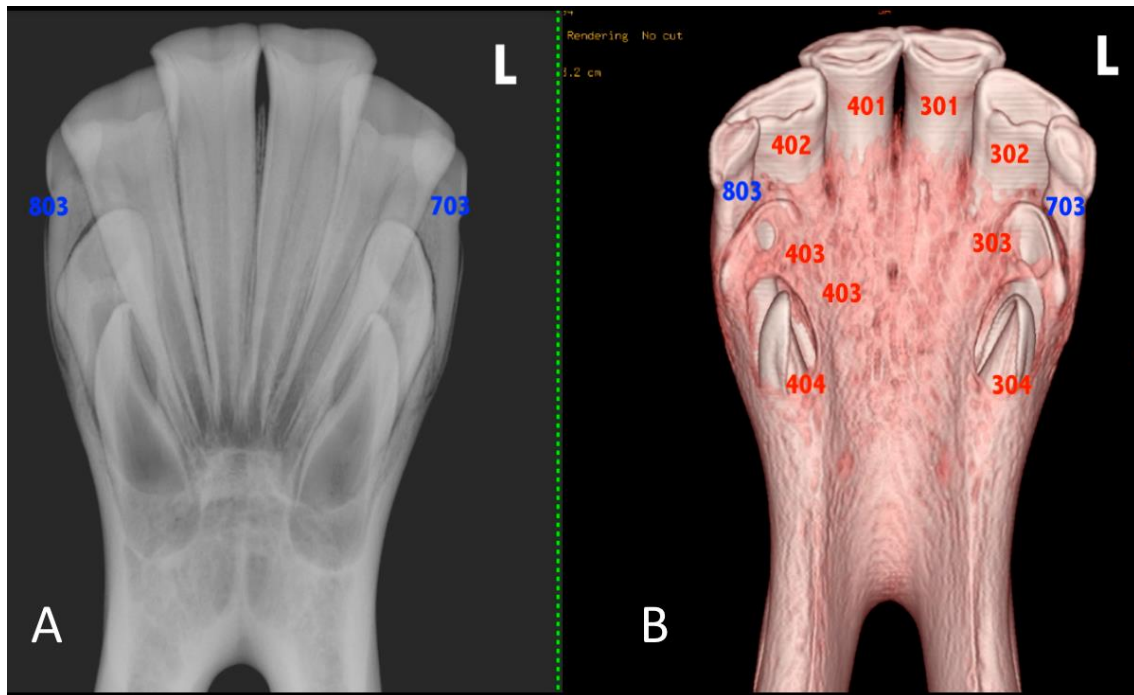


Fig.13 – Intraoral radiograph (A) and three-dimensional reconstruction (B). It´s seen how the central (301 and 401) and middle (302 and 402) permanents are already erupted, the corners (303 and 402) are beginning to erupt and press from the lingual surface to the corresponding deciduous (703 and 803). The canines are also erupted (304 and 404) corresponding to a stallion.

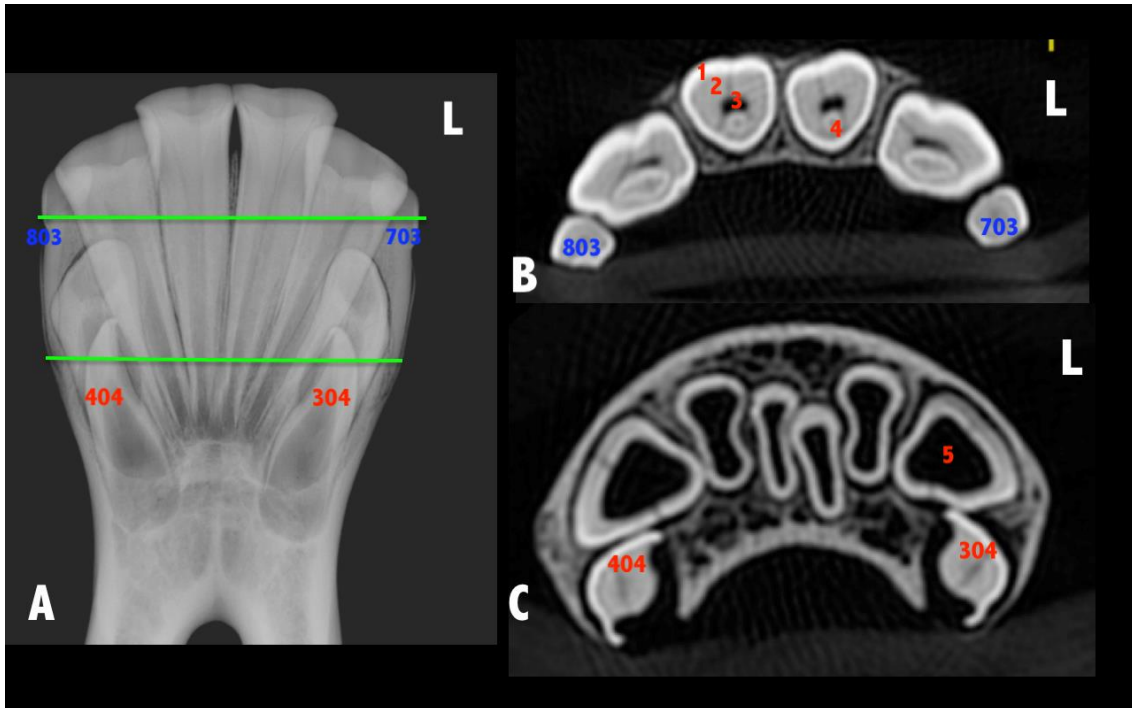


Fig.14 - Intraoral radiograph (A) and axial sections at 703 and 803 (B), and 304 and 404 (C) levels. The permanent internal development is well-developed with 1) peripheral enamel, 2) peripheral cement, 3 and 5) pulp cavity, and 4) infundibular enamel. It can be seen that in the closest portions to the apex, the pulp cavity is highly developed. There aren't still no roots.

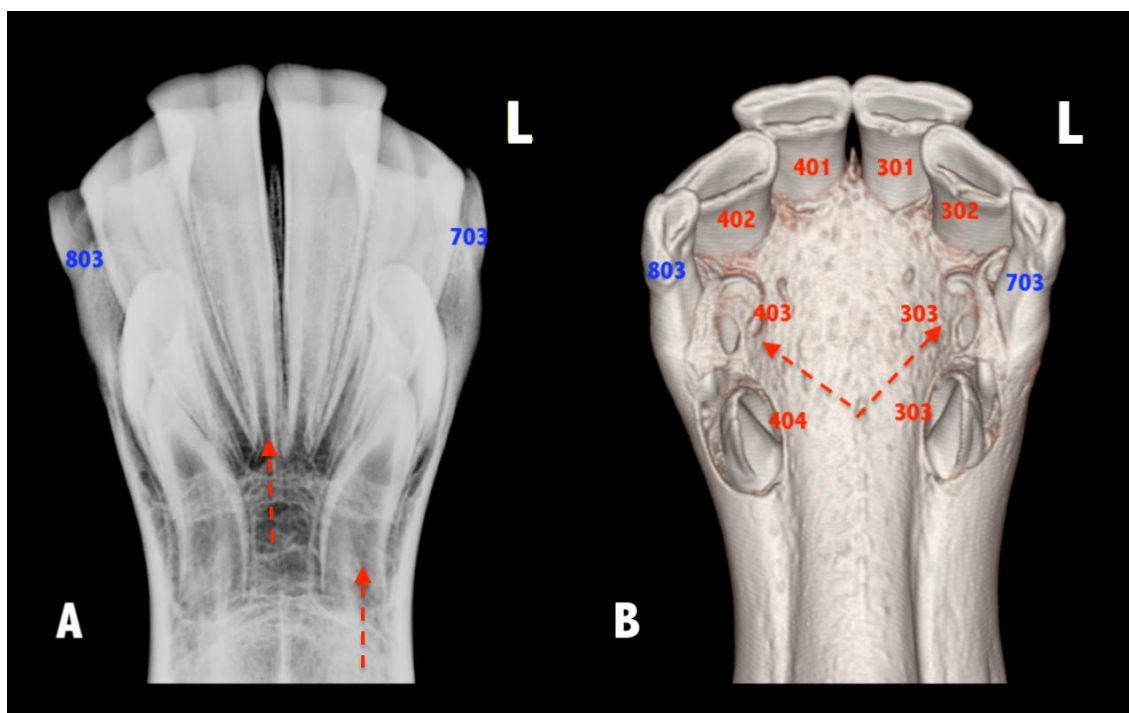


Fig.15 – Intraoral radiograph (A) and three-dimensional reconstruction (B). Although the dental roots haven't yet been formed, the pulp cavity is already very small in the centrals, unlike the canines (arrows). The permanent corners (303 and 403) close to the eruption, are about to expel the deciduous ends (703 and 803) that are already very little developed.

DISCUSSION

One of the work difficulties is that we didn't know the horse breeds of the anatomical pieces studied. It isn't usually an important factor when determining age, although some variants have been described in certain breeds and ponies (Muyllé, 2011).

We also didn't know the sex of the horses studied, although we were able to serve as a reference the canine teeth. Stallions and geldings normally have four permanent canine teeth: two in the maxilla (104, 204) and two in the mandible (304 and 404), that erupt in the interdental space between 4.5-5 years (Muyllé, 2011), or between 4 and 6 years (Dixon and du Toit, 2011). Some authors believe that they're normally present in most horses at the age of 5 years (Easley, 2011). The lower canines are placed more

rostrally than the upper ones, thus avoiding contact of their occlusal surfaces (Dixon and du Toit, 2011).

Canines are absent or rudimentary in mares (Dixon and du Toit, 2011; Muylle, 2011), with a prevalence of between 7.8 and 28% (Dixon and du Toit 2011).

Since we have studied horses below the development time of the canines, the sex determination hasn't been possible. However, in the horses highest age group we have seen some cases of very small elongated structures in the canine position. These cases could be mares with very rudimentary canines (304 and 404), or what Dixon and du Toit (2011) call: canine teeth deciduous (504, 604, 704 and 804), which are vestigial structures in the form of spikes of 0.5 to 1 mm in length, that don't erupt on the level of the gum. These structures appear very clearly on radiographs and CT, and could be vestigial canines (704 and 804) or very rudimentary mare canines (304 and 404). We have also seen some cases with canines that have already erupted. However, the acceptance that there aren't deciduous canines is general, which is why we have considered that in the event that they appeared they were in mares, regardless of their numbering.

The third work difficulty that we had, was to determine the age. Our cases have all been determined by a European Diplomated in Equine Dentistry (Carla Manso) with enormous experience in the determination of horse ages, but we are aware of the limitation that implies the ignorance of the exact age of the mouths studied.

When determining the horse age, the incisors evaluation offers an approximation to it, rather than an exact determination (Muylle, 2011). The cases we have studied correspond to the generally accepted eruption for the central permanent incisors at 2.5 years, the middle incisors at 3.5 years and corners at 4.5 years (Lowder, 1998; Butler *et al.*, 2008; Muylle, 2011; Tremaine, 2012), although variants have been described for some breeds and ponies (Muylle, 2011).

The corners deciduous eruption has been established at 6-9 months (Lowder, 1998; Butler *et al.*, 2008; Muylle 2011; Tremaine 2012), or somewhat later, between 12 and 18 months of age (Muylle, 2011), with an age period, between the eruption of the corner deciduous and the central permanents, where the ages are difficult to determine by

external exploration. It's interesting the period between 1 and 2.5 years, that elapses between the eruption of the corner deciduous and the central permanent ones. Although this age is difficult to determine by dental specialists, radiographs and CT's show a very different dental development within this period, being this information of interest to further determine the dental development of the horse.

Barrat (2013) considers that deciduous and permanent are similar, the cement extends supragingival and the enamel can be identified throughout the extension of the reserve crown. The enamel that covers the infundibulum, is easily identified, and the pulp chamber is compressed labially by this structure.

All the mouths studied corresponded to young animals, and consequently, none of the permanent incisors of the study had reached its maximum length, which is reached at 2-3 years post-emergence, therefore at the ages of 6 years (centrals), 7 years (middle) and 8 years (corners) (Muylle *et al.*, 1999). They also showed no roots, that don't form until 5 years of age (Muylle *et al.*, 1999), covering the enamel all the surface from the occlusal extremity to the apex (Muylle *et al.*, 1999). In general, in our mouths, the right and left arches eruption was similar, with asymmetries in some cases.

Unlike adult horses, there isn't space between the permanent incisors in young horses (Dixon, 2011). The supporting structures of the tooth (lamina dura denta, alveolar ridge, space of the periodontal ligament, cancellous bone) can be seen in the intraoral radiographs in the apical portions of the middle and corner incisors, but not in the central ones since at these ages they present very widths that overlap each other. They're only seen in very early stages, when the dental germs of the central incisors are underdeveloped and because the deciduous of the centrals are opened by their mesial surfaces.

The embryological description of the dental germ development is well explained in the bibliography (Dixon and du Toit, 2011; Tremaine, 2012), but not the young horse teeth evolution, in radiographs and CT's, lacking images that accompany it.

The exfoliation of the deciduous superficial surfaces is stimulated by the eruption of the mature permanent dentition. During a certain time, the arcade will contain teeth in different degree of maturation producing a variable radiographic appearance (Tremaine,

2012). Knowledge of the horse's age especially up to 5 years of age, is essential, when interpreting dental radiographs (Tremaine, 2012). These physiological processes usually occur symmetrically in the left and right arches (Tremaine, 2012).

During the studied period, all the horses had a mixed dentition (with deciduous and permanent incisors) (Barrat, 2011), which is maintained until 4.5-5 years of age (Barrat, 2011), with the permanent ones erupting lingually to the deciduous (Barrat, 2011). The deciduous incisors are more radiolucent, have shorter reserve crowns, and a smaller cross section than the corresponding permanent ones (Barkzai, 2011).

It's well known in radiography, that the radiographic image is larger than the real image. However, given the curvature of the incisors, if the actual size of the incisors is compared with the actual size of each tooth, in the radiographs they appear shorter than they are in reality. The actual incisors are around 1cm, with the largest differences between the central incisors (Muyllé *et al.*, 1999). This concept is important when performing dental repairs and taking decisions about the radiographs.

The developing dental germs produce circular radiolucent areas (in the radiographs) and hypodense (in the CT's) that increase in size and fill up the tooth in formation. These zones appear progressively for the central incisors (301 and 401), middle (302 and 402), and corner incisors (303 and 403). These dental germs on oblique radiographs, can overlap reserve crowns and produce diagnostic errors, especially if oblique radiographs of the mandibular region are made. However, the CT allows a perfect identification of all of them. The dental germs of the middles (302 and 402) don't appear until the centrals (301 and 401) are already developed internally and before the eruption occurs.

Each dental germ of the permanent incisors, produces an exit orifice to the lingual surface of the mandible, and these are disposed rostrally to the corresponding deciduous incisors. In some occasions these orifices can be seen in the radiographs, although given their position, their observation depends on the angle of incidence of the x-ray focus at the time of taking the intraoral radiograph, and can lead to erroneous diagnosis. These orifices are best seen in the oblique projections. All the orifices can be seen in the different CT planes, including the three-dimensional images. They are specially well understood

in sagittal images, where the exact location of each one is appreciated.

The joint study of anatomical pieces, radiographs and CT's, allows to understand well the dental development of the incisors, to describe anatomical structures not previously described, and that if not taken account, can produce diagnostic errors.

Teeth that haven't erupted through the gum, have an apex with a thin layer of enamel that isn't filled with secondary dentin, and isn't covered by cement (Kirkland *et al.*, 1996).

Both in radiographs and in CT's, it's important to evaluate the alveolar bone, which is very flexible and in constant remodelling to accommodate the changes in shape and size of the dental structures it contains: a thin layer of compact bone (radiodense) (the cortex of the alveolus), that covers the alveolus itself, in which the Sharpey fibers are inserted and is called the dental hard lamina (Dixon and du Toit, 2011). This structure isn't appreciated in detail in the intraoral radiographs, but in the oblique ones. The most prominent portion of the alveolar bone that lies between the teeth below the gingival margin, is called the alveolar ridge (Dixon and du Toit, 2011). It's formed by dense cortical bone that's continued with the hard lamina. The crest is really only noticeable at the age of 1-2.5 years, between the central incisors. At these ages, it manifests as an acute opaque tip. It's particularly appreciated because the centrals 701 and 801 show a distal curvature on their mesial surfaces, leaving the alveolar ridge clearly visible. At this age a clearly open interincisive symphysis appears.

All the incisors have a single infundibulum. In a foal, this conical structure with enamel edges, reaches a length of about 10 mm from the occlusal surface of the tooth. It's usually covered incompletely with cement, contains food in a state of putrefaction, and therefore is obscured (Dixon, 2011; Pearce, 2012).

Since the reserve crowns and the apices of the permanent incisors converge with one another, in the intraoral projections, partial overlap occurs between the middle and the corners. To avoid this, slightly angulated radiographs can be made allowing a greater detail of these areas (Barakzai, 2011).

Although not readily apparent on intraoral radiographs, serial sectioning of incisors or computed tomography (CT) studies, reveal a complex pulp chamber anatomy, which in cross section varies from oval just apical to the infundibulum, becoming compressed in the mesiodistal dimension, and often dividing into two root canals apically. For most of the horse's life, normal coronal attrition is compensated for, by continued eruption of the incisor and lengthening of the root, such that the overall length of the tooth in the radiographs appears relatively constant until the horse is older than 25 years (Barrat, 2013).

CONCLUSION

Radiography and CT of young horses, offers a very detailed information not only of the dental germs evaluation inside the mandible, but also of the internal development of the same ones. Something that can't be appreciated by simple oral examination.

During the period of 1-2.5 years, age is difficult to determine by equine specialists. However, the joint study of radiographs and CT, allows to accurately assess the dental germs development and see if the horse's mouths are closer to the age of 1 year or are closer to 2.5 years. However, more similar works are needed with horses of exact known ages.

The joint use of radiographs and CT, allows better understanding of the intraoral radiographs and makes a more attractive teaching of the age of the horse mouth.

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