An anatomical, pathological and clinical study of donkey teeth

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Declaration

I declare that the contents of this thesis are my own work and that they have not been presented to any University other than the University of Edinburgh

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Edinburgh, August 2008

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Dedication

I would like to dedicate this PhD to my husband, Ben, and my parents, George and Suzanne who, even though they didn't quite understand what this project was all about, were always supportive.

Furthermore, I would like to dedicate this project to all the working donkeys in the world. I hope that this project, in even the smallest way, will make a difference to their long term welfare.

Abstract

Eighty normal cheek teeth and 26 normal incisors extracted from 14 donkeys (median age 19 years) at post mortem were anatomically examined including grossly and by computerised axial tomography (CAT) imaging. Decalcified histology was performed on 54 sections from 18 teeth (8 donkeys), undeclacified histology on 16 sections from 7 donkeys and scanning electron microscopy on 10 sections from 10 teeth (3 donkeys). The dental formulae and tooth number was found to be the same as in horses with a higher prevalence (17 %) of canine teeth in female donkeys. A decrease in tooth length, pulp horn length and pulp horn width with age was illustrated, as was an increase in occlusal secondary dentine depth with age, although not all these age changes were statistically significant. Normal histological and ultrastructural features of donkey teeth were identified and found to be similar to equine findings. Enamel was found to be thicker buccally in both maxillary and mandibular cheek teeth. Quantitative measurements of transverse dentine thickness around pulp cavities, dentinal tubule diameters and densities, and enamel prism diameters were made. Left lower incisors (301) were extracted from 7 donkeys and 6 horses for micro-hardness determination of enamel, primary and secondary dentine using a Knoop Hardness indenter. No significant difference between donkey and horse incisor microhardness was demonstrated. Examination of 19 donkey skulls at post mortem examination showed donkeys to have a higher degree of anisognathia (27%) compared to horses (23%).

Post mortem dental examination of 349 donkeys (median age 31) demonstrated a high prevalence of dental disease (93%) and in particular cheek teeth diastemata (85%). Furthermore, age was associated with increasing prevalence of dental disease and diastemata. Diastemata were also associated with the presence of other dental disorders and with colic-related death in affected donkeys. Quantitative measurements of 45 diastemata from 16 donkeys showed no difference in the medial and lateral width of diastemata but periodontal pockets were deeper laterally. The definition of valve and open diastemata were confirmed. Pulp exposure, dental caries and periodontal disease were examined in detail (54 skulls) at post mortem. A total of 19 teeth were extracted for further detailed examination as performed in normal anatomy.

Clinical dental examinations were performed on 357 donkeys in the U.K. that were selected for age distribution, and the prevalence of dental disease in different age groups was found to increase from 28% in the youngest group (age 0-10 years) to 98% in the oldest group (age > 35 years). An increased prevalence of most dental disorders with age was demonstrated as was an association between dental disease and weight loss, poor body condition score, supplemental feeding and previous episodes of colic. Clinical dental examination of 203 working donkeys in Mexico showed similar types of dental disorders as found in the U.K. study, with dental disease present in 62%, of which 18% required urgent dental treatment. There was a significant association between age groups and dental disease, and age groups and body condition score, but there was no association between dental disease and body condition score. However, body condition score was not associated with supplemental feeding or faecal egg counts either.

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Chapter 1: Literature review

1.1 Introduction

Donkeys are well adapted to survival in dry arid environments by their ability to browse (eat and nibble at leaves, tender shoots and other soft vegetation) as well as graze (feed on predominantly grass). Browsing on vegetation that contains high levels of hard silicates results in exposure of their teeth to greater attrition forces than occurs in domestic equidae, indicating the likelihood that donkey teeth are harder and more resistant to wear than those of domestic horses. Differences in microhardness of enamel, primary and secondary dentine in different equine breeds have recently been demonstrated (Muylle *et al.* 1999a) but no such studies have been performed in donkeys. The literature on donkey dental gross anatomy has been very limited and includes two gross anatomical studies, one of which was limited to incisor eruption and occlusal surface anatomy (Bunger and Hertsch 1981; Muylle *et al.* 1999a).The histological anatomy of donkey teeth does not appear to have been described.

Dental disorders have recently been recognised as a significant welfare problem in donkeys in the UK and abroad. A study performed at The Brooke Animal Hospital in India found that 65% of donkeys examined for poor body condition suffered from serious dental disorders (Roy 2002). Similarly, dental disorders have been found to be important in the ageing population of donkeys found on The Donkey Sanctuary Farms in the UK (Crane 2002; Dacre *et al.* 2008a). Unfortunately, very few studies specifically examining donkey dental disease have been performed to date, although pathological changes such as dental displacements, diastemata and periodontal disease have been noted in older donkeys on routine clinical oral examinations (Dacre *et al.* 2008a).

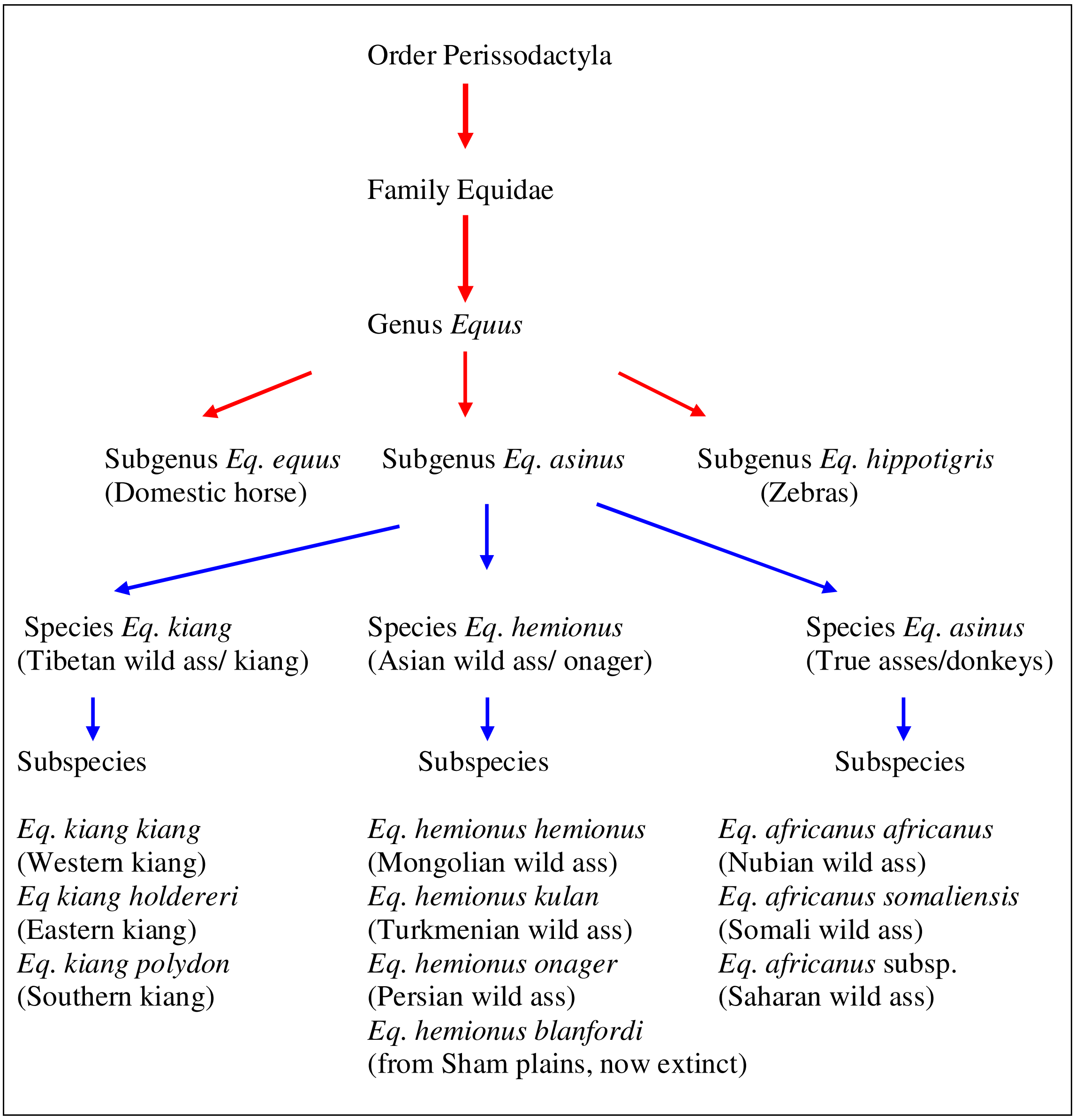
Our understanding of the nature and treatment of equine dental disorders has advanced rapidly in the last few years and has demonstrated the importance of good dental care in the welfare of the horse. Many recent studies have documented equine normal dental anatomy and pathology by gross, radiological, histological and ultrastructural examinations (Dixon and Copeland 1993; Kilic *et al.* 1997d, 1997c, 1997b, 1997a; Dixon *et al.* 1999a; 1999b; Dixon *et al.* 2000a, 2000b; Dixon 2002; Mitchell *et al.* 2003; Dacre 2005b; Dixon 2005).

Most of our limited knowledge of donkey dentistry has developed by extrapolation from research and clinical work in horses, but this may not necessarily be appropriate to the donkey if significant differences exist between donkey and equine dental structure.

1.2 The donkey

Donkeys, *Equus asinus*, are a subgenus of the genus *Equus* (Order Perissodactyla, Family Equidae) while the domestic horse is of the subgenus *Equus equus*. Morphologically, *Eq. asinus* are associated with having tufted tails, counter shaded colouring, chestnuts on the forelimbs only, square occipital crests and wide external auditory meati (Groves and Ryder 2000). Three species of the subgenus *Eq. asinus*, each with a number of subspecies (Figure 1.1) are currently recognised, that make up the large population of donkeys (asses) around the world.

***Figure 1.1*: Phylogenetic tree of the donkey**



Archaeological evidence from Egypt suggests that donkeys were first domesticated about 5000 years ago, although the location of this domestication is unknown (Beja-Pereira *et al.* 2004). Sequencing of mitochondrial DNA from domestic donkeys and from African and Asiatic asses have indicated that the African wild ass, and in particular the Somali wild ass, is the most likely progenitor of the domestic donkey. This suggests that North Africa is the most likely location of evolution of the domestic donkey (Beja-Pereira *et al.* 2004). Donkeys were particularly used along the trade route from the Pacific Ocean to the Mediterranean. It is through this journey, which took several years, that mixing of different donkey and ass breeds occurred (Camac 1997). The donkey was later found to be useful in the Mediterranean for agriculture as well as transport.

With the growth of the Roman Empire, donkeys spread to Northern Europe and eventually to Britain with the Roman invasion. Donkeys only became important in Britain in the sixteenth century, when the advent of war resulted in a scarcity of horses. Following this time, they remained sought after as working farm animals and for transport. Donkeys also gained popularity as show animals and were owned by families for recreational purposes. At the end of the 1800's, with the invention of the combustion engine, donkey numbers in Britain drastically declined along with the massive decrease in horse numbers. This decline was reverted in Britain in the 1960's when donkeys became popular as pets and companion animals (Camac 1997). This increase in donkey numbers has resulted in an increase in veterinary knowledge of the donkey in recent years, and brought about an increased awareness in the health and welfare of the donkey.

The situation is currently very different in the developing world where donkeys are essential to the agricultural and industrial economies of many these countries. Many impoverished families are dependent on their donkey(s) for their livelihood and health. About 95% of the world population of 44 million donkeys live in developing countries (Gebreab 1997; Starkey and Starkey 2004). Donkey welfare problems in these poorer countries appear to be mostly due to ignorance of humane husbandry standards, in particular under-nourishment, overwork and parasitism (Svendsen 1997). Increased veterinary-based objective knowledge will best allow provision of aid that will be most beneficial to the welfare of the donkey worldwide.

The Donkey Sanctuary is a UK based charity that was founded in 1969 by Dr Elisabeth Svendsen MBE for the care, protection and improved welfare of donkeys worldwide.

Donkeys from the UK and Ireland are relinquished to the Donkey Sanctuary for a number of reasons varying from costs, owner illness and retirement of beach donkeys, to one of the 10 farms, and to date the sanctuary has taken in over 12 000 donkeys. The donkeys on these farms are managed in groups, varying from about 20 – 80 per barn according to their individual management needs and have their own dedicated team of farm staff, veterinarians and farriers. The Donkey Sanctuary also works worldwide in many countries in Europe, Asia, Africa and South America, to improve the welfare of working donkeys and mules. These consist of local mobile clinics and sanctuaries providing free clinical treatment and advice, as well as providing funding for projects on national and international disease conditions of donkeys. There are about 55 million donkeys and mules worldwide of which over 95% are found in developing countries, the majority of which will be used for work (Fielding, 1991). As working equines provide more than 50% of global agricultural power needs, their welfare is closely linked with human welfare in these countries (Wilson, 2003).

The Donkey Sanctuary has been working in Mexico since 1991 in collaboration with World Horse Welfare and National Autonomous University of Mexico (UNAM) to provide free veterinary care and improve the welfare of equids. Mobile clinics provide routine 3 – 6 monthly visits to villages and will consist of castrations, dental care, farriery and other veterinary treatment. There are over 3 million donkeys in Mexico where they are used for many purposes in rural areas as pack animals (food, water, wood and other materials), for transport (riding) and hauling carts in rubbish dumps. Donkeys will usually be closely linked to family life and will do most of the work in the early morning and be left to forage in the afternoon. Supplementary feed will be given if available and will depend on the local produce that is available.

The true worth and plight of the donkey has long been recognised, for example De Buffon, Goldsmith and Others wrote in 1810 "[The donkey's] gentleness, patience and perseverance are without example….The services of this useful creature are too often repaid by hard fate and cruel usage".

1.3 Evolution and origin

It is well recognised that *Hyracotherium* (rabbit-like animal), also known as Eohippus or 'dawn-horse' is the oldest member of the Equidae family which lived in the late Eocene period about 55 million years ago (Bennett 1992). The first fossils of *Hyracotherium* were discovered in the United Kingdom in 1838 and 1839, but they weren't initially recognised as the ancestors of the modern horse. Eohippus remains were recovered in North America in 1873, but it was not until 1876 that they were recognised to be the same as *Hyracotherium* (Simpson 1951). Although fossils of *Hyracotherium* were found in both Europe and America, it is believed that they died out in Europe in the mid Oligocene period about 23 – 39 millions years ago. *Hyracotherium* was a small forest-dwelling browser with a short-nosed skull and short, cusped out crowned teeth (brachydont). The diet consisted of succulent shoots and leaves of trees with very little fibrous cellulose (Kozawa *et al.* 1988; Bennett 1992).

Equine evolution was not a linear event (orthogenetic) and had many 'side branches' with the development of many species, some of which became extinct and some surviving species which are closely related to *Equus* (Budiansky 1997; MacFadden 2005b). Due to gradual climatic changes in the Americas, resulting in the formation of large grasslands, one branch of the *Equidae* family adapted to grazing. Various changes in mastication and digestion were required for the *Equidae* family to adapt to grazing on a more fibrous diet containing silica. One adaptation to the more fibrous diet was the development of the caecum for digestion of the cellulose by symbiotic bacteria (hindgut fermenters) (Budiansky 1997).

An increase in the length of the muzzle allowed them to graze while watching for predators (Simpson 1951) and this also resulted in an increase in the size of the physiological diastema between the incisors and the premolars (Bennett 1992; Budiansky 1997). Further adaptive changes to a grazing diet included an increase in the depth of the jaw to allow for more powerful masticatory muscles and a transverse compressive action of the jaw to enable grinding of the food. *Hyracotherium*'s short crowned teeth (brachydont) were then gradually replaced by long crowned teeth (hypsodont) that continually erupt, and the three caudal premolars adapted to resemble molars (Simpson 1951; Kozawa *et al.* 1988; Budiansky 1997; Gorrel 1997). The first premolar still remains in its primitive form in the modern horse with no masticatory function and is termed the 'wolf tooth'. Evolutionary changes have also been demonstrated at histological level where changes in the enamel and dentine structure appear to have increased the strength of the teeth, which actually preceded the macroscopic changes of molarisation (Kozawa *et al.* 1988). Because of their prolonged eruption, the cementum appeared on the erupted (clinical) crown and this cementum outer layer also provided more structural support to the crown, in particular to the enamel infoldings that evolved to increase wear resistance and to provide an irregular, more efficient grinding occlusal surface (Bennett 1992), including the development of infundibular enamel "cups" in the upper cheek teeth and incisors (Vollmerhaus *et al.* 2002).

There was also an increase in body size, reduction in the number of toes to monodactyly, and the development in their locomotory system in order to escape predators (Bennett 1992). Many grazing genera of primitive Equidae lived on the savannahs during the Miocene and Pliocene period, and one of these *Hipparion*, migrated from North America to Eurasia via the Beringian land route that connected Alaska to Russia. It is from this genus that the modern *Equus* had evolved by the Quartenary period, about 2.5 million years ago (Bennett 1992). Recent evidence has suggested that *Equus* can be divided into two deep clades (species from a common ancestor) consisting of the caballines and the zebras/asses, which split about 3 million years ago in North America (MacFadden 2005b). Recent evidence, based on genetic characterisation from equid skeletons from 79 A.D. discovered in a Pompeii stable support the theory that donkeys evolved earlier in the Equidae family than the modern horse (Di Bernardo *et al.* 2004). This may have implications concerning the evolution of donkeys' teeth compared to the horses' teeth. Both clades of *Equus* spread to the Old World, but all Equidae became extinct in North America about 10,000 years ago as a result of multiple factors including climatic changes (MacFadden 2005b). It was not until the discovery of the New World that Equidae were reintroduced to North America by Spaniards.

1.4 Embryology of teeth

1.4.1 Bud, cap and bell stage

The oral cavity develops from the stomatodeum (an ectodermal depression between the first pair of branchial aches) and pharynx (endoderm) and is lined by an epithelial layer (Joseph 1981; Latshaw 1987). The processes involved in dentinogenesis can be divided into initiating, morphogenic and cytodifferentiative phases and result from epithelial-mesenchymal interaction (Fortelius 1985; Ten Cate *et al.* 2003). The first step of tooth development is an accumulation of mesenchymal cells and a plexus of blood capillaries, below the epithelial layer, which starts at about 25 days of gestation in humans (Berkovitz and Moxam 1981; Wiggs and Lobprise 1997b).The oral epithelium proliferates and invaginates into this mesenchymal condensation to form the primary epithelial band, which then develops two distinct ridges: vestibular on the labial side and dental laminae on the lingual side. The dental lamina deepens and lengthens, aligns with the developing jaw, and develops a number of epithelial swellings (tooth buds) surrounded by dense mesenchyme - bud stage of dental development.

The cap stage of development is characterised by the proliferation and invagination of mesenchymal cells into the tooth bud, that takes on an inverted cap shape and forming a structure called the enamel organ (Berkovitz and Moxam 1981; Ten Cate *et al.* 2003). The enamel organ forms all the deciduous teeth and the permanent molars in the horse. The permanent incisors, canines and premolars develop from a separate medial (lingual) extension of the dental lamina (Dixon 2005). During the late cap stage, the central cells of the enamel organ separate and form stellate cells, resulting in the stellate reticulum which contains large quantities of glygosaminoglycans in its intercellular spaces (Ten

Cate *et al.* 2003). The external epithelial layer retains their cuboidal structure, while the inner epithelial cells become more columnar (Latshaw 1987). Proliferating mesenchymal cells surrounding the enamel organs now form the dental follicle (sac). The dental sac gives rise to cementum, periodontal ligament and contributes to alveolar bone formation. Mesenchymal cells beneath the internal epithelium form the dental papilla, which later forms the dentinal and pulpal tissues (Wiggs and Lobprise 1997b). The dental papilla, dental sac and enamel organ collectively make up the tooth germ (Ferguson 1990).

Due to continued growth the enamel organ becomes bell shaped - early bell-stage. The enamel organ consists of four distinct cell layers at this stage: external enamel epithelium, stellate reticulum, stratum intermedium and internal enamel epithelium (Burkitt *et al.* 1993; Ten Cate *et al.* 2003). The external enamel layer has an important role in maintaining the shape of the enamel organ, as well as in the formation of the enamel free roots. The stellate reticulum, that lies centrally, has a nutritive and mechanical function (protection and maintaining enamel organ shape). The stratum intermedium consists of two to three layers of stellate-like cells over the internal epithelium, the cells of which later become ameloblasts. The stratum intermedium cells contribute to protein synthesis. The internal epithelial layer consists of a single layer of columnar epithelial cells separated from the dental papillae by a basement membrane, and is directly involved in enamel production and also induces the formation of odontoblasts in the adjacent cells of the dental papilla.

1.4.2 Dentine formation

The outer layer of the dental papilla (mesenchymal cells) is stimulated to form odontoblasts, which produce the inner layer of unmineralised dentine (predentine) (Fortelius 1985; Ten Cate *et al.* 2003). The cells initially become cylindrical, then tall columnar with the nucleus located at the opposite end from the basement membrane of the inner dental epithelium (Mjor 1984). The dental papilla controls the shape of the tooth germ, and ultimately of the tooth crown, by determining the shape of the enamel-dentine junction (Berkovitz and Shellis 1981; Fortelius 1985).

During dentine formation, odontoblasts develop elongated processes composed of fine cytoplasmic extensions. These odontoblast processes extend the length of the dental tubules while the odontoblast cell bodies remain adjacent to the basement membrane on the pulp cavity periphery (Ten Cate *et al.* 2003). Formation of and mineralisation of dentine, commences at the enamel-dentinal junction and moves inwards, which allows the layer of predentine to be adjacent to the odontoblasts. The first layer of dentine (mantle dentine) that is formed lies against what will become the amelo-dentinal junction (Nanci 2003a). Dentinogenesis begins at the tip of the papilla in canine and incisors. In teeth with multiple cusps, such as equine cheek teeth (CT) (molars and premolars), dentinogenesis commences at the tips of the cusps, before joining up by mineralisation between the cusps.

1.4.3 Enamel formation

Initially, the internal enamel epithelial cells are stimulated by the formation of predentine (mineralised collagen) by odontoblasts to form ameloblasts. These ameloblasts secrete an extracellular matrix of mucopolysaccharides and organic fibres and then migrate away from the dentinal surface, forming the amelo-dentinal junction (ADJ) (Latshaw 1987; Ten Cate *et al.* 2003). Just prior to this migration, ameloblasts change shape and form conical processes (Tomes' processes) at their dentinal interface (Osborn 1981). Hydroxyapatite crystals are formed perpendicular to the secretory surface of the Tomes' process. As the ameloblasts are orientated in different planes this results in changes in crystal orientation and the appearance of enamel prisms (Fortelius 1985). Enamel prisms are formed by secretions from the surface of Tomes' process and interprismatic enamel is formed from proximal secretions. Due to separation of the enamel and dentine during mineralisation, it is likely that the developing enamel is later nourished by the vasculature from the dental sac (Dixon 2005). In equine maxillary CT and incisors, invaginations of the enamel epithelium result in the formation of infundibula (Dixon 2005). Additionally, enamel organs of equine CT develop infoldings that cause infolding of the peripheral enamel. Enamel production ceases once the tooth has reached its full length (Kirkland *et al.* 1996; Dixon 2005).

1.4.4 Cementum and root formation

The root portion is formed by a continuation of the dental papilla and external epithelium. The outer and inner enamel epithelia join at the deepest point of the enamel organ to form the cervical loop which becomes the root sheath of Hertwig. (Burkitt *et al.* 1993; Ten Cate *et al.* 2003). The function of Hertwig's epithelial root sheath is to determine the shape of the roots and stimulate odontoblast differentiation, and hence dentine formation. This epithelial root sheath disintegrates gradually with the deposition of dentine and segments that fail to disintegrate result in the formation of epithelial rests of Malassez in the periodontium of mature teeth (Berkovitz 1981; Burkitt *et al.* 1993; Ten Cate *et al.* 2003). Due to the lack of a stellate reticular layer, no ameloblasts and hence no enamel formation is stimulated in the true roots (Latshaw 1987).

The inner layer of the dental sac comes into direct contact with the dentine of the root and this stimulates the formation of cementoblasts that produce cementum (Berkovitz 1981; Latshaw 1987). The enamel organ in hypsodont teeth degenerates over areas of the crown where enamel mineralisation has occurred, resulting in the formation of cementum by the dental sac over the entire surface of the crown (Latshaw, 1987). The alveolar bone is formed by osteoblasts that originate from the outer layer of the dental sac (Latshaw 1987). The periodontium (periodontal ligament) is formed from fibroblasts and dense bundles of collagen fibres derived from the inner layer and the middle connective tissue layer of the dental follicle (Berkovitz 1981). Root formation in hypsodont teeth usually has not even started at the time of tooth eruption and the unerupted crown cementum attached to the alveolar bone possibly aids the stabilisation of the equid tooth during eruption (Latshaw 1987).

1.4.5 Infundibulae

There are two infundibulae per maxillary cheek tooth and one per incisor, with none in the mandibular cheek teeth. The lengths of infundibulae have shown to range from 89mm in a 4 year old to 2mm in a 30 year old horse i.e. decrease in length with age and CT wear (Fitzgibbons 2007).

The infundibulae are formed by invaginations of the enamel epithelium, and are lined by cementoblasts depositing cementum within these infundibulae. These cells are nourished by the dental sac (Dixon 2005). However, after tooth eruption this blood supply to the infundibular cementum will be lost, with removal of the dental sac, and infundibular cement can then be regarded as inert tissue, with no ability to repair itself. There is evidence to suggest that in some CT there are openings in the apical aspect of an infundibulae, which would allow nourishment of the infundibular cementum from the pulp cavity (N. du Toit and P.M. Dixon 2007, unpublished data). Gross evidence of vascular tissue was demonstrated in 2% (17/786) of infundibulae from 33 horse skulls (Fitzgibbons 2007). Histological examinations from some of these sections have demonstrated viable cementocytes within lacunae and blood vessels within infundibular cementum in younger horses (N. du Toit and P.M. Dixon 2007, unpublished data). More extensive studies to determine the exact prevalence and age of these vascularised infundibular cementum need to be performed.

A recent study examining 786 maxillary cheek teeth from 33 horses (median age 10) on longitudinal section found that only 11% of the infundibulae were completely filled with grossly normal cementum and areas of cemental hypoplasia were observed in 22% of the infundibulae. Cemental hypoplasia of the infundibulae is thought to develop secondary to premature destruction of the dental sac. Discoloured cementum was observed in 72% of the infundibulae and carious lesions were only seen in 8% (Fitzgibbons 2007).

1.5 Equine dental anatomy

1.5.1 Gross anatomy

Equid teeth, like all domestic mammals are diphydont i.e. they have both a deciduous (temporary/primary) and permanent (adult/secondary) set of teeth. Equid teeth are further classed as hypsodont (high crowned) teeth, which are a sub-division of the anelodont group (limited growth time) (Kertesz 1993). Continued eruption is thought to occur due to continued reformation of, and traction by the periodontal ligament (Fortelius 1985). A recent study examining cell proliferation within the periodontium demonstrated that a dynamic process of cell proliferation and migration is involved in the periodontal ligament remodelling associated with continued eruption (Warhonowicz *et al.* 2006). The successful culture of equine periodontal fibroblasts and equine dental cementoblasts recently will result in better elucidation of the exact process of continued eruption (Staszyk and Gasse 2007).

Equid teeth also have different crown patterns (termed heterodont teeth), with three basic groups; incisiform, caniniform and molariform (premolars and molars) teeth (Ten Cate *et al.* 2003). At commencement of eruption there are no true roots present and therefore each tooth is divided into a coronal (crown) and apical regions. The crown is divided into the clinical crown that has erupted and reserve (unerupted) crown. The reserve crown has further been classified into gingival crown (with more active cementum deposition) and alveolar crown (Mitchell *et al.* 2003). Therefore gingival erupted crown development is also often incomplete at the time of eruption (Fortelius 1985). True roots are formed gradually, within 2 years after eruption and are defined as the enamel free apical area (Kirkland *et al.* 1996).

Equids have 24 deciduous teeth and 36-44 permanent teeth represented by the dental formulae (Kertesz 1993):

Deciduous	I_D 3/3, C_D 0/0, P_D 3/3
Permanent	I 3/3, C 0-1/0-1, P 3-4/3, M 3/3

The variation in adult number of teeth is due to the variability of the first premolars (wolf teeth) in males and females, and variability of the presence of canine teeth in females. A prevalence of 27.7% canines in female horses was reported in a *post mortem* study of 173 horses. Of these 48 female horses with canines, only 10 had all four canines present and mandibular canines were found to more common (Colyer 1906). In a study by Bunger and Hertsch (1981), rudimentary canines appeared to be more consistently present in female donkeys. The first premolar is anatomically regarded as being brachydont in horses. The prevalence of first premolars in both sexes was 16.32% in 484 horse skulls with a lower incidence in male horses (14.9%) compared with female horses (21.3%) (Colyer 1906). As premolars have become molariform in equids approximately 35 million years ago, premolars and molars are collectively termed cheek teeth (CT).

The ‘Modified Triadan’ system has recently been widely accepted as a numeric system of identifying equid teeth (Foster 1996; Lowder 1998). In this system the jaw is divided into four quadrants, with the right maxilla numbered one, and continuing clockwise such that the left maxilla is numbered two, the left mandible is numbered three and the right mandible is numbered four. The deciduous teeth are referred to as quadrants five to eight in the same order. The individual teeth are numbered one (central incisor) to eleven (third molar) with the tooth number preceded by the quadrant number.

In horses and donkeys, all three calcified tissues (enamel, dentine and cementum) are exposed on the occlusal surface (Gorrel 1997). This is enhanced by the presence of one infundibulum in each incisor, two infundibula in each maxillary CT and increased enamel infoldings in the mandibular CT (Bunger and Hertsch 1981). These three calcified tissues differ in hardness and durability and therefore wear at different rates, enamel being the hardest and cementum the least hard of the three (Kertesz 1993; Boyde 1997). The areas of dentine and cementum wear down more rapidly resulting in occlusal depressions; however the sizes of these depressions are limited by the presence of adjacent enamel infoldings and infundibula (Kilic *et al.* 1997a). This results in an irregular occlusal surface, which aids grinding of hard silicate matter and coarse fibres (Kertesz 1993). This self-sharpening mechanism was another evolutionary change to compensate for the many hours of mastication required by the equid (average 16 hours a day) (Bennett 1992; Gorrel 1997). However, a study observing working donkeys in Botswana found that days when they were not in work, they only spent 6 to 7 hours a day eating (Aganaga and Tsopito 1998). The rate of dental attrition is determined by a number of factors including tooth morphology, occlusal surface area, chewing mechanism and diet, in particular the dietary content of abrasive particles (Hillson 1986). To remain in wear the rate of eruption equals the rate of wear which is usually cited to be 2-3mm a year (Dixon 2005).

1.5.1.1 Premolars and molars (cheek teeth)

Equines have been shown to have a 23% wider distance between the maxillary CT than the mandibular CT (Taylor 2001). This anatomical feature of a wider maxillary arcade compared to the mandibular arcade is termed anisognathia. In donkeys the mandibles have been subjectively noted to be narrower and more laterally compressed compared with horses (Bunger and Hertsch 1981; Muylle *et al.* 1999a).

It has been reported that after eruption the occlusal surfaces are parallel to each other but due to anisognathia and the medial ‘power’ stroke of mastication (Tremaine 1997), the occlusal surfaces become about 10-15° angled from the palatal/lingual aspect dorsally to the buccal aspect ventrally (Easley 1996; Dixon and Dacre 2005). However two studies were able to demonstrate that there was no change in occlusal angle with age which implies that teeth attain the occlusal angle at or soon after eruption (Carmalt *et al.* 2004; Carmalt *et al.* 2005). Various methods have been used to measure cheek teeth occlusal angles such as the indirect lateral mandibular excursion model assessing incisor separation angle (Rucker 2002; Carmalt 2004) and measurement of a single mandibular

CT (Ralston *et al.* 2001; Carmalt and Carmalt 2004). These studies were limited to mandibular CT occlusal angles and showed that an angle of 6.3º to 19.3º was within the normal range and alterations in occlusal angle did not affect feed digestibility.

However more recently a study measuring all the CT on cadaver studies using a wire method was able to demonstrate that CT occlusal angle varied between maxillary and mandibular teeth and within an arcade (Brown 2006; Brown *et al.* 2008). In horses with no dental abnormalities, the mandibular occlusal angles increased from 19.2 º degrees at the 06 to 30 º at the 11s, while the maxillary CT occlusal angle decreased from the 06 to the 11. The mean of mandibular CT angles was consistenly greater (15.6º - 31.5º) than the maxillary occlusal angles (9.2º - 19.1º). Both the maxillary and mandibular 06s gave the most varied angle and appears to be the most inaccurate tooth to determine the occlusal angle of the entire arcade (Brown 2006). As can be seen from the findings in the study by Brown (2006) the maxillary CT occlusal angle appears to be closer to the range of 10-15º that has previously been reported (Easley 1996; Dixon 2005). Interestingly, Brown (2006) was also able to demonstrate no increase in the mean CT occlusal angles in the presence of some dental disorders (maxillary 9.17 º – 17º; mandibular 14.7º – 26.4º), with only individual abnormal CT having higher occlusal angles.

Recently the movement of the mandible has been shown to be similar among horses using a 3-dimensional kinetics model with differences in the amplitude of movement between individual horses (Bonin *et al.* 2006). The equine chewing cycle has three phases: opening stroke, closing stroke and power stroke. The power stroke forces are about 3.5 times the size of the closing stroke with a mean peak force of 875 N (Staszyk *et al.* 2006). An up and down 'crushing' stroke will predominate when high levels of concentrates are fed (Capper 1992) and with reduced time spent chewing, this will promote a more acute occlusal angle that could become problematic. In contrast horses fed predominantly roughage have a greater degree of lateral excursion during mastication (Bonin *et al.* 2007). Roughage also requires more chewing movements

(3000-3500 per kg consumed) compared with concentrates (800-1200 per kg). The number of chewing movements, per kg roughage and concentrate, has been shown to be even higher in ponies, and the same could be assumed for the donkey (Capper 1992; Frape 2004). There is also a slight rostro-caudal movement during the mastication action, which is also exhibited when the head is elevated and dropped.

The occlusal surfaces also have cusps (elevations), termed transverse ridges that correspond in the maxillary and mandibular teeth. These ridges increase the occlusal surface area for grinding of coarse fibre and are the result of enamel infoldings causing different wear patterns. These ridges are more predominant in young horses, with much individual variation.

In triadan positions 07-10, the maxillary CT have a square shape on transverse section, which is in contrast to the more rectangular shaped mandibular CT. This results in only about one third of the maxillary teeth occlusal surface being in contact with about one half of the mandibular teeth occlusal surface. The upper and lower 06's and 11's are more triangular in shape (Dixon and Copeland 1993). The maxillary CT of equids have a shorter erupted crown, compared with the mandibular CT, and have two small lateral and one large medial root once fully developed. The mandibular CT have two longer roots (rostral and caudal), except for the 11's that have three roots (Dixon and Copeland 1993). To date, one study has demonstrated similar findings in donkeys (Bunger and Hertsch 1981). The maxillary CT also have protruding longitudinal lateral ridges (cingulae), the purpose of which has not been elucidated.

The maxillary CT row tends to be slightly convex in shape on the buccal aspect and mandibular arcade slightly so on the lingual aspect (Misk and Seilem 1999). The clinical crowns of the maxillary and mandibular 06's are orientated caudally on longitudinal section, and the crowns of the 10's and 11's are orientated rostrally. These cause compression of the CT rows and enables each CT row to work as a unit and also inhibits the formation of spaces (diastema[ta]) between the CT (Dixon and Copeland 1993; Misk and Seilem 1999; Dixon 2002). The occlusal surfaces of caudal CT are not level in a rostro-caudal direction, as the caudal aspects of the jaws curves dorsally to a variable extent between breeds. This curve is known as the ‘curve of Spee’.

The long reserve crowns of the maxillary premolars and molars (CT) are embedded within the maxillary bone and sinuses. The rostral and caudal maxillary sinuses are almost completely filled with the apices of the caudal three to four CT in younger horses of less than eight years of age (Dixon *et al.* 1999b; Perkins 2001). As these hypsodont teeth continue to erupt, the reserve crown shortens and the overlying alveolus remodels with a resultant increase in the volume of these sinuses. The locations of these caudal CT are slightly variable between individuals (Perkins 2001), but generally the apices of 08-09’s are in the rostral maxillary sinus and 10-11’s in the caudal maxillary sinus.

1.5.1.2. Incisors and canines

The foal has three deciduous incisors in each quadrant that erupt at birth (01’s), 4-6 weeks (02’s) and 6-9 months (03’s). These deciduous incisors are smaller, whiter and have shallower and wider infundibula than the permanent counterparts, which erupt at about 2 ½ years (01’s), 3 ½ years (02’s) and 4 ½ years (03’s) in horses. In donkeys, the permanent incisors erupt about 3 months later than in horses (Bunger and Hertsch 1981; Muylle *et al.* 1999a). In younger horses the incisors are curved such that they are convex labially. The incisors also taper toward their apex such that with continued eruption in older horses, diastema may form between incisors. The occlusal angle, between maxillary and mandibular incisors, decrease from about 180° after eruption to about 90° by 15 years of age (Dixon 2002).

The exposed surfaces of the incisor infundibula, that are normally incompletely filled with cementum, gradually darken with age as they become stained by food material. The age at which the infindibula wear out varies, dependant on the depth of the infundibula and the rate of incisor wear (Walmsley 1993; Richardson *et al.* 1995; Muylle *et al.*

1996). Similar findings of ageing and variations in the age at which infundibulae wear out have been demonstrated in free ranging Cape mountain zebras (Penzhorn 1987; Penzhorn and Grimbeek 1987). Once the infundibulum is worn out, the distal infundibular enamel ring known as the ‘enamel spot’ remain on the lingual aspect of the incisor for a year or so. In some donkeys, the infundibula of the 03s (corner incisors) is situated so lingually, that it is fused with the peripheral enamel. In these animals with a lingually facing open invagination, no separate ‘cup’ is present on the occlusal surface (Bunger and Hertsch 1981; Muylle *et al.* 1999a). The first known record of ageing of horses by their incisors dates back to 600BC in China (Kertesz 1993). This ‘art’ of ageing was refined over the years and is well illustrated in a text by Sydney Galvayne in 1886. Recently, however, new scientific evidence by many authors has brought this age old technique into dispute and it is now accepted that ageing of horses by their incisors is very inaccurate after 6 years of age (Walmsley 1993; Richardson *et al.* 1994; Muylle *et al.* 1996; Muylle *et al.* 1998, 1999a).

The ‘dental stars’ that appear on the labial aspect of the incisor occlusal surfaces are areas of secondary dentine surrounding small areas of irregular secondary dentine that have been deposited in the former pulp cavities (Dacre *et al.* 2008b). Once again there is variation in the age at which these initial thin brown transverse lines appear, but they generally appear sequentially in the 01’s, 02’s and 03’s. This variation in age of appearance is dependent on the depth of primary dentine above the pulp cavities and the rate of dental wear. In donkeys, these dental stars appear a few months earlier than in horses (Muylle *et al.* 1999a). With continued wear these dental stars become more oval in shape and move more centrally. The shape of the incisor occlusal surfaces also alters with age, changing from oval to round to triangular and oval again.

As noted, the presence of canine teeth are variable (usually absent) in females. The canine teeth are simple brachydont teeth with no coronal cement or enamel infolding. They are not hypsodont and most of the tooth length is unerupted crown. The mandibular canines are more rostrally placed than their maxillary counterparts and so there is no occlusal contact between canines, which is thought to predispose to calculus deposition on these teeth.

1.5.2 Ultrastructure and histology

1.5.2.1. Cementum

Cementum is composed of 45 - 50% inorganic matter which is predominantly hydroxyapatite [$Ca_{10}(PO_4)_6(OH_2)$] crystals (Nanci and Somerman 2003). The rest is composed of organic material, including elastic collagen fibres (predominantly type-I collagen), and water. Due to its higher proportion of organic components, cementum is softer than either enamel or dentine, and appears white to cream in colour. Cementum is formed continuously through the life of the tooth (except in the infundibula) and it can be deposited rapidly in response to various stimuli (Easley 1996; Dixon 2002).

Two main types of cementum can be identified, acellular and cellular. Acellular cementum is formed when cementoblasts deposit cementum and then move away from the amelo-cemental junction (ACJ). In contrast, cellular cementum is formed when cementoblasts become trapped in lacunae within the cementum they have secreteted and become cementocytes (Wiggs and Lobprise 1997b). Cementum has also been classified into primary and secondary cementum, dependant on the time of its formation. Human cementum has further divided into 5 types (Schroeder 1992):

1. Acellular, afibrillar
2. Acellular, extrinsic fibre (primary cementum)
3. Cellular, intrinsic fibre (secondary cementum)
4. Acellular , intrinsic fibre
5. Cellular, mixed fibre (intrinsic and extrinsic fibres)

Intrinsic fibres are formed by cementoblasts and extrinsic fibres are deposited by the periodontal ligament and are termed Sharpey's fibres.

There is increased cementum deposition in the newly erupting clinical crown, which is thought to contribute to the size of the clinical crown (Mitchell *et al.* 2003). Blood supply is maintained in cementum from the periodontal ligament and gingiva until the tooth emerges beyond the gingival margin. Therefore, erupted clinical crown is surrounded by inert cementum. Infundibular cement is nourished by blood vessels from the dental sac, which is destroyed immediately after tooth eruption by normal mastication. Therefore the infundibular cement is inert and cement deposition ceases after eruption. Due to continued cementum production around the roots, older equid teeth are predominantly composed of cementum (Dixon 2002; Mitchell *et al.* 2003).

Two types of cemental hypoplasia have been identified on scanning electron microscopy: central infundibular hypoplasia, occurring within the infundibula, and junctional cemental hypoplasia which occurs in peripheral and infundibular cementum near the amelocemental junctions (Kilic *et al.* 1997d). Central infundibular cemental hypoplasia is though to occur as a result of premature tooth eruption or premature occlusion of the central vascular channel. Infundibular cement hypoplasia is thought to predispose to infundibular caries if areas of hypoplasia are exposed on the occlusal surface. Junctional cemental hypoplasia is proposed to occur as a result of failure of disintegration of the enamel epithelium, or resorption of the enamel surface or rapid cementum deposition. This kind of hypoplasia is regarded as not being clinically significant as it was noted to occur more frequently in equine incisor teeth, which rarely develop caries (Kilic *et al.* 1997d).

Periodontium

The tooth lies within the alveolus which is composed of three distinct layers of alveolar bone. The innermost aspect (cortex) of the alveolar bone that is very compact is called the cribriform plate and is termed *lamina dura denta* radiographically, where it is identified by its radiopacity. It is lined by periodontal ligament and this inner part of the alveolus has Sharpey’s fibres embedded in it. The middle and outer layers of the alveolus consists of spongy, cancellous bone and cortical bone respectively (Wiggs and Lobprise 1997b).

The cementum is firmly but flexibly attached to the alveolar bone by means of the periodontal ligament, which also provide the blood vessels that nourish the cementocytes lying within lacunae (Dixon 2002; Staszyk and Gasse 2005). Sharpey’s fibres run from their attachment in the alveolar bone to a more apical position on the tooth cementum (Burkitt *et al.* 1993). The periodontal ligament is a connective tissue consisting of cells (predominantly fibroblasts), collagenous fibres and an extracellular matrix. The function of the periodontal ligament is to support the tooth (via the Sharpey’s fibres), provide nutrition and have a sensory (pain and proprioceptive) function (Easley 1996; Kilic *et al.* 1997d). Recently 3 distinct types of fibro-vascular arrangements have been identified in horse periodontium: type I – groups of blood vessels in a sheath; type II – individual blood vessels anchored in specific fibres; type III – wide, ballooned venules (Staszyk and Gasse 2005).

1.5.2.2. Enamel

Although enamel is the hardest substance in the body it is also very brittle (Kilic *et al.* 1997b). It is almost translucent, but gets its white colour from the underlying dentine (Wiggs and Lobprise 1997b; Dixon 2002). Enamel has a mineral content of 95-98% by weight, consisting mainly of calcium hydroxyapatite crystals with water and protein making up the rest (Fortelius 1985). These crystals are arranged into enamel prisms and interprismatic enamel, and are classified into different types according to the shape of the prisms, and prismatic and interprismatic enamel arrangement.

Equine enamel type-1 is composed of parallel rows of oval shaped prisms and thick flat interprismatic plates, and is found mainly adjacent to the amelo-dentinal junction. These interprismatic plates makes this type of enamel very hard, but as it contains parallel plates of enamel it is very brittle (Kilic *et al.* 1997b). It is the predominant type of enamel found in the maxillary CT to enable resistance against the repetitive masticatory forces. Equine type-2 enamel is composed of bundles of 'keyhole' to 'horse shoe' shaped enamel prisms with very little interprismatic enamel. The prisms are interwoven in three-dimensional patterns and are mainly found adjacent to the amelo-cemental junction. This type of enamel is very resistant to cracking, but not as hard as type-1. Type-2 enamel is therefore the predominant enamel type found in the incisors, to prevent fractures secondary to the massive shear forces imposed on the relatively small incisors during prehension. Enamel type-3 consists of oval prisms surrounded by 'honeycomb-like' interprismatic enamel. Thin layers of type-3 enamel are inconsistently formed at both the amelo-cemental and amelo-dentinal junction (Kilic *et al.* 1997b).

Enamel crystals grow in a specific orientation to the enamel surface. However, unequal secretion of enamel matrix from the distal pole of ameloblasts can lead to the formation of enamel pits on the surface. This results in abrupt changes in the crystal orientation, forming distinct prism patterns that are species dependent (Boyde *et al.* 1988). Incremental lines, which can be seen on SEM, are secondary to regular cyclic differences in the rate of production enamel components. Decussation, which are zones of prisms in different orientation, leads to the formation of Hunter-Schrerger bands, and are caused by cell movement during enamel secretion (Boyde *et al.* 1988).

Equine CT vary in enamel thickness on transverse section of teeth but not in an apical-occlusal (i.e. longitudinal) plane. Equine enamel is thickest in folds parallel to the long axis of the mandible and maxillae (Kilic *et al.* 1997a). Mandibular CT had more deeply infolded peripheral enamel than maxillary CT and this is thought to compensate for their absence of infundibulae, that are present in maxillary CT (Kilic *et al.* 1997a).

1.5.2.3. Dentine

Dentine is a bone like tissue composed of about 30% organic fibres, mucopolysaccharides, water and 70% hydroxyapatite crystals (Wiggs and Lobprise 1997b). Dentine production commences just prior to enamel production during dentinogenesis and continues throughout the life of the tooth (Ferguson 1990; Dixon 2002). Long odontoblast processes are formed by the odontoblasts within the dentinal tubules as they deposit dentine and retract into the pulp cavity (Kilic *et al.* 1997c; Mueller and Lowder 1998; Muylle *et al.* 2001). These processes are aligned perpendicular to the occlusal surface and horizontal to the amelo-dentinal junction (Kilic *et al.* 1997c). There has been some dispute as to whether these intratubular collagen fibres observed on scanning electron microscopy are truly odontoblast processes and it has been proposed that they are in fact the inner most hypomineralised layer of the intratubular dentine termed laminae limitantes (Muylle *et al.* 2000b).

Dentine can be divided into primary, secondary and tertiary dentine.

1.5.2.3.1 Primary dentine

Primary dentine is formed prior to tooth eruption and consists of odontoblast processes surrounded by dentinal tubules filled with *intra*tubula*r* (peritubular) dentine, which in turn is surrounded by a thin layer of *inter*tubular dentine. Intratubular dentine is present only in primary dentine and has a higher crystalline content than intertubular dentine (Kilic *et al.* 1997c; Wiggs and Lobprise 1997b). The surrounding intertubular dentine confers a honeycomb appearance on phosphoric acid-etched SEM dentine sections (Kilic *et al.* 1997c; Muylle *et al.* 2001). The intratubular dentine and tubular diameter increase in thickness from the amelodentinal junction to the junction of primary and secondary dentine (Kilic *et al.* 1997c). The odontoblast processes, that extend the length of the dentinal tubule, have side branches that extend into lateral canaliculae inhibiting their contraction towards the pulp. Contraction is further resisted by organic fibres of the intratubular dentine that bind the ondontoblast membrane to the dentinal tubular wall (Kilic *et al.* 1997c). Primary dentine formation has been previously proposed to cease approximately three years after dental eruption in horses (Easley 1996). In humans, primary dentine deposition has been reported to cease after the external structure of the tooth or root development has been completed (Nanci 2003a). However, this is not applicable to horses as external development via cementum deposition occurs throughout the life of the tooth (Mitchell *et al.* 2003) and root development does not commence until after eruption (Kirkland *et al.* 1996).

1.5.2.3.2 Secondary dentine

Secondary dentine has been defined as dentine which is laid down after occlusal contact of teeth (Kierdorf and Kierdorf 1992) and eruption have occurred (Wiggs and Lobprise 1997b). This definition of the commencement of secondary dentine deposition after eruption would be the most suitable definition for equids, as was also proposed in horses by Dacre (2006). Secondary dentine differs from primary dentine as it does not contain intratubular dentine. The tubular density of dentinal tubules/unit area in regular secondary dentine increases from the junction with primary dentine to the boundary with irregular secondary dentine on transverse section. However, dentinal tubular thickness and density do not alter in the apical to occlusal plane (Kilic *et al.* 1997c). Secondary dentine can be divided into regular (physiological) and irregular secondary dentine. Regular secondary dentine is continuous with primary dentine and progressively fills the pulp cavity throughout the life of the tooth until the pulp chamber is almost completely filled. As normal attrition wears down the occlusal surface, pulpar exposure on the occlusal surface is prevented by the deposition of secondary dentine. A recent study by Dacre (2005) has shown that irregular secondary dentine is found in all grossly normal equine teeth in the most central part of the pulp cavity and is therefore also a normal physiological dentine that fills the most central portion of the pulp cavity. This differs from the classification by Muyelle *et al.* (2000a) who termed this type of dentine as tertiary dentine.

1.5.2.3.3 Tertiary dentine

Tertiary dentine is defined as the dentine that forms in areas of focal injury in response to a noxious stimulus (Nanci 2003a) and the number of dentinal tubules varies or could be completely absent (Ten Cate *et al.* 2003). Reactionary tertiary dentine is laid down by pre-existing odontoblasts, whereas reparative dentine is formed by pulp derived mesenchymal cells that differentiate into odontoblast-like cells (Magloire *et al.* 1996).

Interglobular dentine are areas of hypocalcified dentine that lie adjacent to the amelo-dentinal junction (Mjor 1984). Granular layers of Tomes dentine are similar areas of hypocalcified dentine found next to the amelo-cemental junction. Areas of intertubular dentine can be seen in decalcified histological sections where dentine deposition has been rapid. Areas where the dentinal tubules are devoid of odontoblast processes and are empty due to traumatically induced cell death are called dead-tract dentine. Sclerotic dentine occurs when the odontoblast processes become replaced by dentinal matrix secondary to insults such as trauma (Wiggs and Lobprise 1997b) or with old age (Mjor 1984). These changes result in the dentine becoming harder, more brittle and translucent and have also been termed secondary intratubular mineralisation.

1.5.2.4. Pulp and endodontic system

The pulp cavity contains blood vessels, lymphatics, nerves and connective tissue that enter it via its apical aspect. The outermost layer of the pulp is lined by odontoblast cells with odontoblast processes extending out towards the tooth periphery. The pulp cavity in young equids is a very large common cavity and divides into smaller pulp horns as the equid ages and secondary dentine deposition commences (Kirkland *et al.* 1996; Dixon 2002). From two to four or five years after eruption, mandibular CT have a distinct pulp chamber that communicates with the pulp horns and root pulp canals (Kirkland *et al.* 1996). Six to eight years after eruption, with the deposition of secondary dentine, the endodontic system is divided into two compartments. Each compartment consists of a root canal, a pulp chamber and two to three pulp horns (Kirkland *et al.* 1996). The CT each have five pulp horns, except the 06s and 11s that have six and seven pulp horns respectively (Dixon and Copeland 1993; Dacre 2005a; Dixon 2005). Exposure of odontoblast processes on the dentinal surface is regarded as pulp exposure in humans and can cause pain, for example if the overlying enamel is destroyed by caries, but is a normal anatomical finding in horses (Kilic *et al.* 1997c). In horses, odontoblast processes are believed to play an important role in regulating secondary dentine deposition in the pulp, or alternatively inducing tertiary dentine formation focally, by transmitting stimuli from the occlusal surface, possibly by fluid changes in the dentinal tubules (Kempson *et al.* 2003).

1.5.2.5. Nerve supply

The trigeminal nerve gives rise to the maxillary and mandibular nerves. The maxillary nerve supplies the maxillary teeth as the superior alveolar nerve and small auxiliary branches. The mandibular teeth are supplied by the lingual, buccal and inferior alveolar branches from the mandibular nerve (Dyce *et al.* 2002). The mandibular nerve exits the mandibular canal as the mental nerve which supplies the external tissues of the rostral mandible and teeth rostral to the first premolar (06) (Dyce *et al.* 2002). These nerves give off small branches that accompany blood vessels to the apical canal of each tooth. These nerves arborise throughout the pulp and form a parietal plexus at the periphery of the pulp cavity called the subodontoblastic plexus of Raschkow (Nanci 2003a). Both myelinated (including Schwann cell covered) and unmyelinated nerve axons end in close apposition to the odontoblast cell bodies and processes but nerve axons can traverse as far as 200 µm into the dentine.

There are three theories as to the conduction of pain through dentine: (a) Direct nerve stimulation; (b) Hydrodynamic theory – fluid movement within the dentinal tubule stimulate nociceptive mechanisms within tubules; (c) Transduction theory – odontoblast processes themselves act as mechanoreceptors to nerve endings located further distally (Avery *et al.* 1984).

1.5.3 Microhardness of enamel and dentine

Due to donkeys having a longer expected lifespan and typically a more coarse diet than horses, it was believed that donkey teeth are harder than horse teeth to ensure slower attrition and a longer functional life. Dental microhardness testing studies have been performed in humans (Craig and Peyton 1958; Collys *et al.* 1992), sheep (Suckling 1979), cattle (Attin *et al.* 1997) and horses (Muylle *et al.* 1999b). In horses, there are breed differences in the rate of wear by attrition (Muylle *et al.* 1997, 1998). More specifically a difference in microhardness of enamel, primary and secondary dentine between horse breeds has been demonstrated (Muylle *et al.* 1999b) and a similar difference in microhardness between donkey teeth and horse teeth is proposed.

The most widely accepted technique for hardness testing of teeth is by using a hardness tester fitted with a Knoop diamond indenter. Microhardness is expressed as the Knoop Hardness Number (KHN) which represents the load required to force the indenter down until $1mm^2$ of the tooth surface is occupied and is expressed in kg/ mm^2. Muylle *et al.* (1999) showed a mean microhardness of 236.8 to 288.6 KHN in enamel, 41.4 to 58.9 KHN in primary dentine and 20.1 – 36.3 KHN in secondary dentine in horse incisors.

The rate of wear is not entirely dependent on tooth microhardness as has been demonstrated by the non-significant difference in enamel and dentine microhardness between trotter and Belgian draft horses (Muylle *et al.* 1999b), despite the differences between these two breeds in incisor wear with age (Muylle *et al.* 1997). Many other factors such as composition of the diet (Carlsson *et al.* 1966), masticatory cycle, enamel thickness and particularly duration of mastication (Dahl *et al.* 1993) have been shown to significantly affect the rate of wear of teeth.

1.6 Equid dental pathology

Dental related work has been reported to account for 10% of equine practice time in the United Kingdom (Anon 1965). A more recent study in the USA showed that dental problems were ranked amongst the top five most common conditions that equine practitioners have to treat (Traub-Dargatz *et al.* 1991). A few post mortem studies have also indicated a high prevalence (up to 80%) of undiagnosed dental conditions that were clinically significant (Honma *et al.* 1962; Wafa 1988; Kirkland *et al.* 1994; Brigham and Duncanson 2000b). Dental disorders have even been noted in 7 out of 37 skulls examined in wild equids (Cape mountain zebras) that died of causes unrelated to dental disease. The abnormalities noted in these zebras included incisor displacements, uneven CT wear, periodontal disease and diastemata (Penzhorn 1984). There is a high prevalence of dental disorders in museum skull specimens from the last two millennia in the Palaeontology museum in Paris (P.M. Dixon, personal communication).

Donkey dental pathology has been poorly documented to date (Rajput *et al.* 1999; Dacre *et al.* 2007). One post mortem study identified abnormalities such as loss of teeth, wave mouth, sharp teeth, focal overgrowths and maxillary osteoporosis (Rajput *et al.* 1999). A more recent clinical study in Egypt identified dental disease in 18 out of 214 donkeys (8.4%). The disorders identified included dental caries, sharp enamel points, diastemata, tartar and dental fractures (Marzok *et al.* 2006). Similar recent reports have identified dental disease in the working donkey in India (Roy 2002; Roy 2006; Shukla 2006), Mexico (Fernando-Martinez *et al.* 2006) and Tunisia (Chabchoub *et al.* 2006). The report of Dacre *et al.* (2008a) describes common dental disease disorders in a predominantly elderly population of donkeys in the UK, based on clinical examination. No comprehensive study appears to have been performed to date on donkey dental disease. The abnormalities described below are based on dental disease that has been described in the horse.

1.6.1 Developmental and eruption abnormalities

Developmental abnormalities are abnormalities that are present prior to, or at the time of tooth eruption whereas acquired conditions occur after eruption. Developmental disorders often lead to acquired dental disorders, resulting in more severe dental disease.

Tooth eruption is based on deposition and absorption of bone, vascular and intraosseous hydrostatic pressure changes and periodontal ligament contraction (Nanci and Somerman 2003; Baker 2005a). The eruption pathway only commences once enamel formation is complete in the developing tooth (Baker 2005a). Eruption of teeth has been divided into 6 phases (Becker 1939):

Stage I: preparatory phase (bony crypt opening)
Stage II: tooth migration towards the oral epithelium
Stage III: emergence of crown tip into the oral cavity (clinical eruption)
Stage IV: first occlusal contact
Stage V: full occlusal contact
Stage VI: continued eruption and movement

Trauma occurring at any stage of the eruption process may lead to developmental disorders. Genetic, teratogenic or viral factors may also result in abnormalities during dental development.

1.6.1.1 Odontodysplasia, oligodontia and polyodontia

Odontodysplasia (abnormal development of dental tissue) has been described in two horses; one was associated with epitheliogenesis imperfecta (Dubielzig *et al.* 1986b) and the other with fluoride toxicosis (Stewart and Genetzky 1984). Odontodysplasia has also been described in other species including dogs (Dubielzig *et al.* 1986a), sheep (Orr *et al.* 1979) and humans (Hamdan *et al.* 2004). The congenital absence of one or more teeth is termed oligodontia and is the result of failure of tooth bud formation. Only a few cases of developmental equine oligodontia with dental dysplasia has been reported and it does not appear to be common (Dixon *et al.* 1999b; Ramzan *et al.* 2001). Single or multiple oligodontia results in the displacement of other CT in the corresponding dental row. This could result in further dental abnormalities in later life.

In contrast polyodontia or supernumerary CT are more common in horses, although the prevalence is still low (Miles and Grigson 1990; Kirkland *et al.* 1994; Dixon *et al.* 1999b; Dixon and Dacre 2005; Quinn *et al.* 2005). Supernumerary CT are more common in the maxillary arcade and typically develop caudal to the normal CT row (Miles and Grigson 1990; Dixon *et al.* 1999b; Baker 2005a). Supernumerary CT may be normal in shape (develop from extra tooth buds) or abnormally shaped and large resulting from trauma causing splitting of a developing tooth bud (Baker 2005a; Quinn *et al.* 2005). Supernumerary CT can result in overgrowths, diastema formation, periodontal food pocketing and disease, potentially leading to apical infections (Dixon *et al.* 1999b; Dixon 2006). Supernumerary CT are often a co-incidental finding, but clinical signs that can be associated with these vary from bit evasion, facial swelling, unilateral nasal discharge and quidding (Quinn *et al.* 2005). Treatment options vary from conservative management of overgrowths to extraction of these teeth (Dixon *et al.* 1999b; Rajput *et al.* 1999; Quinn *et al.* 2005). Of 24 supernumerary CT treated by Quinn *et al.* (2005), 11 out of 13 (85%) cases treated conservatively were successful.

1.6.1.2 Retained deciduous cheek teeth

Between 2 – 4 ½ years of age, equids shed their deciduous CT with the eruption of their permanent 06s, 07s and 08s. It has previously been proposed that prolonged retention of the remnants of the deciduous teeth (‘caps’) may delay the eruption of the underlying permanent CT and can lead to the development of enlarged ‘eruption cysts’, which my lead to peri-apical tooth infections (Barrarion *et al.* 1980). These eruption cysts are often seen on the ventral mandible or rostro-dorsal aspect of the maxilla in 3 or 4 year old horses. However, recently it has been shown that enlarged eruption cysts are not always associated with retained ‘caps’ and are more likely due to CT overcrowding at the time of eruption due to excessive rostro-caudal angulation of the CT in a row and vertical impaction of erupting CT (Dixon *et al.* 1999b; Dixon and Dacre 2005; Dixon 2006). Clinical signs, such as oral discomfort and quidding, may also be associated with loose deciduous ‘caps’, however premature removal of these ‘caps’ may result in loss of the dental sac overlying the permanent tooth and may predispose to infundibular cemental hypoplasia (see section 1.4.5)(Baker 1982; Kilic *et al.* 1997d). Eruption cysts may be predisposed to developing anachoretic pulpitis (Gier and Mitchell 1968; Dixon 2006).

1.6.1.3 Developmental cheek teeth displacements

In young horses, overcrowding during the eruption process of particularly the 08s, which are the last permanent teeth to erupt, may result in these teeth becoming laterally or medially displaced (Miles and Grigson 1990; Baker 2005a; Easley 2006). This is particularly more so when there is excessive rostro-caudal angulation of the dental arcade, and these teeth are often termed to be vertically impacted.

These medial and/or lateral displacements are usually seen in young horses, can be bilateral and are occasionally associated with rotation of the teeth. In a study examining 400 referred equine dental cases by Dixon *et al.* (1999b), 5.8% (23/400) of cases referred suffered from CT displacements of which 69.6% (16/23) were regarded as being of *developmental* origin. Most of these developmental displacements involved the caudal CT, with maxillary 09s and mandibular 10s most commonly affected. Displacements of CT have been noted in previous studies (Colyer 1906; Baker 1979) and Wafa (1988) demonstrated a 7.2% prevalence in 355 skulls examined.

Cheek teeth opposite the displaced teeth often have gross overgrowths where they are not in occlusal contact and the displaced CT can grow into the soft tissues causing chronic pain. More severe problems such as diastema, with secondary food pocketing and periodontal disease are invariably seen with these displacements (Wafa 1988; Dixon *et al.* 1999b). Where very wide diastemata are present adjacent to developmentally displaced teeth, it is believed that the displacement is due to abnormal positioning of the developing dental bud rather than to overcrowding (Dixon *et al.* 1999b). Treatments of displacements range from treating overgrowths to extraction of the affected tooth (Dixon *et al.* 1999b).

Cheek teeth displacements can also be acquired secondary to other conditions such as loss of adjacent teeth resulting in drifting of teeth, severe periodontal disease or exaggerated occlusal angulation of the lower 10s and 11s (Dixon and Dacre 2005). Acquired rostral and caudal displaced CT can also occur secondary to focal dental overgrowths such as seen with overgrowth of the lower 11s or upper 06s (Dixon 2006). The presence of supernumerary CT has also been associated with acquired displacements due to overcrowding (Dixon and Dacre 2005; Quinn *et al.* 2005; Dixon 2006).

1.6.1.4 Diastemata

Diastema is defined as a detectable interdental space between adjacent teeth and CT diastemata was diagnosed as the primary dental disorder in 4% of 400 horses examined for dental disorders by Dixon *et al.* (1999b). This is similar to the 3.6% noted by Wafa (1988). The caudal mandibular CT were more commonly involved, particularly the 09s and 10s (Dixon *et al.* 1999b; Collins and Dixon 2005; Dixon 2006; Dixon *et al.* 2008). Diastemata can be primary developmental and develop due to inadequate rostro-caudal angulation or due to embryonic buds developing too far apart (Collins and Dixon 2005; Dacre 2005a; Dixon 2006). In contrast, if the supporting bones are not large enough to support the developing buds, overcrowding of erupted teeth result in displacement of these CT with subsequent secondary developmental diastemata developing (Dixon 2006). CT taper towards their apices and with continued eruption in aged horses senile diastemata develop between these narrowed CT. Diastemata can also develop secondary to loss of CT or acquired CT displacements, more commonly seen as medial displacement of the mandibular 10s and 11s (Collins and Dixon 2005; Dixon 2006). Interestingly diastemata have also been identified in a survey of free-ranging Cape Mountain Zebras (Equus zebra zebra) (Penzhorn 1984), which indicates that it is unlikely to be a disease of domestication.

Sharp overgrowths or exaggerated transverse ridges may widen diastema and compress food into the diastemata (Dixon *et al.* 1999b; Dixon and Dacre 2005). The presence of diastemata may lead to compression of food between the adjacent teeth, with periodontal pocketing and periodontal disease (Wafa 1988; Dixon *et al.* 2008). In severe cases this progresses to cause lysis and remodelling of alveolar bone and osteomyelitis of the mandibles or maxillae (O'Connor 1950). The most common clinical sign seen with diastemata is quidding and is regarded as one of the most painful dental disorders seen in the horse (Dixon *et al.* 1999b; Collins and Dixon 2005; Dixon and Dacre 2005). Diastemata have been divided into closed/valve diastemata (narrower proximally) and open (same width proximal and distal) diastemata (Carmalt 2003).

Treatment of diastemata depend on the severity of the condition and can range from removal of opposing overgrowths, cleaning out periodontal pockets, diastema widening and even tooth extraction (O'Connor 1950; Collins and Dixon 2005; Dixon and Dacre 2005; Dixon *et al.* 2008). The feeding of a diet of short fibres (< 5mm) that will not become entrapped in diastemata, may reduce food pocketing. In younger horses further eruption of the CT and compression of the CT rows may even result in resolution of the diastemata, provided there is sufficient CT angulation (Barakzai and Dixon 2003).

1.6.1.5 Rostral positioning of the maxillary arcade

Equid maxillae are often slightly more rostrally positioned relative to the mandible. This often results in incisor malocclusion seen as an overjet or even overbite (“parrot mouth”). Simultaneously, this defect results in the maxillary 06s not being in complete occlusion with the mandibular 06s, and small rostral overgrowths (“hooks”) develop on the maxillary 06s. These focal overgrowths are often associated with bitting problems and can restrict normal rostro-caudal movement of the mandibles (Carmalt *et al.* 2003). This disorder often also leads to corresponding caudal overgrowths on the mandibular 11s, however true caudal mandibular overgrowths need to be differentiated from the anatomically normal ‘curve of Spee’. Similar caudal overgrowths can also occur due the presence of supernumerary CT.

1.6.2 Acquired dental disorders

1.6.2.1 Wear abnormalities

Tooth wear starts when opposite teeth come into full occlusion and the opposing teeth occlusal surfaces grind against each other (Hillson 1986). Any asymmetry in the jaw or teeth will result in uneven wear of teeth. Dental overgrowths are often associated with adjacent periodontal disease (possibly due to restricted intra-oral food movement) and soft tissue trauma which may lead to clinical signs such as quidding. In one study, dental related soft tissue trauma, such as mucosal inflammation, ulceration and fibrosis was most commonly observed in horses under 10 years of age (Kirkland *et al.* 1994).

1.6.2.1.1 CT overgrowths

The presence of anisognathia and wider maxillary CT compared to mandibular CT contribute to the development of enamel overgrowths on the buccal aspect of maxillary CT and lingual aspect of CT in the mandibular arcade (O'Connor 1950). These sharp points may lead to soft tissue ulceration of the buccal mucosa and in severe cases equids may exhibit clinical signs such as quidding (O'Connor 1950).

1.6.2.1.2 Shear mouth

If the above generalised CT overgrowths are not managed by routine dental floating, they may increase to the extent that they interfere with the normal side-to-side masticatory action. This further perpetuates the overgrowths and may lead to a condition called shear mouth (Baker 1991; Dixon *et al.* 2000a). This condition results in very steep angled (>45°) CT occlusal surfaces and ineffective grinding of food exhibited as quidding. A prevalence of 0.03% has been reported in a study looking at 30 000 horses (Becker 1962).

1.6.2.1.3 Wave mouth

'Wave-mouth' is uneven wear within a CT row resulting in an undulating occlusal surface of the CT arcade in a rostro-caudal direction. This has been proposed to occur in some CT secondary to periodontal disease which disrupts the normal eruption process (Dixon *et al.* 2000a). Differential rate of CT eruption has also been proposed as a cause of wave-mouth (Becker 1962; Kirkland *et al.* 1996). However the cause is most likely multifactorial.

1.6.2.1.4 Step mouth

Classically, the loss of a cheek tooth will cause 'super-eruption' of the opposing CT, leading to a condition termed '*step mouth'*. Dixon *et al.* (2000a) found 40% of step mouths to occur due to CT maleruptions such as differential eruption of opposing CT with the earlier erupted CT becoming 'dominant' (O'Connor 1950; Becker 1962). In less severe cases this may lead to wave mouth. Overgrown teeth may be rectangular or triangular in shape. These overgrowths can interfere with normal mastication leading to wave-mouth or shear mouth. They may also cause oral pain and may manifest as oral quidding, halitosis and weight loss (Dixon and Dacre 2005). Infundibular cemental hypoplasia or infundibular caries may also weaken maxillary CT resulting in increased wear and overgrowths on the opposing mandibular CT.

1.6.2.1.5 Smooth mouth

In older equids the loss or reduction of enamel ridges is a normal physiological end stage phenomenon of attrition (Lowder and Mueller 1998). This leads to a condition termed "*smooth mouth*" and is characterised by a smooth occlusal surface consisting predominantly of cementum and dentine. This dentine and cementum is no longer protected by the harder enamel and such teeth are ineffective at grinding and are worn away rapidly (Dixon 2002). Once the roots are exposed the teeth may develop hypercementosis, a protective mechanism which is often seen in geriatric equids (Lowder and Mueller 1998; Dixon 2002). Occasionally smooth mouth can develop in younger equids where there is insufficient enamel infolding of peripheral enamel, or absence of maxillary CT infundibulae (Dixon and Dacre 2005) or in cases of enamel dysplasia.

1.6.2.2 Periodontal disease

Periodontal disease describes inflammation of the supporting structures of the tooth i.e. gingiva, periodontal ligaments, cementum and alveolar socket lining and has also been termed paradontal disease, periodontitis, alveolar disease and alveolar periosteitis (Hofmeyr 1960; Wafa 1988; Miles and Grigson 1990). Periodontitis has been shown to be an important disease in dogs and cats (Wiggs and Lobprise 1997c), sheep (Cutress and Healy 1967; Spence *et al.* 1988) and cattle (Ingham 2001).

In 1906 Coyler described periodontitis as a significant disease in the horse with a prevalence of about 33% in the 484 horses examined, however examination of photos of Colyer's specimens (Miles and Grigson 1990) show that the periodontitis described by Coyler (1906) was predominantly secondary to other disorders such as diastemata and displaced teeth (Dixon and Dacre 2005). Other early studies have also reported the presence of periodontal disease in horses (Little 1913; Hofmeyr 1960) and even in zebras secondary to diastemata (Penzhorn 1984).

More extensive studies in horses have also recognised periodontitis as a significant condition (Baker 1970; Wafa 1988; Dixon *et al.* 1999b, 2000a; Dacre 2005b). Baker (1970) and Wafa (1988) found that 60% of horses over 15 and 20 years of age respectively suffered from periodontal disease. However, most often this periodontal disease was secondary to other disorders such as displaced teeth or CT diastema. Both authors also recognised a mild transient periodontitis associated with eruption of teeth with a prevalence of 40 and 52% in immature skulls (Baker 1970; Wafa 1988).

The primary stage of periodontitis in brachydont species is caused by the accumulation of organic dental plaque and adherence of bacteria to teeth (Wiggs and Lobprise 1997c). Later, the plaque may become calcified to calculus that consists of 70-90% minerals. The main component of equine calculus is calcite and it tends to have a chalky appearance, most commonly seen on canine teeth and the buccal aspects of the rostral maxillary CT (excluding the wolf tooth) (Hillson 1986). However accumulation of plaque or calculus does not appear to be a widespread problem in equine CT that do not have intercurrent disorders (Baker 1970; Wafa 1988; Dixon *et al.* 2000a). This absence of calculus on equine CT could be due to the prolonged time horses spend eating as the intra-oral movement of food and saliva deters the formation of plaque (Wiggs and Lobprise 1997c).

Periodontal disease starts with loss of the normal tight gingival attachment between adjacent teeth. This gingiva then becomes inflamed due to mechanical irritation e.g. to impacted food particles or chemical irritation such as bacteria or plaque. As the gingival destruction continues, the gingival opening becomes further impacted with food and the process perpetuates itself with the periodontitis extending deeper into the periodontal ligament and outwards to the buccal and lingual margins forming large periodontal food pockets (Hillson 1986). This inflammation and infection may even extend down into the bone, causing bone remodelling and even bone necrosis and infection. Eventually the tooth becomes loose and may even spontaneously be shed due to loss of supporting structures (Miles and Grigson 1990). More focal apical extension of the periodontal disease may also lead to infection of the pulp, apical infection and ultimately death of the tooth (Little 1913; Wiggs and Lobprise 1997c).

A veterinary periodontal disease index (0-4) has been used in small animals based on the percentage attachment loss (Wiggs and Lobprise 1997c) and could be used in equids:

Stage 0	normal
Stage 1	gingivitis
Stage 2	early periodontal disease (up to 25% attachment loss)
Stage 3	moderate (25-50% attachment loss)
Stage 4	severe (greater than 50% attachment loss)

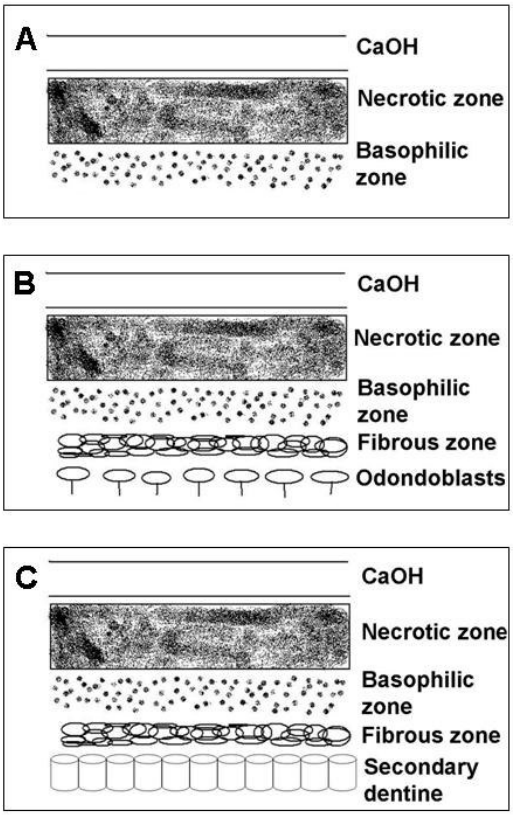
1.6.2.3 Pulpitis

Inflammation of the pulp in humans can be asymptomatic or associated with pain not necessarily localised to a tooth, and occurs most commonly secondary to dental caries (Soames and Southam 1993). Other aetiological factors of pulpitis include bacterial penetration via pulp exposure secondary to attrition, abrasion or trauma; chemicals (e.g. direct application to pulp or diffusion after insertion of restorative material); and thermal damage i.e. heat produced from rasping in particular motorised equipment.

The inflammatory response in the pulp results in oedema and the influx of lymphocytes, plasma cells and macrophages. Due to the pulp being completely encased by dentine, the inflammatory response causes an increase in the pulp pressure and the collapse of the venous microcirculation. This results in areas of hypoxia and anoxia that may lead to localised pulp necrosis. Continued inflammatory response may lead to necrosis of the entire pulp. If time allows reactionary secondary dentine may increase the thickness between the insult and the pulp and result in the resolution of the pulpitis (Soames and Southam 1993).

In cases of acute pulpal exposure (e.g. dental fracture) the application of calcium hydroxide as a ‘pulp-cap’ results in the rapid formation of a necrotic zone adjacent to the calcium hydroxide that has a pH of 11. A basophilic zone consisting of calcium proteinates forms below this necrotic zone (Figure 1.2a). An adjacent fibrous layer and odontoblast cell layer forms within 2 weeks (Figure 1.2b), followed by a layer of early secondary dentine 2 weeks later (Figure 1.2c) (Soames and Southam 1993). The exact mechanism by which calcium hydroxide induces this reparative dentine is unknown.

***Figure 1.2*: The induction of reparative dentine by calcium hydroxide: (A) formation of a basopihilic zone below the necrotic zone; (B) within 2 weeks an adjacent layer of fibrous tissue and odontoblasts form; (C) formation of secondary dentine 2 weeks later.**



1.6.2.4 Occlusal pulpar exposure

Odontoblasts that line the pulp cavity produce secondary dentine that gradually obliterates the pulp cavity circumferentially and occlusally (Nanci 2003a). An imbalance between CT wear and secondary dentine deposition occlusally, e.g. faster wear, will result in exposure of the pulp cavity on the occlusal surface. This may result in food impacting within the exposed pulp cavities, descending infection and ultimately apical infection of the CT (Wafa 1988; Lane 1994; Dacre 2005a). Ultrastructural examination of equine teeth have shown that dentinal tubules are often exposed on the occlusal surface and this may also provide a route of infection of the pulp from the occlusal surface (Kilic *et al.* 1997c). Pulpar exposure has been recognised in equine CT at post mortem (Wafa 1988) and at clinical examination (Dixon *et al.* 2000b). Pulpar exposure of younger teeth with large pulp cavities and a good blood supply are able to respond by stimulation odontoblasts to produce secondary dentine and may prevent apical infections (Wiggs and Lobprise 1997c; Dixon and Dacre 2005). Pulpar exposure has also been noted in CT secondary to pulpal death e.g. after apical tooth infections (Dacre 2005a), due to continued eruption of the tooth that is no longer laying down secondary dentine.

1.6.2.5 Apical infections

Apical infections is a more accurate term to use in equines than "tooth root infection" as it includes infections in young horses prior to root development and also infections in older horses with well developed roots. Infection of the tooth apices is an important disease of horses and may extend to involve the supporting structures including the periodontal ligament, alveolar and supporting bones and paranasal sinuses (Miles and Grigson 1990; Dixon *et al.* 2000b; Dacre 2005a). A study of 400 referred horses with dental disease included 40.5% presented for primary apical infections (92 with maxillary CT and 70 with mandibular CT infections) (Dixon *et al.* 2000b). Apical infections appear to occur most commonly in younger horses, and Dixon *et al.* (2000a) showed that the median age for horses with apical infections of maxillary and mandibular CT was 7 and 5 years respectively.

A commonly proposed aetiology of equine CT apical infections is pulpar exposure (Wafa 1988; Lane 1994; Kilic *et al.* 1997a). A more likely cause of primary apical infections is anachoretic infection i.e. blood or lymphatic borne bacterial infection (Dixon 1997; Dixon *et al.* 2000b). This theory is supported by a recent study that indicated that there are anastomoses between the periodontal vasculature and the maxillary sinus blood vessels (Masset *et al.* 2006). Vertical impactions and hyperemia of the apex may predispose to anachorectic infections (Dacre 2005a) and this theory is supported by the higher incidence of apical infections in younger horses.

Secondary apical infections can occur due to other developmental disorders (polyodontia, dental dysplasia, hypoplasia, diastemata and displacements) by deep periodontal disease, wear disorders or fractures (idiopathic or traumatic). Infundibular hypoplasia with food impaction can predispose to bacterial infection leading to caries either by: weakening the tooth structure resulting in midline sagittal fractures; or occasionally extension into infundibular enamel and then direct spread of the infection into dentine and pulp or directly to the apex (Dixon *et al.* 2000b; Dacre 2005a).

Clinical signs associated with apical infections depend on the tooth involved, the duration of infection and the extent of the infection. The study by Dixon *et al.* (2000a) showed that if the rostral 3 maxillary CT are involved, maxillary swellings and sinus tracts were more common, and nasal discharge was more common if the caudal 3 maxillary CT were involved. Mandibular swellings and sinus tracts were commonly seen in mandibular CT infections (Dixon *et al.* 2000b).

Diagnosis of apical infections requires a complete clinical and oral examination with radiographic evaluation. Radiographic features of apical infections consist of: soft tisuue opacity or fluid lines in the paranasal sinuses; sclerosis, cementosis or clubbing of the tooth apex; disruption of the *lamina dura denta*; and periapical lucencies with surrounding sclerosis (Gibbs and Lane 1987; Weller *et al.* 2001). In some cases, apical infection may need to be confirmed with scintigraphy or by repeated clinical and radiographic examination at a later stage (Weller *et al.* 2001). Computed axial tomography (CAT) examination of the complex three-dimensional head region is the optimal imaging technique. Medical treatment of apical infections is largely unsuccessful and oral extraction is thus far the most successful treatment (Tremaine 1997; Dixon *et al.* 2000b; Dacre 2005a). Recently endodontic treatment has been performed with variable success in early cases (Baker 2005c; Simhofer *et al.* 2006). An initial success rate of 80-85% in the treatment of apical infections by surgical apicoectomy was reported (Simhofer *et al.* 2006). However a longer follow up period (50 months) has shown this success rate to decrease to 64% (Simhofer *et al.* 2007).

1.6.2.6 Dental caries

Caries is characterised by destruction of the hard dental tissue with bacteria as the primary initiator. Bacterial fermentation of carbohydrate release acids that decalcify the inorganic dental components at pH 4 – 5.5 (Hillson 1986). Dentine is demineralised very rapidly once the amelo-dentinal junction is reached and results in the classic black cavity appearance of caries (Wiggs and Lobprise 1997a).

The most common type of dental caries identified in horses is infundibular cemental caries (Colyer 1906; Honma *et al.* 1962; Kilic *et al.* 1997d; Dixon *et al.* 1999a; Brigham and Duncanson 2000b; Dixon *et al.* 2000b). Colyer (1906) observed a prevalence of 13% caries and Honma *et al.* (1962) reported a prevalence of 100% in maxillary CT of horses over 12 years of age. Maxillary CT in older horses are predisposed to developing caries due to cemental hypoplasia in the apical aspect of the infundibula, that only become exposed with age (Baker 1974).

However, a recent study has shown that true infundibular cemental caries only occurred in 8% (62/786) of infundibulae based on gross examination of longitudinally sectioned teeth, with the Triadan 09s accounting for 47% (29/62) of the teeth affected by caries (Fitzgibbons 2007). It is believed that the higher prevalence previously reported was due to misclassification of infundibulae with cemental hypoplasia as caries.

Infundibular caries has been classified by Honma (1962) depending on the extent of caries in the dental tissue. A modified system of classifying caries has been proposed by Dacre (2005b) which is also applicable to peripheral caries:

0 degree: no macroscopic visible caries (can include infundibular hypoplasia)
1st degree: caries only affecting the cementum – from small pitting superficial spots (class 1) to extensive destruction and loss of cementum (class 2)
2nd degree: caries affecting cementum and adjacent enamel
3rd degree: caries affecting cementum, enamel and dentine
4th degree: progressed to affect the integrity of the tooth i.e. development of an apical abscess or secondary tooth fracture.

Peripheral caries has been poorly described (Baker 1979; Easley 1991) and as it may remove coronal and occlusal cementum it can predispose to increased rate of occlusal wear. Infundibular caries may extend to the dentine and result in apical infections or extend concentrically, weaken the tooth and result in saggital fracture of the affected CT (Baker 1974).

1.6.2.7 Dental fractures

Trauma induced dental fractures, particularly of the incisors, are relatively common in horses due to kicks, crib biting, biting hard objects and collisions with solid objects e.g. gates, fences and walls (Hague and Honnas 1998). In a study by Dixon *et al.* (1999a) of referred cases of incisor disorders, 8 of 11 cases of fractures were caused by trauma. Only 7.5% of cases referred for CT dental disease had traumatic fractures, with the majority (71%) being mandibular CT fractures; with traumatic kicks and iatrogenic fractures (use of dental shears) the most common cause. More recently a practice based survey indicated a median prevalence of 0.4% in a mixed population of horses (Taylor and Dixon 2007).

Clinical signs most commonly seen with dental fractures were quidding, followed by bitting and behavioural problems, and halitosis (Taylor and Dixon 2007). Some cases, especially with smaller slab fractures, were asymptomatic and only noted on routine dental examinations (Taylor and Dixon 2007).

A pathological study examining 35 fractured CT found that maxillary CT were more commonly involved than mandibular CT and in particular the maxillary 09s (Dacre *et al.* 2007). Similarly a practice and hospital survey of fractured cheek teeth also showed the maxillary 09s to be most commonly involved (Dixon *et al.* 2007; Taylor and Dixon 2007), but the reason for this is unknown. The most common fracture patterns observed were lateral slab fractures through the two buccally situated pulp cavities (Dacre *et al.* 2007; Dixon *et al.* 2007; Taylor and Dixon 2007). This is thought to be due to mineralised dental tissue being thinner and therefore weakest at this point (Dacre *et al.* 2007). The less common mid-saggital fracture through both pulp cavities occurs secondary to infundibular caries predisposed by infundibular cemental hypoplasia (Dacre *et al.* 2007)

Dental pulps were involved in many maxillary and mandibular slab fractures examined (Dacre *et al.* 2007; Dixon *et al.* 2007; Taylor and Dixon 2007). However, lateral slab fractures have been shown to resolve without development to apical infections (Dixon *et al.* 2000a), implying that the resultant pulpitis remained low grade or was contained by odondoblast-like cells producing tertiary dentine (Magloire *et al.* 1996). Therefore, the initial treatment of lateral slab fractures is removal of the (usually displaced) smaller portion (if still present) and rasping of sharp edges. Midline saggital fractures might require removal of the entire tooth if both fragments are loose (Klugh *et al.* 2001). Prevention of dental fractures secondary to infundibular caries may be attempted with endodontic and restorative treatments although more research needs to be performed in this area in equines to determine its success (Dixon *et al.* 2007).

1.6.3 Iatrogenic dental disorders

Due to increased recognition in the significance of equine dental disease to the welfare and performance of equines, dental treatment has become more popular and led to the development of many manual and motorised dental instruments (Scrutchfield and Easley

2005). Unfortunately this has also led to the increased occurrence of iatrogenic dental diseases such as dental fractures and reduction of normal occlusal ridges resulting in inefficient grinding of food (Ralston *et al.* 2001; Scrutchfield and Easley 2005). It has also been shown that use of power equipment may lead to thermal trauma which may ultimately lead to vascular pulpar injuries and necrosis (Baker 2002; Dacre *et al.* 2005). Similarly aggressive treatment of dental overgrowths may result in sensitive dentine exposure by removal of the protective sclerotic or organic 'smear' layer (Kempson *et al.* 2003) or even cause full pulpar exposure.

1.7 Conclusions

The importance of dental disease in horse welfare and performance has been recognised, and equine dentistry has received renewed interest over the last 15 years. Most of the current literature on equid dentistry is based on research and clinical work performed in horses and no studies have extensively examined normal or abnormal donkey teeth.

The aims of the study were to:

- Determine the normal dental anatomy of donkey teeth using gross examination, computed axial tomography (CAT), decalcified and undecalcified histology and scanning electron microscopy (SEM)
- Compare the microhardness of donkey incisors to horse incisors using a Knoop Hardness Tester
- Identify and categorise donkey dental disease and determine the prevalence of specific dental disorders in donkeys at post mortem from donkeys that have died or were euthansed on humane grounds at The Donkey Sanctuary, Sidmouth, UK
- Describe the radiographical, histological and ultrastructural pathology observed in cheek teeth with caries and pulpal exposure

- Identify donkey dental disease and determine the prevalence of dental pathological conditions in live donkeys from the UK (The Donkey Sanctuary) and Mexico
- Determine epidemiological factors associated with dental disease in the donkey

Chapter 2: Donkey dental anatomy: Gross and computed axial tomography examinations

2.1. Introduction

Donkeys are well adapted to survival in dry arid environments by their ability to browse as well as graze. The literature on donkey dental gross anatomy has been very limited to date and includes two gross anatomical studies, one of which was limited to examination of incisor eruption dates and occlusal surfaces (Bunger and Hertsch 1981; Muylle *et al.* 1999a) and one radiological study on cheek teeth development (Misk and Seilem 1999).

Recently, the use of CAT has enabled detailed examination of maxillary cheek teeth in horses in three-dimensional images, avoiding the superimposition of anatomical structures on radiographs (Henninger *et al.* 2003). CAT is an imaging modality that uses computer geometry processing to create a three-dimensional image of a series of two-dimensional x-ray images that are taken around a single axis. Axial CAT consists of x-ray images taken sequentially along an axis in separate 'slices', whereas in helical computed tomography the x-ray gantry moves continuously following a 'spiral' path. The main advantage of helical compared to axial computed tomography is speed which also reduces motion artefacts, hence has no advantage over axial tomography when scanning exctracted teeth. Most of our limited knowledge of donkey dentistry has developed by extrapolation from basic and clinical research in horses, but this knowledge may not necessarily be appropriate to the donkey.

The aim of this study was to describe the normal dental anatomy of the domestic donkey using gross examination and computed axial tomography in Chapter 2, and decalcified and undecalcified histology, and scanning electron microscopy in Chapter 3. These results were then objectively compared to the previous findings of equine dentition.

2. 2. Gross Anatomy

2.2.1 Measurement of anisognathia

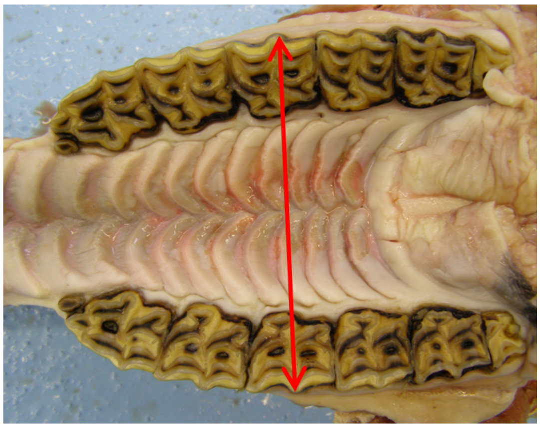
2.2.1.1 Aim of the study

A previous study in horses showed that the distance between the lateral aspects of the maxillary cheek teeth (an indirect measurement of jaw width) were 23.43% wider than this same measurement in mandibular cheek teeth (Taylor 2001). The aim of this study was to confirm the presence of anisognathia in donkeys and to determine the degree of anisognathia as expressed by the percentage of the measurements in the maxillary cheek teeth rows to the mandibular cheek teeth rows.

2.2.1.2. Materials and Methods

Post-mortem measurements were taken from the jaws of 19 donkeys that died or were put to sleep for reasons unrelated to the teeth or jaw at The Donkey Sanctuary. Measurements were taken with digital calipers (Knighton Tool Supplies, Leicester, UK) in millimeters. Measurements were made from the middle of each tooth (at the most buccal aspect) to the corresponding opposite tooth for each of the six cheek teeth per mandible and maxilla (Figure 2.2.1.2). The ratio of each maxillary measurement over corresponding mandibular measurement was determined (i.e. ratio of 06, 07, 08, 09, 10 and 11 for each donkey). If a tooth was displaced or missing that measurement was excluded from that head. Coefficient of variation of the obtained measurements was determined to be less than 15 for all the ratios measured. The distribution of the data were determined to be normal and a one sample t test (Minitab® Release 14) was performed for each ratio to determine if they were significantly different from the 23% as determined in the horse.

Figure 2.2.1.2: **Maxillary cheek teeth rows of a donkey skull illustrating where jaw width measurements were taken across the 08 cheek teeth.**



2.2.1.3. Results

The age distribution of the donkeys from which measurements were taken is illustrated in Table 2.2.1.3.1. The age range was from 7 to 40 years with a median age of 26 years.

***Table 2.2.1.3.1*:** **Age ranges of donkey from which jaw widths were measured.**

Age ranges	**0 - 10**	**11 – 20**	**21 -30**	**31 – 40**
Number of donkeys	1	4	11	3

The results of the one sample t tests are presented in Table 2.2.1.3.2. The ratios varied from 25.37% at the level of CT 10s to 31.35% at the level of CT 07s. This ratio at the 11s was in fact smaller than 23% at 21.69%. The ratios of CT 06-10 were all significantly greater than 23% (Ratio 06-09, $P<0.001$; Ratio 10, $P<0.01$) indicating a higher degree of anisognathia in donkeys as compared to horses.

***Table 2.2.1.3.2.*: Mean (%) (and standard deviations) of the ratios of maxillary to mandibular jaw width at different cheek teeth levels.**

Teeth Measured	Number of measurements	Mean degree of anisognathia (%)
06s	17	28.09 (3.8)
07s	17	31.35 (2.2)
08s	18	29.45 (2.9)
09s	17	26.75 (3.0)
10s	19	25.37 (3.6)
11s	17	21.69 (4.0)

The combined ratio of all 6 measurements (mean 27.09%) also show a significantly ($P < 0.001$) greater degree of anisognathia than in the horse. Gross examination also revealed that maxillae curved slightly such that they are convex laterally and mandibulae are straight that narrow towards each other as they join at the rostral symphysis i.e. triangular shaped.

2.2.2. Measurement of cheek teeth peripheral enamel infolding

2.2.2.1. Aim of the study

The aim of this study was to determine the degree of peripheral enamel infolding in maxillary and mandibular donkey cheek teeth, and to examine if the degree of enamel infolding was greater in mandibular than maxillary cheek teeth, to compensate for the absence of infundibula. Further to this, the contribution of infundibular enamel would be considered in maxillary CT.

2.2.2.2. Materials and methods

Collection, classification and fixation of teeth

A total of 82 intact cheek teeth and 26 incisors were extracted at routine post mortem examinations from 14 donkeys that died or were put to sleep for humane reasons at The Donkey Sanctuary, Sidmouth, UK and were then fixed in 10% buffered formalin. The median age of these donkeys was 19 years (range 6-21). The age and history of each donkey were recorded, and each tooth was labelled with its Triadan number. Only cheek teeth that appeared grossly normal were extracted and were selected randomly at routine post mortems for fixation (Table 2.2.2.2.1).

Table 2.2.2.2.1: **The number of cheek teeth in each Triadan positions that were extracted randomly at routine post mortems and age of the donkeys from which the cheek teeth were extracted.**

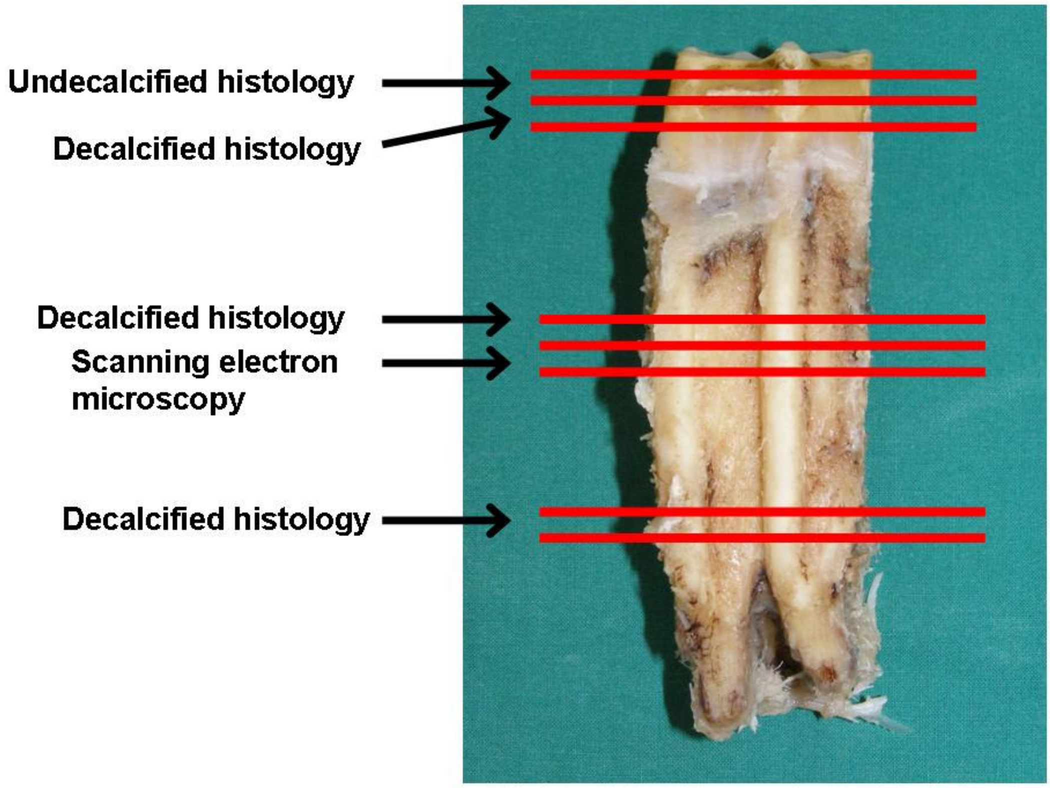
Triadan number	Total number of CT	Age of donkey 0-5 years	6-10 years	11-15 years	16-20 years	20+ years
106	3		1		1	1
107	7		2	2	3	
108	5		1	2	2	
109	4			3	1	
110	7		1	3	3	0
111	4		1		3	
209	5				3	2
210	5				3	2
307	3		1	1	1	0
308	5			2	2	1
309	5			2	2	1
310	9		1	3	4	1
311	8			1	6	1
406	1					1
407	4		1		2	1
408	1				1	
409	2			1	1	
410	2				1	1
411	2		1			1
Total number of teeth	82	0	10	20	39	13

Teeth Sectioning

All teeth were sectioned in transverse planes using a water-cooled 99-TS230M tile-saw (Buehler, Coventry, UK) with an 8” thin CR diamond blade (Malvern Lapidary, Malvern, Worcester, UK). As was noted by Dacre (2005a), due to the variable curvature of normal teeth in the longitudinal plane, longitudinal sectioning was not useful for quantitive dental analysis and was not performed in this study. All (transverse) cut sections were digitally photographed (Olympus SP-500UZ).

The total number of sections obtained from each tooth could not be standardised for all teeth, as older teeth were too short to allow all the sections to be acquired. Where possible, five sections were obtained from each tooth, including 3 for decalcified histology, one for undecalcified histology and one for SEM (Figure 2.2.2.2.1). Sub-occlusal, mid and apical sections of 5 mm thickness were obtained for decalcified histology. A 2-3 mm thick sub-occlusal section was obtained for undecalcified histology and 8mm thick sections mid tooth for SEM. Scanned JPEG images of the 5 sections were obtained using a HP Scanjet 4850 (Hewlett Packard) and saved on a computer.

***Figure 2.2.2.2.1:* Location of sites of transverse sections on a maxillary cheek tooth for scanning electron microscopy, decalcified and undecalcified histology.**



Images of subocclusal transverse sections of 37 mandibular teeth and 34 maxillary teeth were used to determine the degree of peripheral enamel infolding. Table 2.2.2.2.2 demonstrates the Triadan position of the cheek teeth examined.

Table 2.2.2.2.2: **Triadan positions of donkey teeth used to examine peripheral enamel infolding on subocclusal transverse sections.**

Tooth	107	108	109	110	111	209	210	307	308
Number used	6	4	4	7	3	5	5	4	4
Tooth	**309**	**310**	**311**	**406**	**407**	**408**	**409**	**410**	**411**
Number used	4	8	8	1	3	1	1	2	1

Measurements of the total tooth perimeter and enamel perimeter were taken, using an image analysis computer programme (ImageJ, Image analysis and processing in JAVA, http://rsb.info.nih.gov/ij/, USA) in millimeters (Figure 2.2.2.2.2). Each image was taken with a standard 1 cm marker which was then used to determine the image scale.

Figure 2.2.2.2.2: **Cut section of maxillary (right) and mandibular (left) cheek teeth illustrating the tooth peripheral enamel perimeter (yellow) and actual enamel perimeter (red) as measured using the ImageJ programme**

Degree of enamel infolding

The one sample t-test (Minitab® Release 14) was used to determine whether the ratio of enamel perimeter to total tooth perimeter was greater than one i.e. enamel perimeter was greater than tooth perimeter, with P < 0.05 denoting a statistical significance.

Maxillary compared with mandibular enamel infolding

Paired t-tests were used in 10 donkeys to determine if enamel infolding in 2 mandibular teeth were greater than in corresponding or equivalent 2 maxillary teeth in each donkey. Consequently, only teeth from donkeys that had corresponding or equivalent maxillary and mandibular cheek teeth were chosen for the paired t-test (Table 2.2.2.2.3).

Table 2.2.2.2.3**: Maxillary and mandibular cheek teeth of 10 donkeys used for the paired t-test to asses degree of enamel infolding.**

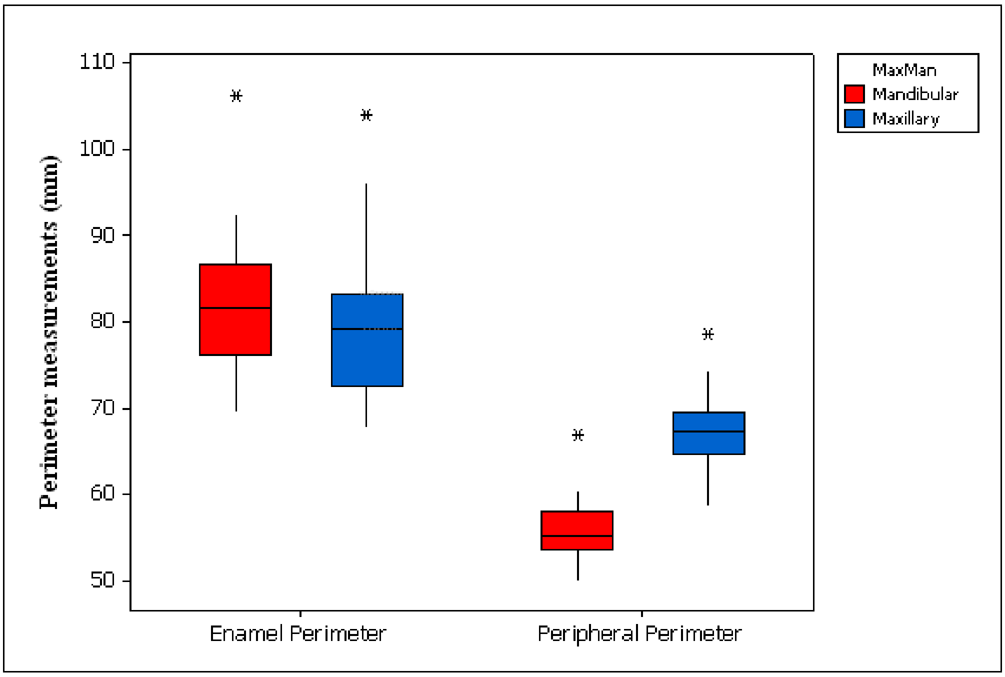
Donkey	Maxillary Cheek Teeth	Mandibular Cheek Teeth
1	209 & 210	407 & 410
2	110&111	310 &311
3	209 & 210	409 & 410
4	109 & 110	309 & 310
5	109 & 110	309 & 310
6	209 & 210	307 & 308
7	209 & 210	309 & 310
8	107&110	307 & 310
9	209 & 210	308 & 310
10	110&111	310 & 311

2.2.2.3. Results

Degree of enamel infolding

The results of the one sample T test clearly indicated a significant increase in enamel perimeter compared with total tooth perimeter ($P < 0.001$), with the mean ratio of 1.47 for mandibular teeth and of 1.17 for maxillary teeth (Figure 2.2.2.3.1). The mean ratio for maxillary CT enamel perimeter, including the infundibular enamel perimeters, compared to total tooth perimeter was 1.87.

Figure 2.2.2.3.1: **Boxplot illustrating infolded enamel perimeter and peripheral enamel perimeter in maxillary and mandibular CT[a]**

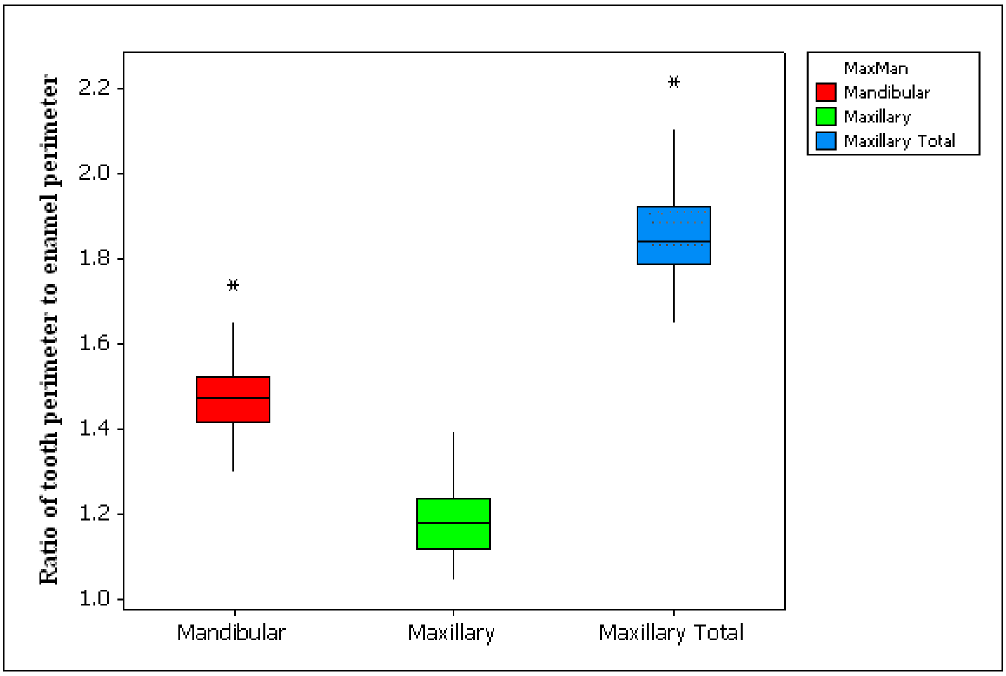


[a]Box represents interquartile range; vertical lines represent range; horizontal line represents median; * represents outliers

Comparison of maxillary and mandibular enamel infolding

The paired t test indicated a significantly greater degree of enamel infolding in mandibular cheek teeth compared to maxillary teeth ($P < 0.001$; Figure 2.2.2.3.2). The mean enamel perimeter to tooth perimeter in mandibular and maxillary cheek teeth was 1.47 and 1.17 respectively. However, when the infundibular enamel was included in maxillary CT they had a significantly greater ratio (1.87) of exposed enamel folds to tooth perimeter (P < 0.001; Figure 2.2.2.3.2).

***Figure 2.2.2.3.2*: Mandibular and maxillary cheek teeth ratio of enamel infolding to peripheral enamel perimeter. Maxillary total = with infundibular enamel perimeter; Maxillary = without infundibular enamel perimeter.**



2.2.3 Peripheral cementum

2.2.3.1 Aims of the study

The aim of the study was to determine if there is an increase in peripheral cementum area from the apical to the occlusal transverse section of donkey CT.

2.2.3.2 Materials and methods

The transverse sections of 26 maxillary and 26 mandibular CT of those used for enamel infolding determination were examined and scanned (HP scanjet 4850) to obtain digital images which were measured (ImageJ, Image analysis and processing in JAVA, http://rsb.info.nih.gov/ij/, USA). Three transverse sections were measured per tooth at different longitudinal levels (A = subocclusal; B = mid-tooth; C = apical third). The total transverse area of the tooth and peripheral cementum area were measured and the ratio calculated to determine the proportion of peripheral cementum at each level. Data were recorded (Microsoft Office Excel) and linear mixed effect models were performed (R V2.3.1, R Foundation for Statistical Computing) with $P < 0.05$ denoting a statistical significance. Individual donkeys and teeth were entered as random effects to take account of the fact that there could be individual donkey and specific tooth variation. The data were log 10 transformed to normalise the residuals.

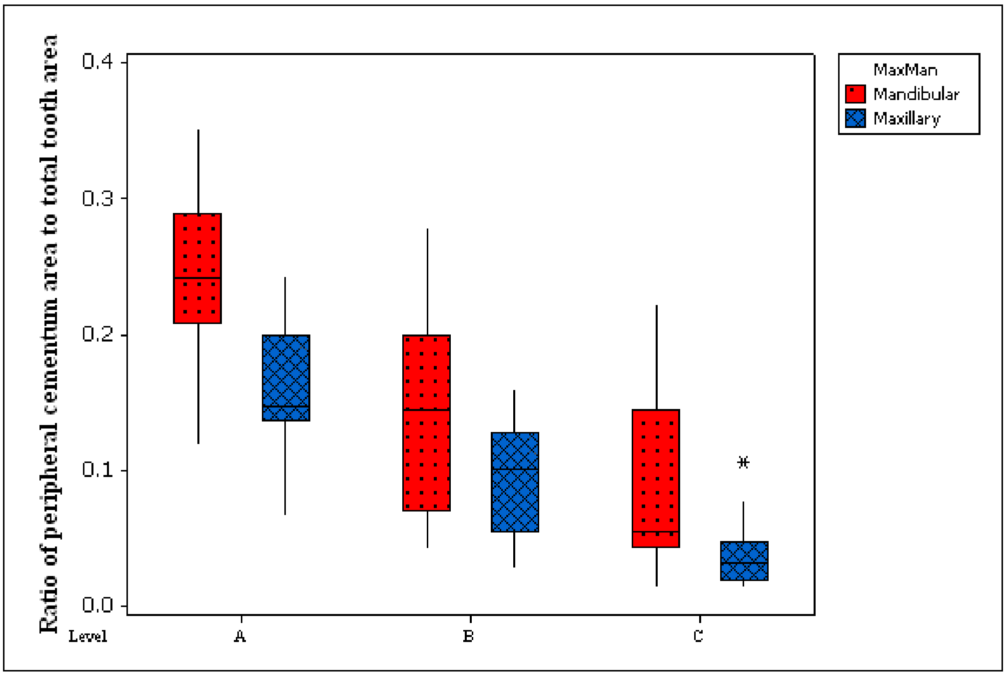
2.2.3.3 Results

The mean, median, range and coefficients of variation in maxillary and mandibular CT are shown in Table 2.2.3.3 and Figure 2.2.3.3.

Table 2.2.3.3: **Summary of peripheral cementum area to total tooth area ratio in maxillary and mandibular CT in level A, B and C. A = subocclusal; B = mid-tooth; C = apical third.**

Max/Mand	Level	Mean	Median	Standard deviation	Range	Coefficient of Variation
Maxillary	A	0.16	0.15	0.04	0.07 – 0.24	26.80
Maxillary	B	0.09	0.10	0.04	0.03 – 0.16	41.54
Maxillary	C	0.037	0.032	0.02	0.01 – 0.11	60.65
Mandibular	A	0.25	0.24	0.06	0.20 – 0.35	25.07
Mandibular	B	0.14	0.14	0.08	0.04 – 0.28	53.92
Mandibular	C	0.08	0.05	0.02	0.01 – 0.22	67.80

Figure 2.2.3.3: **Maxillary and mandibular CT peripheral cementum to total tooth area ratios in levels A, B and C. A = subocclusal; B = mid-tooth; C = apical third.**



In maxillary CT there was a significant difference in the ratio of peripheral cementum to total tooth area between the longitudinal levels A & B, A & C and B & C ($P < 0.0001$; $F_{1, 25} = 40.30$; $F_{1, 25} = 289.29$; $F_{1, .25} = 66.46$, respectively). In mandibular CT there was also a statistically significant difference between the longitudinal levels A & B, A & C and B & C ($P < 0.0001$; $F_{1, 25} = 47.82$; $F_{1, 25} = 147.56$; $F_{1, 25} = 42.04$ respectively). There was also a statistically significant difference between maxillary and mandibular CT ($P < 0.001$; $F_{1, 38} = 33.71$) peripheral cementum ratios. The decrease seen from level A to B to C was not statistically different in maxillary or mandibular CT ($P = 0.25$; $F_{2, 100} = 1.41$).

2.2.4 Prevalence of canine teeth

2.2.4.1 Aims of the study

The aim of the study was to determine the prevalence of canine teeth in male and female donkeys.

2.2.4.2 Materials and methods

A total of 187 male and 168 female donkeys were examined for the presence of canine teeth at the live survey (UK).

2.2.4.3 Results

One or more canines were seen in 31% of female donkeys with only 17.3 % having all 4 canines. One or more canines were observed in 78.6% of the male donkeys. The results of findings are summarised below (Table 2.2.4.3).

Table 2.2.4.3: **The number of maxillary and mandibular canine teeth present in 168 female and 187 male donkeys.**

Sex	Female	Male
Maxillary canines	32	142
Mandibular canines	33	145
Maxillary and mandibular canines	29	142
Number with all 4 canines	29 (17.3% prevelance)	139 (74.3% prevelance)

2.2.5 Discussion

Our observations have shown that normal donkey maxillae have a convex curvature, while their mandibles are straight, with the rostral aspect of the cheek teeth rows closer together than their caudal aspects. As a result of these different shapes of the upper and lower cheek teeth rows, there is no single area on the maxillae and mandible that is representative of the degree of anisognathia of the entire donkey jaw. It is for this reason that multiple measurements were taken along the length of the jaws corresponding to each CT. The results of this study have clearly indicated a high degree of anisognathia (27%) which is greater than that found in the horse (23%) (Taylor 2001). The reason why donkeys have a greater degree of anisognathia than horses is speculated to be due to their relative smaller body size. Further studies to determine the degree of anisognathia in ponies might be able to support this theory that smaller body size results in a greater degree of anisognathia.

The measurements at the different CT levels also illustrate the convex shape of the maxillary arcade and the widening of the mandibular jaw as the ratio gets smaller further caudally with the lowest degree of anisognathia found at the level of the 11s. Due to the anisognathia the palatal third of the maxillary cheek teeth are in contact with the buccal half of the mandibular cheek teeth in the neutral position (Dixon 2002). Furthermore it is likely that the degree of anisognathia will have an effect on the masticatory action and thus the masticatory forces applied to the teeth. Therefore this characteristic could contribute to conditions such as shear mouth and cheek teeth displacements.

It has previously been proposed that enamel infolding in equid cheek teeth increase the enamel occlusal surface area, thereby decreasing the rate of normal attrition by protecting the adjacent softer dentine and cementum (Kilic *et al.* 1997b). It has also been proposed that enamel infolding is greater in mandibular teeth to compensate for the absence of infundibulae as found in maxillary teeth. This study has clearly demonstrated that the perimeter of donkey peripheral enamel is significantly greater than the perimeter of the whole tooth thereby increasing the amount of enamel exposed on the occlusal surface. This study has also shown that the degree of peripheral enamel infolding in mandibular cheek teeth is significantly greater than in maxillary cheek teeth. However, the presence of infundibular enamel in maxillary CT has been shown to more than compensate for the lesser degree of peripheral enamel infolding. Ultimately both the peripheral enamel infolding and the infundibular enamel contribute to increasing wear resistance in donkey maxillary CT.

The deposition of cementum at the gingival crown with an increase in the transverse area of occlusal cementum has been demonstrated in horses (Mitchell *et al.* 2003). This study has also shown an increase in peripheral cementum in donkeys' CT closer to the occlusal surface compared to the alveolar apical sections. This increase in peripheral cementum did not differ between maxillary and mandibular CT although mandibular CT have a significantly greater amount of peripheral cementum at all levels. This confirms that cementum is a dynamic tissue that continues to be deposited after eruption and increases the occlusal surface area of donkey CT.

It has been well recognized in horses that females have small rudimentary or absent canines (Hillson 1986; Miles and Grigson 1990).The results from this study indicate that female donkeys have a higher prevalence of canine teeth (17.3% had all 4 canines) than female domesticated horses, where the reported incidence of canine teeth is 7.8% (Miles and Grigson 1990). The study by Colyer (1906) in horses demonstrated prevalence of one or more canines to be 27.7% which is still less than the 31% in the female donkeys in this study, with the prevalence of all 4 canines only 5.8% in horses. The reason for the higher prevalence of canine teeth in female donkeys could be due to donkeys being more primitive or might be due to behavioural differences in donkeys and horses, but this is purely speculative. Similar to equine canine teeth, donkeys tend to have a slightly higher prevalence of mandibular canines only.

2.3. Computed axial tomography anatomy and measurements

2.3.1 Endodontic anatomy

Recently an endodontic identification system has been proposed in horses by Dacre (2005b) (Figure 2.3.1.1 and 2.3.1.2). This pulp nomenclature has facilitated accurate identification and iagnosis of dental disorders involving the pulp horns in horses. Prior to systematic study of donkey dental pathology, the normal pulp morphology, including the degree of pulp horn communication needs to be established in donkeys, as has been performed in horses (Dacre 2005b).

Figure 2.3.1.1: **The endodontic identification numbering system of equine maxillary cheek teeth. B = buccal; P = palaptal; R = rostral; C = caudal (Dacre 2005b).**

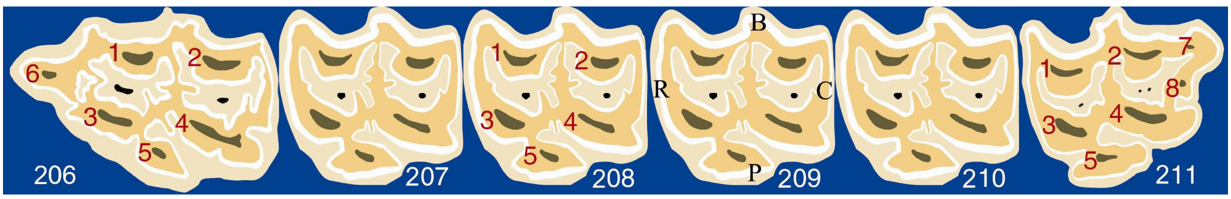
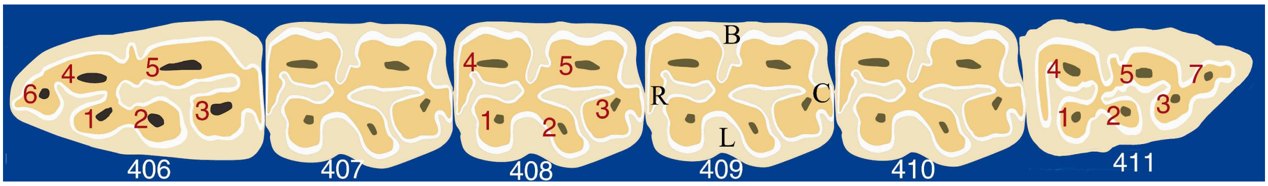


Figure 2.3.1.2: **The endodontic identification numbering system of equine mandibular cheek teeth; B = buccal; L = lingual; R = rostral; C = caudal (Dacre 2005b).**



2.3.1.1 Aim of the study

The aim of this study was to describe the normal endondontic anatomy in donkey cheek teeth.

2.3.1.2 Materials and methods

All teeth (42 mandibular and 40 maxillary), were imaged using a Somatom Esprit CAT scanner (Siemens AG, Munich, Germany) at the Scottish Agricultural College, Bush Estate, Midlothian. A spiral series of images (1.5mm in depth and 0.75mm overlap) was taken of each tooth and analysed using Syngo A40A software (Siemens AG, Munich, Germany). Attenuated density of CAT was measured in Hounsfield units (HU).

The following measurements taken in centimetres except densities:

Total length of tooth (excluding roots if present)

Length of roots (enamel free apical area)

Rostro-caudal and medio-lateral width of teeth at occlusal surface

Length of pulp cavities identifiable on CT

Depth of secondary dentine at occlusal surface of pulp cavities

Latero-medial width of pulp cavities at (a) sub-occlusal, (b) mid and (c) pre-apical level

Densities (in HU) of enamel, peripheral and infundibular cementum, primary and secondary dentine and pulp

Measurements from each tooth were recorded on an Excel sheet (Microsoft Office Excel) and CAT image data were stored on CD in DICOM format (NEMA) for further re-evaluation and image capturing using Osiris software.

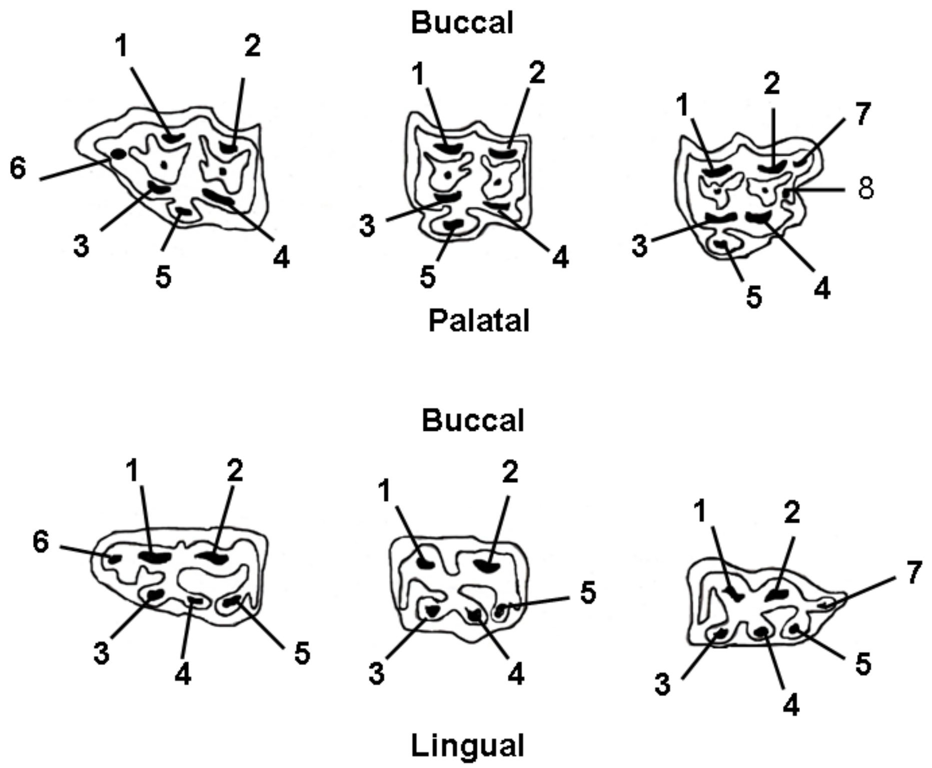
CAT images were examined for the presence of pulp horns which were identified on transverse sections as consistent longitudinal areas of least density (Kirkland *et al.* 1996). The number of pulp horns were noted and correlated with that seen on gross examination of the occlusal surface.

2.3.1.3 Results

The number of pulp horns identified was consistent with equine findings. Five pulp horns were consistently identified in maxillary and mandibular cheek teeth in Triadan position 07-10. Six pulp horns were identified in CT 06 and 11, with 7 pulp horns identified in one maxillary 11. These are similar numbers of pulp horns to those described in the horse, apart from Triadan position 11 where there was only one cheek tooth with 7 pulp horns in the donkey CT.

The location of these pulp horns correlated with the pulp horn number classification system that has been proposed in the horse (Dacre 2005b). However, a new classification system for the mandibular CT has been proposed and used in this study to provide a more consistent numbering system to correspond to the maxillary CT endodontic numbering. In the proposed numbering system mandibular CT pulp cavity numbering starts on the buccal aspect as shown in Figure 2.3.1.3.1.

Figure 2.3.1.3.1: **The proposed endodontic numbering system for maxillary (top) and mandibular (bottom) equid CT; examples of CT in Triadan positions 06 (left), 07-10 (middle) and 11 (right).**



2.3.2 Prevalence of pulp horn communication

2.3.2.1 Aim of the study

The aim of this study was to determine the prevalence of communication between the individual pulp horns in donkey cheek teeth.

2.3.2.2 Materials and Methods

The communication of pulp horns in donkey CT was determined using images of 36 mandibular and 38 maxillary teeth obtained by a CAT scanner. Three levels of cheek teeth (subocclusal, mid and pre-apical) were examined in transverse sections. The number and position of pulp horns and rostro-caudal width of the individual pulp chambers and the presence of pulp chamber communications were determined at each of the 3 levels on the images.

2.3.2.3 Results

The median age of the donkeys for mandibular CT was 20 and maxillary CT was 18.The number and type of pulp communications at different levels in 38 maxillary and 36 mandibular cheek teeth are described in Table 2.3.2.3.1 and 2.3.2.3.2.

***Table 2.3.2.3.1*: Total number of individual pulp horn communications (using the modified endodontic numbering system) at the mid and pre-apical level of donkey cheek teeth as determined in CAT images.**

Pulp chamber communication		1&2	2&4	1&4	4&5	3&5	3&4	2&5	1&3	1&6
Mandibular CT	Mid						1	8	5	1
	Pre-Apical	2		11		1	13	25	14	
Maxillary CT	Mid									
	Pre-Apical		2		4	11			4	

***Table 2.3.2.3.2*: Number and position (pulp number) of single and multiple pulp horn communications observed in donkey cheek teeth as determined in CAT images.**

Mandibular	
Pulp horn communication	Number of CT
2&5	3
2&5; 1&3	12
2&5; 3&4	5
2&5; 3&4; 1&4	4
2&5; 1&4	1
3&4; 1&3	2
3&4; 3&5; 1&2	1
1&4; 1&2	1
1&3; 1&4	1
1&3; 1&4; 2&5	2

Maxillary	
Pulp horn communication	Number of CT
3&5	10
1&3	2
3&5; 1&3	1
1&3; 2&4	1
2&4	1
4&5	4

In the maxillary cheek teeth examined, 19/38 (50%) had communications between their pulp horns, with communication between pulp horns 3 & 5 being the most common pattern (10/19), and comprised 53% of all maxillary pulp horn communications. The next most common pulp horn communication was between pulp horns 4 & 5, within 4 of 19 cheek teeth (21%).

In the mandibular cheek teeth 32/36 (89%) of cheek teeth had pulp horn communications. The most common pulp horn communications occurred between both pulp horns 2 & 5, and 1 & 3, with 38% (12/32) mandibular cheek teeth having both these communications. The next most observed pulp horn communications observed were between 2 & 5, and 3 & 4 in 5 of 32 CT (16%) and in 4 CT between 2 & 5, 3 & 4 and 1 & 4 (13%).

2.3.3 Tooth length, rostro-caudal and latero-medial width

2.3.3.1 Aims of the study

The aim of this study was to establish a range of normal values of tooth length, rostro-caudal and latero-medial width of donkeys' cheek teeth and validate the accuracy of measurements taken from CAT images. Values of these measurements were also compared between donkeys that were older (> 15 years) and younger (≤ 15 years).

2.3.3.2 Materials and methods

The measurements of tooth length and infundibulae in maxillary cheek teeth on longitudinal images, and rostro-caudal and latero-medial width at the occlusal surface were obtained from CAT images using Syngo A40A software (Siemens AG, Munich, Germany). Tooth length, rostro-caudal and latero-medial width were also grossly measured using digital electronic callipers (Knighton Tool Supplies, Leicester, UK) These measurements were compared using paired t-tests (Minitab® Release 14) with a value of $P < 0.05$ denoting significance, to determine the accuracy of CAT dental measurements. Tooth length was measured from the mid-occlusal surface to the beginning of the root (enamel free apex) (Figure 2.3.3.2.1). Rostro-caudal and latero-medial widths were measured at the occlusal surface (Figure 2.3.3.2.2). Root lengths were not measured on incisors as it was too difficult to visualise the true root.

Values of teeth from older and younger donkeys were compared using linear mixed effect models (R V2.3.1, R Foundation for Statistical Computing) with donkey skull entered as random effects to take account of the fact that there were multiple donkeys. Younger (≤ 15 years) and older (> 15 years) were entered as fixed effects. Normal values were square rooted to normalise the residuals and zero values were discarded as we could not be convinced that zero values were truly 0. Statistical significance was taken as $P < 0.05$ and degrees of freedom are shown as subscripts.

Figure 2.3.3.2.1: **Colour enhanced CAT image of a maxillary cheek tooth displayed in longitudinal section. The red line illustrates where the measurement of tooth length was taken in CAT images. Colour codes represent density differences with purple the least dense and red the most dense.**

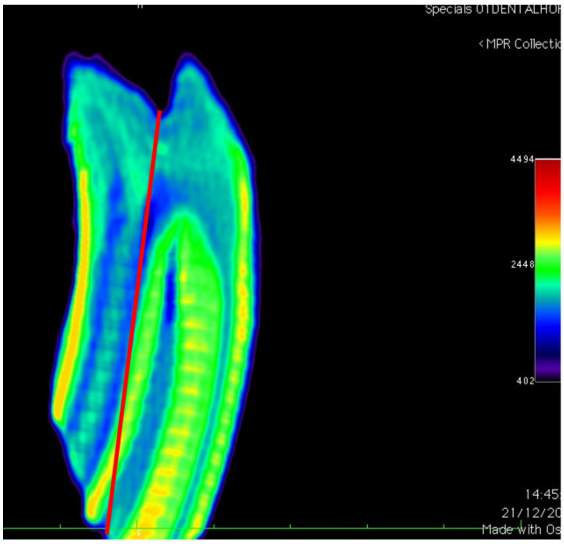
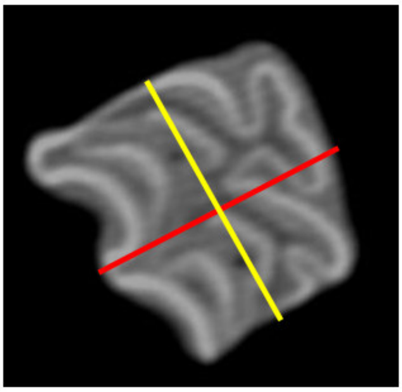


Figure 2.3.3.2.2: **CAT image of a maxillary cheek tooth at sub-occlusal level illustrating where measurements were taken of rostro-caudal (yellow) and latero-medial (red) width in CAT iamges.**



2.3.3.3 Results

Maxillary cheek teeth

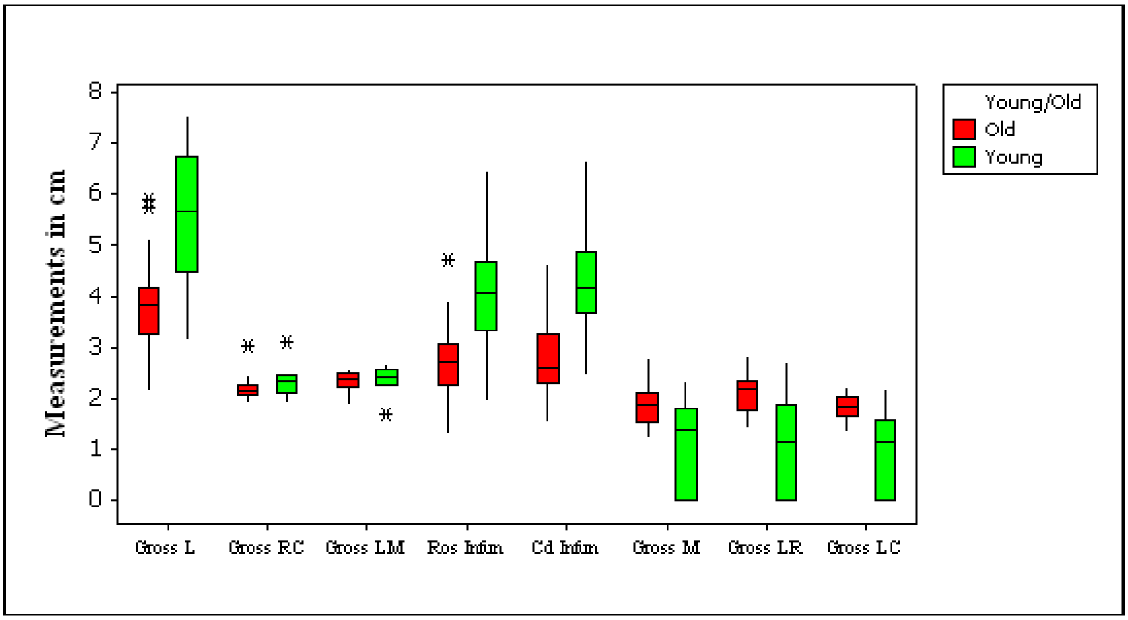
Table 2.3.3.3.1: **Summary of maxillary teeth length, rostro-caudal and medio-lateral widths as measured on CAT images and by gross examination respectively (cm). RC = rostro-caudal; LM = latero-medial; Coef of Var = coefficient of variation.**

Length	Number of CT	Mean	Minimum	Median	Maximum	Coef of Var
Total Length	40	4.57	2.78	4.57	7.43	24.95
Gross Total Length	40	4.57	2.16	4.17	7.55	29.91
RC width	40	2.25	1.91	2.21	3.06	11.71
Gross RC width	40	2.25	1.92	2.21	3.11	11.07
LM width	40	2.29	1.63	2.33	2.61	8.65
Gross LM width	40	2.35	1.67	2.40	2.67	9.18

There was no significant difference between values of CAT image and gross measurements in the tooth length ($P = 0.919$) or rostro-caudal width ($P = 0.963$), but there was a significant difference between latero-medial width measurements ($P = 0.024$) in maxillary cheek teeth. There was also a significant variation in both tooth length measurements as illustrated by the coefficients of variation of > 15.

There was a statistically significant difference in the values of tooth length, rostral infundibulum, caudal infundibulum, and caudal lateral root in maxillary CT of young and old donkeys ($P \leq 0.041$; Figure 2.3.3.3.1; Table 2.3.3.3a; Appendix 2).

Figure 2.3.3.3.1: **Difference between gross measurements of younger and older donkey maxillary CT. Gross L = tooth length; Gross RC = rostro-caudal width; Gross LM = latero-medial width; Ros Infun = rostral infundibulum; Cd Infund = caudal infundbulum; Gross M = medial root; Gross LR = rostro-lateral root; Gross LC = caudo-lateral root.**



Mandibular cheek teeth

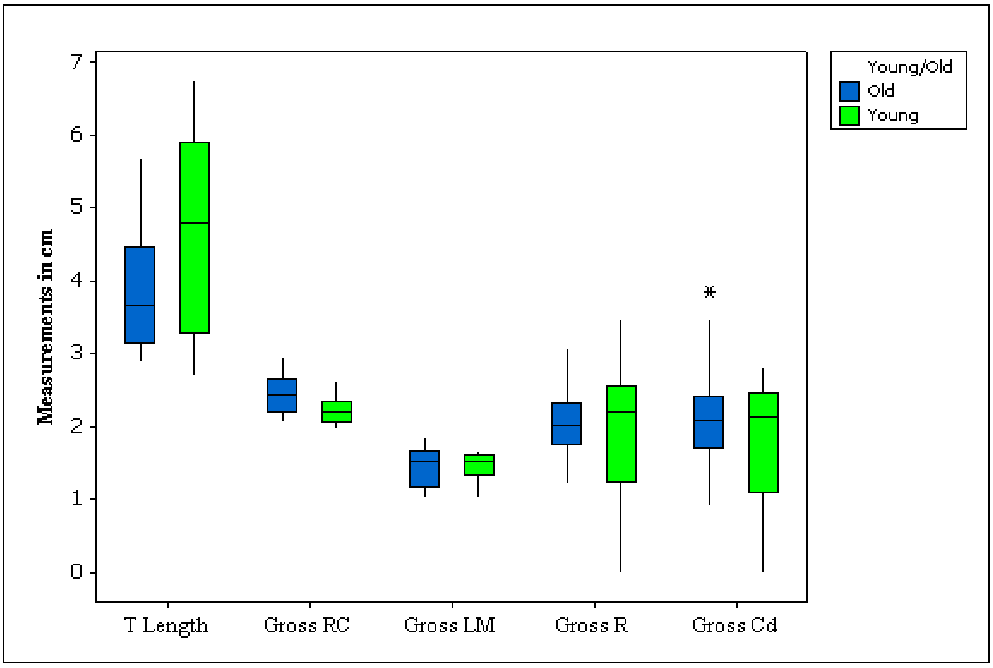
Table 2.3.3.3.2: **Summary of tooth length, rostro-caudal and medio-lateral widths measured on CAT images and gross examination respectively (cm). RC = rostro-caudal; LM = latero-medial; Coef of Var = coefficient of variation.**

Length	Number	Mean	Minimum	Median	Maximum	Coef of Var
T Length	42	4.14	2.71	3.82	6.75	26.39
Gross Length	42	4.07	2.55	3.55	6.96	28.13
RC width	42	2.39	1.97	2.34	2.99	10.61
Gross RC width	42	2.37	1.98	2.29	2.96	10.58
LM diameter	42	1.40	0.95	1.43	1.88	16.19
Gross LM width	42	1.46	1.03	1.51	1.86	17.42

There was no significant difference between CAT image and gross measurements in the tooth length (P = 0.49), rostro-caudal width (P = 0.33), but there was a significant difference in latero-medial width (P = 0.014) in mandibular cheek teeth. There was significant variation in both tooth length and latero-medial width measurements as illustrated by the coefficients of variation of > 15.

There was a significant difference in gross measurements of tooth length and rostro-caudal width (P < 0.018; Table 2.3.3.3b, Appendix 2) between young and old donkey mandibular CT (Figure 2.3.3.3.2). All other measurements were not significantly different.

***Figure 2.3.3.3.2*: Difference in gross values young and old donkey mandibular CT. Gross L = tooth length; Gross RC = rostro-caudal width; Gross LM = latero-medial width; Gross = rostral root; Gross Cd = caudal root.**



Incisors

Table 2.3.3.3.3: **Summary of tooth length, rostro-caudal and medio-lateral width as measured on CAT images and gross examination respectively (cm). RC = rostro-caudal; LM = latero-medial; Coef of Var = coefficient of variation.**

Length	Number	Mean	Minimum	Median	Maximum	Coef of Var
Length	25	4.52	3.65	4.59	5.40	11.36
Gross Length	26	4.926	0.83	4.99	6.91	29.86
RC width	26	1.075	0.88	1.10	1.46	0.88
Gross RC width	26	1.01	0.56	1.03	1.34	122.5
LM width	26	1.14	0.87	1.14	1.60	0.64
Gross LM width	26	1.22	0.81	1.24	1.60	112.5

There was no significant difference between CAT image and gross measurements in the tooth length (P = 0.157), rostro-caudal width (P = 0.081) or latero-medial width (P = 0.236) in incisors. There was a significant level of variation in the gross incisor measurements as illustrated by the coefficients of variations (all >15).

Table 2.3.3.3.4**: P values of two samples t tests comparing gross and CAT image measuremensts in donkey maxillary and mandibular CT.**

Measurement	Maxillary CT	Mandibular CT	Incisors
Tooth Length	0.919	0.49	0.157
Latero-medial width	0.024	0.014	0.081
Rostro-caudal width	0.963	0.33	0.236

2.3.4 Pulp horn lengths and occlusal thickness of secondary dentine

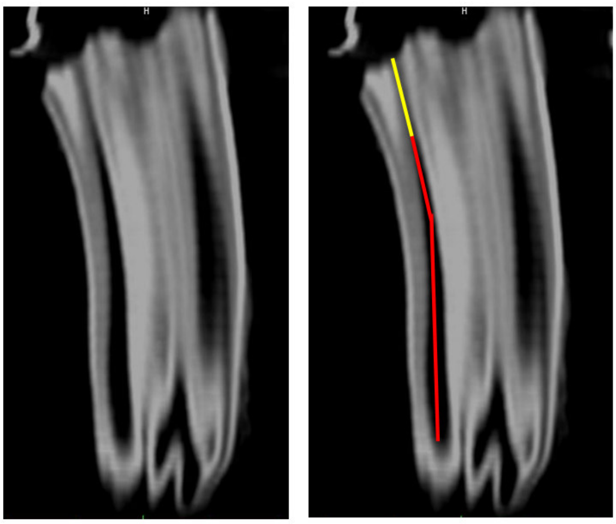
2.3.4.1 Aims of the study

The aim of this study was to establish normal values for donkey pulp horn lengths and the occlusal depth/thickness of secondary dentine and compare these values in older (> 15 years) and younger (≤ 15 years) donkeys.

2.3.4.2 Materials and methods

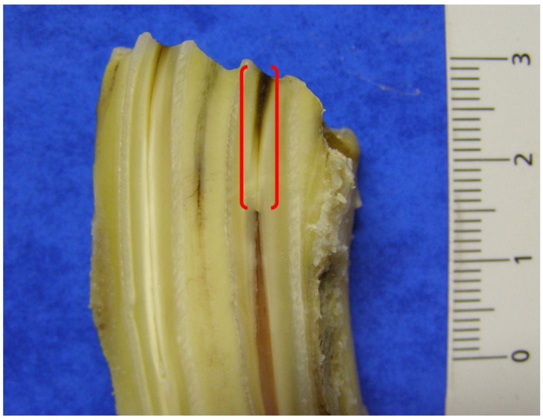
Pulp horn lengths were measured on longitudinal section with the teeth rotated on a three dimensional plane to accurately show up each individual pulp horn as a consistent hypoattenuated area (black on image) (Figure 2.3.4.2.1). Similarly the depth of the secondary dentine was taken as the area of secondary dentine hypodensity above the visible pulp horn up to the occlusal surface (Figure 2.3.4.2.1).

Figure 2.3.4.2.1: **Longitudinal CAT image of a maxillary CT rotated to fully outline an individual pulp horn to illustrate the measurement of its pulp length (red lines) and also secondary dentine depth (yellow line).**



Eight CT (4 maxillary and 4 mandibular CT) were sectioned longitudinally after the CAT scan and the depth of secondary dentine measured as the dentine visible above the pulp cavity (Figure 2.3.4.2.2). Due to curvature of the teeth and the narrow width of the pulp horns, not all pulp horns were sectioned such that secondary dentine occlusal depth could be measured.

***Figure 2.3.4.2.2*: Longitudinally sectioned donkey maxillary cheek tooth illustrating depth of occlusal secondary dentine (red brackets).**



One sample t tests were performed on the difference between gross and CAT image secondary dentine depth measurements with significance taken as $P < 0.05$ (Minitab 14). Values of older and younger donkeys were compared using linear mixed effect models (see section 2.3.3.2).

2.3.4.3 Results

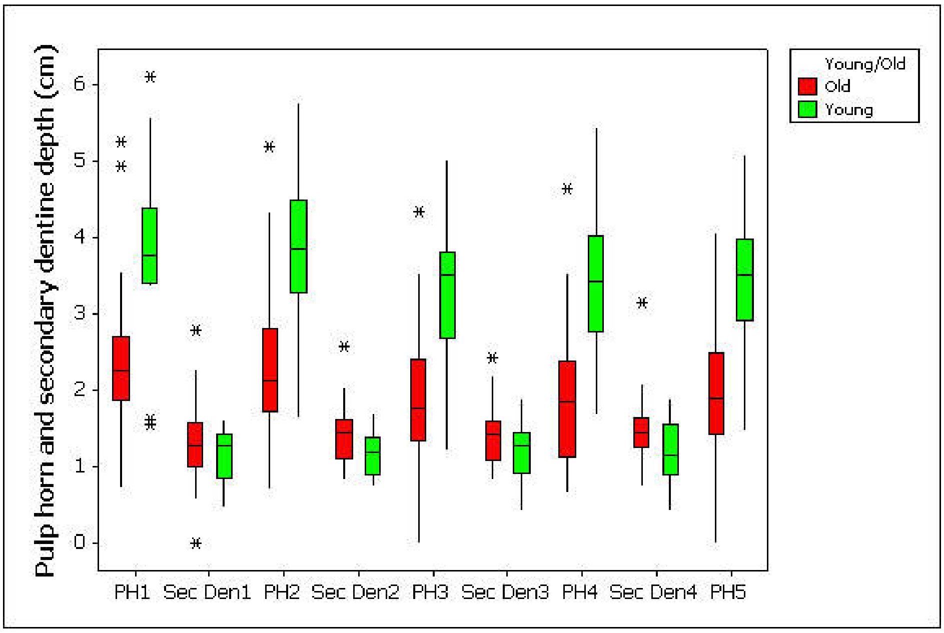
Maxillary cheek teeth

The Table (Table 2.3.4.3.1) and Figure (Figure 2.3.4.3.1) below summarises the maxillary cheek teeth results. Zero values of pulp horn lengths were indicative of complete secondary dentine deposition filling the pulp horn. The pulp horn lengths varied from 0 – 6.10 cm with a mean of 2.56 cm and secondary dentine thickness from 0.42 - 3.22 cm with a mean of 1.34 cm.

Table 2.3.4.3.1: **Summary of length of maxillary cheek teeth individual pulp horns (PH 1-5) and depth of secondary dentine (Sec Den 1 – 5) (cm) as determined on CAT images.**

Pulp horn	Number of PH	Mean	Minimum	Median	Maximum	Coef of Var
PH1	40	2.99	0.73	2.68	6.10	42.70
Sec Den1	40	1.27	0	1.28	2.79	39.37
PH2	40	2.95	0.70	2.76	5.76	43.87
Sec Den2	40	1.32	0.75	1.26	2.58	29.77
PH3	40	2.47	0	2.32	5.01	48.99
Sec Den3	40	1.34	0.43	1.34	2.42	29.24
PH4	40	2.54	0.66	2.37	5.44	47.13
Sec Den4	40	1.36	0.42	1.36	3.15	34.70
PH5	40	2.52	0	2.43	5.08	48.04
Sec Den5	40	1.31	0.61	1.20	3.22	46.55

Figure 2.3.4.3.1: **Lengths of pulp horns and depths of secondary dentine in pulp horns 1- 5 of maxillary cheek teeth (cm) in younger (≤ 15 years) and older (>15 years) donkeys as determined on CAT images. PH = pulp horn; Sec Den = secondary dentine.**



The mean pulp horn length in teeth from older (> 15 years) donkeys (2.1cm [std dev 0.9]) were shorter than younger (≤ 15 years) donkeys (3.6cm [std dev 1.1]). However, this was only statistically significant different in the lengths of pulp horns 2, 3, 4 and 5. Mean secondary dentine depth was greater in older donkeys (1.4cm [std dev 0.5]) than younger donkeys (1.1cm [std dev 0.3]), but this was only statistically significant in pulp horn 4 ($P < 0.041$; Table 2.3.3.4.a; Appendix 2). There was a tendency for the lengths of pulp horns to decrease with age and secondary dentine thickness to increase with age (Figure 2.3.4.3.1). The ratio of all secondary dentine depths to pulp horn lengths were significantly ($P = 0.03$) greater in older donkeys (median 0.66; range 0 – 6.2) than younger donkeys (median 0.31; range 0.12 – 0.85), indicating an increase in secondary dentine with age.

Mandibular cheek teeth

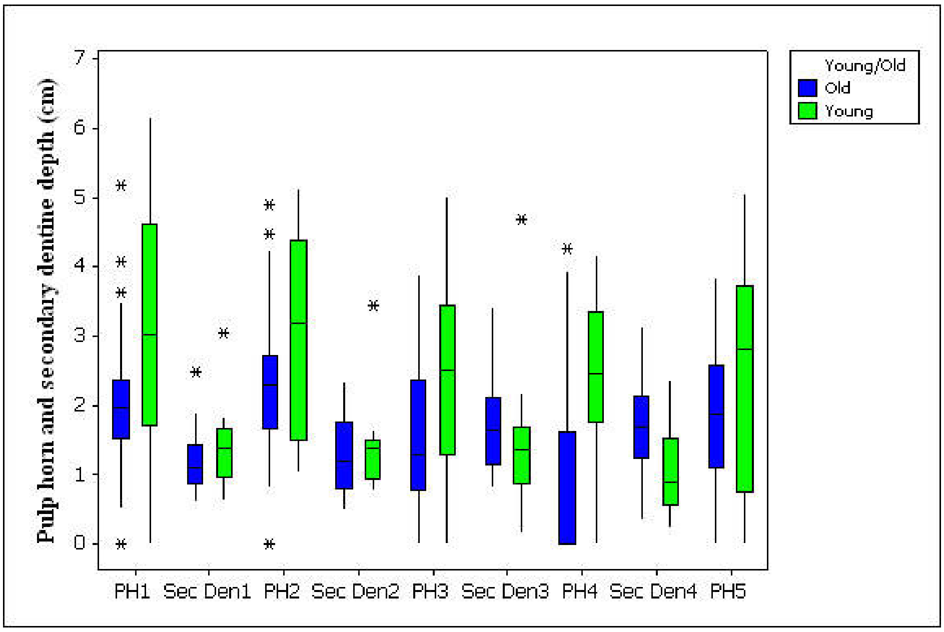
The Table (Table 2.3.4.3.2) and Figure (Figure 2.3.4.3.2) below summarises the mandibular cheek teeth results. Zero values of pulp horn lengths were indicative of complete secondary dentine deposition. The pulp horn lengths varied from 0 – 6.16 cm with a mean of 1.93 cm and secondary dentine thickness from 0.17 – 4.68 cm with a mean of 1.46 cm.

***Table 2.3.4.3.2:* Summary of mandibular cheek teeth individual pulp horn (PH 1-5) lengths and depth of secondary dentine (Sec Den 1- 5) (cm) as determined on CAT images.**

Pulp horn	Number	Mean	Minimum	Median	Maximum	Coef of Var
PH1	42	2.43	0	2.32	6.16	58.48
Sec Den1	42	1.28	0.62	1.17	3.06	44.29
PH2	41	2.62	0	2.49	5.13	47.04
Sec Den2	41	1.34	0.56	1.28	3.44	41.86
PH3	42	1.83	0	1.51	5.02	72.00
Sec Den3	42	1.63	0.17	1.48	4.68	48.46
PH4	42	1.41	0	1.35	4.27	103.0
Sec Den4	42	1.47	0.23	1.50	3.15	50.18
PH5	41	1.99	0	2.03	5.07	70.46
Sec Den5	41	1.41	0	1.35	4.33	54.67

The mean pulp horn lengths in teeth from older (> 15 years) donkeys (1.7cm [std dev 1.2]) were shorter than younger (≤ 15 years) donkeys (2.7cm [std dev 1.5]). This was only statistically significantly different in the lengths of pulp horns 1, 3 and 5. In contrast, the mean secondary dentine depth was greater in older donkeys (1.5cm [std dev 0.6]) than younger donkeys (1.4cm [std dev 0.8]), but this was only statistically significant in pulp cavity 4 ($P < 0.047$; Table 2.3.3.4b; Appendix 2) (Figure 2.3.4.3.2). All other values were not significantly different in young and old donkeys although there was a general trend for pulp horn lengths to decrease and secondary dentine occlusal depths to increase with age. The ratios of secondary dentine depths to plulp horn lengths in older donkeys (median; range 0.15 – 3.03) were greater than younger donkeys (median 0.37; 0.11 – 4.29), but this was not statistically significant (P = 0.18).

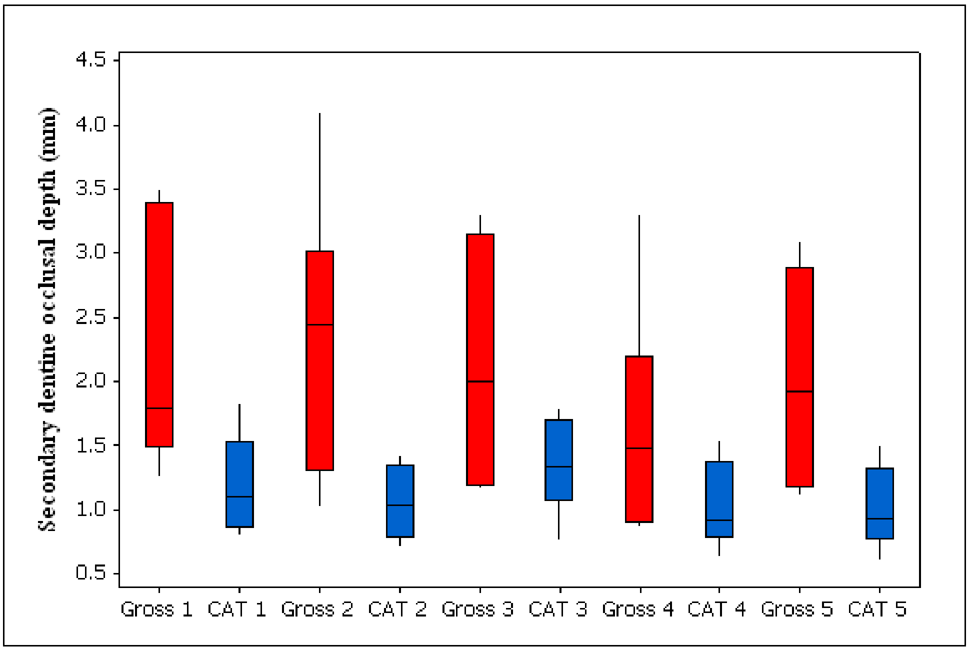
Figure 2.3.4.3.2: **Lengths of pulp horns and depth of secondary dentine in pulp horns 1- 5 of mandibular cheek teeth (cm) in younger (≤ 15 years) and older (> 15 years) donkeys. PH = pulp cavity; Sec Den = secondary dentine.**



Secondary dentine depth validation

The length of the secondary dentine measured with CAT images and gross measurements varied greatly with pulp horns 1, 2 and 5 being significantly different (P < 0.05) and pulp horns 3 and 4 not being significantly different (P = 0.063 and 0.072). The gross measurements were usually larger than the CAT image measurements (Figure 2.3.4.3.3).

Figure 2.3.4.3.3: **The difference in secondary dentine occlusal pulp horn depth between gross and CAT image measurements in eight donkey CT.**



2.3.5. Pulp horn widths

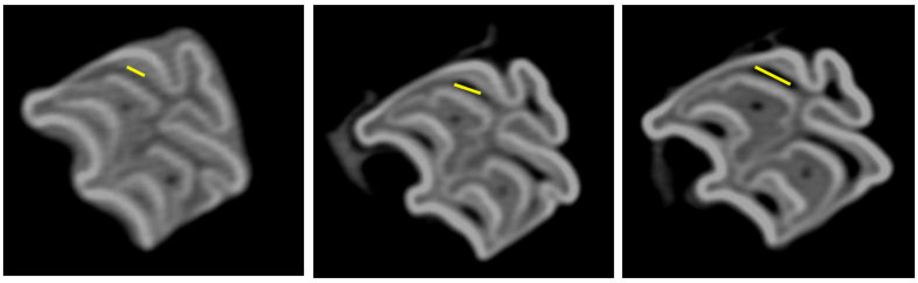
2.3.5.1 Aim of the study

The aim of this study was to establish normal values for pulp horn width at sub-occlusal, mid and pre-apical transverse sections levels of maxillary and mandibular CT.

2.3.5.2 Materials and methods

Measurements of pulp horn width were taken in a rostro-caudal direction from CAT transverse images taken at three different longitudinal levels of each CT (Figure 2.3.5.2.1).

Figure 2.3.5.2.1: **Transverse CAT images of subocclusal (left), mid (middle) and pre-apical (right) levels of a maxillary cheek tooth, illustrating the site of the pulp horn width (pulp horn three) measurement (yellow lines).**



Values of older and younger donkeys were compared using linear mixed effect models (see section 2.3.3.2).

2.3.5.3 Results

Maxillary cheek teeth

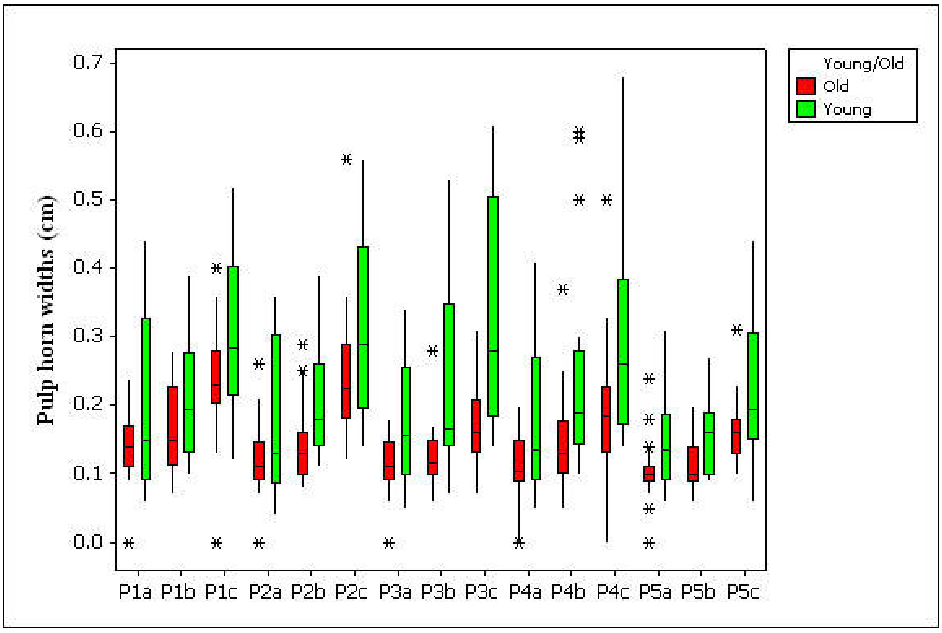
Table 2.3.5.3.1 and Figure 2.3.5.3.1 below summarise the pulp horn widths measurements in maxillary cheek teeth. Pulp horn widths varied from 0 – 0.68 cm. The mean width at subocclusal level was 0.15 cm, at mid level 0.17cm and pre-apical level 0.25cm.

Table 2.3.5.3.1: **Summary of maxillary cheek teeth pulp horn latero-medial widths at sub-occlusal (a), mid (b) and pre-apical (c) level (cm)**

Pulp horn	Number of PH	Mean	Minimum	Median	Maximum	Coef of Var
PH1a	40	0.17	0	0.14	0.44	56.76
PH1b	40	0.19	0.07	0.16	0.39	41.68
PH1c	40	0.26	0	0.26	0.52	37.89
PH2a	40	0.15	0	0.12	0.36	58.22
PH2b	40	0.17	0.08	0.14	0.39	43.82
PH2c	40	0.28	0.12	0.27	0.56	42.75
PH3a	40	0.14	0	0.12	0.34	50.39
PH3b	40	0.17	0.06	0.14	0.53	66.85
PH3c	40	0.23	0.07	0.19	0.61	57.39
PH4a	40	0.14	0	0.11	0.41	63.70
PH4b	40	0.19	0.05	0.16	0.60	66.79
PH4c	40	0.23	0	0.20	0.68	63.06
PH5a	40	0.12	0	0.11	0.31	47.37
PH5b	40	0.13	0.06	0.12	0.27	37.04
PH5c	40	0.19	0.06	0.17	0.44	44.25

Although there was a general trend of pulp horn widths to decrease in older donkey maxillary CT this was only found to be statistically significant in pulp horn 3 at mid and pre-apical tooth level ($P < 0.03$; Table 2.3.3.5a; Appendix 2) (Figure 2.3.5.3.1).

Figure 2.3.5.3.1: **Latero-medial widths of maxillary cheek teeth pulp horns 1- 5 at subocclusal (a), mid (b) and pre-apical (c) transverse sections (cm) in younger and older donkeys. P = pulp horns; a = subocclusal; b = mid-tooth; c = pre-apically.**



Mandibular cheek teeth

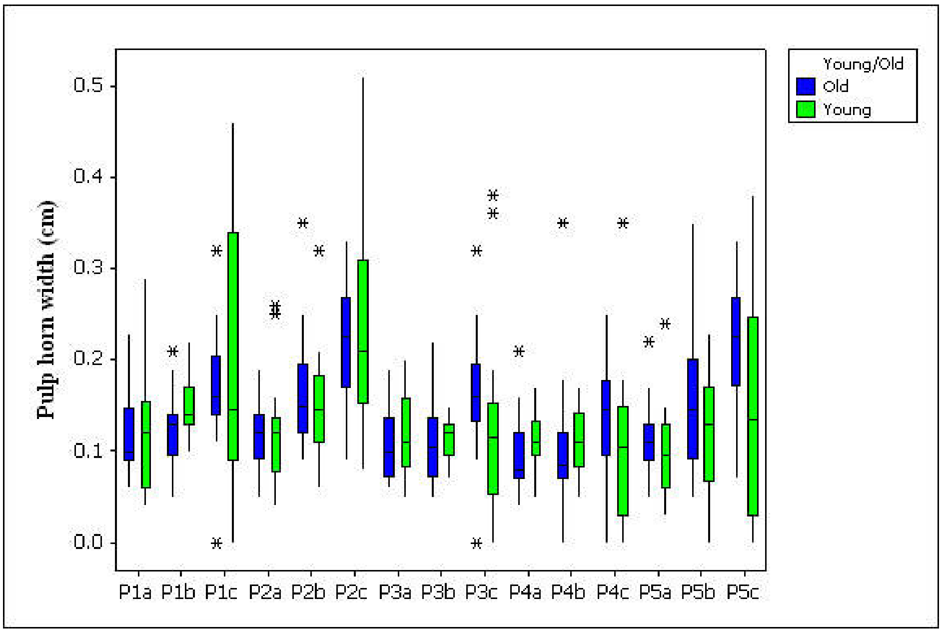
Table 2.3.5.3.2 and Figure 2.3.5.3.2 below summarise the pulp horn widths measurements in mandibular cheek teeth. Pulp horn widths varied from 0 – 0.58 cm. The mean width at subocclusal level was 0.12 cm, at mid level 0.18cm and pre-apical level 0.18 cm.

Table 2.3.5.3.2: **Summary of mandibular cheek teeth pulp horn latero-medial widths at sub-occlusal (a), mid (b) and pre-apical (c) level (cm)**

Pulp horn	Number of PH	Mean	Minimum	Median	Maximum	Coef of Var
PH1a	42	0.12	0.04	0.11	0.29	41.75
PH1b	42	0.13	0.05	0.14	0.22	29.60
PH1c	42	0.17	0	0.16	0.46	56.20
PH2a	42	0.12	0.04	0.12	0.26	39.09
PH2b	42	0.16	0.06	0.15	0.35	38.33
PH2c	42	0.22	0.08	0.22	0.51	36.36
PH3a	42	0.11	0.05	0.10	0.20	38.66
PH3b	42	0.11	0.05	0.11	0.22	35.28
PH3c	42	0.15	0	0.14	0.38	55.66
PH4a	42	0.10	0.04	0.10	0.21	38.08
PH4b	42	0.10	0	0.10	0.35	55.04
PH4c	38	0.13	0	0.14	0.35	68.64
PH5a	41	0.11	0	0.11	0.24	40.35
PH5b	42	0.14	0	0.14	0.35	48.02
PH5c	42	0.20	0	0.22	0.58	47.85

Although there was a general trend of smaller pulp horn widths in older donkey mandibular CT there were no statistically significant differences ($P > 0.11$; Table 2.3.3.5b; Appendix 2) (Figure 2.3.5.3.2).

Figure 2.3.5.3.2: **Latero-medial widths of mandibular cheek teeth pulp horns 1- 5 at subocclusal (a), mid (b) and pre-apical (c) transverse sections (mm) in young and old donkeys. P = pulp horns; a = subocclusal; b = mid-tooth; c = pre-apically.**



2.3.6. Radiographic densities (Hounsfield units) of dental tissues

2.3.6.1 Aim of the study

The aim of this study was to determine the range of radiographic densities as determined on CAT images in donkey dental tissues and to compare these values with those found in horses.

2.3.6.2 Materials and methods

Densities (in Hounsfield units) of enamel, primary and secondary dentine, peripheral and infundibular enamel were established on subocclusal transverse section CAT images.

The densities were determined using computerised programme, Syngo A40A software (Siemens AG, Munich, Germany), by holding the pointer over the centre of the area of interest. The maximum density recorded was limited by the software programme to 3071 Hounsfield units. Values of older and younger donkeys were compared using linear mixed effect models (see section 2.3.3.2).

2.3.6.3 Results

Maxillary cheek teeth

The results of dental tissue density measurements (HU) in donkey maxillary cheek teeth is summarised in Table 2.3.6.3.1 and Figure 2.3.6.3.1 below. The median density of enamel was 3041 HU, primary dentine 2158 HU, secondary dentine 1634 HU, peripheral cementum 1977 HU and infundibular cementum 1838 HU.

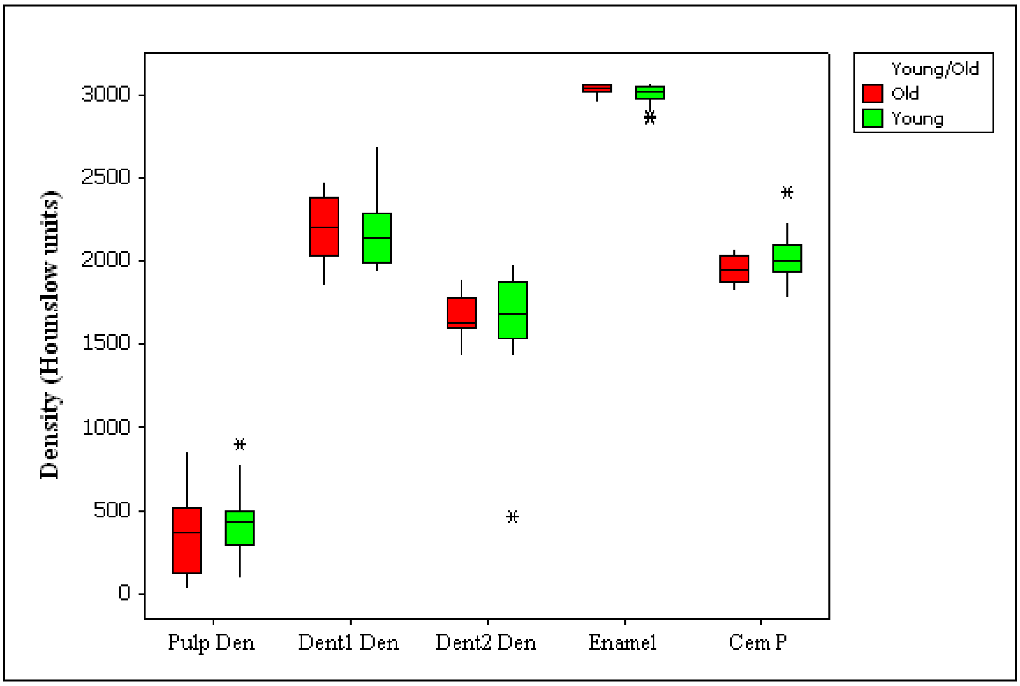
Table 2.3.6.3.1: **Summary of density mesaurements (in Hounsfield units) for pulp, primary dentine, secondary dentine, enamel, peripheral cementum and infundibular cementum in donkey maxillary cheek teeth.**

Tooth Structure	Number	Mean	Minimum	Median	Maximum	Coef of Var
Pulp	40	387.1	34.0	395.5	904.0	58
Primary dentine	40	2195.9	1852.0	2158.0	2686.0	9.1
Secondary dentine	40	1684.5	471.0	1634.0	1977.0	14.65
Enamel	40	3022.6	2862.0	3041.0	3070.0	1.71
P[1] cementum	40	1979.5	1780.0	1976.5	2420.0	6.18
I[2] cementum	40	1852.1	1545.0	1837.5	2376.0	8.31

[1]P Cementum = peripheral cementum; [2]I Cementum = infundibular cementum

There were no statistically significant differences in maxillary CT dental tissue densities between young and old donkeys (P > 0.1; Table 3.3.3.6a; Appendix 2) (Figure 4.6.3.1)

Figure 2.3.6.3.1: **Density measurements of dental tissues in Hounsfield units in younger and older donkey maxillary cheek teeth. Dent1 = primary dentine; Dent2 = secondary dentine; CemP = peripheral cementum; CemI = infundibular cementum.**



Mandibular cheek teeth

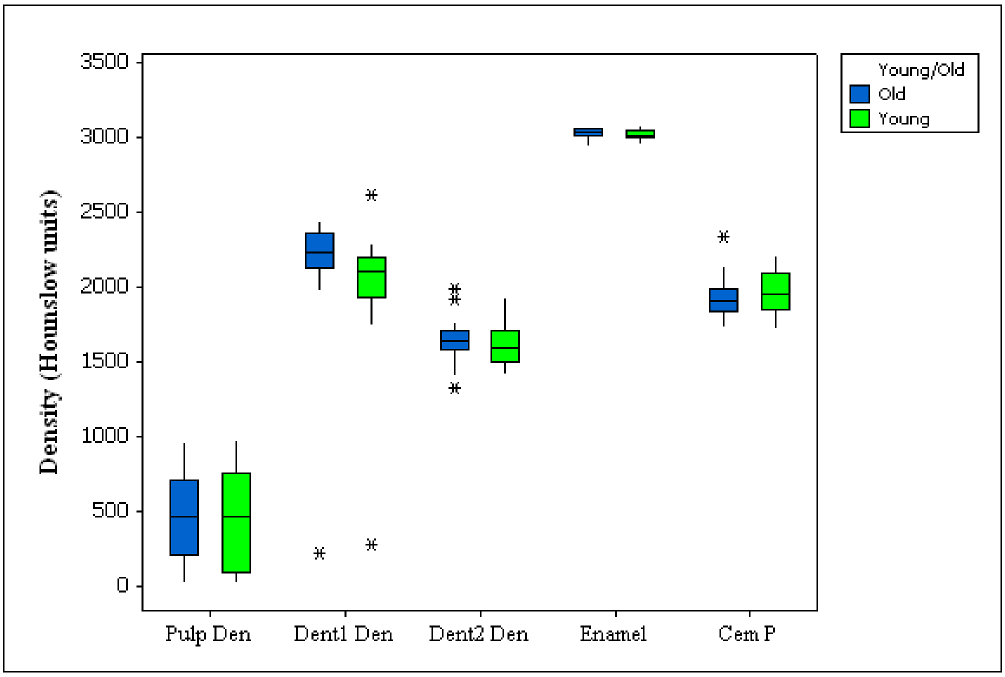
The results of dental tissue density measurements in donkey mandibular cheek teeth is summarised in Table 2.3.6.3.2 and Figure 2.3.6.3.2 below. The median density of enamel was 3035 HU, primary dentine 2190 HU, secondary dentine 1626 HU and peripheral cementum 1921 HU.

Table 2.3.6.3.2: **Summary of density measurements (Hounsfield units) for pulp, primary dentine, secondary dentine, enamel and peripheral cementum in mandibular CT. [1]P cementum = peripheral cementum.**

Structure	Number	Mean	Minimum	Median	Maximum	Coef of Var
Pulp Den	42	463.1	26.0	466.0	984.0	63.98
Primary dentine	42	2107.2	227.0	2190.0	2617.0	21.54
Secondary dentine	42	1645.8	1332.0	1625.5	1994.0	8.99
Enamel	42	3027.2	2944.0	3035.5	3083.0	1.23
[1]P cementum	42	1943.5	1723.0	1921.0	2345.0	6.97

There were no statistically significant differences in maxillary CT dental tissue densities between younger and older donkeys ($P > 0.32$; Table 2.3.3.6b; Appendix 2) (Figure 2.3.6.3.2).

Figure 2.3.6.3.2: **Density measurements of dental tissues in Hounsfield units in mandibular CT in younger and older donkeys. Dent1 = primary dentine; Dent2 = secondary dentine; CemP = peripheral cementum; CemI = infundibular cementum.**



2.3.7. Incisors

2.3.7.1. Aim of the study

The aim of this study was to determine normal ranges for donkey incisor pulp horn depth, secondary dentine thickness/depth, pulp horn width and dental tissue densities in Hounsfield units as measured by CAT.

2.3.7.2 Materials and methods

The same methods used in cheek teeth measurements (4.4.2, 4.5.2, 4.6.2) were repeated in 26 incisors from the same 13 donkeys as CT were obtained.

2.3.7.3 Results

Table 2.3.7.3.1: **Depth of pulp horn and subocclusal secondary dentine (cm) in incisors as determined on CAT images.**

Thickness	Number	Mean	Minimum	Median	Maximum	Coef of Var
Pulp horn	26	2.75	1.24	2.57	4.69	31.67
Secondary Dentine	26	0.94	0.26	0.86	2.02	52.49

Figure 2.3.7.3.1: **Lengths of pulp horns and depths of subocclusal secondary dentine of incisors (cm); Den2 – secondary dentine.**

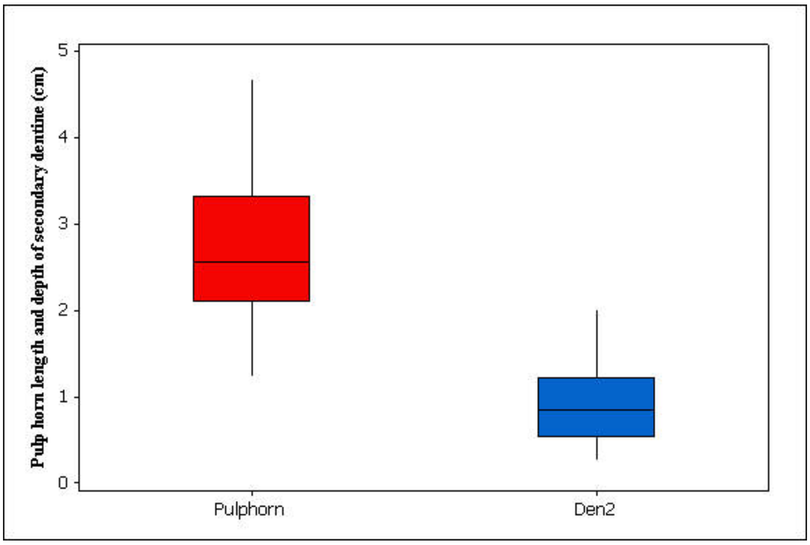


Table 2.3.7.3.2: **Summary of incisor pulp horn latero-medial widths at sub-occlusal (a), mid (b) and pre-apical (c) level (cm) as determined on CAT images.**

Pulp horn	Number	Mean	Minimum	Median	Maximum	Coef of Var
PHa	24	0.21	0	0.16	0.54	97.67
PHb	24	0.20	0.09	0.18	0.61	52.28
PHc	24	0.16	0.07	0.13	0.38	48.54

Figure 2.3.7.3.2: **Latero-medial widths of incisor pulp horns (PH) at subocclusal (a) mid, (b) pre-apical, and (c) transverse sections (cm) as determined on CAT images.**

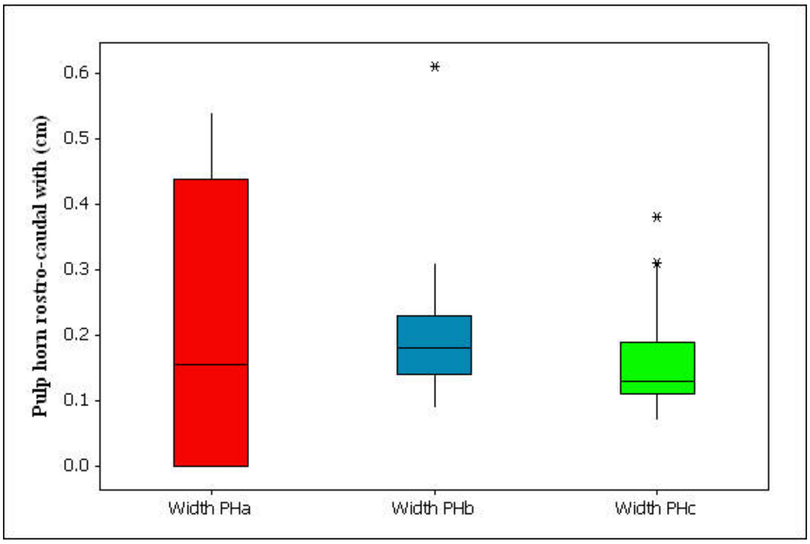
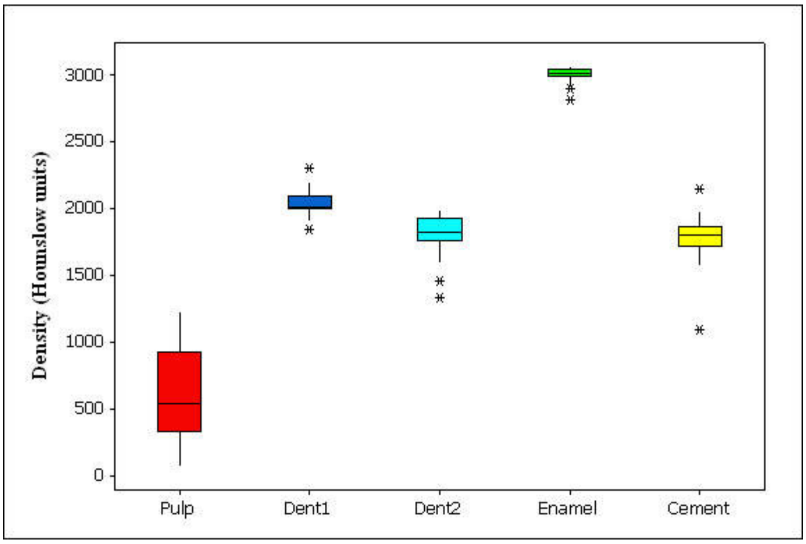


Table 2.3.7.3.3: **Summary of density measurements (Hounsfield units) for incisor pulp, primary dentine, secondary dentine, enamel, peripheral cementum and infundibular cementum.**

Structure	Number	Mean	Minimum	Median	Maximum	Coef of Var
Pulp	26	606.7	72.0	538.5	1230.0	53.43
Primary dentine	26	2043.9	1850.0	2014.5	2311.0	4.64
Secondary dentine	26	1799.1	1330.0	1827.0	1988.0	8.99
Enamel	26	3010.3	2818.0	3020.0	3067.0	1.98
Cementum	25	1775.1	1093.0	1802.0	2152.0	10.65

Figure 2.3.7.3.3: **Density measurements of incisor dental tissues in Hounsfield units as determined on CAT images.**



2.3.8 Infundibular cemental hypoplasia

A recent study (Fitzgibbons 2007) on equine maxillary cheek teeth found infundibular cemental hypoplasia and discoloration in 55% of all infundibula in 33 horse skulls.

2.3.8.1. Aim of this study

The aim of this study was to determine the prevalence of infundibular hypoplasia in the non-diseased maxillary cheek teeth of 12 donkeys utilising CAT examination.

2.3.8.2 Materials and methods

In total 36 maxillary cheek teeth (and 72 infundibula) were examined on CAT for the presence of areas of cemental hypoplasia within the infundibula i.e. areas of hypodensity compared to normal cementum (Figure 2.3.8.1). The presence of normal circular vascular channels in the centre of the cementum were not regarded as evidence of hypoplasia (Figure 2.3.8.2).

Figure 2.3.8.1: **CAT image of transverse section (left) and longitudinal section (right) of a donkey maxillary cheek tooth illustrating cemental hypoplasia within the infundibula (solid arrows). Also note the enlarged, hollowed pulp horn indicating likely incidental pulpar exposure (open arrow head) that was not visible on gross examination.**

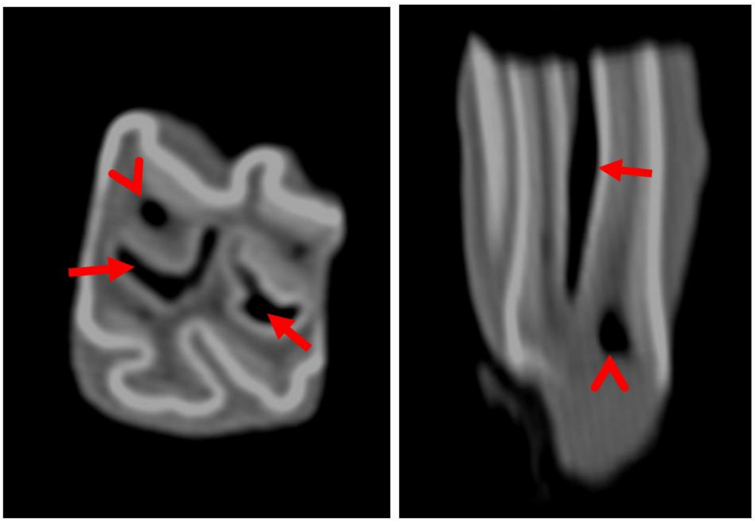
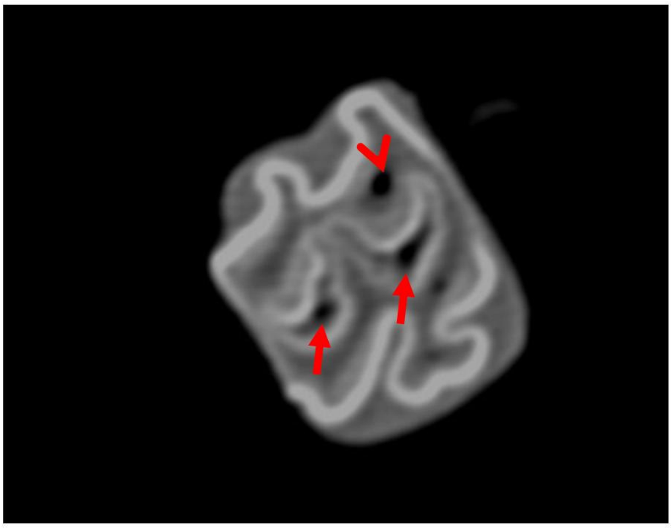


Figure 2.3.8.2: **CAT image of maxillary cheek tooth transverse section illustrating the small circular hypodense central appearance of the remnant of the vascular channel within the infundibula (solid arrows). Once again note the enlarged pulp horn indicating likely incidental occlusal pulpal exposure (open arrow head).**



2.3.8.3 Results

Of the 36 maxillary cheek teeth examined, 20 (50.5% of teeth) had infundibular hypoplasia in one or both infundibula. All 12 donkeys examined had at least one maxillary cheek tooth that was affected. In total 15 rostral (41.6%) and 17 caudal (47.2%) of infundibula had hypoplasia on CAT images. In total 32 out of 72 infundibula (44.4%) were affected with cemental hypoplasia. The Triadan 09s had the highest percentage of infundibular cemental hypoplasia (77.8%; Table 2.3.8.1).

Table 2.3.8.1: **The number of maxillary cheek teeth in each Triadan position examined, the number of rostral and caudal infundibula with hypoplasia, total number of infundibula with hypoplasia and percentage with hypoplasia in each Triadan position.**

Triadan position	No. of teeth examined	No. of rostral infundibulae with hypoplasia	No. of caudal infundibulae with hypoplasia	Total number of infundibulae with hypoplasia	Percentage hypoplasia per Triadan position
06	2	1	1	2	50%
07	6	3	2	5	41.6%
08	4	0	1	1	12.5%
09	9	6	8	14	77.8%
10	12	5	4	9	37.5%
11	3	0	1	1	16.6%
Total	36	15	17	32	NA

2.3.9 Discussion

The number and positions of pulp horns in normal donkey CT were identified and was similar to that which has been described in the horse (Dacre 2005b). There are five pulp horns in CT 07-10 with six in 06 and 11 positions and very rarely a 7th in the 11. It is proposed that for ease of use, the endodontic numbering system described in horses (Dacre 2005b) is altered such that the mandibular CT nomenclature correlates with the maxillary CT nomenclature i.e. starts on the buccal aspect.

Most of the pulp horn communications observed were found in the pre-apical areas. This could in fact indicate the merging of the pulp horns as they near the common pulp chamber. Using a dissecting microscope, Dacre (2005a) observed pulp horn communication in the sub-occlusal and mid- transverse sections. However, the age of the horses used in Dacre’s (2005a) study was much younger than the donkeys used in this study, therefore comparison of the findings of both studies is difficult. Nevertheless, there was a higher degree of pulp communication in donkey mandibular, compared to maxillary CT, as was also observed in equine teeth. This is an important factor that needs to be considered during endondontic treatment, as pulp horn communications of an affected pulp horn will result in a greater chance of treatment failure (Emily *et al.* 1997). It is also worth noting that the most common pulp horn communications in maxillary and mandibular cheek teeth in horses and donkeys are between pulp chambers 3&5 and 2&5 respectively.

Comparisons of tooth length and rostro-caudal width of the measurements from CAT images and gross evaluation of CT were not statistically significantly different, validating the accuracy of CAT image measurements. However, the latero-medial width measurements were significantly different. This is believed to be due to inability to measure at exactly the same mid point of the occlusal surface for each tooth. There were larger apparent differences between physical and CAT image measurements in the incisors, although these differences were not statistically significant. The reason for this variation between physical and CAT incisor teeth measurements could be two fold: incisors are quite curved which complicates both CAT image and gross measurements and secondly, the precise location of the junction of the roots (enamel free apex) and the crown is particularly difficult to distinguish on gross evaluation which is emphasised by the high coefficient of variation (> 15). All CT and incisor length measurements coefficients of variation were greater than 15 indicative of high variation, which may be due to tooth length variation with age and size of the donkey.

The comparison between older (> 15 years) and younger (≤ 15 years) teeth did show that there was a significant difference in: tooth length (maxillary and mandibular CT), infundibula (maxillary CT), lateral roots (maxillary CT) and rostro-caudal width (mandibular CT). The difference in tooth length, infundibula and roots are as expected as teeth undergo a normal degree of attrition. Tooth root development also only starts a few years after eruption and continues throughout the life of the tooth with cementum deposition increasing the length of the roots with age (Dixon and Copeland 1993).

This study clearly illustrates the great degree of variation of pulp horn length and secondary dentine depth within donkey cheek teeth (coefficients of variation >15). Contrary to previous reports in horses where depths of secondary dentine have been reported to be 6 – 8mm (Baker 2002), the depth of subocclusal secondary dentine thickness appeared to be greater than 10mm in both mandibular and maxillary teeth. The mean secondary dentine thickness (1.14cm) was less in younger donkeys (≤ 15 years) than older (> 15 year) donkeys (1.44cm), although this difference was not statistically significant. Similarly the ratios of secondary dentine depth to pulp horn length were greater in older donkeys, although this was only statistically significant in maxillary cheek teeth. Unfortunately the number of teeth used in the validation study were too few to definitely determine the validity of the CAT image measurements of this parameter. Furthermore, CAT images would likely not detect any partially mineralised secondary dentine, and are likely to underestimate the depth of secondary dentine. Recently, a study examining the secondary depths of cheek teeth pulp horns from 17 horse skulls showed that the mean occlusal secondary dentine depth was 10.76 and 9.03 mm in mandibular and maxillary CT respectively (White 2008), which supports the results from this study in donkeys.

This study has provided a range of normal pulp horn widths on transverse section, but has shown that there is great variation between donkey cheek teeth in the same donkey or between donkeys in the subocclussal, mid or pre-apical transverse sections. As expected, there appears to be a gradual increase in pulp horn width apically. There were no statistically significant differences in pulp horn width between young and old donkeys. There was as expected a general trend of a decrease in pulp horn width in older donkeys' teeth. The reason for the findings of this study being statistically non-significant could be attributed to a number of factors. Firstly the age of distinguishing between young and old donkeys (15 years of age) in this study was randomly chosen based on the population of donkeys available for this part of the study. It is expected that soon after eruption CT will have large pulp horns only lined with primary dentine and secondary dentine deposition will commence rapidly (Kierdorf and Kierdorf 1992;

Kirkland *et al.* 1996). However once root formation starts (about 2-4 years after eruption) (Dixon and Copeland 1993) the secondary dentine deposition will occur to prevent occlusal pulpal exposure but not complete obliteration of the pulp horn. Furthermore, the donkey population at the donkey sanctuary are not the same breed and therefore vary in size and contribute to the variations in measurements obtained here.

Apart from the density of pulp, the density measurement of calcified dental tissue did not vary significantly between sites on tooth, individual teeth or between donkeys (coefficient of variation <15). It can therefore be concluded that the measurements are an accurate reflection of the CAT densities in Hounsfield units. The median density of the different dental tissues also correlated with the range recorded in horses (cementum 500 – 2400 HU, dentine 950 – 3000 HU and enamel 2000-3071 HU) (Dacre 2005a). In conclusion enamel is the densest tissue, followed by primary dentine, cementum, secondary dentine and pulp. In horses it has been proposed that secondary dentine becomes denser with age due to mineralisation (sclerosis) of secondary dentine tubules (Baker 2005b). However, this study showed that there was no statistically significant difference in the density of dental tissue in old and young donkeys' teeth.

Incisor measurements showed a great variation in the length, rostro-caudal and latero-medial width, as well as the length and width of pulp horns and depth of subocclusal secondary dentine as indicated by the coefficients of variation (>15). The measurements of dental tissue density (except pulp) did not show such a great variation (coefficient of variation < 15). However it has to be remembered that these samples have been taken from a small population of donkeys. This is a limited population with a median age of 20 and there are many other factors, such as age and size of donkeys that can influence measurements of length of pulp horns and depth of occlusal secondary dentine.

The number of rostral (41.6%), caudal (47.2%) and total (44.4%) infundibula with hypoplasia were more than that observed in horses (24, 20 and 22% respectively) (Fitzgibbons 2007). This could be due to older age of donkeys that were used for CAT examination in this study. Considering that most of the infundibular hypoplasia in the horse study (Fitzgibbons 2007) was observed in the apical third of the infundibulae, this area is most likely to be retained in older donkeys, even with extensive CT wear. Furthermore, donkeys with infundibular caries were excluded from this study, which would also decrease the prevalence of CT with marked cemental hypoplasia observed here. In conclusion, this study correlated with the results of the study in horses by Fitzgibbons (2007) that infundibular cemental hypoplasia can be regarded as a normal anatomical variation of equid maxillary cheek teeth infundibula.

Chapter 3: Donkey dental anatomy: Histological and scanning electron microscopy examinations.

3.1. Histology

3.1.1 Histological anatomy

3.1.1.1. Aims of the study

The aim of this study was to establish the normal histological structure of donkey dental tissues using decalcified and undecalcified histology.

3.1.1.2. Materials and methods

Decalcified histology

Fifty four sections (from 18 teeth obtained from 8 donkeys) were placed in individual histokinette chambers (Simport Plastics, Beloil. Canada) and washed in tap water to remove some of the formalin fixative (+/- 20 minutes). The sections within the histokinette chambers were then placed in Surgipath Decalcifier II (Surgipath Europe Ltd, Peterborough, UK) for 72 hours, with the decalcifying solution replenished at 24 and 48 hours. After removal from the decalcifying solution, the sections were washed in tap water for 20 minutes prior to processing in a Shandon Pathology Centre automated tissue processor. In this processor the sections were dehydrated in a series of graded alcohol and xylene solutions, prior to being impregnated in Paraplast Plus tissue embedding medium (Oxford Labware, St Louis, USA). Sections were then cast into paraffin wax blocks.

Tissue blocks were placed on ice for one hour prior to sectioning on a microtome. Sections were cut 4-5µm thick and mounted on Xtra adhesive slides (Surgipath Europe Ltd, Peterborough, UK). The slides were then dried at 37°C for 48 hours prior to routine staining with haematoxylin and eosin.

During the histological processing the decalcification and loss of enamel resulted in the dentine losing its attachment to the outer cementum and some samples sections were incomplete. This resulted in only 12 teeth having complete sets i.e. subocclusal, mid and pre-apical sections. For statistical purposes, to minimise variables, cheek teeth in positions 09 and 10 were used for histological measurements from 5 maxillary and 5 mandibular cheek teeth from the 5 youngest donkeys.

Undecalcified histology

Sixteen sub-occlusal, 2mm thick transverse sections from 7 donkeys were dehydrated through a series of graded acetone (10 minutes each in 50%, 70%, 90%, and three times in 100% acetone solutions) and air dried for 24 hours. Sections were then placed in a foil mould and using Epo-thin resin with 2% petroxy blue dye (Buehler, Coventry, UK), were set into blocks at room temperature for 24 hours.

Excess resin was then trimmed away with a diamond saw and one side of the section was hand ground flat using silicon carbide grit paper (240, 400, 600, 800, 1200, 2500, 4000 grit sequentially) (Buehler, Coventry, UK) and Ilocut 430 (Castrol UK Ltd, Swindon, UK) as a non-water based lubricant. After a final clean rinse with acetone, the hand ground surface was bonded to a glass slide using Epi-thin resin and placed in a spring loaded jig (Logitech Ltd, Poole, Dorset) for 24 hours.

The mounted teeth sections were then cut to 500µm thick with a Petrothin cut-off saw (Buehler, Coventry, UK) with a diamond blade and vacuum mount. A Logitech rotary lapping machine (Logitech Ltd, Poole, Dorset) was then used to thin the sections further.

This was achieved using 600 grit silicon carbide powder (Peter Wolters U.K. Ltd, London, UK) in conjunction with a constant drip of ethylene glycol onto the contact surface. Finally, the sections were hand-lapped on a glass plate using 800 grit silicon carbide paper to give a fine finish and thickness of 80µm. A glass cover slip was then placed over the finished surface using Eukitt adhesive (VWR International Ltd, Poole, Dorset, UK).

Sections were then viewed using a Nikon, Alphashot -2 Microscope and measurements taken using a stage micrometer. The sections were examined using a light microscope (Nikon, Alphashot-2 YS2, Nikon UK Ltd, Surrey, UK) and the normal histological features identified.

3.1.1.3 Results

The ages of the donkeys and teeth used for decalcified histology are tabulated below (Table 3.1.1.3.1). The mean age of donkey for maxillary CT decalcified histology was 13 years and mandibular CT was 8 years.

Table 3.1.1.3.1: **Cheek teeth (Triadan position and age of donkeys) used for decalcified histology measurements.**

Age	Maxillary cheek teeth	Mandibular cheek teeth
4		309; 409
6	110	410
11	109	309
15	110	310
16	209; 210	

Only 13 sections from 4 donkeys were adequate for undecalcified histological evaluation. The age of donkeys and teeth used for undecalcified histology is tabulated in Table 3.1.1.3.2. The mean age of donkey for maxillary CT undecalcified histology was 14 years and mandibular CT was 11 years.

Table 3.1.1.3.2: **Cheek teeth (Triadan position and age of donkey) used for undecalcified histology.**

Age	Maxillary cheek teeth	Mandibular cheek teeth
4		309; 409
11	109; 110	309; 310
15	109;110	309; 310; 310
16	209;210	

Decalcified histology

Primary, secondary dentine and cementum have been identified histologically. The normal histology of donkey cheek teeth appears to be the same as that which has been described in the horse (Dacre 2005a). The classification of donkey dentine used here is that used by Dacre (2005a), with normal secondary dentine divided into regular and irregular secondary dentine (Figure 3.1.1.3.3 and 3.4.1.3.4). This is in contrast to older classifications where irregular secondary dentine was classified as tertiary dentine in horses (Kilic *et al.* 1997c; Muylle *et al.* 2002) and humans (Nanci 2003a). Irregular secondary dentine is predominantly found in the occluded pulp horn and is found in normal teeth. No true tertiary dentine (dentine laid down in response to noxious stimuli) has been identified during examination of normal donkey teeth histological sections in this study. This use of intact cheek teeth did restrict penetration of formalin into the pulp via the apical foramina, and hence fixation of the pulp tissue. Therefore, histology of pulp was very limited.

Figure 3.1.1.3.3: **Decalcified subocclusal section of a maxillary cheek tooth illustrating primary dentine (PD), regular secondary dentine (RSD) and irregular secondary dentine (ISD) (x 100).**

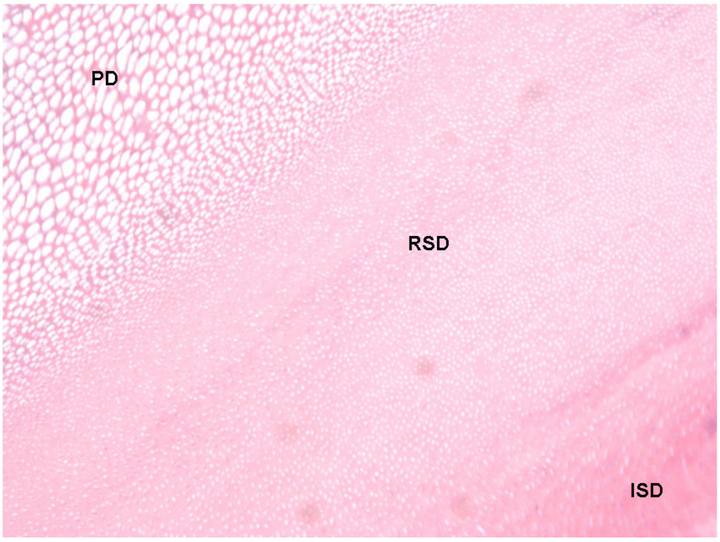
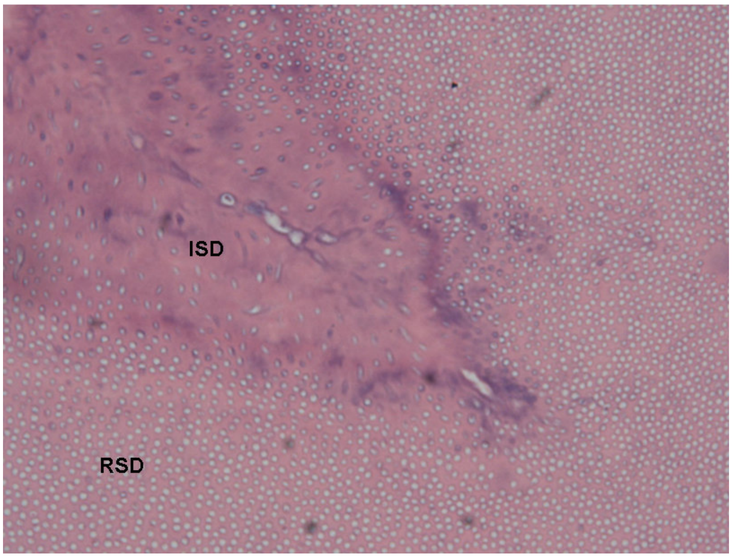
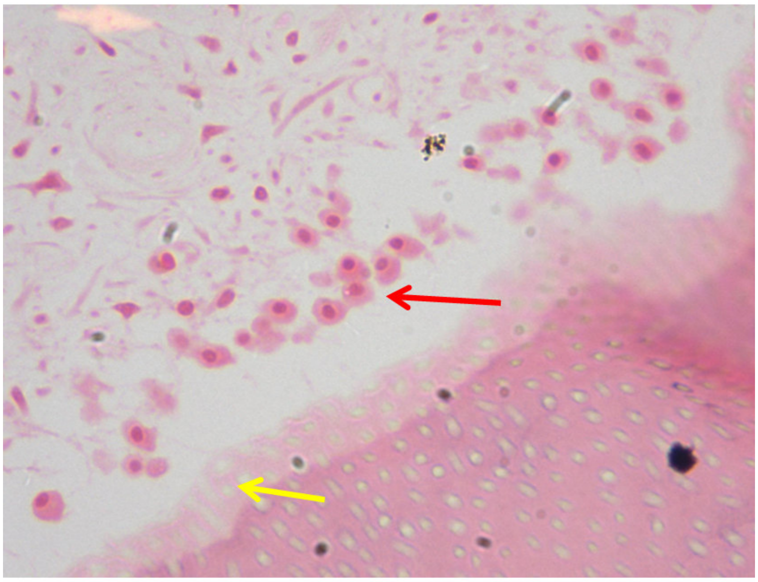


Figure 3.1.1.3.4: **Decalcified subocclusal section of maxillary cheek tooth illustrating regular secondary dentine (RSD) and irregular secondary dentine (ISD) occluding the pulp cavity (x 100).**



The initial dentine that is laid down is not calcified and is termed predentine which has been identified here in donkey CT (Figure 3.1.1.3.5).

Figure 3.1.1.3.5: **A decalcified mid-tooth section of a mandibular cheek tooth showing odontoblasts (red arrow) lining the periphery of the pulp (artefactually separated from dentine), laying down a layer of predentine (yellow arrow) (x 200).**



Other features that were identified on donkey dental histology included the presence of intertubular dentine (Figure 3.1.1.3.6), interglobular dentine (Figure 3.1.1.3.7) and pulp stones. Pulp stones are identified as 'free stones' within a viable pulp cavity and as 'attached stones' surrounded by irregular secondary dentine as deposited during physiological pulp occlusion (Nanci 2003a) (Figure 3.1.1.3.8).

Figure 3.1.1.3.6: **Decalcified H&E transverse mid-tooth section illustrating areas of intertubular dentine (arrows) present in primary dentine. Intertubular dentine deposition is associated with rapid dentine deposition in other species (x100).**

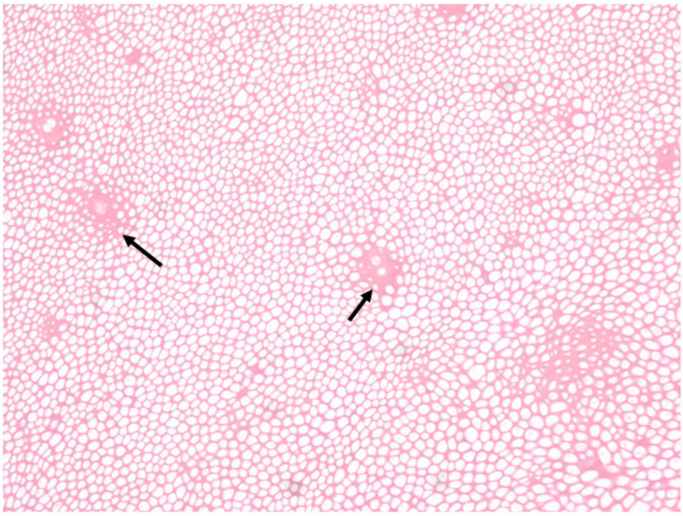


Figure 3.1.1.3.7: **Decalcified H&E histological mid-tooth section showing areas of interglobular dentine (arrows). These are areas of partially mineralised dentine (x 100).**

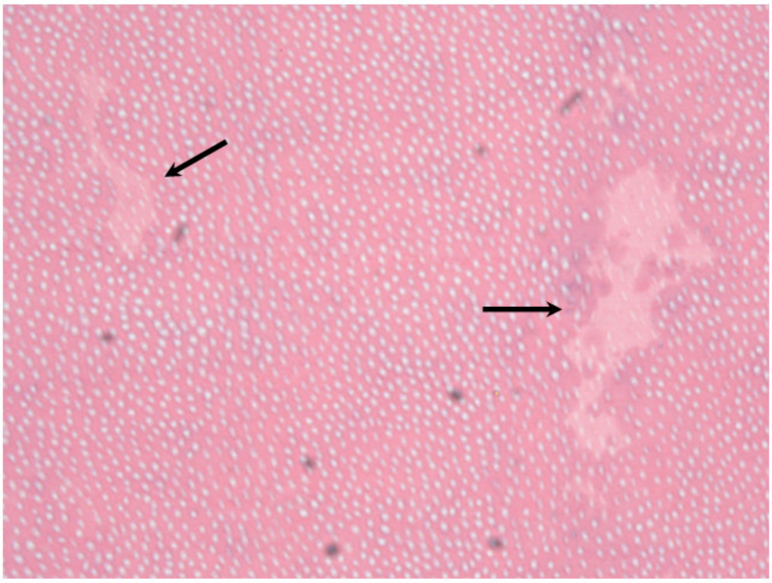
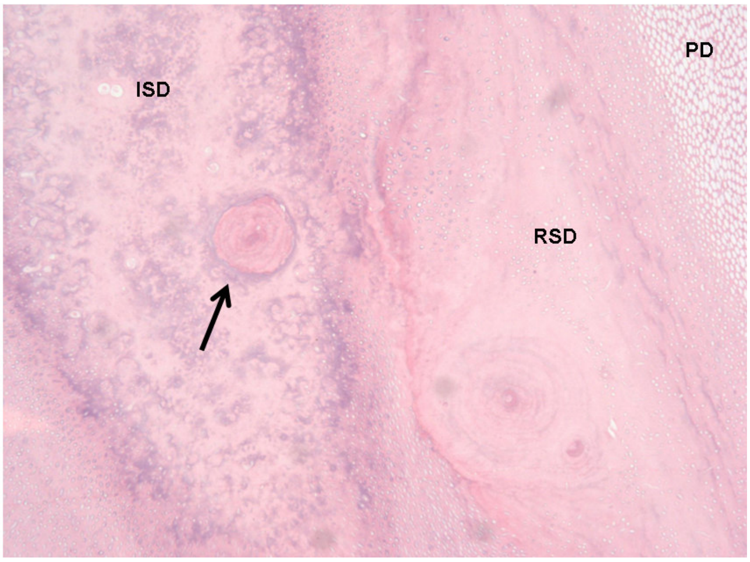
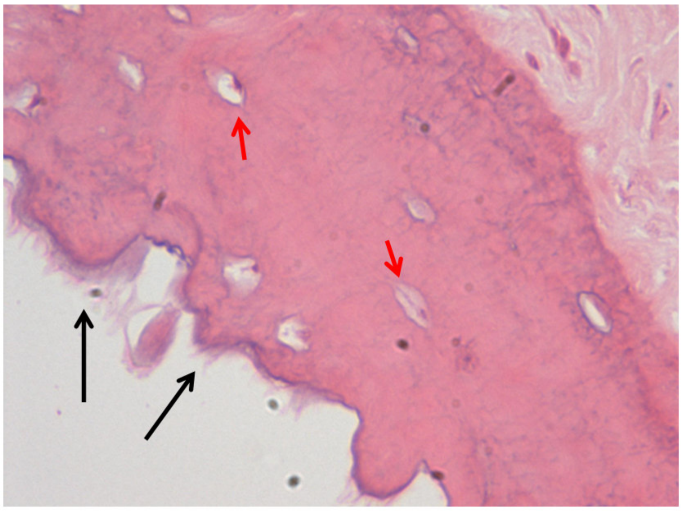


Figure 3.1.1.3.8: **Decalcified H&E histological subocclusal section illustrating a pulp stone (arrow) within the occluded pulp horn. ISD = irregular secondary dentine; RSD = regular secondary dentine; PD = primary dentine (x 40).**



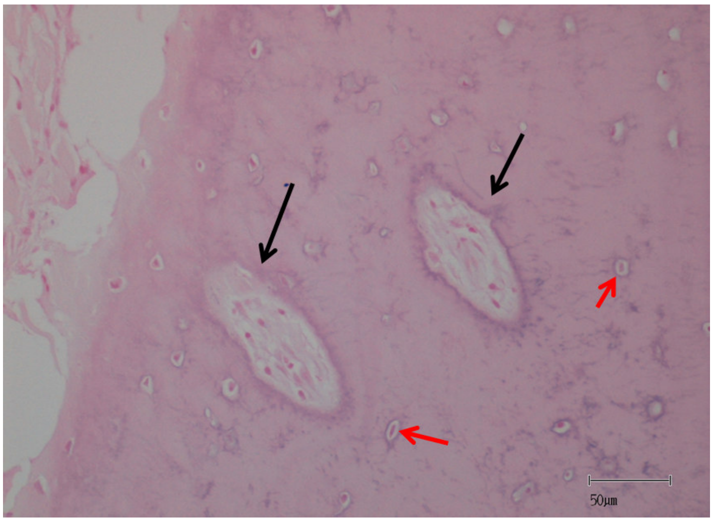
Peripheral cementum identified contained lacunae, some of which contained visible cementocytes (Figure 3.1.1.3.9 and 3.1.1.3.10). At the ACJ a fibrillar hyaline layer was visible where the enamel had been removed during decalcification (Figure 3.1.1 3.9).

Figure 3.1.1.3.9: **Decalcified pre-apical section at the ACJ illustrating the fibrillar hyaline layer where the enamel had been attached (black arrows). Also note the lacunae with cementocytes (red arrows) (x 200).**



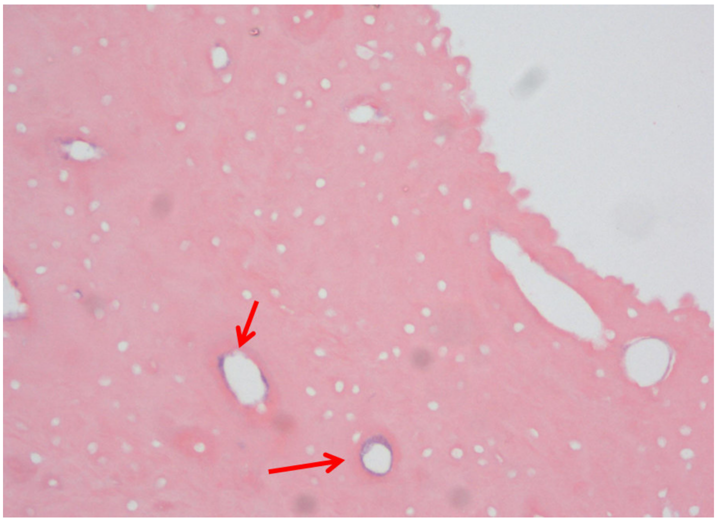
Nerve bundles and blood vessels were also identified in the peripheral cementum (Figure 3.1.1.3.10), confirming that peripheral cementum is cellular and viable.

Figure 3.1.1.3.10: **Decalcified sub-occlusal section illustrating nerve bundles in peripheral cementum (black arrows). Cementocytes can be clearly visualised within the lacunae (red arrows) (x 200).**



Remnants of the vascular channels were identified in infundibular cementum (Figure 3.1.1.3.11).

Figure 3.1.1.3.11: **Decalcified pre-apical section of maxillary cheek tooth illustrating possible blood vessels within the infundibular cementum (red arrows) (x 200).**



Undecalcified histology

Undecalcified histology allows identification of enamel which is lost during decalcification. Normal undecalcified histological appearance of enamel, dentine and cementum was identified (Figure 3.1.1.3.12, 3.1.1.3.13 and 3.1.1.3.15). Enamel spindles have not previously been described in equids, but have been identified in these sections (Figure 3.1.1.3.14).

Figure 3.1.1.3.12: **Undecalcified subocclusal section of a maxillary cheek tooth illustrating the appearance of enamel (E), dentine (D) and cementum (C) (x 40).**

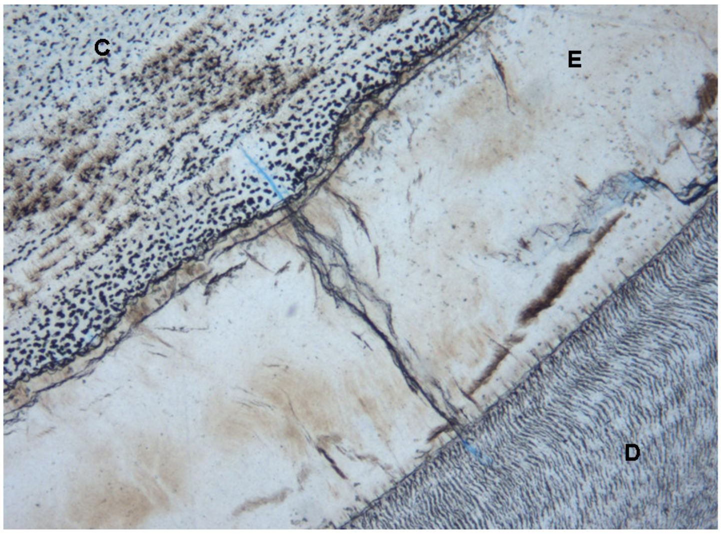


Figure 3.1.1.3.13: **Undecalcified sub-occlusal section of a maxillary cheek tooth illustrating appearance of primary dentine (A) and regular secondary dentine (B) (x 200).**

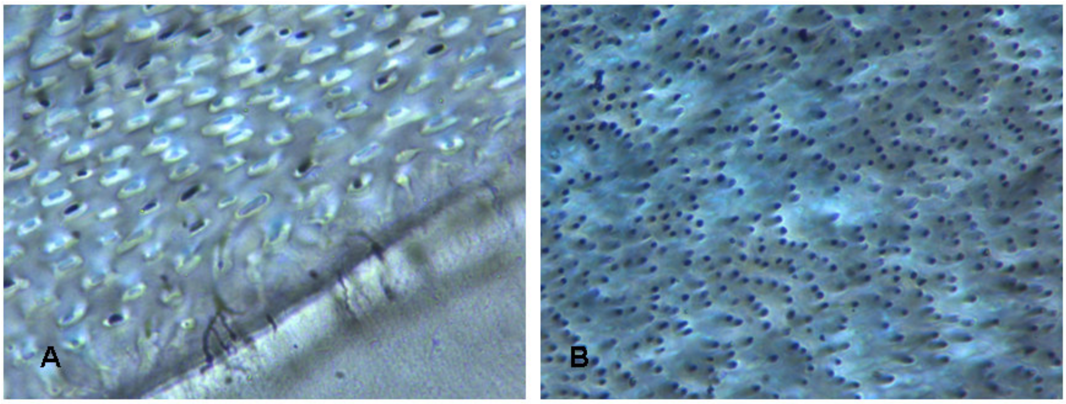


Figure 3.1.1.3.14: **Undecaclcified sub-occlusal section showing enamel spindles (arrows) extending from dentine into enamel at the amelo-dentinal junction (x 200).**

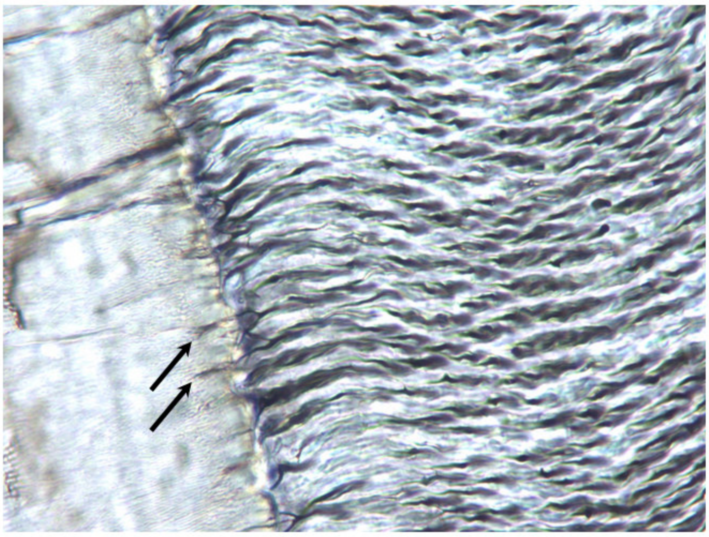
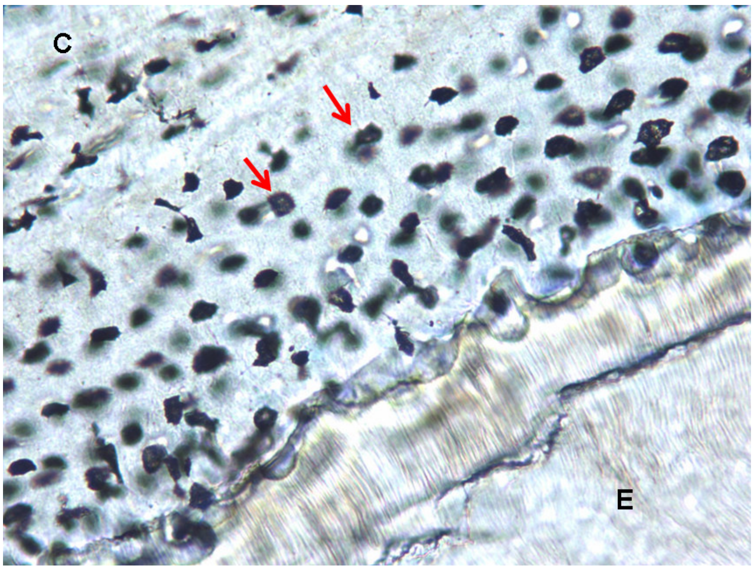


Figure3.1.1.3.15: **Undecalcified sub-occlusal section illustrating the appearance of cementum and lacunae (red arrows); C = cementum, E = enamel (x 200).**



3.1.2 Quantitive analysis of decalcified histology

3.1.2.1. Aims of the study

The aim of this study was to quantitatively evaluate decalcified histology sections of donkey cheek teeth to establish normal values for dental primary and secondary dentine thickness around pulp horns.

3.1.2.2 Materials and Methods

Measurements of primary and secondary dentine thickness in buccal and lingual/palatal directions around each pulp horn were taken using a stage micrometer and light microscope (Nikon, Alphashot-2 YS2, Nikon UK Ltd, Surrey, UK) in micrometers (µm) (Figs. 3.1.2.2.1 and 3.1.2.2.2). Pulp horns were identified using the numbering system as described in Figure 2.3.1.

Figure 3.1.2.2.1: **A decalcified subocclusal section of a maxillary cheek tooth (H&E) illustrating the site of measurements of dentine around each pulp cavity in buccal (green arrow) and palatal (blue arrow) directions.**

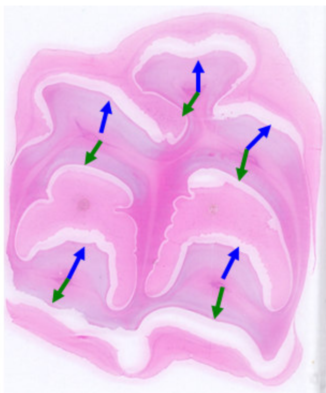
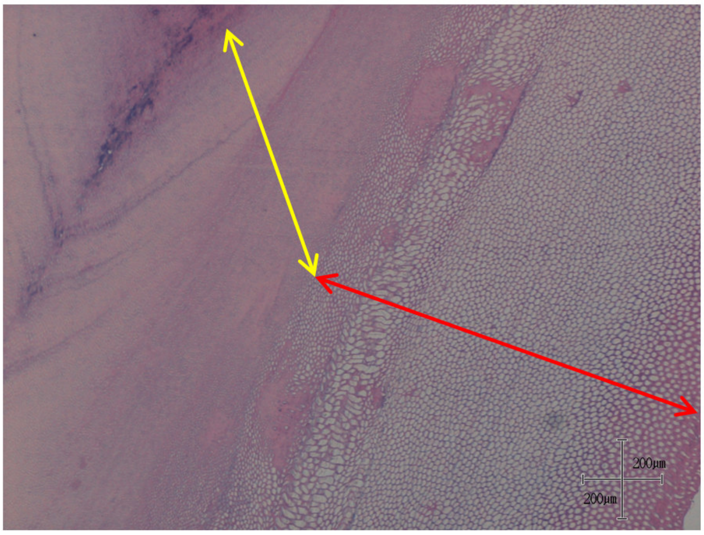


Figure 3.1.2.2.2: **Decalcified subocclusal section of a maxillary cheek tooth illustrating the histological differentiation between primary (red line) and secondary (regular and irregular) (yellow line) dentine from the dentino-enamel junction (on right side of red arrow) to the midline of the former pulp cavity (to left of yellow arrow) (x40).**



Linear, mixed-effect models were performed (using R V2.3.1, R Foundation for Statistical Computing) comparing buccal and lingual/palatal primary and secondary dentine thicknesses on transverse cheek teeth sections. Histological section level (sub-occlusal, mid or pre-apical) nested within each (former) pulp horn nested within tooth, nested within donkey were entered as random effects to take account of the fact that there are multiple sections per tooth and multiple pulp horns per tooth and multiple teeth per donkey. Histological section level (a, b or c), specific pulp horns (1-5) and location of pulp horns (buccal or lingual/palatal) were then entered as fixed effects to determine their statistical significance. $P < 0.05$ was taken to indicate the statistical significance and degrees of freedom are indicated by subscripts.

3.1.2.3. Results

The histological measurements and results of the linear mixed effect models are tabulated in tables 3.1.2.3.1. and 3.1.2.3.2.

Table 3.1.2.3.1: **Summary of histological measurements of dentine thickness in a buccal and lingual/ palatal direction around all pulp horns in µm.**

Dentinal Thickness	Maxillary cheek teeth			Mandibular cheek teeth		
	Mean (Median)	Coefficient Variation	Range	Mean	Coefficient Variation	Range
Primary Lingual/Palatal	1768.8 (1190)	34.1	90-1875	922.3 (780)	51.34	275-2450
Primary Buccal	1305.1 (10870	49.75	0-3050	1102.7 (725)	70.83	725-3350
Secondary Lingual/Palatal	557.9 (350)	90.23	40-2875	272.7 (250)	70.62	0-660
Secondary Buccal	445.0 (275)	82.41	0-2000	242.3 (210)	81.61	0-850

Table 3.1.2.3.2: **Results of linear, mixed effect models showing degrees of freedom and P values for overall difference in buccal and palatal/lingual dentine thickness and differences between individual pulp horns, level of tooth or pulp horn location (bold values are significant).**

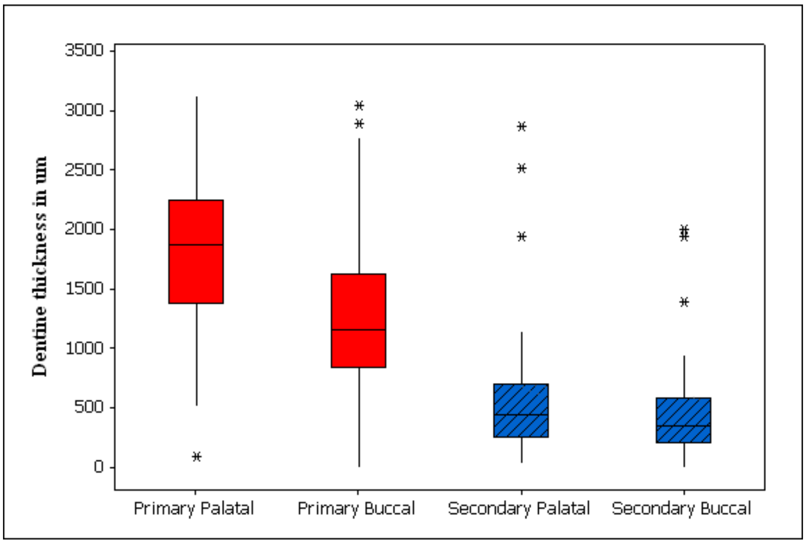
	Maxillary cheek teeth		Mandibular cheek teeth	
Dentine	**Primary**	**Secondary**	**Primary**	**Secondary**
Difference between buccal and lingual/palatal thickness	$F_{1.54}$ = 22.49; P **< 0.001**	$F_{1.54}$ = 3.3; P = 0.07	$F_{1.58}$ = 1.70; P = 0.198	$F_{1.58}$ = 1.69; P = 0.198
Pulp horns	$F_{4.50}$ = 1.95; P = 0.12	$F_{4.50}$ = 4,89; P **< 0.01**	$F_{4.54}$ = 9.15; P **< 0.001**	$F_{4.54}$ = 0.32; P = 0.866
Level	$F_{2.62}$ = 0.72; P = 0.49	$F_{2.62}$ = 1.16; P = 0.32	$F_{2.66}$ = 0.31; P = 0.73	$F_{2.66}$ = 0.32; P = 0.73
Buccal or palatal/lingual pulp horn	$F_{1.53}$ = 1.34; P = 0.25	$F_{1.53}$ = 18,44; P **< 0.001**	$F_{1.53}$ = 38.06; P **< 0.001**	$F_{1.57}$ = 0.02; P = 0.89

Maxillary cheek teeth

Primary dentinal thickness was significantly different between palatal and buccal directions around all pulp horns in maxillary cheek teeth (P < 0.001; Figure 3.1.3.1; Table 3.1.2.3.2). This buccal/palatal dentine thickness difference did not significantly differ between the different pulp horns, between different levels or between buccally and palatally situated pulp horns (All P > 0.12; Table 3.1.2.3.2).

Secondary dentine thickness was not significantly different between palatal and buccal directions around all pulp horns in maxillary cheek teeth (P = 0.07; Figure 3.1.2.3.1; Table 3.1.2.3.2). This buccal/palatal dentine thickness difference was significantly different between the different pulp horns and between buccally and palatally situated pulp horns (P < 0.01; Table 3.1.2.3.2). This buccal/palatal dentine thickness difference was not significantly different between different levels (P = 0.32; Table 3.4.2.3.2).

Figure 3.1.2.3.1: **Boxplot showing maxillary cheek teeth primary and secondary dentine thickness in buccal and palatal direction around the pulp horns.**

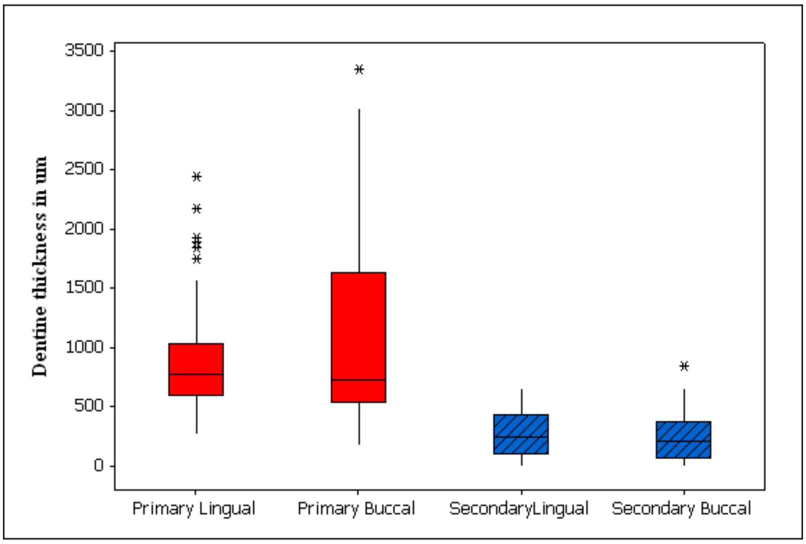


Mandibular cheek teeth

Primary dentine thickness was not significantly different between palatal and buccal directions around pulp horns (P = 0.198; fig.3.1.2.3.2; Table 3.1.2.3.2) in mandibular cheek teeth. This buccal/palatal dentine thickness difference was not significantly different between different levels on the same cheek tooth (P = 0.73; Table 3.1.2.3.2), but was significantly different between individual pulp horns and between buccally and palatally situated pulp horns (P < 0.001; Table 3.1.2.3.2).

Secondary dentine thickness was not significantly different in palatal and buccal directions around all pulp horns (P = 0.198; Table 3.1.2.3.2) in mandibular cheek teeth. This buccal/palatal dentine thickness difference was not significantly different between the different pulp horns, between different levels or between buccally and palatally situated pulp horns (All P > 0.73; Table 3.1.2.3.2).

Figure 3.1.2.3.2: **Mandibular cheek teeth primary and secondary dentine thickness in buccal and palatal direction around the pulp horns**



3.1.3 Discussion

Histologically, donkey dental tissue is very similar to what has been described in the horse (Dixon 2002; Dacre 2005b). In particular the classification of secondary dentine into regular and irregular secondary dentine as used by Dacre (2005b) was used in this work, as irregular secondary dentine was seen in the normal physiological occlusion of the pulp horn in all teeth. Previously this dentine has been defined as tertiary dentine (Kilic *et al.* 1997c; Muylle *et al.* 2002; Nanci 2003a), however no true tertiary dentine as described by Dacre (2005b) was identified in these grossly normal teeth. This finding supports the proposal by Dacre (2005a) that tertiary dentine is produced focally by the pulp in response to specific noxious stimuli and the results of this study support this classification system.

Histological features such as intertubular dentine, interglobular dentine and pulp stones were identified in donkey teeth. The finding of viable peripheral cementum with blood vessels and nerve bundles also confirms the viability of this dental tissue in donkey teeth as has been described in the horse (Mitchell *et al.* 2003). The structure of donkey enamel was similar to equine enamel and in addition the enamel spindles were identified on undecalcified histology. Enamel spindles are formed by odontoblast processes extending into the ameloblast layer prior to enamel formation, that later become trapped when enamel is deposited (Nanci 2003b).

Thus far in this study, quantitative examination of decalcified histological sections of donkey teeth appears to be similar to those in equine teeth (Dixon 2002; Dacre 2005b). There was a great variation on these measurements that could have been affected by age, size and Triadan position of teeth. Dacre (2005b) showed that reduced thickness of dentine was a feature of some equine dental diseases, including reduced thickness of secondary dentine, with apical infections and idiopathic cheek teeth fractures – in many cases due to pulpar death and cessation of deposition of secondary dentine. It is for this reason that normal ranges of dentinal thickness in donkey cheek teeth were determined, prior to embarking on pathological studies in donkey teeth.

In maxillary cheek teeth there was a significant difference in buccal and palatal primary dentine thickness with palatal dentine thickness being thicker as was determined in equine teeth (Dacre 2005b). In contrast, in maxillary cheek teeth secondary dentine thickness did not differ between the buccal and palatal aspect of pulp horns as in the horse (Dacre 2005b). This non-significant difference in donkey teeth could be due to the small sample size (76 pulp horns) used in this study compared to the study in horses (376 pulp horns) (Dacre 2005b). In mandibular cheek teeth there was no significant difference between primary and secondary dentine thickness between buccal and lingual aspects. This was similar to the study in horses (Dacre 2005b). In all teeth buccal and palatal dentine thickness difference did not alter between the longitudinal levels of the tooth examined. This could indicate that these apparently normal cheek teeth examined had not experienced any altered attrition forces or altered dentinal stimulation during secondary dentine deposition. The difference in buccal and palatal primary dentine thickness in maxillary CT would suggest an inherited development as primary dentine is primarily laid down prior to tooth eruption (Kierdorf and Kierdorf 1992). Therefore this difference in primary dentine thickness is not an adaptation to attrition forces. The position of the pulp horn did however have an effect in the dentine thickness in mandibular CT but not maxillary CT. The reason for this is unknown.

3.2 Scanning Electron Microscopy

3.2.1. Peripheral enamel thickness

Previous studies by Kozawa (1992) and Kilic (1997a) showed that cheek teeth peripheral enamel thickness in the modern horse (*E. caballus*) is 5-6 times thicker than that of the primitive horse as recorded from fossils. Kilic *et al.* (1997a) also demonstrated an increase in thickness of the enamel parallel to the long axis of the jaw and a greater variation in maxillary enamel thickness with peripheral enamel thickness greater on the buccal aspect of maxillary and mandibular CT.

3.2.1.1. Aims of the study

The aim of this study was to use the method employed by Kilic (1995) to determine normal enamel thickness values of donkey teeth and to compare them to the values found by Kozawa (1992) and Kilic *et al*. (1997a).

3.2.1.2. Materials and Methods

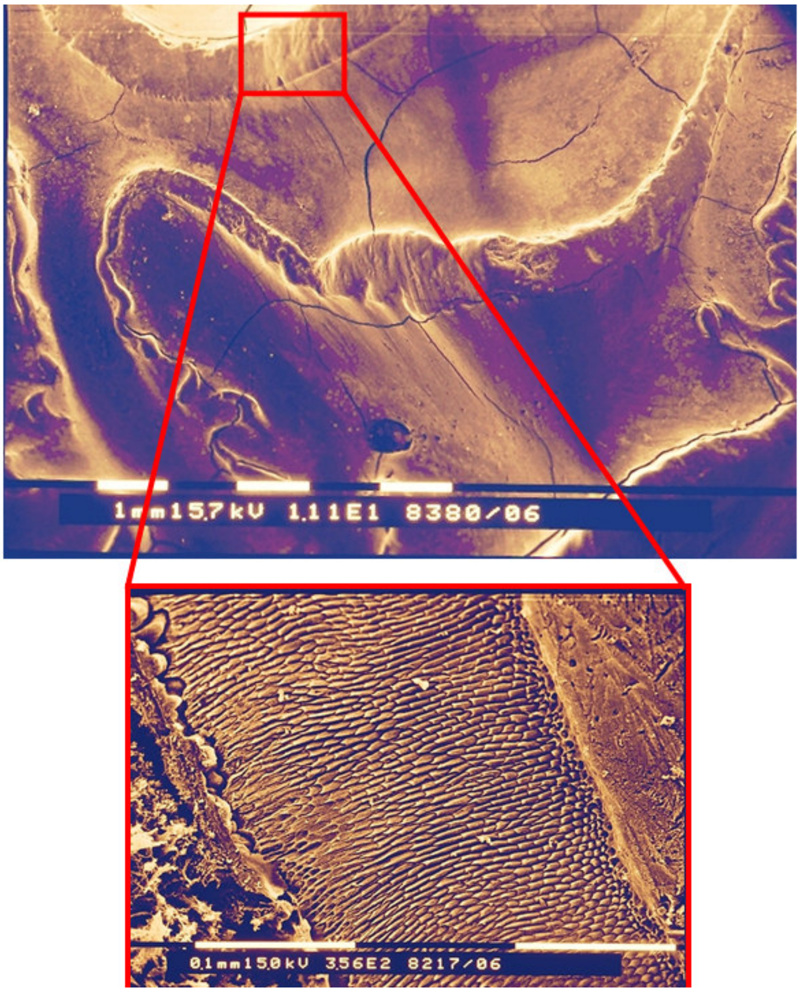
Mid-tooth, 8mm thick transverse sections of 10 teeth from 3 donkeys and 2 mid-transverse tooth sections from 1 horse for comparison were prepared for scanning electron microscopy (SEM). The 8mm thick tooth sections were hand ground with grit silicon carbide paper (200, 400, 600, 800, 1200 sequentially) to smooth the tooth section surface and erase any artefactual lines caused by the diamond saw. The smoothed surface was then acid etched using 2.5M phosphoric acid (Fischer Scientific UK Ltd, Loughborough, UK) for 45 seconds (Kilic 1995), rinsed in running tap water after which 2% hypochlorite solution (Milton Chemicals, UK) was dripped on the surface for 30 seconds.

The sections were then washed in cold running tap water for 5 minutes prior to dehydration in a series of graded acetone solutions (10 minutes each in 50%, 70%, 90%, and twice in 100% acetone solutions). Dental sections were then placed in wire baskets and critically point dried for 24 hours. After drying, sections were mounted on aluminium stubs (Agar Scientific Ltd., Stansted, UK) with double sided tape and sputter coated with 20nm T8843 Gold (60)/Palladium (40) (Emtech Ltd., South Stour Avenue, Ashford, Kent, UK) in a SC500 sputter coater (EMScope Laboratories Ltd, Ashford, Kent, UK).

Two occlusal sections were also examined to define the occlusal anatomy. Neither of these sections were hand ground, and one occlusal section was etched and the other was left unetched to visualise the organic pellicle. Prepared tooth sections were then viewed in a Philips SEM 505 Scanning electron microscope (FEI UK Ltd, Cambridge, England). Images were recorded using a Pentax camera and the negatives were later scanned into a computer using a HP Scanjet 4850. The horse dental sections were used as controls of section processing.

The ages of the donkeys and Triadan identification of the cheek teeth used are tabulated in Table 3.2.1.2. SEM magnification varied from x 50.5 to x 341 depending on the thickness of the enamel. Images were taken with an Olympus camera and the black and white negatives were scanned into a computer (HP Scanjet 4850). An image analysis programme (ImageJ, Image analysis and processing in JAVA, http://rsb.info.nih.gov/ij/, USA) was used to obtain measurements of enamel thickness. Every image obtained on SEM had a scale bar showing 0.1mm to 10μm, dependent on the magnification, which was used to set a different scale for each image (Figure 3.2.1.2.1).

Figure 3.2.1.2.1: **SEM picture of maxillary cheek tooth enamel width (red box) as measured in mm (magnification x 11.1). In the close up picture (magnification x 263), the enamel is bordered by dentine to the top right and cementum to the bottom left.**



Set anatomical reference points, as used by Kilic (1995), were used to take measurements of each tooth. Eighteen points were measured on the maxillary teeth (Figure 3.2.1.2.2) (90 measurements) and 12 (60 measurements) on the mandibular teeth (Figure 3.2.1.2.3). These reference points were also separated into points perpendicular and parallel to the long axis of the jaw.

Figure 3.2.1.2.2: **Transverse section of a maxillary cheek tooth illustrating the 18 anatomical reference points used to take SEM measurements for enamel (P = palatal; B = buccal; R = rostral; C = caudal).**

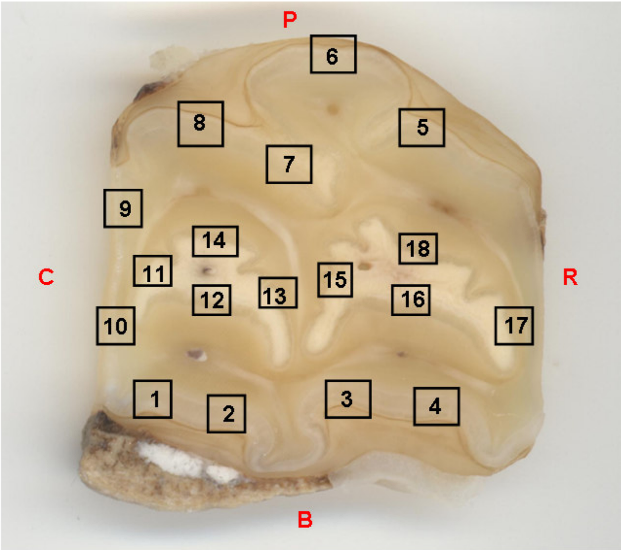
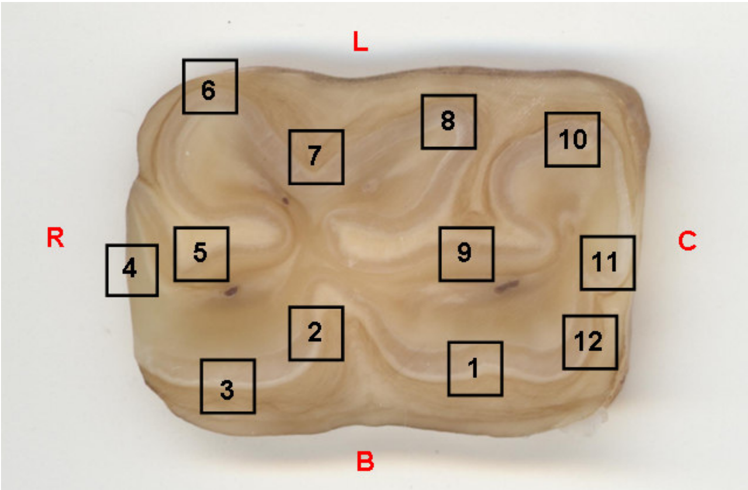


Figure 3.2.1.2.3: **Transverse section of a mandibular cheek tooth illustrating the12 anatomical reference points used to take SEM measurements for enamel (L = lingual; B = buccal; R = rostral; C = caudal).**



Linear mixed effect models were used on mean enamel thickness with the tooth (09 or 10) nested within donkey, entered as random effects, to take account of the fact that there are multiple teeth per donkey. Perpendicular/parallel or buccal/lingual enamel thickness data were entered as the fixed effects for the different studies. Enamel thickness values did not need to be transformed to individualise residuals as the data were normally distributed. Statistical significance was taken as $P < 0.05$ and degrees of freedom are indicated by subscripts.

3.2.1.3. Results

Table 3.2.1.3: **Ages of donkeys and Triadan identification of teeth used for measuring enamel thickness using SEM images.**

Age of donkey (years)	Triadan Number of Teeth
6	109, 110, 409, 410
11	109, 110, 309, 310
15	110, 310

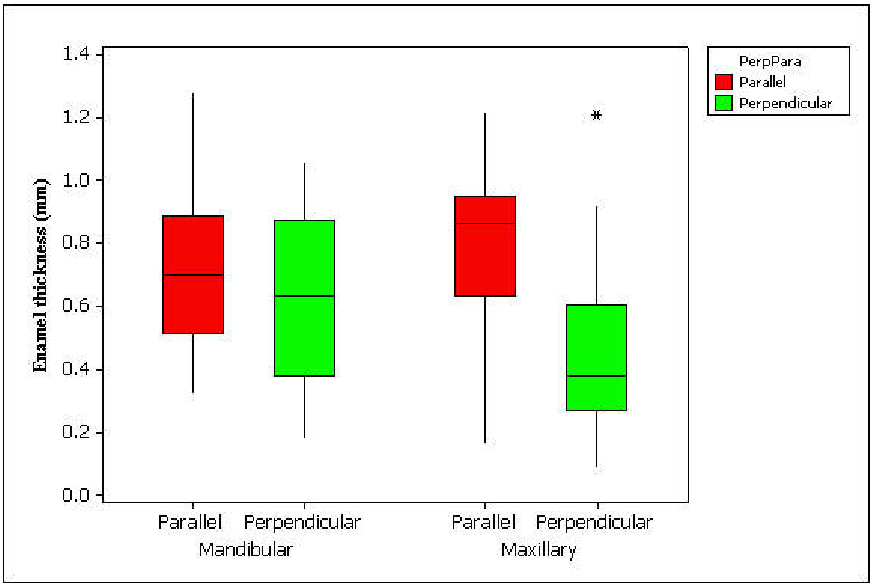
Cheek teeth enamel thickness

The overall mean of the enamel thickness measured in mandibular teeth was 0.68 mm (Table 3.2.1.3.1). The mean of the perpendicular and parallel enamel thickness were 0.63mm and 0.73mm respectively (Table 3.2.1.3.1). The overall mean of the enamel thickness of measure in maxillary teeth was 0.63 mm (Table 3.2.1.3.1). The mean of the perpendicular and parallel enamel thickness were 0.46mm and 0.81mm respectively (Table 3.2.1.3.1).

Table 3.2.1.3.1: **Comparison of equine (as found by Kilic 1995) and donkey enamel thickness on ultrastructural images in areas parallel and perpendicular to the long axis of the jaw in maxillary and mandibular teeth (median and range in mm).**

Enamel thickness Area	**Donkey**		**Horse (Kilic 1995)**
	Mean and Std deviation	**Median and Range**	**Median and Range**
Maxillary parallel	0.81 (0.25)	0.80 (0.77-0.83)	1.07 (0.7-1.45)
Maxillary perpendicular	0.46 (0.26)	0.48 (0.39-0.49)	0.36 (0.21-1.16)
Mandibular parallel	0.73 (0.26)	0.75 (0.61-0.84)	0.79 (0.33-1.03)
Mandibular perpendicular	0.63 (0.29)	0.57 (0.56-0.80)	0.29 (0.22-0.49)
Maxillary total	0.69 (0.27)	(0.39-0.83)	0.15-1.62
Mandibular total	0.67 (0.30)	(0.56-0.84)	0.18- 1.2

Figure 3.2.1.3.1: **Enamel thickness in points perpendicular and parallel to the long axis of the jaw in mandibular and maxillary teeth.PerpPar = perpendicular and parallel.**



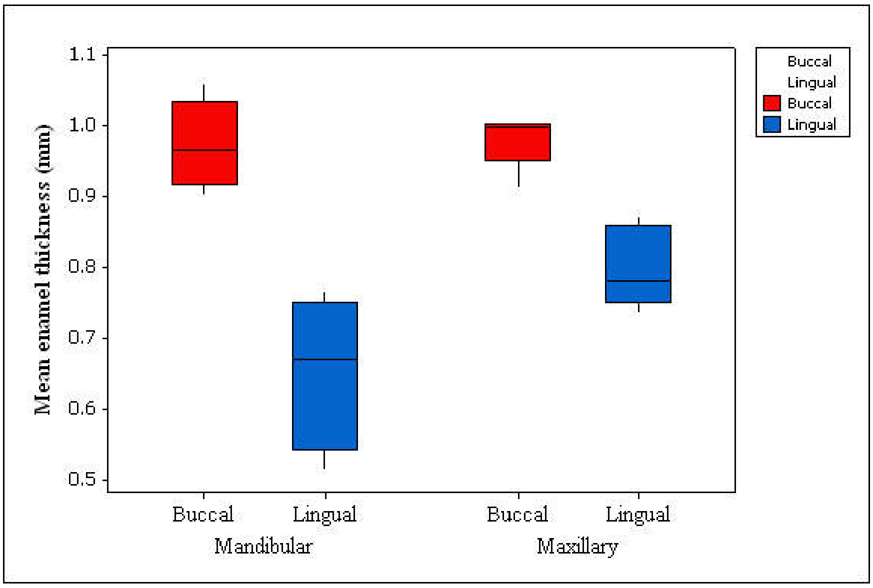
Enamel thickness perpendicular and parallel to the long axis of the jaw

Maxillary and mandibular perpendicular and parallel enamel thickness were compared. In maxillary teeth there was a significant difference between perpendicular and parallel enamel thickness ($F_{1,4}$ = 250.3; P<0.001; Figure 3.2.1.3.1), this difference was not statistically significant in mandibular teeth ($F_{1,4}$ = 6.2; P=0.067; Figure 3.2.1.3.1). There was also a statistically significant difference ($F_{1,4}$ = 11.72; P<0.001; Figure 3.2.1.3.1) between mandibular and maxillary perpendicular enamel thickness. However, comparison between maxillary and mandibular parallel enamel thickness showed no statistically significant difference ($F_{1,4}$ = 5.2; P = 0.084; Figure 3.2.1.3.1)

Enamel thickness on the buccal and lingual aspect

Buccal enamel thickness was compared with lingual enamel width (mean value per tooth) in maxillary and mandibular teeth. In maxillary teeth the buccal enamel thickness (mean 0.98mm) was significantly thicker than the palatal enamel thickness (0.80mm) (P<0.05; $F_{1,4}$ = 34.2; Figure 3.2.1.3.2). Similarly in mandibular cheek teeth, buccal enamel thickness (mean 0.99mm) was significantly thicker than lingual enamel thickness (0.67mm) (P<0.05; $F_{1,4}$ = 45.6; Figure 3.2.1.3.2).

Figure 3.2.1.3.2: **Buccal and lingual mean enamel thickness in maxillary and mandibular cheek teeth.**



3.2.2. Ultrastructural anatomy of donkey dental tissue

3.2.2.1. Introduction

The SEM ultrastructure of dental tissues has been defined by various authors in horses (Kilic *et al.* 1997a, 1997b, 1997c, 1997d; Muylle *et al.* 2000a, 2000b, 2001, 2002), as well as in other species (Listgarten 1968; Jones and Boyde 1987; Boyde *et al.* 1988; Kierdorf and Kierdorf 1992; Pfretzschner 1992), but no such studies have been performed in the donkey.

3.2.2.2. Aims of the study

The aim of this study was to determine the ultrastructural anatomy of the calcified dental tissues, namely enamel, denine and cementum, in the donkey.

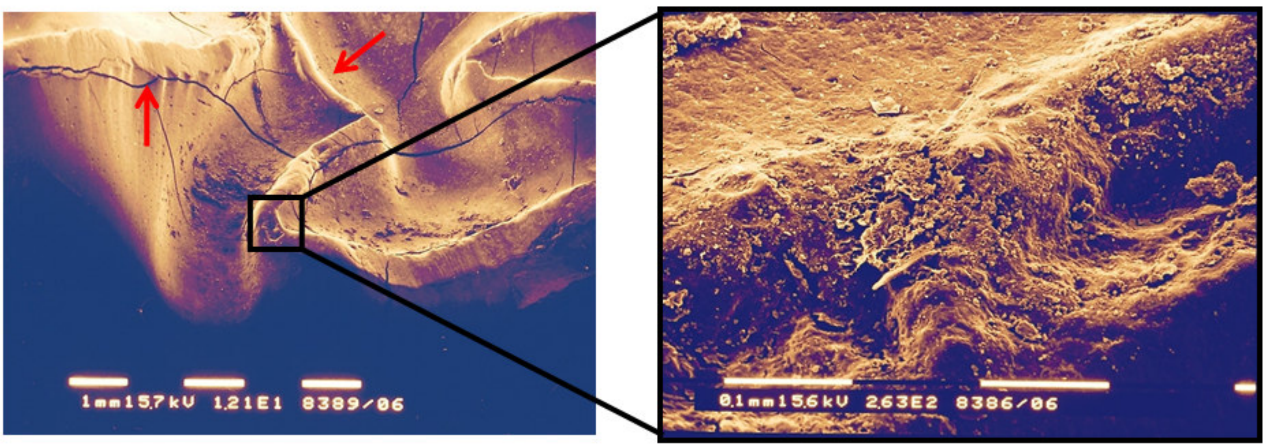
3.2.2.3. Materials and methods

The ten mid-transverse sections used to measure enamel thickness (Table 3.2.1.2), were re - examined on SEM and anatomical features of interest were recorded and photographed.

3.2.2.4. Results

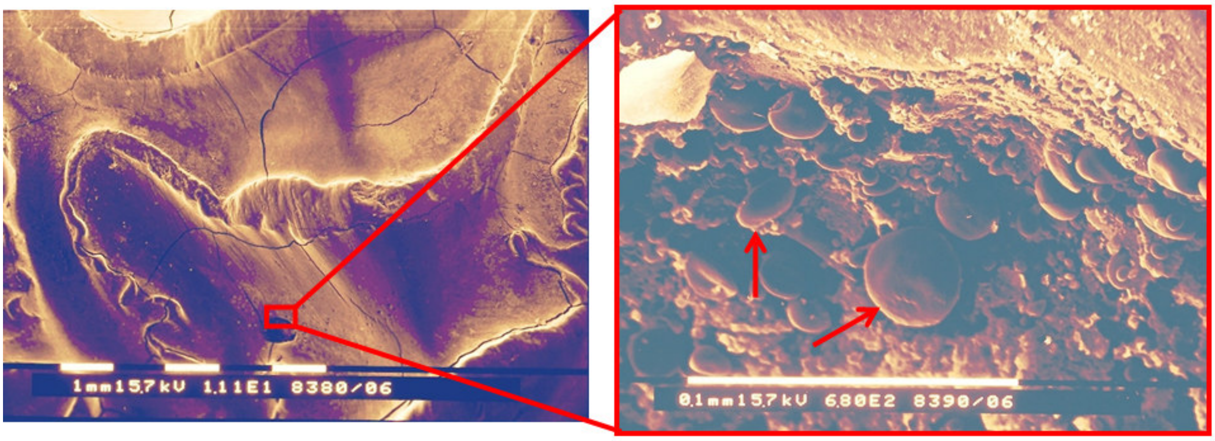
Unetched occlusal sections were examined first and these examinations allowed visualisation of an organic pellicle layer on the surface on the tooth (Figure 3.2.2.4.1). Enamel ridges were prominent on the occlusal surface protruding beyond the adjacent dentine and cementum (Figure 3.2.2.4.1) as also described in equine teeth (Kilic *et al.* 1997a). This is an important feature in ensuring an irregular occlusal surface to enable more efficient grinding.

***Figure 3.2.2.4.1:* SEM picture of donkey maxillary cheek tooth illustrating the organic pellicle and the enamel ridges (red arrows) on the occlusal surface (magnification x 12.2). Inset is higher magnification (x 263) of the organic pellicle on the enamel ridge.**



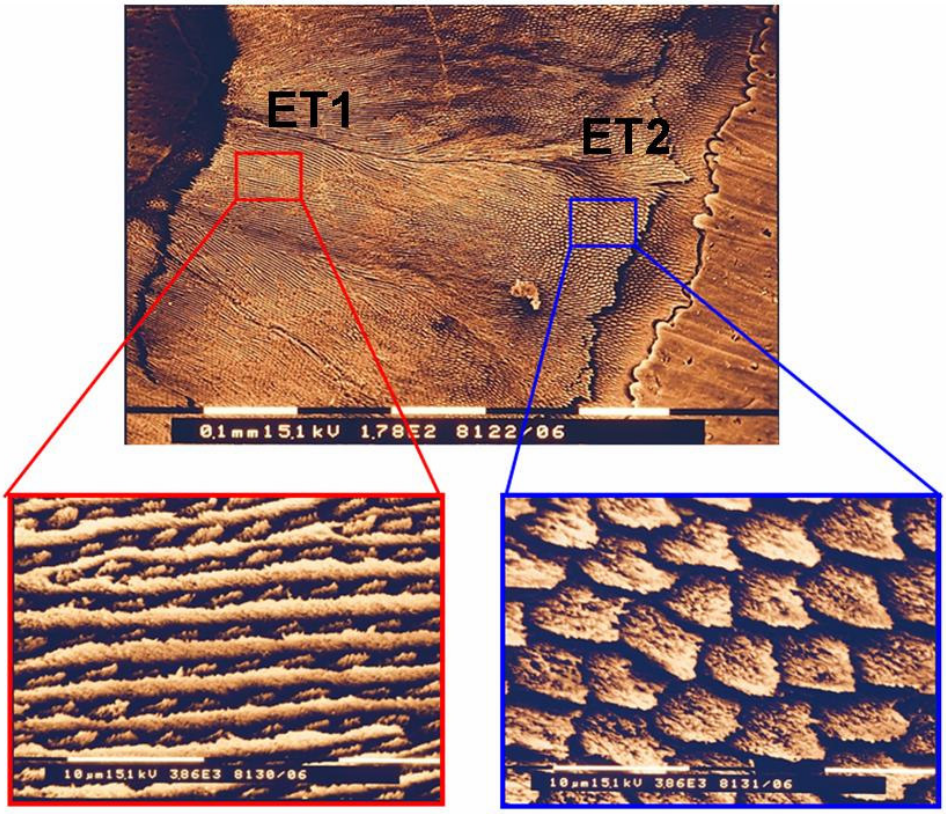
Examination of the occlusal surface of unetched section showed the organic pellicle, enamel ridges and remnants of the infundibular central vascular channel of a 110 in a 6 year old donkey (Figure 3.2.2.4.2).

Figure 3.2.2.4.2: **SEM picture of unetched occlusal surface of a 6 year old donkey maxillary 110, illustrating the infundibular vascular channel (left; x11.1), and round structures thought to be artefactual lipid droplets (red arrows) visualized within the vascular channel (right; x 600).**



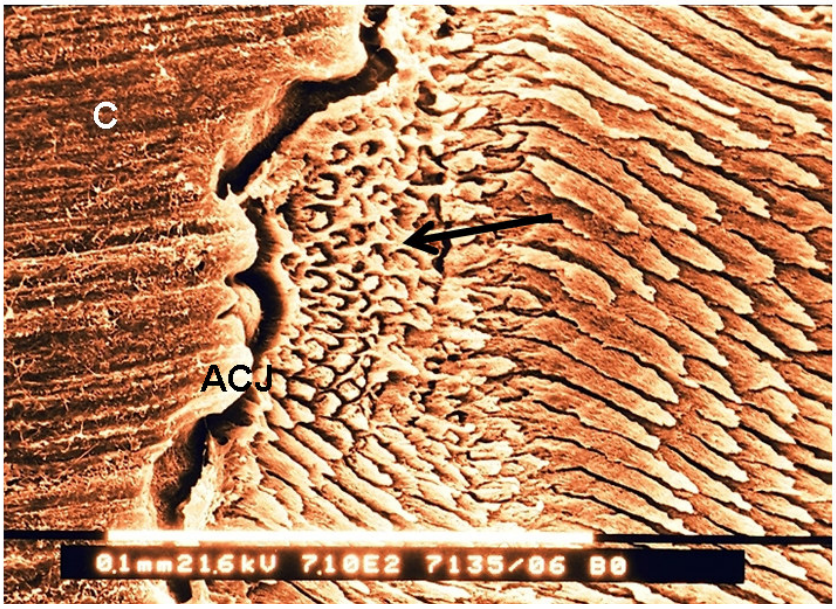
The different enamel types were identified on ultrastructural examination by the transverse appearance of their prisms according to Kilic's (1997b) equine enamel classification system. Equine type-1 enamel consists of small ovoid shaped prisms that are arranged in parallel rows separated by thick interprismatic enamel plates (Figure 3.2.2.4.3). As in equine teeth, this enamel composed the inner layer of enamel adjacent to the amelo-dentinal junction (ADJ) in donkey cheek teeth. On transverse sections, equine type-1 enamel prisms and plates were found to be obliquely oriented to the occlusal surface. Equine type-2 enamel was identified by the absence of interprismatic enamel plates and the presence of horseshoe or keyhole shaped prisms on transverse section (Figure 3.2.2.4.3). This type of enamel was seen predominantly in the more peripheral layer adjacent to the amelo-cemental junction (ACJ) in the donkey.

Figure 3.2.2.4.3: **SEM picture of donkey mandibular cheek tooth enamel illustrating equine type-1 (ET1) and type-2 enamel (ET2) (x 178). Insets illustrate equine type-1 enamel (red) and type-2 enamel (blue) at x 3860 magnification.**



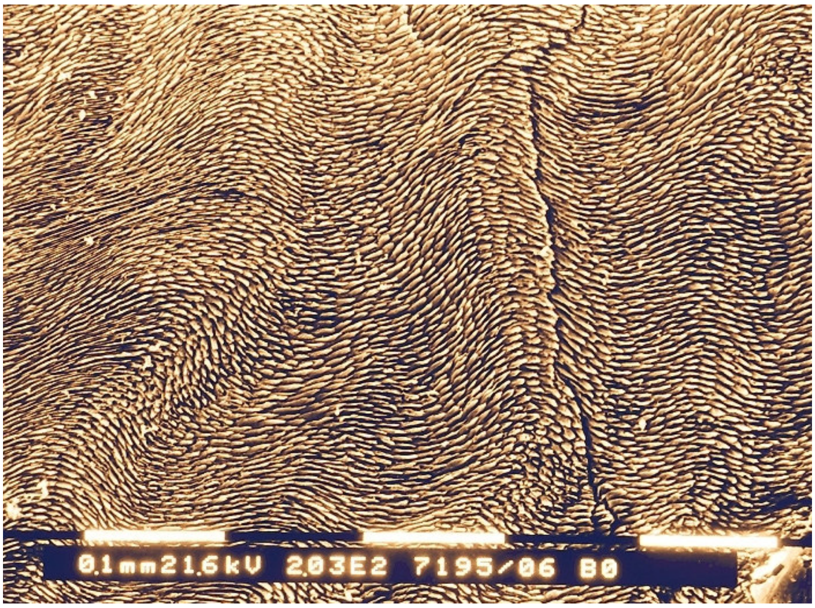
Equine type-3 enamel was also identified in areas of the ADJ and ACJ as areas where tapered prisms were completely surrounded by interprismatic enamel giving equine type-3 enamel a honeycomb appearance on transverse sections (Figure 3.2.2.4.4).

Figure 3.2.2.4.4: **SEM image illustrating the thin honeycombed appearance of equine type-3 enamel (black arrow) at the amelo-cemental junction (ACJ); C = cementum (x 710).**



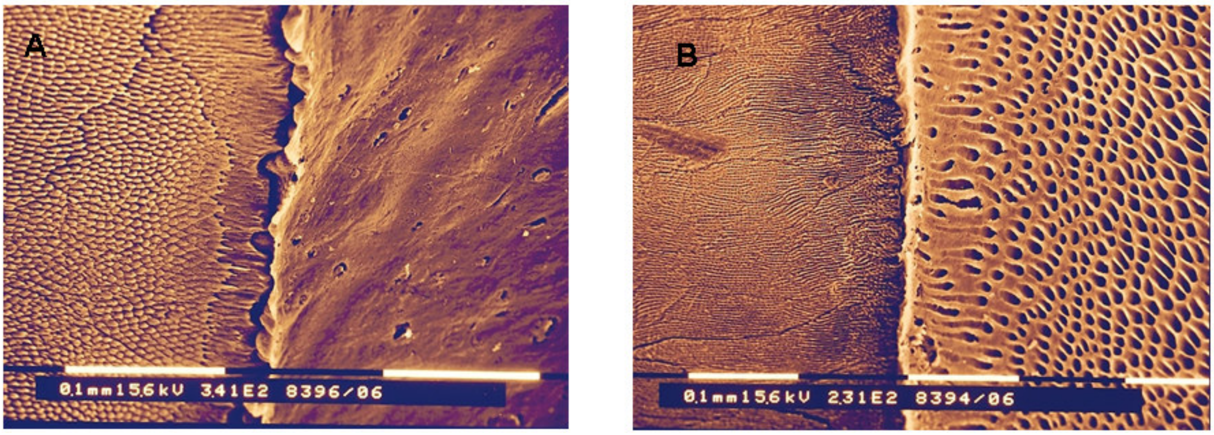
In the transverse sections from donkey incisors, enamel type-1 was only identified in a small area from one section. It is for this reason that no quantitative analysis could be performed on incisor enamel type distribution. It can therefore be concluded that donkey incisor enamel is composed of predominantly equine type-2 enamel. Incisor enamel also consistently had a high degree of prism decussation (Figure 3.2.2.4.5). These findings are similar to what has been demonstrated in horses by Kilic (1997b).

Figure 3.2.2.4.5: **SEM picture illustrating prism decussation of equine type 2 enamel in a transverse section of a donkey incisor (x 203).**



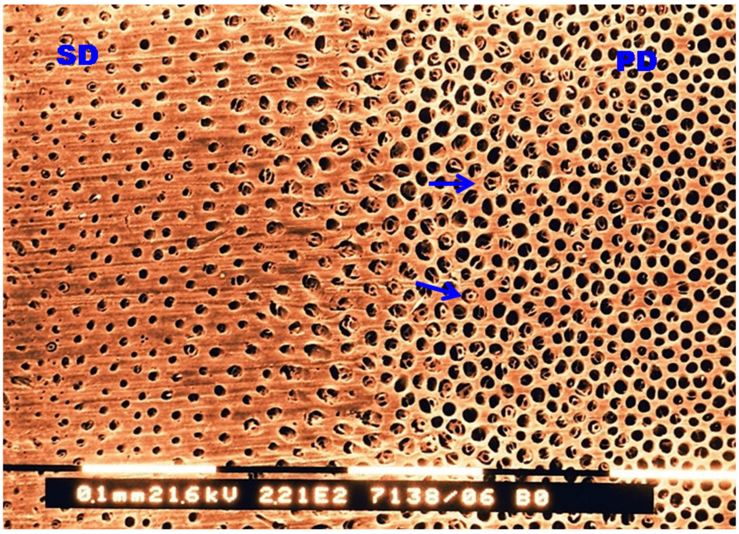
The ADJ is a straight and smooth junction compared to the amelo-cemental junction which has a scalloped appearance (Figure 3.2.2.4.6) increasing the surface area. As the number of enamel prisms remain constant from the ADJ to the ACJ (Fortelius 1985), the increase in enamel prism size (see section 3.2.3) is accommodated for by the greater surface area of the ACJ.

Figure 3.2.2.4.6: **SEM pictures illustrating the difference between amelo-cemental junction (A) (x 341) and amelo-dentinal junction (B) (x 231).**



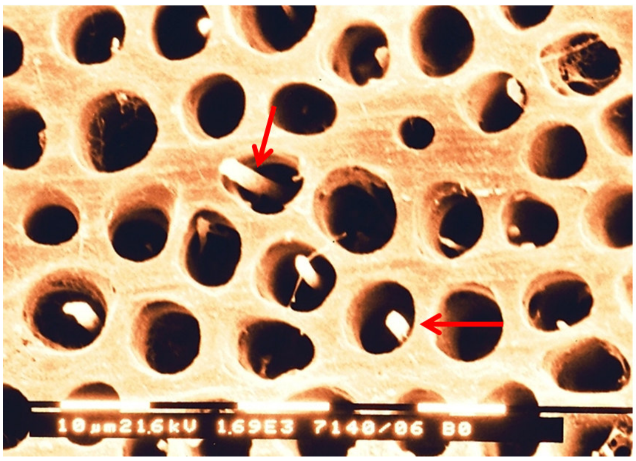
A similar pattern of dentine types, (primary, regular and irregular secondary dentine) as identified in horses was observed in the donkey cheek teeth sections examined by SEM in this study. The area adjacent to the ADJ consistently had an area devoid of dentinal tubules, where the tubules terminated prior to the ADJ, with dentinal tubules adjacent to this area being small and irregular in shape. This finding has also been observed in equine teeth (Kilic *et al.* 1997c; Muylle *et al.* 2000a). Due to acid etching during preparation of the sections for SEM, the intratubular dentine of primary dentine was removed, and only irregular remnants could be seen (Figure 3.2.2.4.7).

Figure 3.2.2.4.7: **SEM picture of dentine at magnification X 2210 illustrating primary dentine (right, PD) and secondary denine (left, SD) as seen on transverse section. Remnants of intratubular dentine in the primary dentine can be seen in the primary dentinal tubules (blue arrows) (x221).**



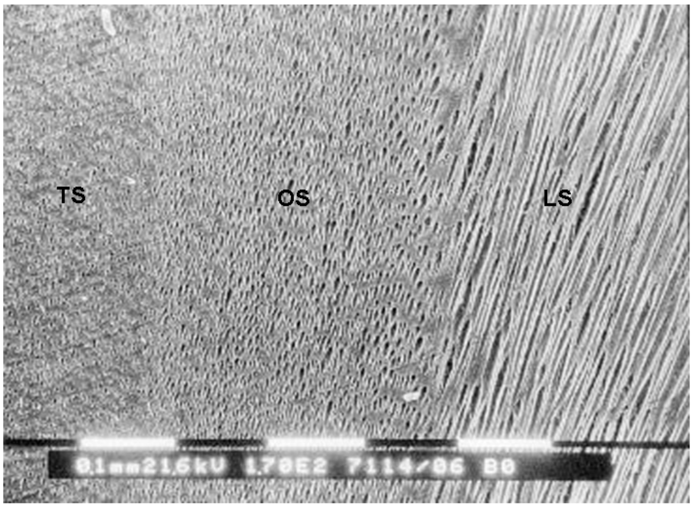
Odontoblast processes within the dentinal tubules were also identified in a few of the sections (Figure 3.2.2.4.8).

Figure 3.2.2.4.8: **SEM of dentine illustrating odontoblast processes (red arrows) within dentinal tubules (x1690).**



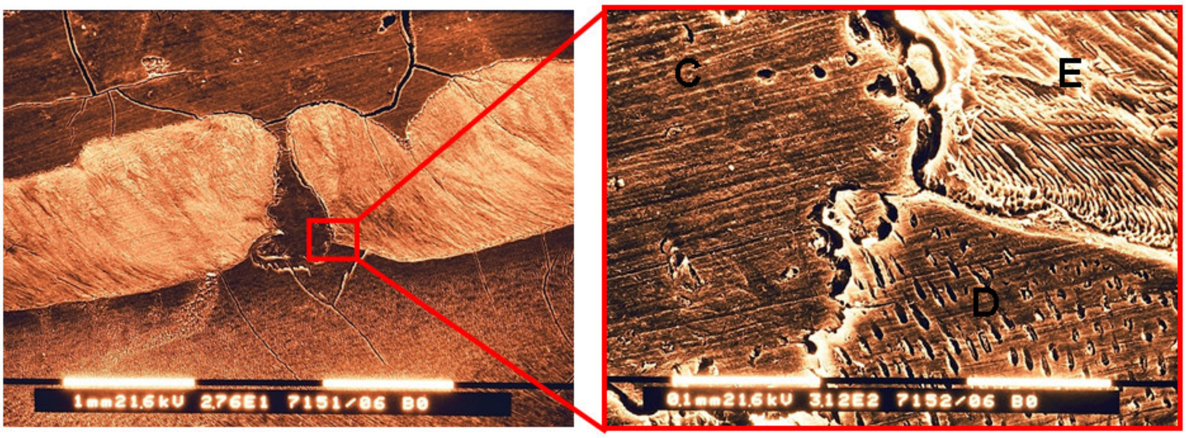
On SEM, donkey dentine appears to be very similar in ultrastucture to equine dentine (Kilic *et al.* 1997c). Transverse sections resulted in dentine being sectioned in different planes, transveresly, obliquely and longitudinally, according to the areas within the tooth. Dentinal tubules in areas closer to the pulp cavity were generally sectioned transversely, with the area closer to the ADJ sectioned longitudinally and the area between the pulp and the ADJ sectioned obliquely (Figure 3.2.2.4.9).

Figure 3.2.2.4.9: **SEM picture showing different planes of dentine in a transverse section. The pulp cavity is to the left of the picture and the ADJ to the right; TS = transverse section; OS = oblique section; LS = longitudinal section (x 170).**



In one section of a 20 year old donkey a fault or gap was noted in the enamel. It appeared that this gap had been ‘repaired’ by the filling in of cementum (Figure 3.2.2.4.10), with no changes noted in the tooth indicative of disease. This ‘reparative’ function of cementum has been proposed before (Mitchell *et al.* 2003; Dacre 2005a), and it reaffirms the proposal that cementum is a dynamic vital tissue.

Figure 3.2.2.4.10: **SEM of a transverse section of maxillary tooth from a 20 year old donkey illustrating the gap in enamel filled with cementum (x 27.6). The inset clearly shows all 3 dental tissues lying adjacent to each other (x 312); C = cementum; E = enamel; D = dentine.**



3.2.3. Quantitative analysis on enamel types

3.2.3.1. Aims of the study

Kilic (1995) performed quantitative analysis on enamel and showed that equine enamel prism size increased from the ADJ (equid type-1 enamel) towards the ACJ (equid type-2 enamel) with a concurrent decrease in interprismatic distance. The aim of this study was to determine normal values for enamel prism diameters and enamel interprismatic distances in donkey type-1 and type-2 enamel and to determine if the enamel prism diameters increase and interprismatic distances decrease from the ADJ to the ACJ as it does in cheek teeth enamel of domestic horses.

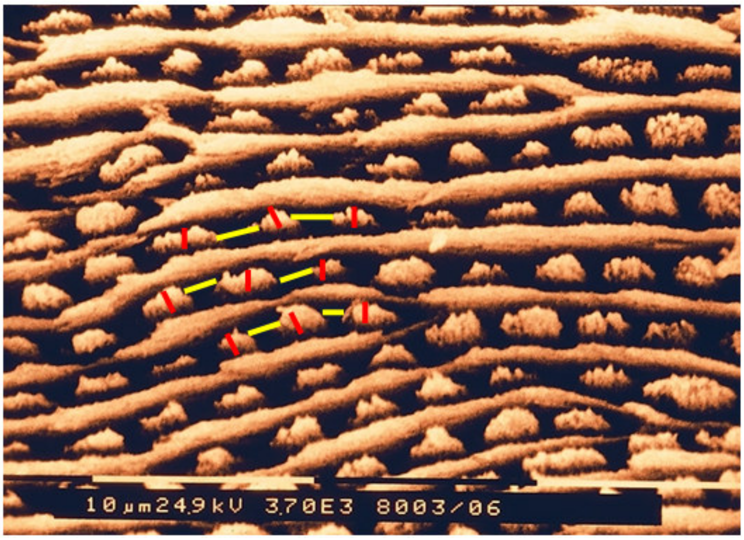
3.2.3.2. Materials and methods

The same ten mid-transverse sections used to measure enamel thickness (Table 3.5.1.2.1), were used to determine the ratio of the two main enamel types (EqT1 and

EqT2) per anatomical reference point measured (18 maxillary and 12 mandibular points per tooth). The same images that were obtained for enamel thickness, were found to be suitable to determine the area of the different equid enamel types per point. Measurements were recorded in mm^2 and the ratio of EqT1 to EqT2 was calculated per point. Descriptive statistics were performed to determine the mean, medians and ranges of the different enamel type ratios. Four mid transverse sections from four incisors were examined on SEM to determine the ratio of enamel types.

Eight transverse sections from the cheek teeth of two donkeys were used to measure enamel prism diameters and interprismatic distances. All the same points were used but two images were obtained per point at a magnification of x 3860 with SEM (144 images for maxillary CT and 96 for mandibular CT). EqT1 enamel was measured at points about 10 µm from the ADJ and EqT2 enamel measurements were obtained adjacent to the ACJ. At each point, 9 enamel prism diameters and their 6 interprismatic distances were measured (Figure 3.2.3.3.1).

Figure 3.2.3.3.1: **SEM picture of equid type-1 enamel prism diameter (red lines) and interprismatic distance (yellow lines) measurement in a donkey maxillary cheek tooth (x3700).**



Linear mixed-effect models were performed on the data using R V2.3.1, R Foundation for Statistical Computing, with tooth points nested within tooth (09 or 10), nested within donkey, entered as random effects to take account of the fact that there are multiple points per tooth and multiple teeth per donkey. Enamel prism diameter means and interprismatic distances means were log10 transformed, to normalise the residuals and equid enamel type-1 and type-2 were entered as positive fixed effects. Statistical significance was taken as $P < 0.05$ and degrees of freedom are indicated by subscripts.

3.2.3.3. Results

3.2.3.3.1 Distribution of enamel types

The ratio of equid enamel type-1 to equid enamel type-2 was just below 1 overall in mandibular and maxillary teeth indicating slightly lower proportions of equid type-1 enamel in all donkey teeth (Table 3.2.3.3.1). Enamel that was perpendicular to the long axis of the maxillae and mandible contained slightly higher proportions of type-1 enamel (Table 3.2.3.3.1).

Table 3.2.3.3.1**: The ratio of equid type-1 enamel to equine type-2 enamel in maxillary and mandibular cheek teeth.**

Maxillary or Mandibular	**Parallel/ Perpendicular/ Overall**	**Mean**	**Range**	**Median**
Maxillary	Parallel	0.96	0.24 – 2.41	0.90
	Perpendicular	1.04	0.13 – 3.00	0.94
	Overall	0.99	0.13 – 1.19	0.90
Mandibular	Parallel	0.94	0.45 – 1.89	0.83
	Perpendicular	1.01	0.26 – 2.42	0.94
	Overall	0.97	0.27 – 1.19	0.87

3.2.3.3.2 Enamel prism size and enamel interprismatic distance

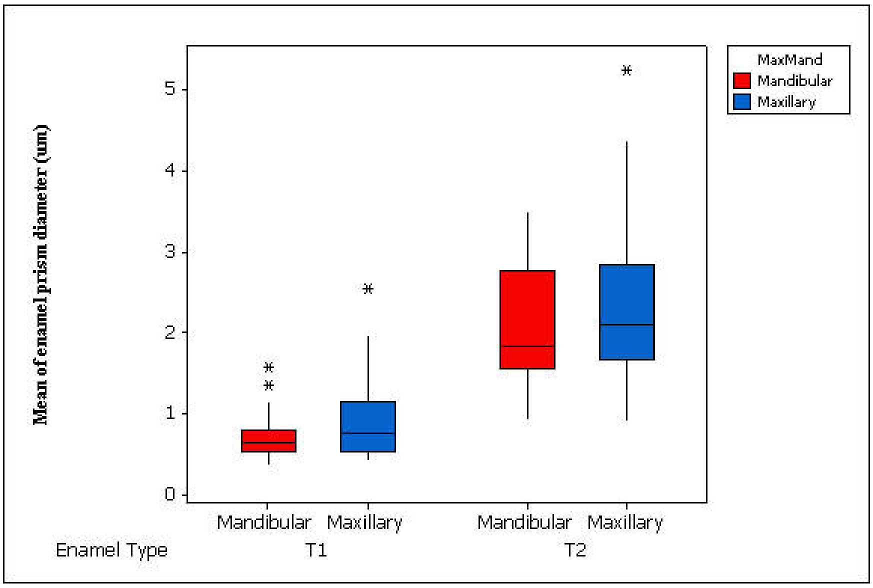
In maxillary and mandibular cheek teeth combined, mean interprismatic distance in equine type-1 enamel was 1.42 µm (median = 1.41; range 0.69 – 2.59) and equid enamel type 2-was 0.96 µm (range0.54 – 3.23). Mean enamel prism diameter in equid enamel type-1 was 0.82µm (range 0.36 – 2.55) and equid enamel type-2 was 2.20 µm (range 0.91 – 5.25). Separate values for maxillary and mandibular CT are shown below in Table 3.2.3.3.2.

***Table 3.2.3.3.2*: The mean (and standard deviation) and ranges for the enamel prism diameters and interprismatic distances in equid enamel type-1 and type-2, in maxillary and mandibular teeth.**

Teeth	**Enamel type**	**Prism diameter (µm)**		**Interprismatic distance (µm)**	
		Mean	**Range**	**Mean**	**Range**
Maxillary	1	0.91 (0.46)	0.42 - 2.55	1.45 (0.33)	0.72 – 2.32
Mandibular	1	0.69 (0.24)	0.36 – 1.58	1.40 (0.40)	0.69 – 2.59
Maxillary	2	2.30 (0.90)	0.91 - 5.25	0.93 (0.35)	0.54 – 3.23
Mandibular	2	2.07 (0.72)	0.93 – 3.50	1.012 (0.29)	0.61 – 1.62

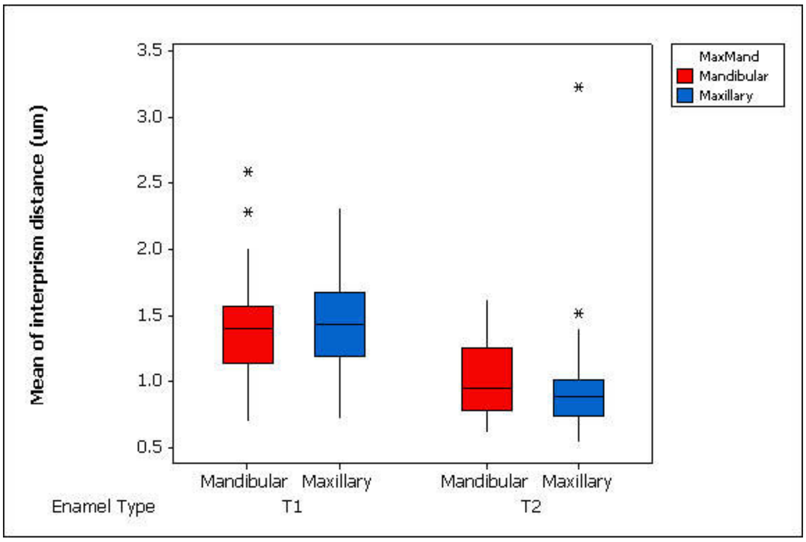
There was a statistically significant difference between equid enamel type-1 and type-2 enamel prism diameters in maxillary CT ($F_{1,69}$ = 239.2; P < 0.001; Figure 3.2.3.3.1) and mandibular teeth ($F_{1,46}$ = 281.5; P < 0.001; Figure 3.2.3.3.1). There was also a statistically significant difference between maxillary and mandibular teeth enamel type-1 prism diameter ($F_{1,44}$ = 15.1; P < 0.001; Figure 3.2.3.3.1), but not type-2 ($F_{1,44}$ = 1.9; P = 0.176; Figure 3.2.3.3.1).

Figure 3.2.3.3.1: **Mean enamel prism diameters (µm) of equid enamel type-1 (T1) and equid enamel type-2 (T2) in maxillary and mandibular teeth.**



There was a statistically significant difference between equid enamel type-1 and type-2 interprismatic distance in maxillary CT ($F_{1,69} = 144.26$; $P < 0.001$; Figure 3.2.3.3.2) and mandibular CT ($F_{1,45} = 44.57$; $P < 0.001$; Figure 3.2.3.3.2). There was a significant difference between maxillary and mandibular teeth interprismatic distance in type-2 enamel ($F_{1,44} = 4.97$; $P = 0.031$; Figure 3.2.3.3.2) but not in type-1 enamel ($F_{1,44} = 1.24$; $P = 0.27$; Figure 3.2.3.3.2).

***Figure 3.2.3.3.2:* Mean interprismatic distance (µm) in equine enamel type-1 (T1) and equid enamel type-2 (T2) in maxillary and mandibular cheek teeth.**



Comparison of enamel types in maxillary and mandibular teeth showed that enamel prism diameter of equid type-1 enamel was significantly smaller than the enamel prism diameter of equid enamel type-2 ($F_{1,116} = 493.1$; $P<0.001$; Figure 3.2.3.3.3). In contrast, equid type-1 enamel interprismatic distance in maxillary and mandibular CT was significantly bigger than the interprismatic distance in equid type-2 enamel in maxillary and mandibular teeth ($F_{1,115} = 178.7$; $P<0.001$; Figure 3.2.3.3.4).

Figure 3.2.3.3.3: **Mean enamel prism diameters (µm) of equid enamel type-1 (T1) and equine enamel type-2 (T2) in maxillary and mandibular cheek teeth.**

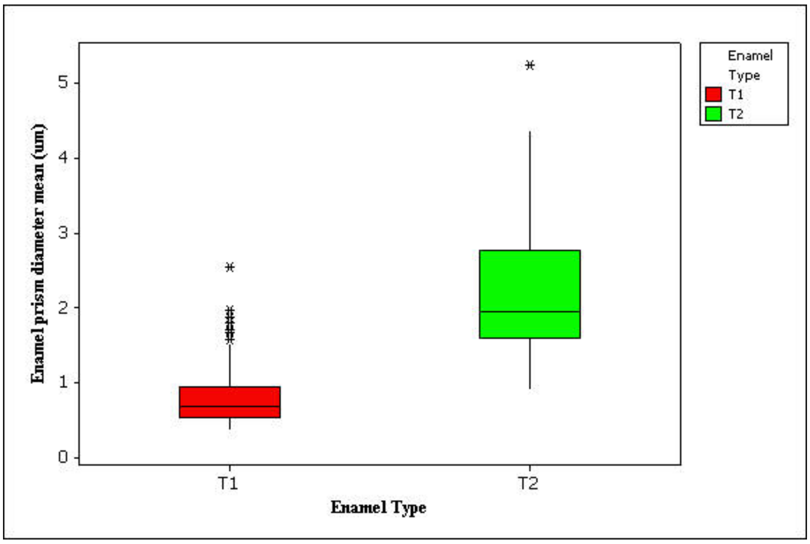
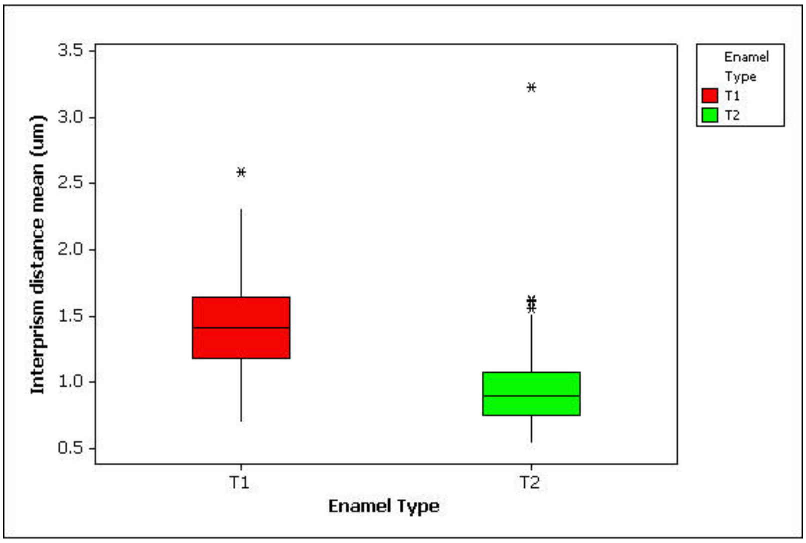


Fig. 3.2.3.3.4: **Interprism distance mean (µm) in equid enamel type-1 (T1) and equine enamel type-2 (T2) in maxillary and mandibular cheek teeth.**



3.2.4. Quantitive analysis of dentine

3.2.4.1. Introduction

The study by Kilic et al. (1997c) determined normal values for ultrastructure of horse dentine on SEM. The density (numbers per fixed area) of dentinal tubules and dentinal tubule diameters of primary, secondary, and primary dentine at the junction between primary and secondary dentine (termed primary transitional dentine) were measured. The term intratubular dentine was used instead of the previously used peritubular dentine (Kilic *et al.* 1997c), as this term describes the highly mineralized dentine lining the inside of the dentinal tubule, as shown by Muylle (2000). Dentinal tubular diameter in this study was defined as the outer aspect of the tubule as acid etching resulted in demineralisation and loss of the intratubular dentine. Kilic *et al*. (1997c) measured the inner aspect of the intratubular dentine and thus no comparison could be made between these two studies.

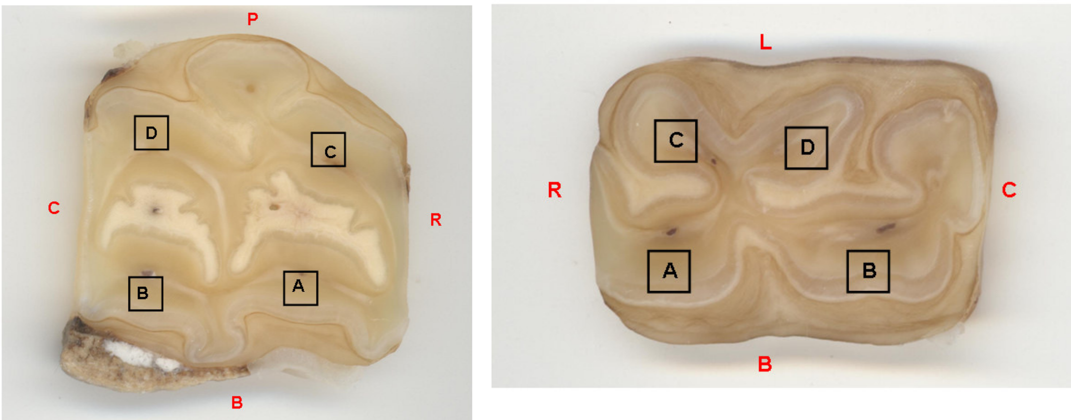
3.2.4.2. Aims of the study

The aims of this study were to determine normal values for donkey dentine density (tubules per set area) and the diameter of dentine tubules in primary, primary transitional and secondary dentine, and to determine if there is an increase in dentinal tubule density and a decrease in dentinal tubule diameter from primary to secondary dentine as observed in equine CT.

3.2.4.3. Materials and methods

The same 10 transverse sections that were used for enamel quantitative analysis (Table 3.2.1.3) were used for quantitative dentinal analysis. Four anatomical reference points were examined in each CT, where 3 SEM images were taken of: (1) the primary dentine, (2) primary transitional dentine and (3), regular secondary dentine at a magnification of x 2020. To enable a direct comparison with the work done by (Kilic *et al.* 1997c) the number of dentinal tubules per 2202 μm^2 area was counted and the diameters and perimeters of 9 dentinal tubules were measured in four areas of each tooth (Figure 3.2.4.3.1). Three images were obtained per anatomical reference point (60 images each for maxillary and mandibular CT), stored and analysed as previously described.

Figure 3.2.4.3.1: **Maxillary (left) and mandibular (right) transverse teeth sections illustrating the 4 areas that were examined for SEM dentine measurements (P = palatal; L = lingual; B = buccal; R = rostral; C = caudal).**



Linear mixed-effect models were used to determine the difference between dentinal tubule densities and dentinal diameter and perimeter measurements in primary, primary transitional and secondary dentine. Linear mixed effect models were performed with area nested within tooth; nested within maxillae/mandible; and nested within donkey entered as random effects to take account of the fact that there are multiple areas per tooth and multiple teeth per donkey. Dentine type (primary, transitional and secondary), tubule density, tubule diameter and mean dentinal tubule diameter were entered as the fixed effect. Dentinal tubule density data were log ^{10} transformed and dentinal tubule diameter (µm) data were square root transformed to normalise the data. Mean dentinal tubule perimeter did not need transformation to normalise the residuals. Statistical significance was taken as $P < 0.05$ and degrees of freedom are indicated by subscripts.

3.2.4.4. Results

3.2.4.4.1 Dentinal tubule density

The mean, median and range of dentinal tubule density in different dentine types in donkey teeth are tabulated in Table 3.2.4.4.1.

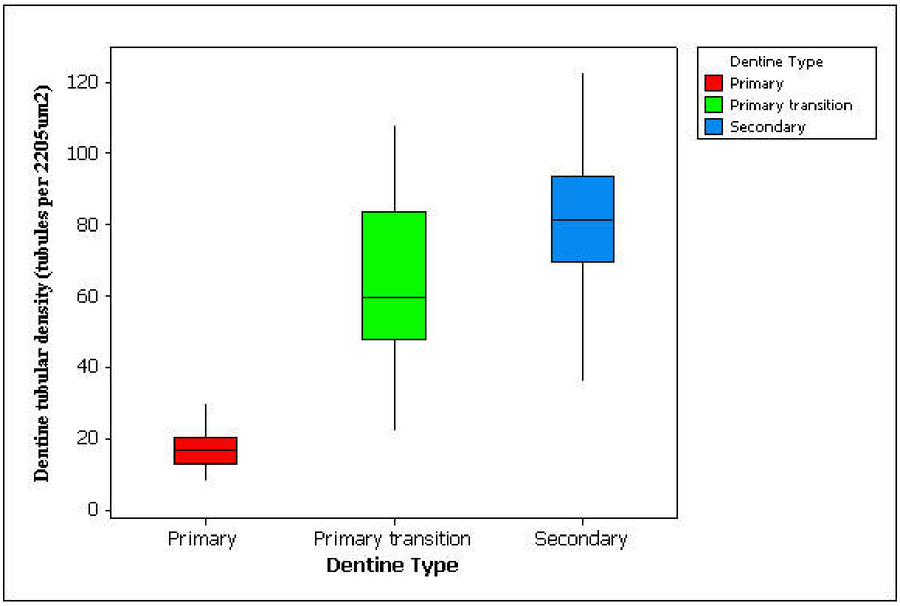
Table 3.2.4.4.1: **The mean number of dentinal tubules per 2202 µm^2 in primary, primary transitional and secondary dentine as determined on ultrastructural iamges.**

Dentine type	Mean (Median)	Standard deviation	Range	Horse median
Primary	17.30 (17)	5.65	8 – 30	19
Primary transitional	63.34 (59.5)	21.00	22 – 108	25
Secondary	82.09 (81.5)	20.17	36 – 123	128

This study showed that there was a significant difference in the density of dentinal tubules between the different dentinal types ($F_{2.78} = 227.3$; P<0.001; Figure 3.2.4.4.2).

Post hoc tests comparing different dentinal types confirmed a significant difference in dentinal tubule density between primary and primary transitional dentine ($F_{1,47} = 223.7$; P<0.001; Figure 3.2.4.4.2), primary transitional and secondary dentine ($F_{1,47} = 16.1$; P<0.002; Figure 3.2.4.4.2) and primary and secondary dentine ($F_{1,47} = 411.2$; P<0.001; fig.3.2.4.4.2).

***Figure 3.2.4.4.2*: Dentinal tubule number per 2202 μm^2 in different dentine types as determined on ultrastructural images.**



3.2.4.4.2 Dentinal tubule diameter

Mean dentinal tubule diameter is summarised in Table 3.2.4.4.2.

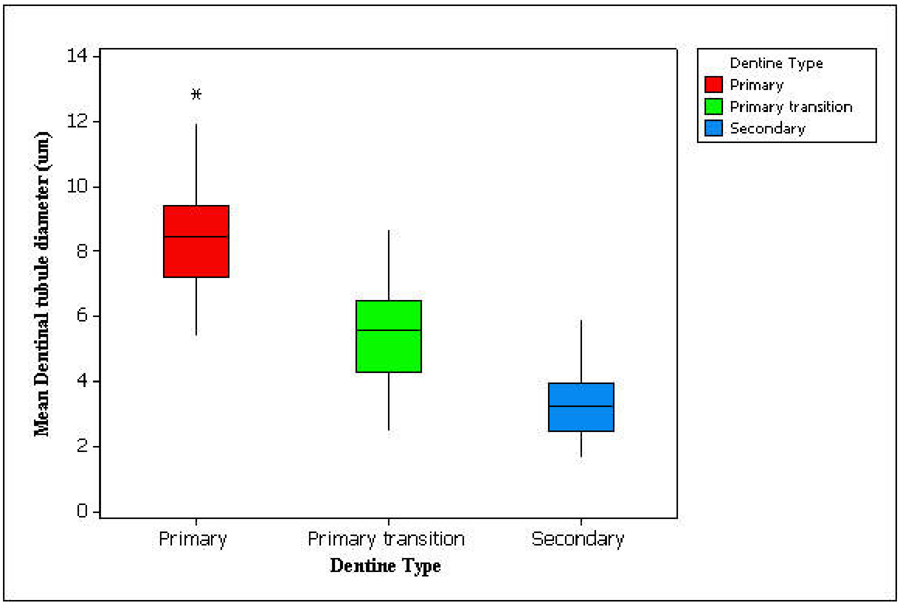
***Table 3.2.4.4.2:* Mean and range of dentinal tubule diameter (μm) as measured on ultrastructural images.**

Dentine type	Mean	Standard deviation	Range
Primary	8.45	1.93	4.35 – 10.84
Primary transitional	5.45	1.69	1.85 – 9.80
Secondary	3.28	1.14	1.07 – 7.62

This comparison showed a significant difference in dentinal tubule diameter between the different dentinal types (primary, primary transitional and secondary) ($F_{2,66}$ = 137.4; P<0.001; Figure 3.2.4.4.3).

Post hoc testing showed a similar significant difference in dentinal tubule diameter between primary and primary transitional dentine ($F_{1,35}$ = 64.6; P<0.001), primary transitional and secondary dentine ($F_{1,35}$ = 102.4; P<0.001) and primary and secondary dentine ($F_{1,35}$ = 277.4; P<0.001; Figure 3.2.4.4.3).

***Figure 3.2.4.4.3*: Dentinal tubule diameter mean (µm) in different types of dentine as determined on ultrastructural images.**



3.2.4.4.3 Dentinal tubule perimeter

Mean dentinal tubule perimeter is summarised in Table 3.2.4.4.3.

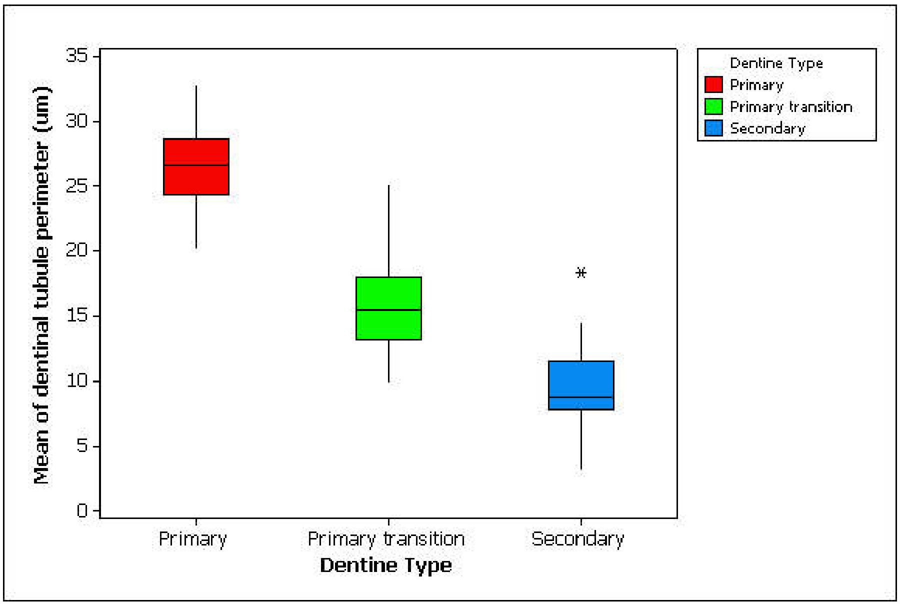
Table 3.2.4.4.3: **Mean and range of dentinal tubular perimeter values (µm) as measured on ultrastructural images.**

Dentine type	Mean	Range
Primary	26.44	10.04 – 37.78
Primary transitional	15.84	5.83 – 31.28
Secondary	9.35	2.21 – 20.55

Linear, mixed-effect models showed a significant difference in the mean dentinal tubule perimeter between dentine types (primary, primary transitional and secondary). ($F_{2.66}$ = 243.6; P<0.001; Figure 3.2.4.4.4).

Post-hoc testing showed a similar significant difference in dentinal tubule perimeter between primary and primary transition dentine ($F_{1.35}$ = 171.9; P<0.001), primary transition and secondary dentine ($F_{1.35}$ = 76.6; P<0.001) and primary and secondary dentine ($F_{1.35}$ = 530.8; P<0.001; Figure 3.2.4.4.4).

Figure 3.2.4.4.4**: Mean dentinal tubule perimeter values (µm) in different dentine types as determined on ultrastructural images.**



3.2.5 Discussion

This study used a similar technique to that used by Kilic *et al*. (1997a) in equine teeth to measure enamel thickness in donkey cheek teeth. It was previously believed that linear measurements of enamel are inaccurate and that measurement of enamel volume utilizing the surface area of the ADJ is a better indicator of enamel thickness in brachydont teeth (Martin 1985). However, due to the complex and hypsodont nature of equid teeth with enamel embedded within cementum and dentine it would be extremely difficult to measure either the enamel volume or the ADJ surface area. Kilic (1995) showed that the problems of variation in linear enamel thickness can be overcome by taking multiple measurements at set points along the enamel folds in the transverse plane. Only one transverse section was used per tooth (mid section) to measure the enamel thickness as Kilic (1995) had shown that enamel thickness was constant throughout the longitudinal length of the tooth.

This study has shown similar enamel thickness patterns to that demonstrated in horses. Enamel thickness was shown to be significantly thicker ($P<0.001$) in enamel parallel to the long axis of the jaw in maxillary CT but not in mandibular CT ($P = 0.067$). A greater degree of variation in the enamel thickness in maxillary CT compared with mandibular teeth was found, as also noted in equine CT (Kilic *et al.* 1997a). Enamel thickness is greater in the area of higher attrition forces in the horse as noted by thicker enamel width buccally in maxillary and mandibular CT (Kilic et al. 1997a). Similarly, thicker buccal enamel was found in this study in donkey maxillary and mandibular CT.

Kozawa (1992) showed that enamel thickness was greatly increased in the modern horse compared with enamel thickness recorded from fossils of primitive horses. Both maxillary and mandibular cheek tooth enamel thickness were smaller in the donkey than horse CT as shown by Kilic (1997a).This would support the theory that the donkey, with a thinner CT enamel thickness, is a more primitive member of the Equidae Family, as shown by Di Bernardo *et al.* (2004) by genetic characterization. However, this needs to be quantified relative to body size as the thinner enamel could just be due to the smaller body size of donkeys.

This study has examined the normal ultrastructural anatomy of donkey dental tissue. It has been shown that the findings are very similar as to what has been described in the horse (Kilic *et al.* 1997a, 1997b, 1997c, 1997d). As the ultrastructural enamel types identified in the horse can be used for the donkey, it seems likely that other members of the Equidae family will have a similar enamel pattern (although further studies will be needed to confirm this). Therefore the term *Equid* enamel type should be used to describe enamel in equids. The same enamel types have been identified in donkeys in a similar distribution pattern to that found in the horse. However it does appear that there is a greater proportion of equid type-2 enamel in donkeys than what has been reported in the horse (Kilic *et al.* 1997b). Equid type-3 enamel was identified irregularly at the ADJ and ACJ in donkey CT. Equid type-3 enamel is more abundant in primitive animals and is regarded as a less evolved enamel than equid types 1 and 2 (Fortelius 1985). It has also been proposed that type-3 enamel is in fact a transitional enamel seen at enamel junctions (Nanci 2003b).

The above study shows that there is a similar trend in donkey enamel appearance to that recorded in equine enamel, with enamel prism diameters increasing in size from equid type-1 to type-2 enamel i.e. from ADJ to ACJ, and enamel interprism distance decreasing from type-1 to type-2 equid enamel. This inverse relationship between increasing enamel prism diameter size and decreasing interprismatic distance is not proportional, but the number of prisms has been shown to be constant and this has been accommodated by the larger ACJ which has a greater surface area. Kilic (1997b) obtained measurements from 3 transverse levels per tooth, finding significant differences in prism and interprismatic measurements between different levels of CT. However, despite this variation that is dependent on the level of the transverse section and on the site of the section within the 3 different levels, the general trend of increasing enamel prism diameter and decreasing interprismatic distance remained the same. It is for this reason that it was felt measurements at a single longitudinal level were adequate to examine donkey enamel.

The mean enamel prism diameter of donkey enamel at the same transverse section were consistently less than that found in the horse by Kilic et al. (1997b). Mean enamel type-1 prism diameter in equine maxillary CT and mandibular CT were 2.35 µm (donkey mean 0.91 µm) and 2.16 µm (donkey mean 0.69 µm), respectively. Mean enamel type-2 prism diameter in equine maxillary CT was 4.65µm (donkeys mean 2.30µm) and mandibular CT 3.71µm (donkey mean 2.07µm).

Only mid-section transverse sections of donkey teeth were used in this study for quantitative analysis of dentine, as it has been shown that the dentinal tubular diameter and density of equine dentine does not change from the apical to the occlusal region, i.e. along the longitudinal length of the tooth (Kilic *et al.* 1997c). No quantitative analysis were performed on the presence or absence of odontoblast processes or intratubular (peritubular) dentine as it was shown in an equine study that intratubular (peritubular) dentine and odontoblast processes can only be observed in unetched sections (Kilic *et al.* 1997c).

In this study primary donkey dentine had the largest dentinal tubules, as demonstrated by the mean dentinal tubule diameter and perimeter, and contained relatively little intertubular dentine. Dentinal tubular diameter and perimeter sizes of primary dentine decreased towards the junction with secondary dentine, with a concurrent increase in intertubular dentine and dentinal tubule density per 2202 μm^2. A similar pattern of increasing intertubular dentine has demonstrated in the horse by Kilic *et al.* (1997). However due to a difference in interpretation of dentinal tubule diameter between the current and Kilic *et al*. (1997c) studies, no direct comparison can be made between the two studies on tubule diameter. As the etching process used on the transverse tooth sections for SEM examinations in this study had resulted in the destruction of most of the intratubular dentine, the diameter was measured as the clearly visible dentinal tubule outer diameter.

This study also clearly demonstrated an increase in dentinal tubule density from the ADJ to the border of the pulp horn. Donkey primary dentine had a similar dentinal tubule density (median = 17 per 2202 μm^2) as in the horse (median = 19). The dentinal tubule density in donkey primary transitional dentine (median = 59.5) was higher than in the equine dentine (median = 25) and donkey secondary dentine tubule density was lower (median = 81.5) than in equine dentine (median = 128). This difference between donkey and horse dentinal tubule density could be partially due to operator difference. Kilic *et al*'s (1997c) study did not clearly demonstrate where the measurements of primary transitional and secondary dentine were taken, thus variations in the sites examined could have contributed to the observed variations in dentinal tubule density. Despite this possible difference, an important clinical finding of this study is that donkey dentine ultrastructure conforms to the general pattern of dentine observed in the domestic horse.

It is believed that the blood supply to the infundibular cementum from its occlusal surface is lost with removal of the dental sac at the time of first occlusal wear (Dixon 2005). In this study remnants of the infundibular cemental vascular channels were demonstrated on histological sections. It has been proposed that an alternate blood supply from the apex or via lateral channels is still present to the infundibular cementum after eruption and occlusal contact (Dixon 2005). The findings in this study do not support this theory but further investigations looking at the apical aspect of infudibula in younger teeth will be required to confirm this.

3.3 Microhardness of donkey enamel, primary and secondary dentine

3.3.1 Introduction

Due to donkeys having a longer expected lifespan than horses, it has been proposed that donkey teeth are harder than horse teeth to ensure slower attrition and thus longer functional longevity. Dental microhardness testing studies have been performed in humans (Craig and Peyton 1958; Collys *et al.* 1992), sheep (Suckling 1979), cattle (Attin *et al.* 1997) and horses (Muylle *et al.* 1999b). In horses there are breed differences in the rate of dental wear caused by attrition (Muylle *et al.* 1997, 1998) and a difference in microhardness of enamel and secondary dentine has been demonstrated between horse breeds (Muylle *et al.* 1999b). It is proposed that a similar difference in microhardness is present between horse and donkey teeth.

The aim of the study was to determine the microhardness of the enamel, primary and secondary dentine in donkey incisors. A comparison of horse and donkey dental microhardness was also performed to determine if there was a difference in dental microhardness between these two equid subspecies.

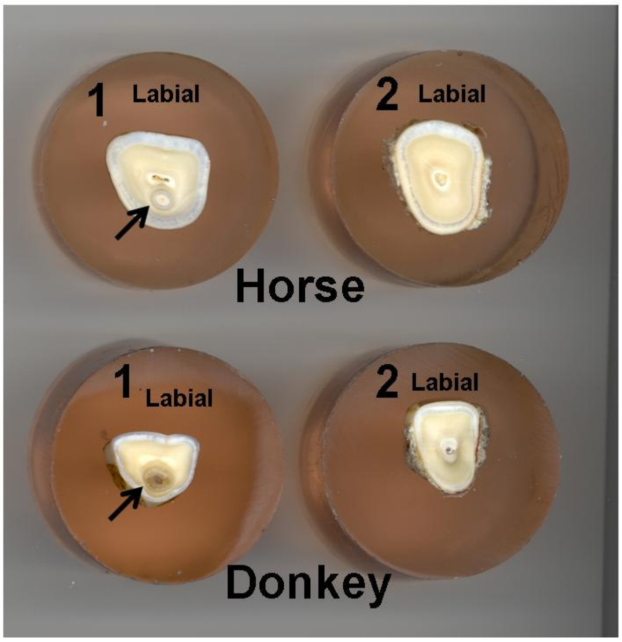
3.3.2 Materials and Methods

Samples

Incisors 301s (left central lower) were extracted from 13 age-matched donkeys and Thoroughbred-cross horses that died or were euthanased on humane grounds for non-dental disorders (Table 3.3.3.1). The extracted incisors were immediately placed in airtight containers containing a thymol solution soaked gauze for 2 to 8 weeks. All teeth were later sectioned in the transverse plane using a water-cooled 99-TS230M tile-saw (Buehler, Coventry, UK) with an 8" thin CR diamond blade (Malvern Lapidary, Malvern, Worcester, UK). A 2-3 mm thick transverse section was obtained from each tooth just below the occlusal surface (level 1) and a further section was obtained midway between the occlusal surface and the mid tooth (level 2). These sections were stored in airtight plastic bags (to prevent dehydration) for 48 hours until further processing.

Sections were mounted in a clear set polyester casting resin (MetPrep Ltd, Coventry, UK) and ground on a Metaserv hand grinder (Buehler Ltd, Illinois, U.S.A.) using 4 grades of abrasive paper (220, 320, 400, 600) (English Abrasives and Chemicals Ltd, London, UK) using tap water as a lubricant. Sections were then polished on a Metaserv universal polisher using Metadi II 1 micron diamond polishing paste (Buehler Ltd, Illinois, U.S.A.), again using tap water as a lubricant (Figure 3.3.2.1).

Figure 3.3.2.1: **Four mounted transverse sections of a horse and a donkey incisor. Arrows indicate infundibula in level 1. Note the obvious difference in the size of these incisors.**



Care was taken to minimise the exposure of prepared sections to excessive humidity, alcohols or other chemicals. Prepared samples were stored at indoor atmospheric conditions (20 ^{o}C, ~50% humidity) and hardness measurements were obtained within 24 hours.

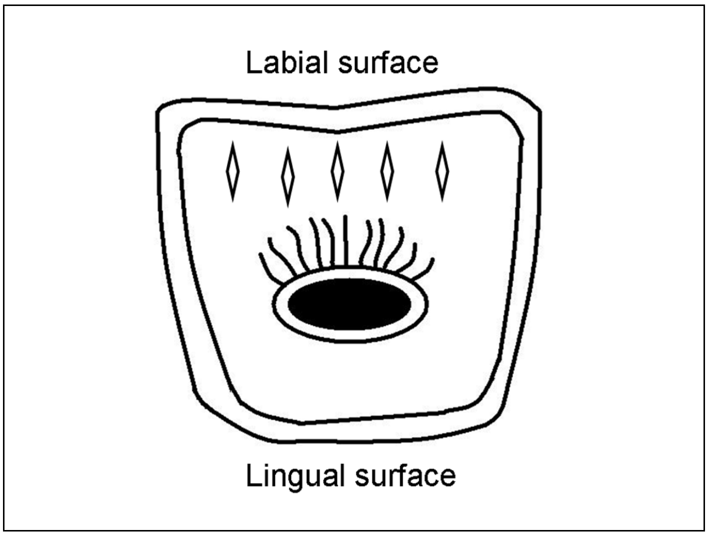
Hardness measurement

Dental hardness was measured using a micro-hardness measurement instrument fitted with a Knoop indenter (Wilson Wolpert Gmbh, Heerlen, Netherlands) (Figure 3.3.2.2).

***Figure 3.3.2.2*: Microhardess tester fitted with a Knoop indenter as used for microhardness testing of donkey and horse teeth.**

Five readings were taken for enamel, primary dentine and regular secondary dentine labial to the pulp cavity at both tooth levels (1 and 2). The Knoop hardness indenter makes a rhomboidal (length to width ratio circa 7:1) shaped indentation on the surface being measured. Readings were taken with the long axis of the rhomboidal indentation running in a rostral to caudal direction (Figure 3.3.2.3.). Microhardness is expressed as the Knoop Hardness Number (KHN) which represents the load required to force the indenter down until 1mm^2 of the tooth surface is occupied by it and is expressed in kg/mm^2 (the ratio of load (kg) over indented area (mm^2)). Area is calculated by the length of the long diagonal of the indentation (mm) multiplied by a constant for the indenter (0.07028). Loads used were based on the loads used in a previous study on the same incisors in horses (Muylle *et al.* 1999b). Readings for enamel were obtained at a load of 500 grams and readings for both primary and regular secondary dentine at a load of 100 grams. All measurements were taken over a constant load period of 15 seconds and 5 readings were taken per dental tissue.

Figure 3.3.2.3: **Diagram illustrating orientation of Knoop hardness indenter relative to labial surface of the tooth. The rhomboidal shaped prisms are not to scale for illustrative purposes.**



Statistical analyses

Linear mixed effect models were performed to compare donkey and horse incisor microhardness in enamel, primary and regular secondary dentine. Individual animals and section of tooth were entered as random effects. Horse/donkey, tooth level and horse/donkey with level were taken as fixed effects. Significance was taken as P < 0.05 and degrees of freedom are indicated by subscripts.

3.3.3 Results

***Table 3.3.3.1*: Number of donkeys and horses from which an incisor was extracted at post mortem in each matched age group for micro-hardness testing.**

Age Group (years)	Donkeys	Horses
6-10	2	2
11-15	4	3
16-20	1	1

The coefficient of variation for the KHN values of enamel, primary and regular secondary dentine was < 15 % in all donkey and horse dental tissues examined, except donkey secondary dentine (15.23%) (Table 3.3.3.2)

***Table 3.3.3.2*: Mean, ranges and coefficient of variation (CoV) of enamel, primary and regular secondary dentine microhardness in donkey and horse incisors in Knoop Hardness Number (KHN).**

Species	Enamel		Primary dentine		Secondary dentine	
	Mean (range)	CoV	Mean (range)	CoV	Mean (range)	CoV
Donkey	264.61 (163.70 – 315.40)	12.64	63.00 (54.76 – 74.50)	5.79	53.64 (36.61 – 70.86)	15.23
Horse	276.45 (236.70 – 329.10)	7.22	61.68 (51.82 – 71.07)	7.94	51.64 (36.96 – 61.18)	11.62

There was no significant difference between dental microhardness of donkey and horse enamel, primary or regular secondary dentine (P > 0.36; Table 3.3.3.3; Figure 3.3.3.1).

Figure 3.3.3.1: **Donkey and horse dental microhardness (Knoop Hardness Number) values in enamel, primary dentine and regular secondary dentine.**

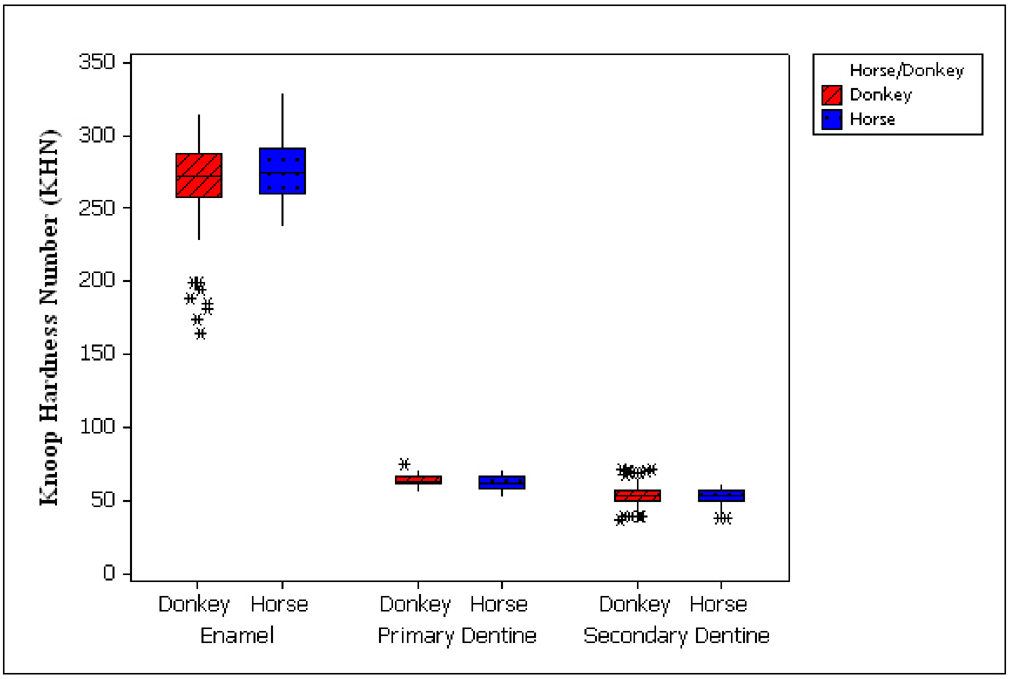
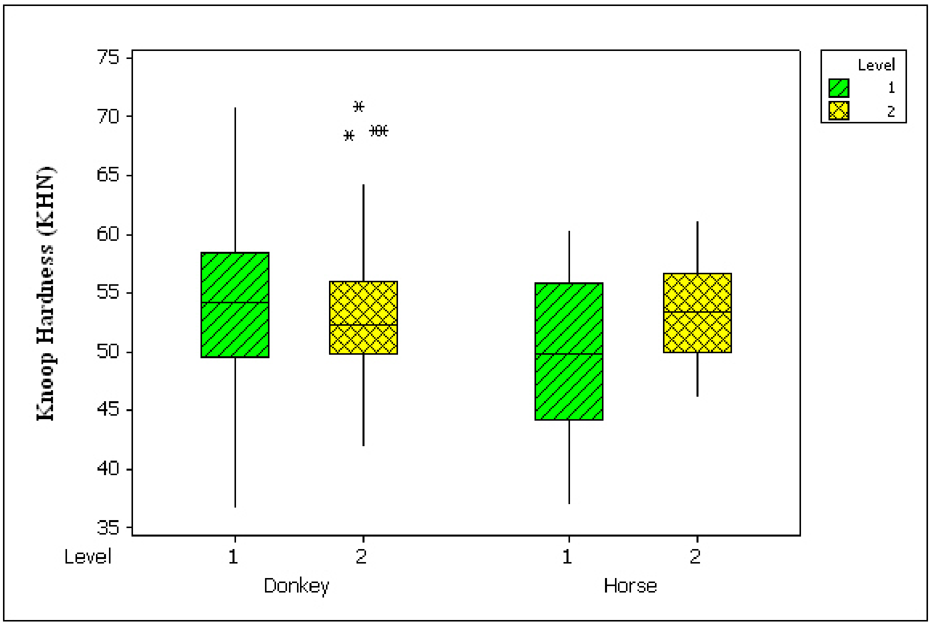


Table 3.3.3.3: **Results of linear mixed effect models comparing Knoop Hardness Number in enamel, primary dentine and regular secondary dentine in horses and donkeys,**

Comparison	Enamel	Primary Dentine	Secondary dentine
Horse vs Donkey	P = 0.40; $F_{1, 11}$ = 0.78	P = 0.36; $F_{1, 11}$ = 0.93	P = 0.60; $F_{1, 11}$ = 0.30
Tooth level (1 vs 2)	P = 0.71; $F_{1, 115}$ = 0.14	P = 0.77; $F_{1, 125}$ = 0.084	**P = 0.019**; $F_{1, 115}$ = 5.32
Difference in tooth levels between horse and donkey	P = 0.38; $F_{1, 115}$ = 0.78	P = 0.40; $F_{1, 125}$ = 0.71	**P = 0.012**; $F_{1, 115}$ = 6.58

There was no significant difference in the dental microhardness of enamel and primary dentine between different teeth levels, however there was a significant difference in microhardness between different tooth levels ($P < 0.05$) in regular secondary dentine in donkey and horse incisors. Regular secondary dentine was harder in level 1 in donkeys and harder in level 2 in horses. The difference in regular secondary dentine hardness in different tooth levels was statistically significant between donkeys and horses ($P < 0.05$; Figure 3.3.3.2).

Figure 3.3.3.2: **Microhardness in Knoop Hardness Number (KHN) of secondary dentine in donkeys and horses at different levels of incisor teeth (level 1: just below occlusal surface; level 2: midway between occlussal surface and mid-tooth).**



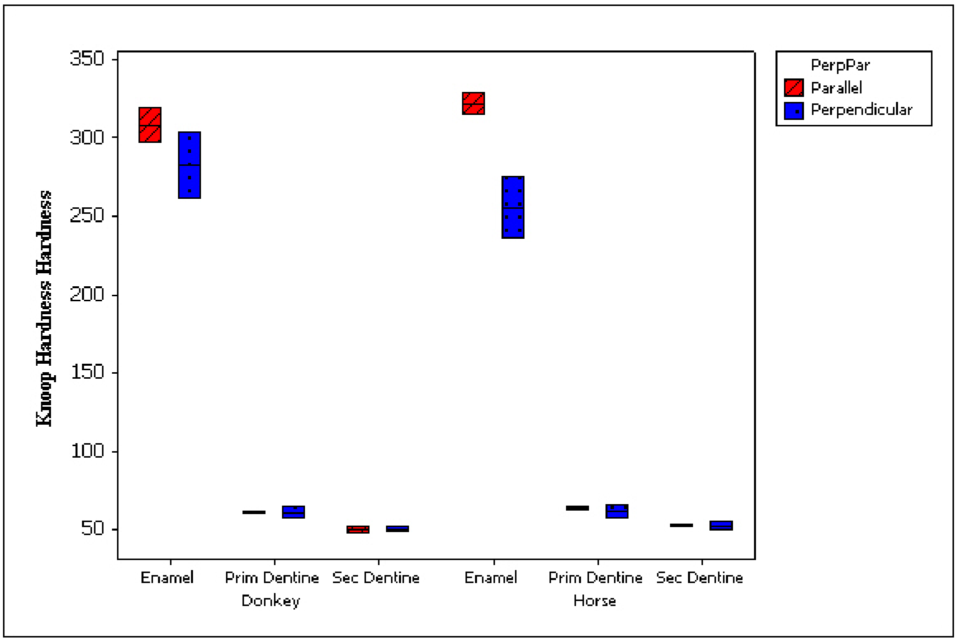
Effect of measurement method

The above microhardness measurements (as used for statistical analysis) were all performed with the rhomboid shape indenter perpendicular to the labial surface. For comparison, measurements were repeated in one horse and one donkey incisor with the rhomboid indentation made parallel to the labial surface. Consistent small differences were observed in enamel measurements of both species. There was no apparent difference in values obtained with the Knoop indenter perpendicular or parallel to the labial surface in primary and secondary dentine (Table 3.3.3.4; Figure 3.3.3.3), but due to the small sample size, no statistical analyses were possible on these values.

Table 3.3.3.4: **Mean Knoop Hardness Number of enamel, primary dentine and secondary dentine measured with the rhomboidal indenter perpendicular (perp) and parallel to the labial surface of the tooth in one horse and one donkey incisor.**

	Level	Enamel		Primary dentine		Secondary dentine	
		Perp	**Parallel**	**Perp**	**Parallel**	**Perp**	**Parallel**
Donkey	**1**	262.30	294.84	57.73	54.92	50.00	50.67
	2	303.90	305.24	65.20	60.22	52.26	55.07
Horse	**1**	275.50	315.40	66.30	62.82	55.93	65.22
	2	236.70	329.20	57.58	65.22	50.12	52.90

Figure 3.3.3.3: **Knoop Hardness Number (KHN) in enamel, primary dentine and secondary dentine measured with the rhomboidal shaped indenter perpendicular and parallel to the labial surface in a donkey (A) and horse (B).**



3.3.4 Discussion

The most widely accepted method for hardness testing in teeth all species is the technique used in this study i.e. hardness tester fitted with a Knoop diamond indenter (Sweeney 1942). The loading weights used for measurements in this study are similar to those used in previous human, equine and bovine teeth measurements. In particular, the loads used in this study were based upon a previous study in 3 different horse breeds (Muylle *et al.* 1999b). However, KHN varies as a function of the load and therefore KHN cannot be considered as a constant value when measured using loads less than 100g, which is probably due to inaccuracies in measuring small indentation lengths (Collys *et al.* 1992). Consequently 100g was the minimum weight used as for assessing both primary and secondary dentine in this study, rather than a 50g load for secondary dentine, as used by Muyelle *et al.* (1999b).

Muylle *et al.* (1999b) found the microhardness values of equine incisor teeth enamel to range from 236.8 - 288.6 KHN; primary dentine to range from 41.4 - 58.9 KHN and secondary dentine to range from 20.1 – 36.3 KHN. The KHN of enamel in Muylle's study is similar to the values obtained in donkeys (264.61 KHN) and horses (276.45 KHN) in the current study, although there was a greater range of hardness values in donkey enamel (163.70 - 315.40 KHN). Despite replicating the Knoop microhardness technique previously used in horses, the hardness values of horse and donkey dentine in this study, and in particular of secondary dentine were greater than values recorded in equine incisors previously (Muylle *et al.* 1999b). Similarly, much variation has been recorded in the microhardness of human enamel and dentine between and within studies (Craig and Peyton 1958; Collys *et al.* 1992; ten Bosch and Coops 1995). This variation may be due to different loads, different techniques or different teeth used in these studies. For this reason both donkey and horse incisors were simultaneously examined for dental microhardness in this study.

The previous study comparing dental Knoop microhardness in horses demonstrated higher enamel and secondary dentine microhardness in Arabian horses over 12 years of age, as compared to trotter horses and Belgian draft horses (Muylle *et al.* 1999b). The present study has however shown no significant difference between horse and donkey enamel, primary or regular secondary dentine microhardness. The only significant difference observed in this study between donkey and horse dental hardness was between level 1 and level 2 of incisor regular secondary dentine. Interestingly, the secondary dentine in horses was harder in level 2 (than level 1) similar to the findings of Muyelle *et al*. (1999b). This is in contrast to donkey teeth, where secondary dentine was harder in level 1 just below the occlusal surface. The reason for this difference in secondary dentine microhardness at different levels of incisors is unknown.

However, the rate of dental wear is not entirely dependent on tooth microhardness because there are no significant differences in enamel and dentine microhardness between trotter and Belgian draft horses (Muylle *et al.* 1999b) and yet age related differences in incisor wear occurs between these breeds (Muylle *et al.* 1997). Many other factors such as diet composition (Carlsson *et al.* 1966), masticatory cycle, enamel thickness and particularly, duration of mastication (Dahl *et al.* 1993) have a significant effect on the rate of dental wear. In particular, a study determining the age of donkeys by clinical examination of the incisors demonstrated an earlier appearance of secondary dentine (“dental stars”) and later disappearance of infundibular enamel (“cups”) in donkeys, despite later incisor eruption times compared to horses (Muylle *et al.* 1999a). This latter finding could be attributed to larger enamel infoldings i.e. longer infundibulae in donkeys rather than slower dental attrition.

Non working donkeys in a natural environment spend only 6-7 hours a day grazing and browsing (Aganaga and Tsopito 1998), which is much less than the 16-18 hours/day reported in horses (Tyler 1972; Capper 1992; Frape 2004). In view of recent findings where donkey dental gross and microscopic anatomy has been shown to be almost identical to that of the horse (du Toit *et al.* 2008c, 2008d), it appears that decreased physiological occlusal attrition, contributes to the increased longevity of donkey teeth and may indirectly contribute to the longer life spans of donkeys as compared to horses.

In conclusion, the normal attrition of teeth is dependent on many factors but this study has shown that the differences in the rate of attrition between donkey and horse incisors cannot be attributed to a difference in the microhardness of their calcified dental tissues.

Chapter 4: Post mortem survey of dental disorders in 349 donkeys from an aged population

4.1. Introduction

Dental disorders have been recognised in working donkeys (Rajput *et al.* 1999; Roy 2002; Chabchoub *et al.* 2006; Fernando-Martinez *et al.* 2006), however no extensive studies of donkey dental disease appeared to have been performed to date. The aim of this study was to determine the prevalence and common sites of occurrence of specified dental disorders by post-mortem examination of donkeys that died or were euthanased on humane grounds. Furthermore, the epidemiology of dental disease in donkeys has not been investigated in detail to date, and thus the aim of the second part of this study was to examine possible relationships between specific dental disorders identified in donkeys to: other dental disorders; age; body condition score; the provision of supplemental feed, time since last dental treatment and the type of illness that necessitated euthanasia or caused death.

4.2. Materials and Methods

4.2.1 Descriptive data

A prospective study was performed on routine post mortem data from 349 donkeys that died or were euthanised on humane grounds at the Donkey Sanctuary from February 2005 to September 2006. As part of the routine post mortem they were evaluated for the presence of dental disease. Dental disorders were recorded by The Donkey Sanctuary veterinary pathologist, John Gallagher, as per the pre-agreed criteria after examination of several donkey skulls with P.M. Dixon and the author. Recorded data included number and location of missing teeth, number and location of displaced teeth, number and location of diastemata, number and location of worn teeth, prevalence of dental-related soft tissue trauma, dental calculus, shear mouth, wave mouth and step mouth.

Skulls with fewer teeth than the standard equid dental formulae were classified as having missing teeth and the sites of dental loss were recorded. Displaced CT were defined as teeth which were not in normal latero-medial or rostro-caudal alignment with the cheek teeth row. Diastema (pleural diastemata) was defined as an abnormal space between adjacent CT with or without food pocketing. Worn teeth were defined as teeth with excessive wear which varied from senile excavation i.e. loss of infundibula or enamel infolding and resultant excessive wear of the central part of the tooth, to teeth worn fully down to the gingival margin, with only remnants of the apex, most usually roots visible within the alveolus. Focal overgrowths were defined as overgrowths on part of a CT, usually corresponding to an area of absent occlusal contact with the opposing tooth. Calculus of CT was defined as areas on the surface of CT that were covered in obvious calculus. Shear mouth was defined as excessive angulation of the CT occlusal surfaces (> 45º) in the buccolingual plane (Brown *et al.* 2008), wave mouth as an undulating appearance of the occlusal surface in a rostro-caudal plane and step mouth as individual CT supra-eruptions, classically rectangular-shaped (Dixon *et al.* 2000a). Sharp enamel points on individual teeth were noted as dental disorders, but sharp buccal maxillary and lingual mandibular enamel overgrowths (“enamel points”) that are usually associated with normal dental wear were not included in the definition of dental disease.

4.2.2 Epidemiology of dental disorders and association with systemic disease

Other recorded data from these 349 donkeys included data such as body condition score (BCS) and age at the time of death, whether extra feed rations were given (i.e. concentrate feeds to supplement *ad lib* roughage) and the illness. As only a small percentage of the donkeys examined had precise dates of birth, the estimated age (including using dental ageing when acquired at a young age, donor information when older donkeys were acquired and complete medical records since acquired by The Donkey Sanctuary), was also divided into age groups that would be more accurately estimated and biologically plausible i.e. Young (0-14 years old), Middle aged (15-21); Old (22-28); Very old (29-35); Geriatric (≥36). This age-grouping was then used to verify the statistical results found with age in the analyses.

Specific dental disorders investigated included the presence or absence of diastemata, displaced teeth, missing teeth and worn teeth ("smooth mouth" "senile excavation"). The illness necessitating euthanasia (hereafter referred to as illness) was categorised into 6 disease categories: colic (impaction colic, large and small intestinal disorders, peritonitis); liver/pancreatitic/metabolic (hepatopathy, hyperlipaemia, pancreatitis); musculoskeletal (laminitis, arthritis and fractures); respiratory (pulmonary fibrosis, bacterial infections, guttural pouch disease); cardiovascular (cardiac disease, haemorrhages, thrombi) and miscellaneous (neoplasia, nephropathy, urolithiasis, ocular disease, sarcoids). As only colic and liver/pancreatic/metabolic deaths could be directly related to dental disease, these were the only two illness categories that were further investigated statistically in this study. The prevalence of dental disease was so high in this group of donkeys that the relationship between illness and donkeys without dental disease could not be investigated.

4.2.3 Statistical analyses

ANOVA was used to investigate possible relationships between estimated age of donkeys and illness. Two ANOVA were used for this analysis: one including and one excluding the miscellaneous illness group. For any statistically significant results post hoc multiple comparisons of the mean values using Tukey contrasts were undertaken. Two sampled t-tests were performed to investigate the relationship between age and the presence/absence of dental disease and diastemata. The relationship between BCS and the presence/absence of diastemata and supplemental feed were determined using Mann-Whitney tests. The relationship between estimated age and BCS was investigated using

Spearman rank correlation analysis. To investigate possible relationships between age group and disease, general linear models with binomial errors were used, excluding the miscellaneous illness group. Chi squared tests were also performed to investigate the relationship between age group and the presence/absence of dental disease and diastemata. Post hoc multiple comparisons of the prevalence of dental disease and diastemata in different age groups using Tukey contrasts were undertaken for any statistically significant results. The relationship between age group and BCS was investigated using a Kruskal-Wallis test.

When investigating the possible association between the presence or absence of dental disease and the illness, general linear models with binomial errors were used. These models were also used to investigate the association between different dental disorders. Multivariable analyses were then performed to determine whether the interaction of explanatory variables altered their effect on the response variable, as it likely that many of the factors are not mutually independent. The models were selected on initial univariate screening statistical significance ($P \leq 0.2$) and biological plausibility of the data used. Odds ratios (OR) and their 95% confidence intervals were determined for each explanatory variable to determine their association with response variables. $P < 0.05$ was taken to be statistically significant in the final multivariate analyses. R V2.3.1 (R Foundation for Statistical Computing) was used for statistical analyses.

4.3. Results

4.3.1 Descriptive analysis

Of the 349 donkeys examined at post mortem only 23 (6.6%) had no gross evidence of dental disease i.e. prevalence of 93.4%. A total of 43.6% (152) of donkeys had abnormalities of dental wear (worn teeth and/or focal overgrowths) (Figure 4.3.1.1).The mean estimated age of all the donkeys was 31.1 (median 31) (range 6-52). The age of donkeys with and donkeys without dental disease was statistically significantly different

(P < 0.001), with a median age of 31 in donkeys with dental disease and 24 without dental disease (Figure 4.3.1.2). When comparing the prevalence of dental disease and diastemata in different age groups there was also a statistically significant difference between age groups (P < 0.001). Post hoc comparisons of age groups showed that the prevalence of dental disease in the young, middle and old age groups were statistically significantly different from donkeys in the geriatric age group (Table 4.3.1.1). Due to the low prevalence of diastemata in the young age group, post hoc comparisons of diastemata prevalence was performed with the young and middle age groups combined. Only the diastemata prevalence in the young/middle age groups was significantly different from the geriatric age group.

Figure 4.3.1.1: **Severe dental disease in this 28 year old donkey including missing teeth, diastemata, displaced teeth and periodontal disease in both maxillary and mandibular cheek teeth.**

Figure 4.3.1.2: **Boxplot illustrating the age (years) difference between donkeys with (Y) and without (N) dental disease.**

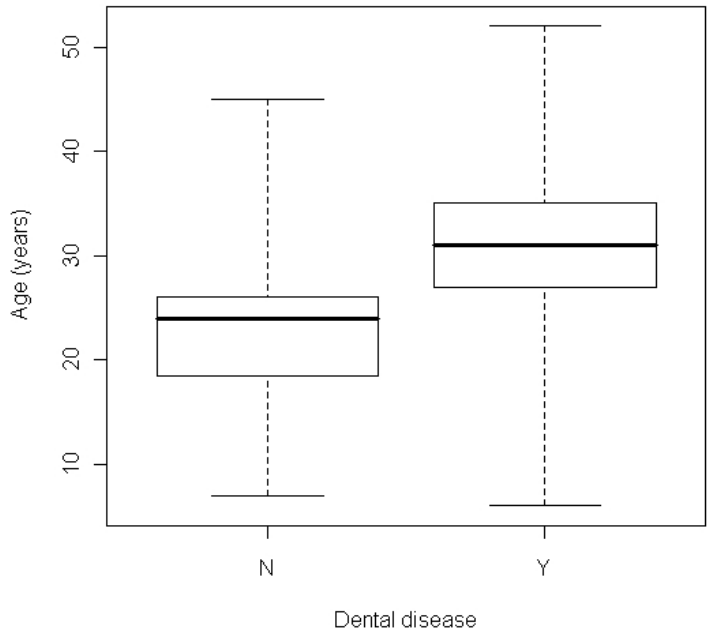


Table 4.3.1.1: **The number of donkeys and prevalence (%) of dental disease in each age group, and the odds ratio (with 95% confidence interval) relative to the prevalence of dental disease in the geriatric group.**

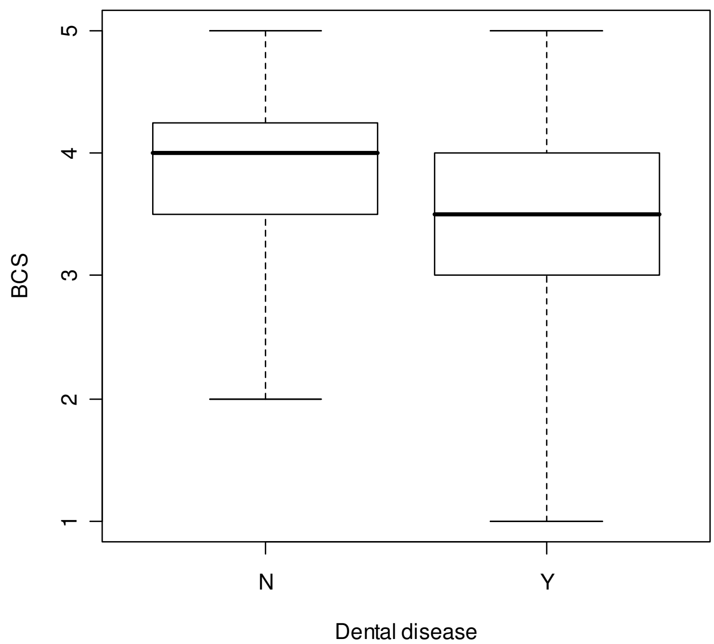
Age Group	No dental disease	Dental disease	P value	Odds ratio
Young	4 (66.7%)	2 (33.3%)	P < 0.001	0.0007 (0 – 0.92)
Middle aged	4 (17.4%)	19 (82.6%)	P = 0.015	0.06 (0.0007 – 0.61)
Old	12 (12.5%)	84 (87.5%)	P = 0.025	0.095 (0.01 – 0.75)
Very old	2 (1.3%)	147 (98.7%)	P = 0.99	0.99 (0.09 – 11.2)
Geriatric	1 (1.3%)	74 (98.7%)	-	-

Table 4.3.1.2: **The number of donkeys and prevalence (%) of diastemata in each age group, and the odds ratio (with 95% confidence interval) relative to the prevalence of diastemata in the geriatric group.**

Age Group	No diastemata	Diastemata	P Value	Odds ratio
Young	6 (100%)	0	-	Combined with middle
Middle aged	7 (30.4%)	16 (69.6%)	P < 0.001	0.11 (0.04 – 0.33)
Old	16 (16.7%)	80 (83.3%)	P = 0.09	0.44 (0.16 – 1.18)
Very old	18 (12.1%)	131 (87.9%)	P =0.35	0.63 (0.24 – 1.67)
Geriatric	6 (8%)	69 (92%)	-	-

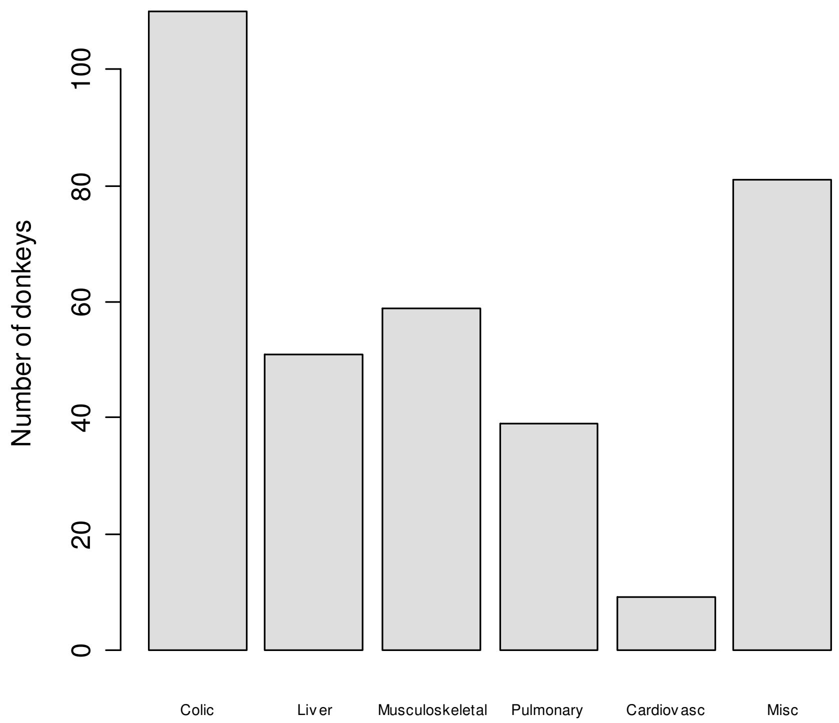
The BCS of donkeys with dental disease was 3.5 and without dental disease was 4 (Figure 4.3.1.3) which was statistically significantly different (P = 0.01).

Figure 4.3.1.3: **Boxplot of body condition score (BCS) in donkeys with (Y) and without (N) dental disease.**



Colic-related disease was the most common disorder (31.3%) causing death or necessitating euthanasia of these 349 donkeys (Figure 4.3.1.4). The second largest group was the miscellaneous illness (23.2%), followed by musculoskeletal (16.9%), liver (14.6%), pulmonary (11.2%) and cardiovascular disease groups (2.6%).

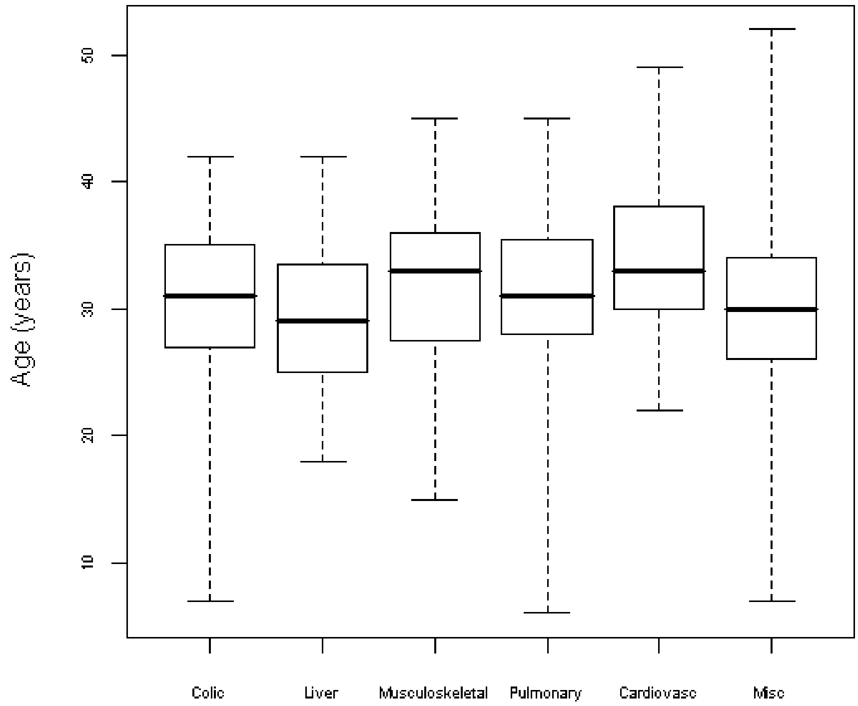
Figure 4.3.1.4: **A bar chart illustrating the number of donkeys categorised into 6 disease groups.**



There was no relationship of age to disease including to the miscellaneous group (P = 0.076; Figure 4.3.1.5). However, when the miscellaneous group was excluded, a significant relationship (P = 0.047) existed between age and the remaining disease groups. Multiple comparisons of means using Tukey contrasts indicated that this significance was due to the difference in mean age between donkeys that died of liver/pancreatic/metabolic disease and cardiovascular related disease (P = 0.06, all other

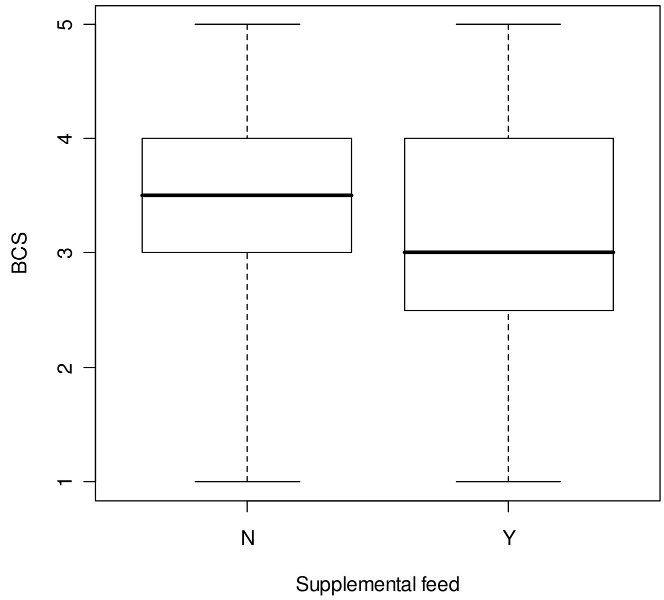
P values > 0.15). It can therefore be deducted that there was no real significant relationship between age and disease. No statistically significant association was found between age group and the type of disease excluding the miscellaneous group (P = 0.16; $\chi^2_1 = 6.58$).

Figure 4.3.1.5: **Boxplot illustrating the age of donkeys at the time of death for each illness group. Boxes = interquartile range; vertical lines = range; horizontal lines = median.**



The BCS of donkeys ranged from 1-5 (median of 3.5). The BCS for donkeys not given extra feed was 3.5 and of those given extra feed was 3.0 and this difference was statistically significant (P = 0.008; Figure 4.3.1.6).

***Figure 4.3.1.6*: Boxplot of body condition score (BCS) in donkeys that were given extra feed (Y) and that were not given extra feed (N). Boxes = interquartile range; vertical lines = range; horizontal lines = median.**



The Spearman rank correlation test indicated a statistically significant but small correlation between age and BCS ($P = 0.005$; $r = -0.15$; Figure 4.3.1.7), with older donkeys tending to have lower BCS (Table 4.3.1.3).

***Figure 4.3.1.7*: Boxplot of ages and body condition score of donkeys at the time of death.**

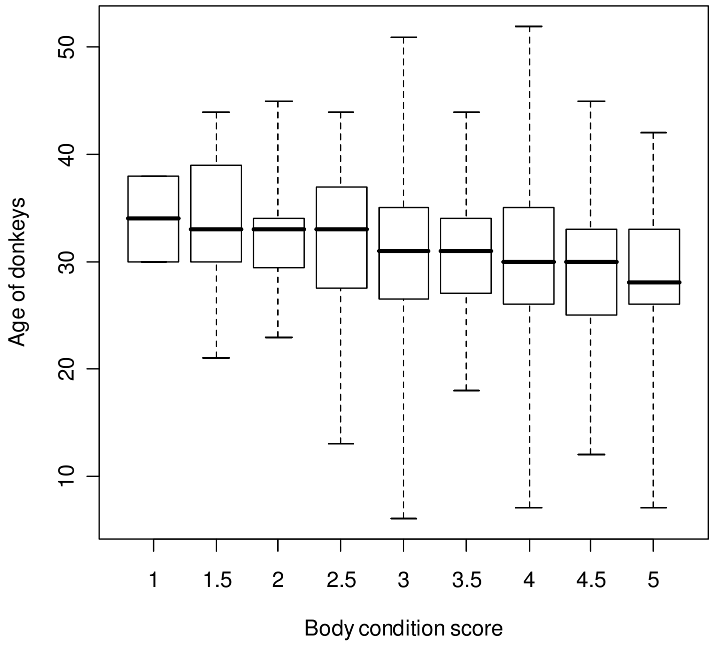
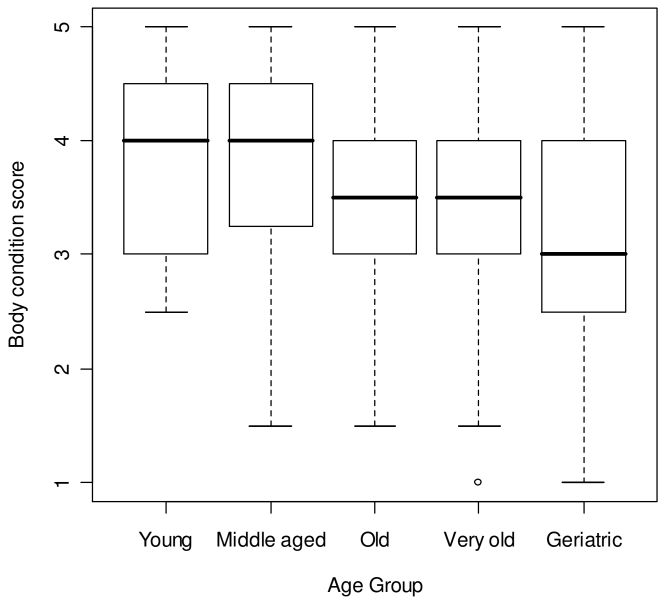


***Table 4.3.1.3:* Mean ages (and standard deviation) of donkeys in each body condition score as recorded at the time of death.**

Body Condition Score	Number of donkeys	Mean age (years)	Standard deviation
1	2	34	5.66
1.5	10	32.9	7.29
2	22	32.73	5.68
2.5	28	32.14	6.87
3	91	30.44	6.63
3.5	57	30.33	5.50
4	78	30.53	7.78
4.5	37	29.35	7.61
5	22	27.95	7.61

Similarly, there was also a statistically significant, but weak correlation between age group and BCS (P = 0.032), with donkeys in the older age group tending to have lower BCS (Figure 4.3.1.8).

***Figure 4.3.1.8*: Boxplot of age group and body condition score of donkeys at the time of death.**



There was no significant association between donkeys given extra feed and dental disease (P = 0.12; $\chi^2_1 = 2.43$). Dental disease was seen in 92.5% of donkeys that were given supplemental feed and 96.8% of donkeys not given supplemental feed.

4.3.1.1 Diastemata

The Triadan position of the CT on either side of all diastemata was recorded.
The prevalence of CT diastemata was 85.1% (297/349) with 24 (8.1%) donkeys having only maxillary CT diastemata, 102 (34.3%) donkeys having only mandibular CT diastemata and 171 (57.6%) donkeys having both maxillary and mandibular CT diastemata. In affected donkeys, the mean number of diastemata noted in the maxillary CT row was 2.6 (median 2; range 1-10) and in the mandibular CT row 3.2 (median 3; range 1-9).

Figure 4.3.1.1.1: **Multiple valve mandibular diastemata in a 12 year old donkey made visible by the small amount of grass impacted in each diastemata.**

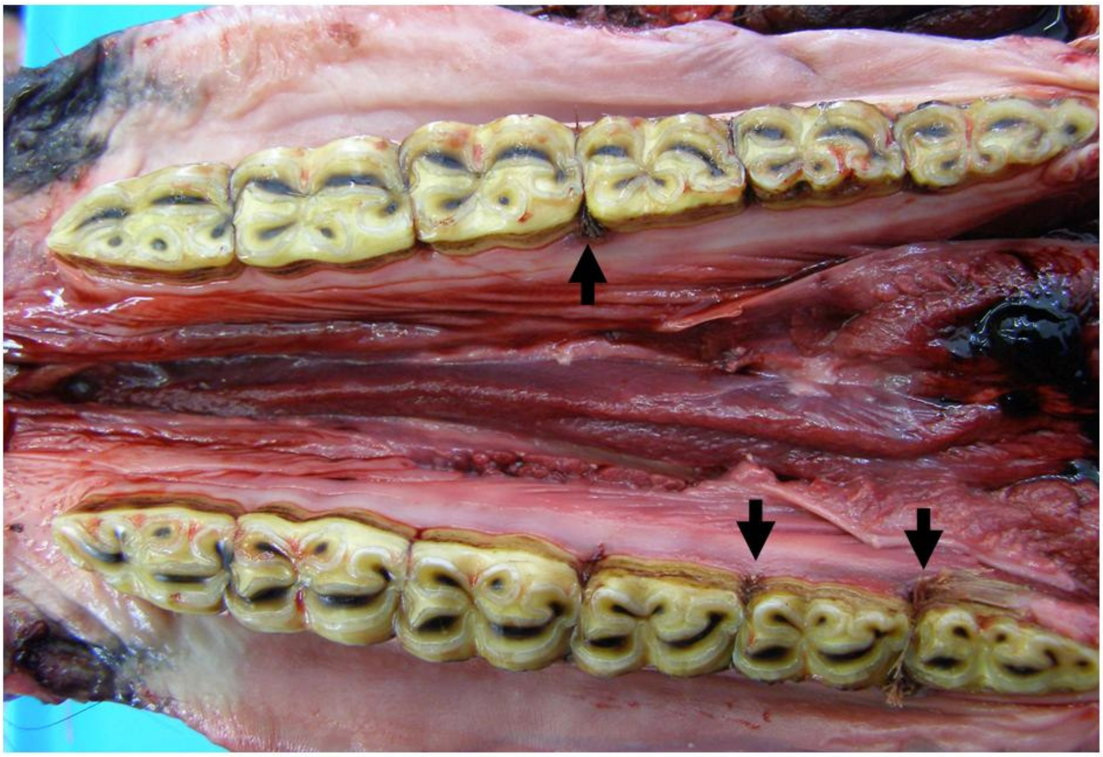


Figure 4.3.1.1.2: **Multiple large open mandibular diastemata with food pocketing in a 31 year old donkey.**

In total, 1360 CT diastemata were present in 297 donkeys (mean of 4.6 diastemata /donkey) with 861 (63.3%) mandibular CT and 499 (36.7%) maxillary CT diastemata. In the maxillary CT, the most common site for diastemata was between Triadan positions 06/07 (26.5%) and 07/08 (24.7%). In contrast, the most common site for mandibular CT diastemata was between 09/10 (22.5%) and 10/11 (22.5%), followed by diastemata in the 06/07 position (20.4%) (Table 4.3.1.1.1, 4.3.1.1.2 and Figure 4.3.1.1.3).

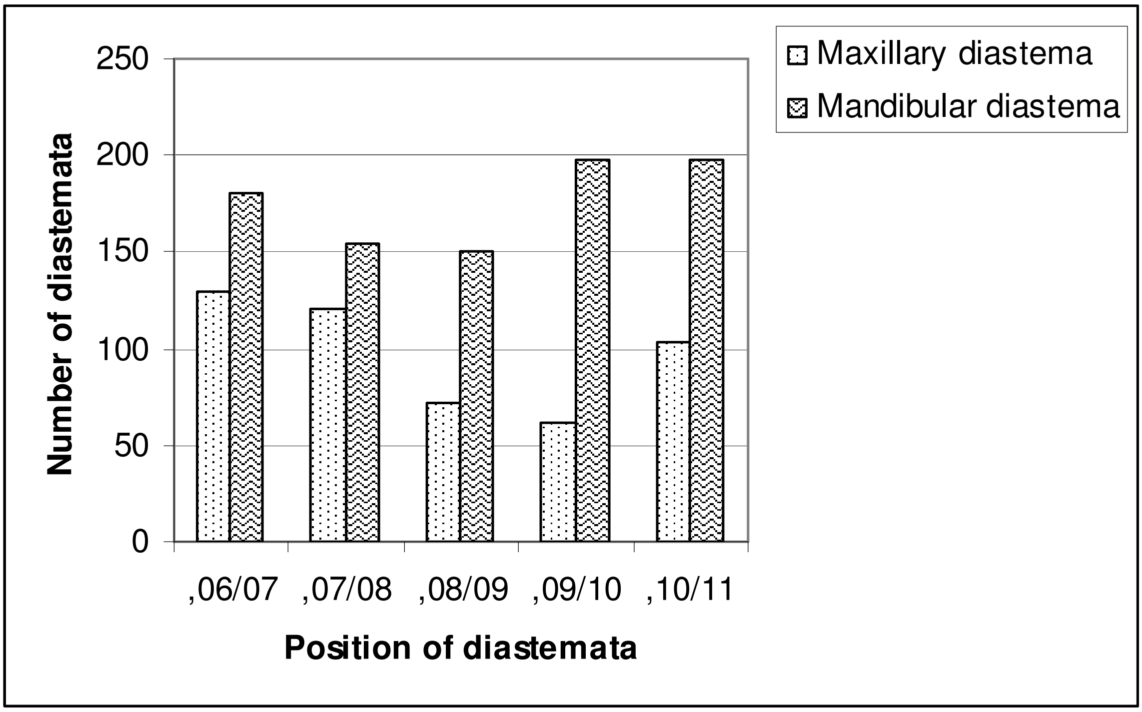
***Table 4.3.1.1.1:* The number of diastemata in the five interdental spaces as defined by the Triadan position of the teeth on either side of diastemata for each dental quadrant (1 - 4).**

Triadan postion	06/07	07/08	08/09	09/10	10/11
Quadrant 1	67	64	31	24	54
Quadrant 2	65	59	41	39	53
Quadrant 3	82	79	67	99	98
Quadrant 4	94	72	81	95	96

***Table 4.3.1.1.2:* Numbers and percentage of total diastemata between Triadan positions in mandibular and maxillary cheek teeth.**

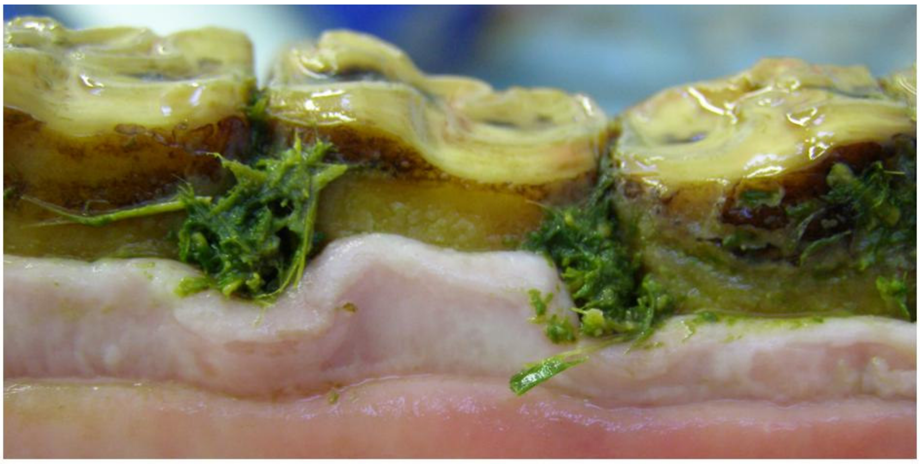
Diastemata	Maxillary		Mandibular	
	Number	Percentage	Number	Percentage
06/07	132	26.5%	176	20.4%
07/08	123	24.7%	151	17.5%
08/09	74	14.8%	147	17.1%
09/10	63	12.6%	194	22.5%
10/11	107	21.4%	194	22.5%

Figure 4.3.1.1.3: **Number of diastemata between the different maxillary and mandibular Triadan positions.**



Of the 297 donkeys with CT diastemata, 138 (46.5%) also had displaced CT, 167 (56.2%) had missing CT, 74 (24.9%) had displaced and missing CT and 30 (10.1%) had no displaced or missing teeth. These missing and displaced teeth were not necessarily adjacent to the diastemata.

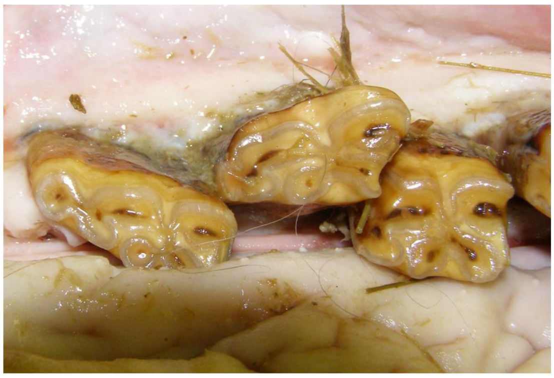
***Figure 4.3.1.1.4:* Close up view of large, open mandibular diastemata with periodontal food pockets in a 31 year old donkey.**



4.3.1.2 Cheek teeth displacements

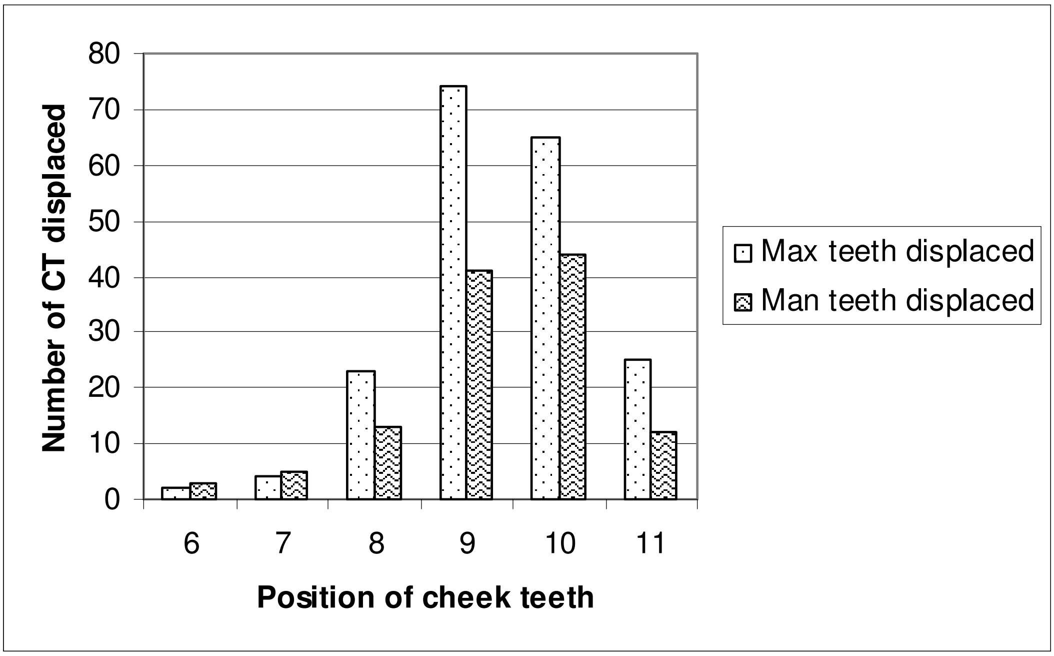
Most CT displacements were in a medial or lateral direction (as opposed to rostral or caudal). A total of 149 of the 349 donkeys had CT displacements (42.7%). Of these, 75 (21.5%) had only maxillary CT displacements, 35 (10%) had only mandibular CT displacements and 39 (11.2%) had both maxillary and mandibular CT displacements.

***Figure 4.3.1.2.1:* Maxillary (left) and mandibular (right) lateral cheek teeth displacements in a 43 and 20 year old donkey respectively.**



Of the donkeys with maxillary CT displacements (114), 58 (51%) had only one tooth displaced, 43 (37.7%) had two maxillary CT displaced, and the remaining 13 cases (11.4%) had between 3 and 6 maxillary CT displaced (Figure 4.3.1.2.2). Of the 74 donkeys with mandibular CT displacements (74), 41 (55.4%) had only one tooth displaced, 25 (33.8%) had 2 CT displaced with the remaining 8 cases (10.8%) having 3 or 4 teeth displaced (Figure 4.3.1.2.2).

Figure 4.3.1.2.2: **The number of donkeys, Triadan positions and numbers of maxillary (Max) and mandibular (Man) CT displacements**.



A total of 311 displaced CT were found in the 149 donkeys, of which 193 (62.1 %) were maxillary CT and 118 (37.9%) were mandibular CT. The most common Triadan position for maxillary CT displacements were the 09s (38.3%) and 10s (33.7%). Similarly, the mandibular 10s (37.3%) and 09s (34.7%) were the most commonly displaced mandibular CT (Table 4.3.1.2.1 and Figure 4.3.1.2.2). Bilateral CT displacements were present in 13.2 % (41/311) of the displaced CT (Figure 4.3.1.2.3).

Table 4.3.1.2.1: **Number and percentage of maxillary and mandibular CT displacements in each Triadan position.**

Displacements	Maxillary		Mandibular	
	Number	Percentage	Number	Percentage
06	2	1%	3	2.5%
07	4	2%	5	4.2%
08	23	11.9%	13	11%
09	74	38.3%	41	34.7%
10	65	33.7%	44	37.3%
11	25	13%	12	10.2%

Figure 4.3.1.2.2: **The number of CT displacements in each maxillary and mandibular Triadan position.**

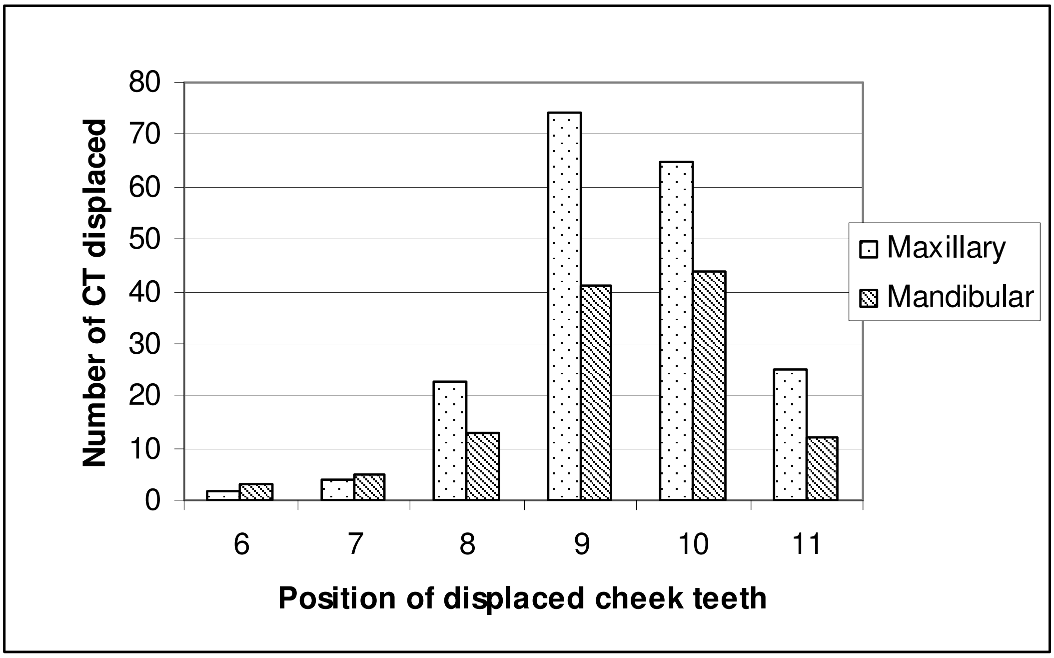


***Figure 4.3.1.2.3*: Severe bilateral lateral displacements of mandibular 10s in a 38 year old donkey. Also note the laterally displaced 407.**

4.3.1.3 Missing teeth

Canine (04s) and wolf teeth (05s) were not included in this part of the study, because of their physiological variability. A total of 1151 teeth were missing in 55.6% (194/349) of the skulls examined, of which 23.5% (271/1151) were incisors, 23.0% (265) were maxillary CT and 53.4% (615/1151) were mandibular CT. The total number of teeth missing per skull varied from 1-34 teeth (Figure 4.3.1.3.1 and Figure 4.3.1.3.2). The 5 worst affected donkeys (24 – 34 teeth missing) had all 24 of their CT missing, in addition to a variable number of missing incisors.

Figure 4.3.1.3.1: **Three maxillary cheek teeth (208, 209 and 210) were missing in this 32 year old donkey. Also note the diastemata between 107 and 108, and the lack of a rostral infundibula in the 108 due to age related wear.**

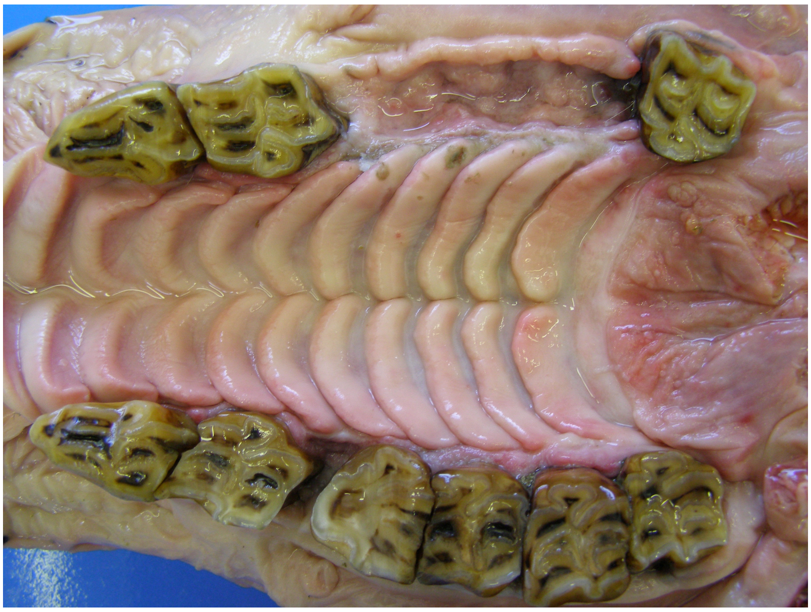
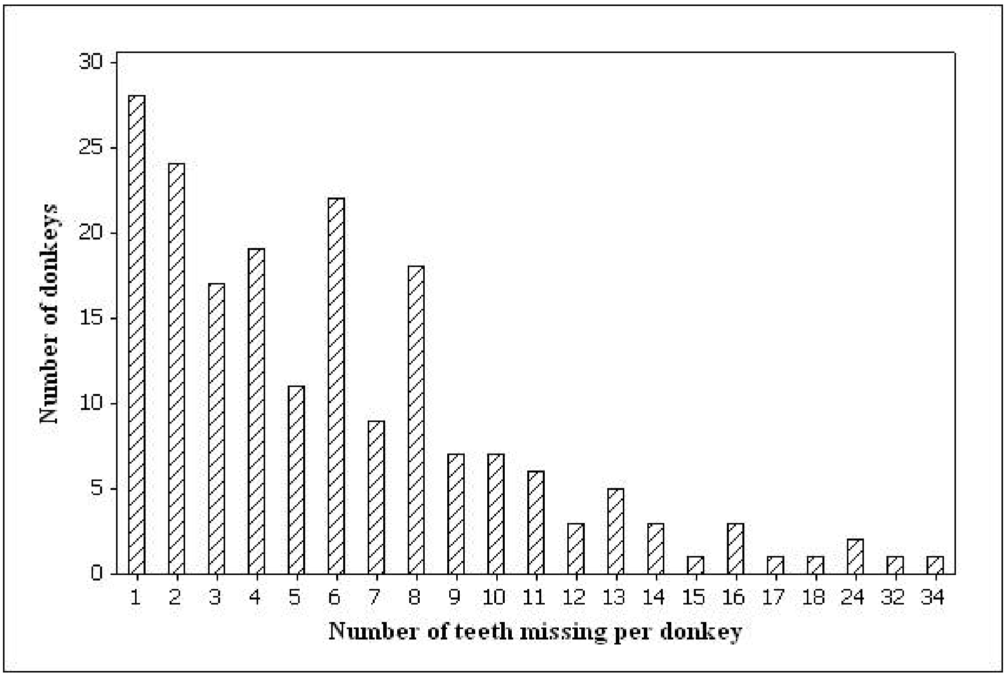


Figure 4.3.1.3.2: **Number of donkeys with specified number of teeth missing.**



The number and Triadan position of missing CT were recorded, in order to determine the most common CT missing (Table 4.3.1.3.1). There was predominantly more loss of mandibular CT (55.5%) compared with maxillary CT (20.6%). The 10s (26%) were the most common maxillary CT missing and the 09s (22.8%) were the most common mandibular CT missing (Table 4.3.1.3.2 and Figure 4.3.1.3.3). There was no difference between the different sides (right and left) for missing teeth.

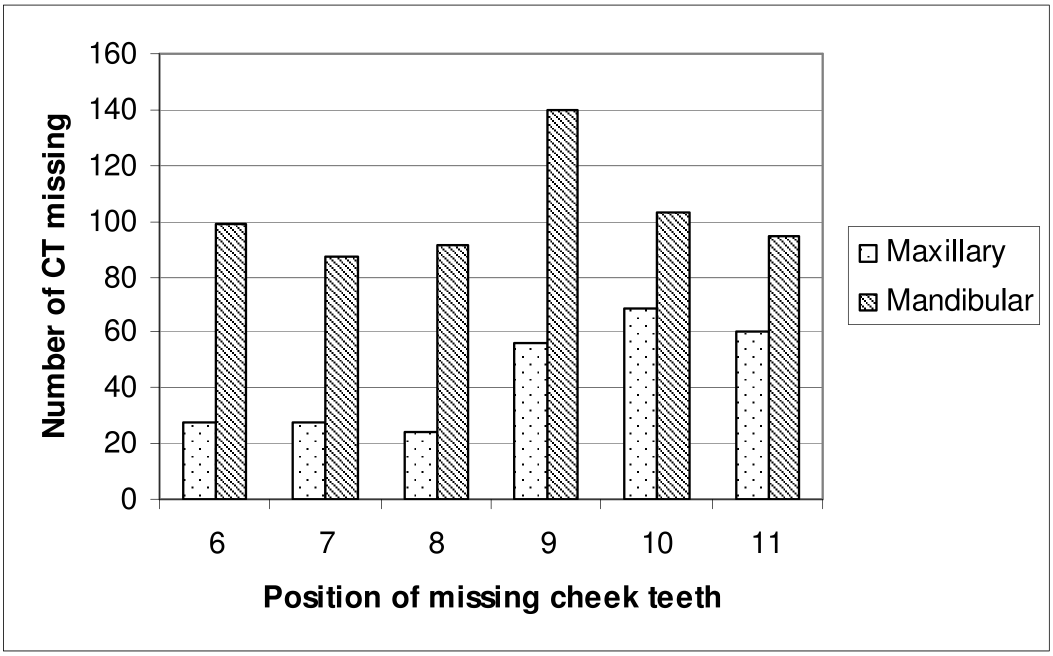
Table 4.3.1.3.1: **Number of CT missing per Triadan position.**

Triadan position	**06**	**07**	**08**	**09**	**10**	**11**
Quadrant 1	14	15	13	28	38	30
Quadrant 2	14	13	11	28	31	30
Quadrant 3	63	43	43	71	50	54
Quadrant 4	36	44	48	69	53	41

Table 4.3.1.3.2: **Number and percentage of CT missing in maxillary and mandibular Triadan positions.**

Triadan number	**06**	**07**	**08**	**09**	**10**	**11**	**Total**
Number of **maxillary** CT missing	28 (10.6%)	28 (10.6%)	24 (9.1%)	56 (21.2%)	69 (26%)	60 (22.6%)	265
Number of **mandibular** CT missing	99 (16%)	87 (14.1%)	91 (14.8%)	140 (22.8%)	103 (16.7%)	95 (15.5%)	615

Figure 4.3.1.3.3: **Number of maxillary and mandibular CT missing per Triadan position.**



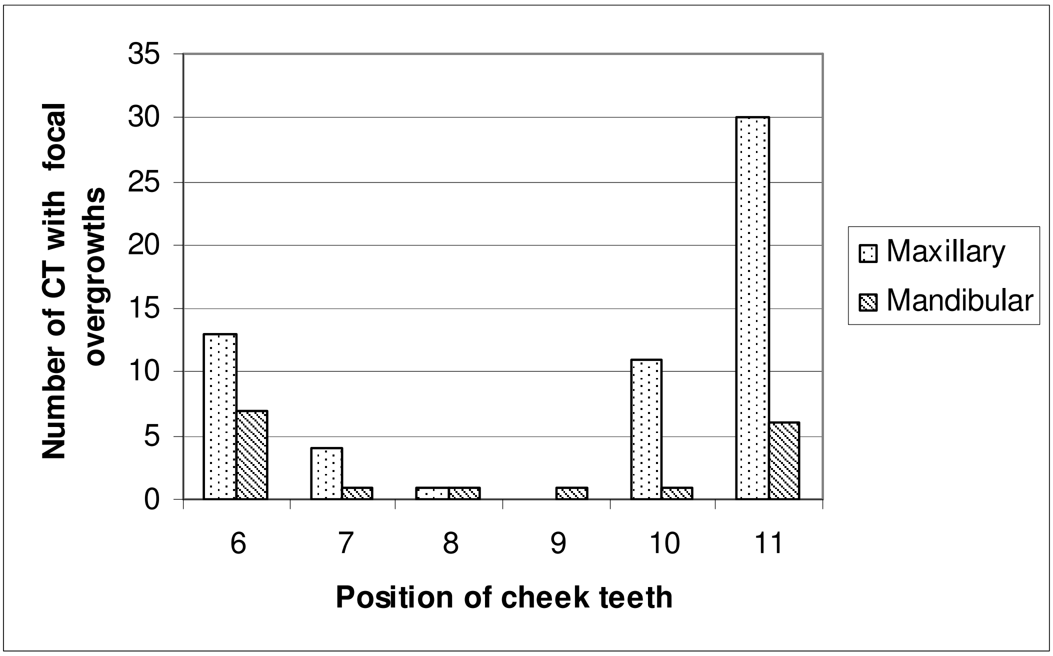
4.3.1.4 Focal overgrowths of cheek teeth

Only 13.5% (47/349) of donkeys had focal CT overgrowths. In these 47 donkeys there were a total of 76 CT focal overgrowths. The maxillary CT (59/76; 77.6%) had many more focal overgrowths than the mandibular CT (17/76; 22.4%). Most of these focal overgrowths were on the maxillary 11s (39.5%; 30/76), maxillary 06s (17.1%; 13/76), maxillary 10s (14.5%; 11/76), mandibular 06s (9.2%; 7/76) and mandibular 11s (7.9%; 6/76). Other teeth that had focal overgrowths included maxillary 07s and 08s, and mandibular 07, 08, 09 and 10s (Table 4.3.1.4.1 and Figure 4.3.1.4.1).

Table 4.3.1.4.1: **Number and Triadan position of maxillary and mandibular CT with focal overgrowths.**

Triadan number	**06**	**07**	**08**	**09**	**10**	**11**	**Total**
Number of **maxillary** teeth with focal overgrowths	13 (22%)	4 (6.8%)	1 (1.7%)	0	11 (18.6%)	30 (50.8%)	59
Number of **mandibular** teeth with focal overgrowths	7 (41.2%)	1 (5.8%)	1 (5.8%)	1 (5.8%)	1 (5.8%)	6 (35.3%)	17

Figure 4.3.1.4: **Number of maxillary and mandibular CT with focal overgrowths per Triadan position.**



4.3.1.5 Calculus of cheek teeth

The prevalence of calculus on the donkey skulls examined was 19.5% (68/349), with 67 donkeys having calculus on their maxillary CT and one donkey having calculus on its mandibular CT (411). A total of 217 maxillary CT had calculus in the 67 affected skulls.

The most common Triadan positions of maxillary CT with calculus were the 08s (34.6%) and 07s (30.9%) (Table 4.3.1.5.1 and Figure 4.3.1.5.1).

Table 4.3.1.5.1: **The number of maxillary CT in each Triadan position with calculus.**

Maxillary Triadan number	06	07	08	09	10	11	Total
Number of CT with calculus	35 (16.1%)	67 (30.9%)	75 (34.6%)	32 (14.7%)	6 (27.6%)	2 (1%)	217

Figure 4.3.1.5.1: **The number of maxillary CT with calculus in each Triadan position.**

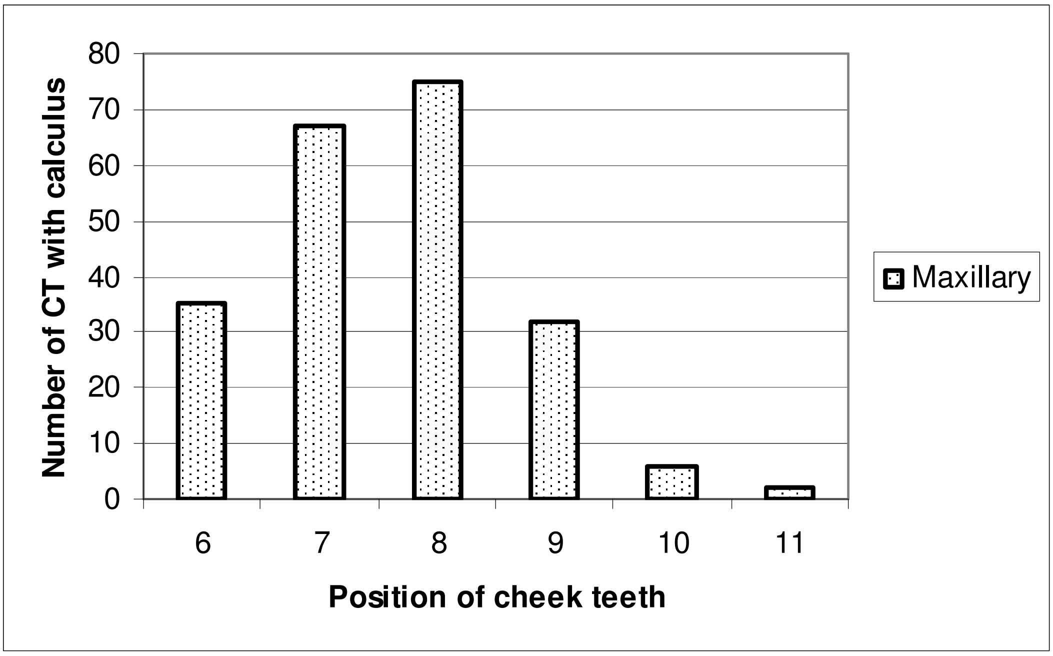
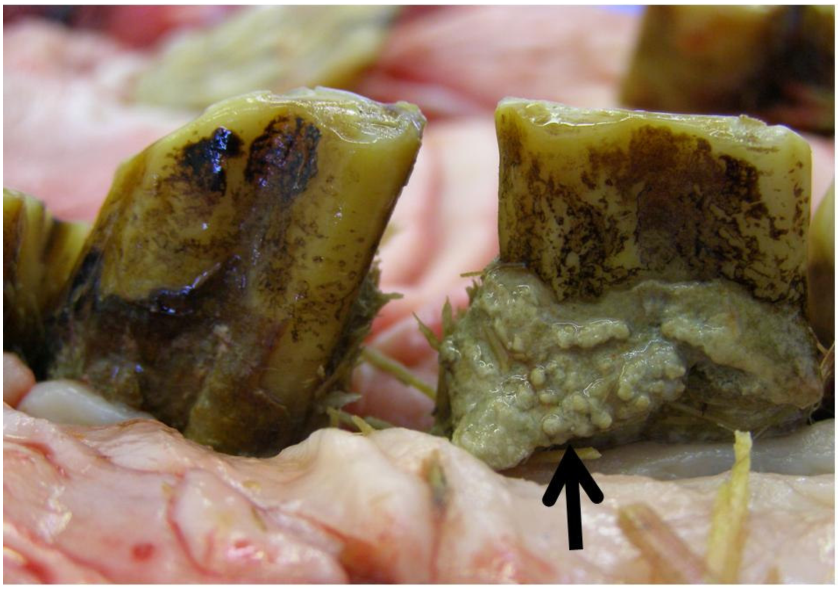


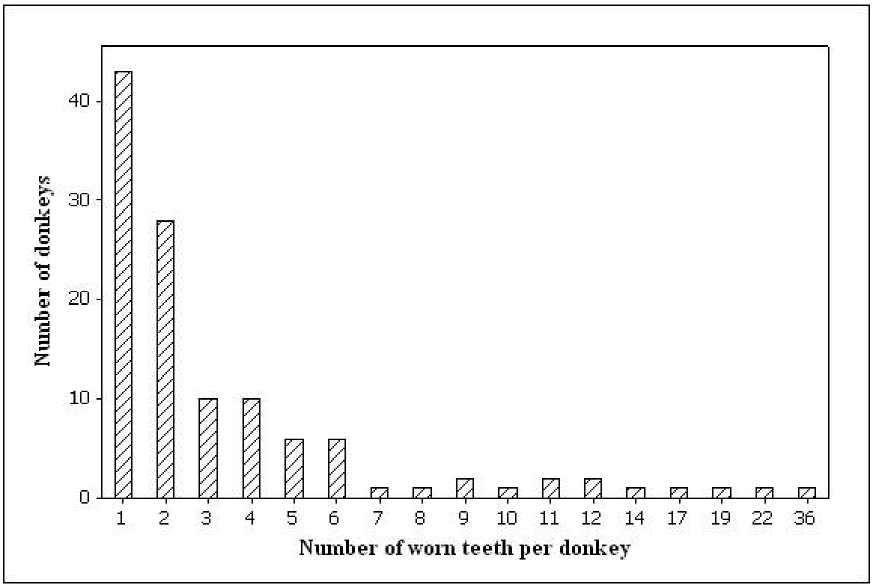
Figure 4.3.1.5.2: **Large calculus accumulation (arrow) on the buccal aspect of a maxillary cheek tooth in a 28 year old donkey. Also note that the 2 maxillary cheek teeth in the picture are drifting together after loss of the tooth between them.**



4.3.1.6 Worn teeth

A total of 118 donkeys (33.8%) had worn teeth (incisors and cheek teeth). The number of worn teeth per donkey varied from 1 – 36 (Table 4.3.1.6.1) with most donkeys only having a single worn tooth (36.4%; 43/118).

Figure 4.3.1.6.1: **The distribution of number of donkeys with specified number of worn teeth.**



One donkey had all its incisors and cheek teeth (36 teeth in total) worn. A total of 420 teeth were worn in all skulls. Incisors accounted for 20.5% of these (86/420), of which maxillary incisors (86%) were the preferentially worn. Only two donkeys had mandibular incisors worn and in these donkeys all the mandibular incisors, as well as the maxillary incisors were worn. In 9 out of 14 (64.3%) donkeys that had worn maxillary incisors, all the maxillary incisors were worn (54 incisors)

A total of 334 CT were worn, which constituted 79.5% of all worn teeth. Mandibular CT (214/334-64.1%) were more commonly worn than maxillary CT (35.9%). The most commonly worn CT was the maxillary 11 and the most common mandibular CT worn was the 06, followed by the 08 and 11 (Table 4.3.1.6.1; Figure 4.3.1.6.2; and Figure 4.3.1.6.3).

Table 4.3.1.6.1: **Number and Triadan position of worn maxillary and mandibular CT (and percentage of the number of worn teeth).**

Triadan number	**06**	**07**	**08**	**09**	**10**	**11**	**Total**
Number of **maxillary** teeth worn	24 (20.7%)	7 (6.0%)	4 (3.4%)	2 (1.7%)	5 (4.3%)	74 (63.8%)	116
Number of **mandibular** teeth worn	48 (22%)	34 (15.6%)	40 (18.3%)	25 (11.5%)	33 (15.1%)	38 (17.4%)	218

Figure 4.3.1.6.2: **Number and Triadan position of worn CT.**

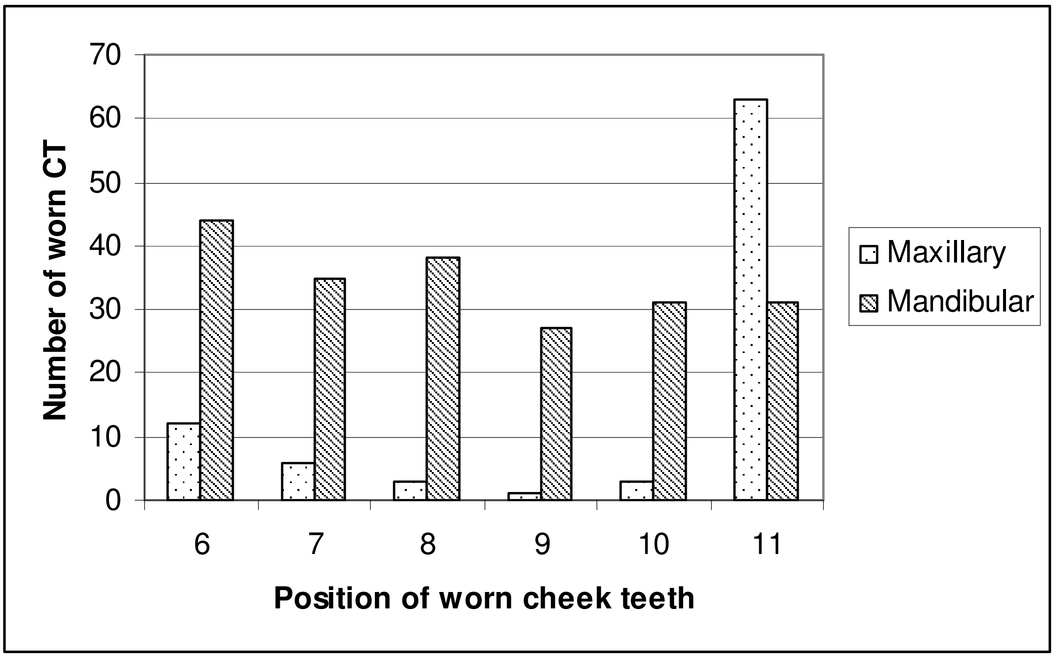


Figure 4.3.1.6.3: **Skull of a 28 year old donkey with excessive wear of both maxillary 11s. There is also a small diastema and food impaction associated with the worn 211. Note the lateral displacements of the 110 and 208.**

4.3.1.7 Sharp teeth, fractured teeth, soft tissue trauma and wear patterns

Significantly sharp teeth were only noted in 12 (3.4%) of the 349 donkey mouths examined. Sharp edges observed were predominantly present in individual teeth and were associated with overgrown CT caused by absence or displacement of the opposing teeth.

Fractured teeth (n = 17) were observed in 12 (3.4%) of the donkeys, with one fractured CT in 10 donkeys, two fractured CT in one donkey and 5 fractured teeth in the other donkey. These CT fractures consisted predominantly of small lateral or medial fragments. Eleven of the 17 fractured CT (64.7%) were mandibular CT and 6 (35.3%) were maxillary CT, with the mandibular 11s (n = 4) and 07s (n = 3) most commonly affected.

Dental-related oral soft tissue trauma was observed in 29 (8.3%) donkeys. These soft tissue injuries consisted predominantly of chronic cheek ulcers adjacent to sharp dental edges, displaced and overgrown teeth. Step mouth was observed in 18 (5.2%) and wave mouth in 15 (4.3%) of the donkeys examined.

4.3.2 Epidemiology of dental disease and association with systemic disease

4.3.2.1 Diastemata

Due to the high prevalence of CT diastemata in this population and also of other dental disorders that could predispose to diastemata (Collins and Dixon 2005; Dixon 2006), the relationship of diastemata to other dental disorders was investigated. The univariate analysis of diastemata and possibly inter-related CT disorders are summarised in Table 4.3.2.1.1.

***Table 4.3.2.1.1*: Numbers of donkeys with diastemata, and the dental disorders, age, time since last dental examination and feed management, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (with 95% confidence interval) for categorical variables and slope for numerical variables.**

	Total	**Donkeys with diastemata**	**%**	**χ^2 & P value**	**Odds ratio (95% CI)/ Slope**
No. of donkeys	349	297	85.1		
Binary variables					*Odds ratio*
Missing teeth	186	166	89.25	$\chi^2_1 = 6.09$;**P = 0.015**	2.11 (1.15 – 3.85)
No missing teeth	163	131	80.37		
Displaced teeth	148	138	93.24	$\chi^2_1 = 15.41$; **P < 0.001**	3.76 (1.81 – 7.77)
No displaced teeth	201	159	79.10		
Worn teeth	115	107	93.04	$\chi^2_1 = 10.11$;**P = 0.004**	3.19 (1.44 – 7.03)
No worn teeth	234	190	81.20		
Hooks	40	35	87.50	$\chi^2_1 = 0.27$; P = 0.61	1.29 (0.49 – 3.46)
No focal overgrowths	309	262	84.79		
Extra feeding	94	82	87.23	$\chi^2_1 = 0.49$; P = 0.48	1.28 (0.64 – 2.56)
No extra feeding	254	215	84.65		
Numerical variables					*Slope*
Number of missing teeth				$\chi^2_1 = 0.096$; P = 0.76	-0.009
Number of displaced teeth				$\chi^2_1 = 16.75$; **P < 0.001**	0.68
Number of worn teeth				$\chi^2_1 = 0.54$; P = 0.50	0.038
Time since last dental examination				$\chi^2_1 = 1.01$; P = 0.32	-0.022
Age				$\chi^2_1 = 20.59$; **P < 0.001**	0.099

There was a significantly positive association between the presence of diastemata and the presence of missing teeth, displaced teeth and worn teeth ($P < 0.15$). There was also a significant association with the number of displaced teeth and age ($P < 0.001$). There was no association between the time since the last dental treatment and the presence of diastemata ($P = 0.32$) (Table 4.3.2.1.1). Multivariable analyses were performed on the interaction of the significant factors identified in univariate analysis i.e. presence of displaced, missing, worn teeth, number of displaced teeth and age (Table 4.3.2.1.2).

***Table 4.3.2.1.2*: Multivariable models with diastemata as the response variable and missing teeth, displaced teeth, worn teeth, number of displaced teeth and age as the explanatory variables based on P value < 0.2 in the univariate analysis. The model was repeated with either the presence of displaced teeth or number of displaced teeth or both included as explanatory variables.**

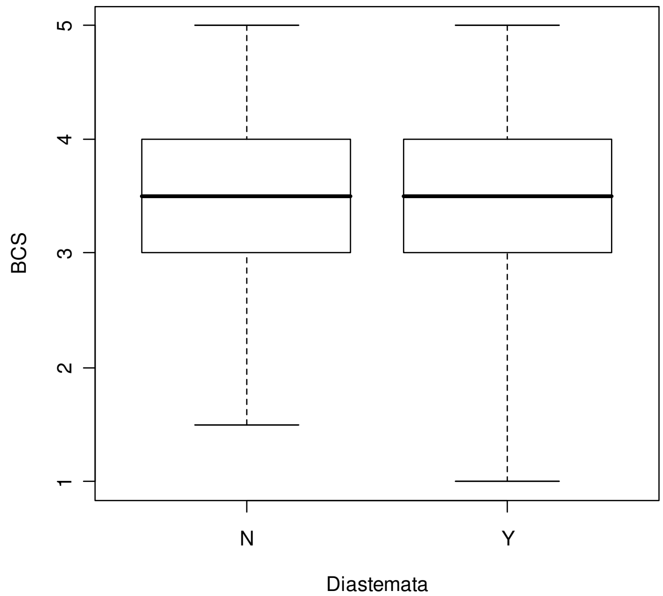
	P value of multivariable with displaced teeth	P value of multivariable with number of displaced teeth	P value of both	Final model
Binary variables				
Missing teeth	**0.014**	**0.014**	**0.014**	**0.014**
Displaced teeth	**<0.001**	NA	**< 0.001**	**< 0.001**
Worn teeth	**0.001**	**0.004**	**0.001**	**0.001**
Numerical variables				
Number of displaced teeth	NA	**< 0.001**	0.12	0.11
Age	**0.001**	**0.001**	**0.001**	**0.001**
Interactions				
Missing:Displaced	**0.02**	NA	**0.023**	**0.023**
Missing:Worn	1	0.75	0.61	NA
Missing: No. of displaced	NA	**0.02**	0.71	1
Missing: Age	**0.03**	0.54	0.43	0.65
Displaced: Worn	0.4	NA	0.48	NA
Displaced: No. of displaced	NA	NA	0.64	NA
Displaced: Age	0.9	NA	0.88	NA
Worn: No. of displaced teeth	NA	0.37	0.35	NA
Worn: Age	0.18	0.07	0.06	NA
No. of displaced teeth: Age	NA	0.65	0.74	NA

The presence of missing (P = 0.014), displaced (P < 0.001), worn teeth (P = 0.001) and age (P = 0.001) remained statistically significant in the multivariate analysis. The interaction between missing teeth and displaced teeth remained significant (P = 0.023), with diastemata in 68.8% of the skulls without any missing or displaced teeth, but 87.6% diastemata seen in the skulls with missing teeth but no displaced teeth.

When age group was used instead of estimated age exactly the same significantly associated variables and interactions between variables was found with a significant association of diastemata to age group ($P < 0.001$; $\chi^2_1 = 30.89$).

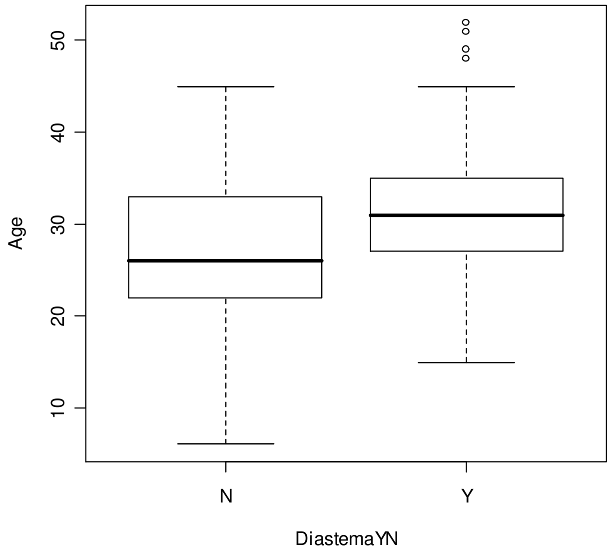
The median BCS for donkeys with and without diastemata was 3.5 and the Mann-Whitney test confirmed that there was no statistically significant difference ($P = 0.94$; Figure 4.3.2.1).

***Figure 4.3.2.1*: Boxplot of body condition score (BCS) of donkeys with (Y) and without (N) diastemata (dark horizontal lines represent median and boxes represent interquartile range).**



The mean age of donkeys with diastemata was 31 years and without diastemata was 27 years. This difference in age between donkeys with and without diastemata is statistically significant ($P < 0.001$; Figure 4.3.2.2; Table 4.3.2.1).

***Figure 4.3.2.2*: Boxplot of age of donkeys at the time of death that had diastemata (Y) and did not have diastemata (N).**



4.3.2.2 Colic

The univariate analysis of colic and related factors are summarised in Table 4.3.2.2.

Table 4.3.2.2.1: **A summary of the relationship between whether a donkey died of a colic-related death and specific dental disorders, age, time since last dental examination and feed management, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (with 95% confidence interval) for categorical variables and slope for numerical variables.**

	Total	**Colic-related death**	**%**	**χ^2 & P value**	**Odds ratio (95% CI)**
No. of donkeys	349	110	31.34		
Binary variable					*Odds ratio*
Diastemata	296	100	33.78	χ^2_1 = 5.01 ;**P = 0.04**	2.19 (1.06 - 4.56)
No diastemata	53	10	18.87		
Missing teeth	186	66	35.48	χ^2_1 = 2.92 ;P = 0.09	1.49 (0.94 – 2.36)
No teeth missing	163	44	26.19		
Displaced teeth	148	52	35.14	χ^2_1 = 1.55 ;P = 0.21	1.36 (0.85 – 2.11)
No displaced teeth	210	58	28.86		
Worn teeth	115	41	35.65	χ^2_1 = 1.34 ;P = 0.25	1.33 (0.82 – 2.13)
No worn teeth	224	69	30.80		
Focal overgrowths	40	9	22.50	χ^2_1 = 1.80; P = 0.18	0.60 (0.25 – 1.31)
No focal overgrowths	309	101	32.69		
Extra feed	94	31	32.98	χ^2_1 = 0.11; P = 0.74	0.68 (0.51 – 0.90)
No extra feed	254	79	31.10		
Numerical variables					*Slope*
Number of diastemata				χ^2_1 = 3.73; P = 0.05	0.07
Number of missing teeth				χ^2_1 = 3.83; P = 0.05	0.05
Number of displaced teeth				χ^2_1 = 0.04; P = 0.83	0.02
Number of worn teeth				χ^2_1 = 0.09; P = 0.77	0.01
Time since last dental examination				χ^2_1 = 0.17; P = 0.68	0.01
Age				χ^2_1 = 0.01; P = 0.94	-0.001

There was a statistically significant positive association between the presence of diastemata and colic as a disease (P = 0.04) (Table 4.3.2.2). No other factors (other dental disorders or time since last dental treatment) had a statistically significant influence on the prevalence colic (P > 0.05; Table 4.3.2.2). The univariate P value of the association of colic to age group was also statistically non-significant and not included in the multivariable (P = 0.93; $\chi^2_1 = 0.01$). The interaction of the presence of diastemata, missing teeth, focal overgrowths, number of diastemata, number of missing teeth and age were also included in the interaction with diastemata as the P value of the univariate analyses were < 0.2 (Table 4.3.2.2.1).

Table 4.3.2.2.2**: Multivariable models with colic as the response variable and diastemata, missing teeth, focal overgrowths, number of diastemata, number of missing teeth and age as the explanatory variables based on P value < 0.2 in the univariate analysis. The model was repeated with either the presence of diastemata and missing teeth or number of diastemata and number of missing teeth or all four variables included as explanatory variables.**

	P value with diastema and missing teeth	P value with number of diastemata and missing teeth	P value of all variables	Final model
Binary variables				
Diastemata	**0.03**	NA	**0.03**	**0.03**
Missing teeth	0.15	NA	0.15	NA
Focal overgrowths	0.24	0.18	0.24	NA
Numerical variables				
Number of diastemata	NA	**0.04**	0.22	0.37
No. of missing teeth	NA	**0.03**	0.07	**0.02**
Age	0.24	0.25	0.24	NA
Interactions				
Diastemata: Missing	0.91	NA	0.6	NA
Diastemata: Focal overgrowths	0.2	NA	0.22	NA
Diastemata: No. of diastemata	NA	NA	1	NA
Diastemata: No. of missing	NA	NA	0.13	NA
Diastemata: Age	0.33	NA	0.39	NA
Missing: Focal overgrowths	0.41	NA	0.30	NA
Missing: No. of diastemata	NA	NA	0.15	NA
Missing:No. of missing	NA	NA	0.09	NA
Missing: Age	0.10	NA	0.06	NA
Focal overgrowths: No. of diastemata	NA	0.41	0.15	NA
Focal overgrowths: No. of missing	NA	0.20	0.71	NA
Focal overgrowth: Age	0.06	**0.04**	0.05	NA
No. of diastemata: Age	NA	**0.01**	0.05	NA
No. of missing	NA	0.54	0.65	NA

The presence of diastemata remained statistically significantly associated with colic related illness in the multivariate analysis (P = 0.03) with all other variables still statistically insignificant (P > 0.15). However in the multivariate analysis of the numerical variables (number of diastemata and number of missing teeth), the number of missing teeth became statistically significant (P = 0.02).There was no significant interaction with the presence of diastemata and missing teeth (P = 0.74), displaced teeth (P = 0.44), worn teeth (P = 0.79) or the presence of focal overgrowths (P = 0.23) as associated factors with colic as a disease. However the interaction between missing teeth and displaced teeth was significant (P = 0.03) with 17.3% of the donkeys that died of colic related death having no missing or displaced teeth but 35.5% of the donkeys having missing teeth with no displaced teeth. All other interactions were statistically non-significant (P > 0.53).

4.3.2.3 Death from liver/pancreatic/metabolic disorders

The univariate analysis of liver disease-related death and related factors are summarised in Table 4.3.2.3.

Table 4.3.2.3: **A summary of the relationship between whether a donkey died of liver related death and specific dental disease disorders, and associated factors, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope for numerical variables.**

	Total	**Liver-related death**	**%**	**χ^2 & P value**	**Odds ratio**
Number of donkeys	349	52	14.90		
Diastemata	296	41	13.85	χ^2_1 = 1.57; P = 0.21	0.61(0.29 – 1.29)
No diastemata	53	11	20.75		
Missing teeth	186	23	12.37	χ^2_1 = 2.01; P = 0.16	0.65 (0.36 – 1.18)
No missing teeth	163	29	17.79		
Displaced	148	23	15.54	χ^2_1 =0.08; P = 0.77	1.09 (0.60 – 1.98)
No displaced teeth	201	29	14.43		
Worn teeth	115	13	11.30	χ^2_1 = 1.82; P = 0.177	0.64 (0.33 – 1.25)
No worn teeth	234	39	16.67		
Focal overgrowths	40	6	15.00	χ^2_1 = 0 ; P = 0.99	1.01 (0.4 – 2.55)
No focal overgrowths	309	46	14.89		
Extra feed	94	15	15.96	χ^2_1 =0.10; P = 0.75	1.11 (0.58 – 2.14)
No extra feeding	254	37	14.57		
Number of diastemata				χ^2_1 = 1.36; P = 0.24	-0.06
Number of displaced teeth				χ^2_1 = 0.63; P = 0.43)	0.09
Number of worn teeth				χ^2_1 = 0.08; P = 0.257	0.006
Number of missing teeth				χ^2_1 = 0.97; P = 0.89	-0.038
Time since last dental treatment				χ^2_1 = 0.004; P = 0.95	-0.001
Age				χ^2_1 = 2.84; P = 0.092	-0.036

There was no statistically significant association between liver disease as an illness and the presence of dental disorders, age, supplemental feed and time since previous dental treatment ($P > 0.092$; Table 4.3.2.3). Multivariate analysis was only performed on presence of missing and worn teeth ($P < 0.2$) and they remained statistically insignificant ($P > 0.16$) with no significant interaction ($P = 0.58$).

4.4. Discussion

The prevalence of dental disorders in horses has been estimated to be between10 to 80% (Anon 1965; Baker 1979; Uhlinger 1987; Wafa 1988; Kirkland *et al.* 1994), which was lower than the 93% prevalence of dental disorders recorded in this donkey study. The prevalence of abnormalities of CT wear in horses has been recorded as 17% (Wafa 1988) and 29% (Baker 1979), which is also lower than the 43.6% CT wear (worn teeth and focal overgrowths) abnormalities observed here. It has to be remembered that the median age of the population studied here (31 years) is much older than in the above two equine studies looking at abnormalities of dental wear (median age < 20). Another study examined 500 horse skulls with an age range of 6 months to 30 years and found dental disorders present in over 80% (Kirkland *et al.* 1994) which is not dissimilar to the prevalence of 93% observed in this donkey study.

Diastemata were very common in this population of older donkeys with a prevalence of 85%. Diastemata were recorded in 4.6% of 349 referred younger horses with CT disorders and were most commonly observed between 09/10 (Dixon *et al.* 1999b). The prevalence of diastemata in horses was reported to be about 20% at post mortem examination (Brigham and Duncanson 2000b). Most donkeys with diastemata had diastemata in both maxillary and mandibular arcades (47.1%), although mandibular diastemata were more common (64.4% of diastemata). The most common sites for diastemata in the maxillary arcade was between 06/07 and 07/08 whereas the most common site in the mandibular arcade was between 09/10 and 10/11. Most of the diastemata (77.3%) were associated with CT displacements and/or missing CT.

A total of 42.5% of donkeys had CT displacements, with most having only one CT displaced and maxillary CT displacements were more common (62.1% of CT displacements). In a study of 355 horse skulls, Wafa (1988) found a prevalence of 7.2% displaced CT. A study of 349 referred horses with CT dental disorders found that 6.6% had displaced CT (Dixon *et al.* 1999b), with mandibular CT displacements more common (70% of displacements) The Triadan10s were the most commonly displaced mandibular CT and the 09s in the maxillary CT (Dixon *et al.* 1999b). Donkeys appear to have a similar pattern of displacements with the 09s and 10s most commonly displaced in both the maxillary and mandibular rows. Developmental displacements are more commonly seen in the 09-11s as they have no precursor teeth that open up the space for eruption (Miles and Grigson 1990), and this could account for the high number of displaced 09-11 CT observed here. The possibility that many of the displacements observed here in donkeys were acquired, including secondary to periodontal disease, must also be considered.

The 10s were the most common maxillary CT missing whilst the 09s were the most commonly affected mandibular CT. This is to be expected as the 09s and 10s erupt first and hence should wear out before the other CT. As well as loss due to natural wear, loss of teeth is often associated with advanced periodontal disease, which is more common in horses over 12 years of age (Baker 1979; Kirkland *et al.* 1994). A small study of dental disease in the Indian ass also observed missing CT in 2 out of 3 skulls examined (Rajput *et al.* 1999).

Only 47 (13.4%) donkey skulls had focal CT overgrowths, which were more common in maxillary CT (77.6%) with the Triadan 11 followed by the 06 positions most commonly affected. A clinical survey of 100 horses found a higher prevalence (37%) of focal dental overgrowths than the current study, with maxillary 06s and mandibular 11s most commonly affected (Brigham and Duncanson 2000a). It has also been noted that focal overgrowths and sharp points are more commonly identified in horses under 10 years of age (Kirkland *et al.* 1994). The study by Dixon *et al.* (1999) observed a 5.6% prevalence of focal dental overgrowths in 349 referred horses with CT disorders with the maxillary 06s and mandibular 11s most commonly affected. This is due to the fact that many horses have a slight overjet or overbite that results in the maxillary CT rows being situated slightly rostral to the mandibular CT rows, in turn leading to the caudal mandibular 11s and rostral maxillary 06s developing focal overgrowths (Dixon *et al.* 1999a; Dixon *et al.* 1999b). It is interesting to note that this study found maxillary CT 11 overgrowths to be very common. More surprisingly, these overgrowths often occurred on the more rostral aspect of the 11 with the caudal aspect of the CT worn to gingival level. This maxillary 11 wear abnormality could be partly attributed to the marked curve of Spee observed in donkey mandibular arcades.

Calculus was recorded on the CT in 3.4% horse skulls (12 /355) (Wafa 1988), which is lower than the prevalence of 19.4% noted in donkeys in this study. Calculus was predominantly present on the maxillary CT with mandibular CT calculus only observed in 1 out of 68 donkeys with this disorder. Calculus was most commonly present on the 08s (34.6%) and 07s (30.9%). Likewise, the most common site of calculus deposition in horses was on the maxillary premolars (06s - 08s) with only 2 cases noted on the maxillary molars (9s-11s) and none on mandibular CT (Wafa 1988). It is believed that these teeth were most commonly involved as they are close to the salivary duct opening which will provide the minerals for calculus formation. Furthermore, this is also where food is likely to accumulate in donkeys that have impaired masticatory action secondary to concurrent dental disease.

A total of 118 (33.6%) donkeys had worn teeth, with usually only a single tooth affected. The maxillary incisors were more commonly worn than mandibular incisors, and affected donkeys usually had all their maxillary incisors affected. Mandibular CT accounted for 70.1% of CT worn with 06s, 07s and 08s most commonly affected. In contrast the 11s were the most common maxillary CT showing this disorder. A high prevalence of worn teeth was expected in this population of aged donkeys as cupping out of teeth has been described in old horses where enamel infoldings towards the centre of the tooth or infundibula have worn away and the softer dentine gets worn away quickly (Becker 1962; Lowder and Mueller 1998; Dixon 2002).

Other dental disorders such as individual sharp teeth, fractured teeth, step mouth, wave mouth and dental induced oral soft tissue trauma had a low prevalence in this population (< 8.3%). The prevalence of individual sharp points noted here is much lower than the 72% sharp points reported equine post mortem studies (Becker 1962; Brigham and Duncanson 2000b), but the sharp points observed in these horses would have included the sharp enamel points on the buccal aspect of maxillary CT and lingual aspect of the mandibular CT associated with wear of CT during mastication. It has been noted that sharp points and soft tissue ulceration is more likely to occur in younger horses (Kirkland *et al.* 1994), which is confirmed by low prevalence of sharp enamel points observed in this aged population of donkeys.

Colic-related disease was the most common illness that necessitated euthanasia of donkeys at The Donkey Sanctuary over the 18 month period of this study. Previous studies have shown a relationship between the presence of dental disease and colic (White 1990; Cox *et al.* 2007). This study has shown that the presence of diastemata was highly associated with colic-related disease ($P = 0.03$, $OR = 2.194$). In contrast, the presence of missing, displaced or worn teeth were not significantly associated with colic as a disease ($P > 0.09$), and furthermore, there was no interaction between these three dental disorders and diastemata associated with colic as a illness. This study confirms the importance of diastemata as a major dental disorder in equids, not only as a painful oral condition but also as a likely contributing factor to colic. This relationship might be confounded by the need for supplemental feeding in equids with diastemata, because supplemental feeding has also been shown to be a risk factor for colic (Tinker *et al.* 1997). The population of donkeys examined this study was very old (median age 31) and age has also been shown to be a large risk factor for colic in equids (Reeves *et al.* 1989; Tinker *et al.* 1997) and so it is very likely that age also contributed to the large number of colic- related deaths recorded in this study. The association of dental disease to liver/pancreatic/metabolic disease was also investigated because hyperlipaemia is very common in donkeys and causes liver changes that are apparent at post mortem. Any factor which contributes to anorexia may result in hyperlipaemia, however no association with dental disease and liver disease was found in this study.

Missing teeth, displaced teeth and worn teeth were also significantly associated with diastemata ($P < 0.014$). In addition there was a positive association between the number of displaced maxillary CT and diastemata ($P < 0.001$) and number of worn teeth ($P = 0.013$) and diastemata. There was also an interaction between missing and displaced teeth associated with diastemata, as there was a significantly higher percentage of diastemata in donkeys with missing teeth but no displaced teeth. The loss of teeth allows drifting of the remaining CT in rostral and caudal directions and thus can predispose to diastemata formation (Collins and Dixon 2005; Dixon and Dacre 2005; Dixon 2006). Similarly, a displaced CT creates a space between it and the adjacent, normally aligned CT and so allows for the impaction of food material and the formation of clinically significant secondary diastemata at such sites (Collins and Dixon 2005; Dixon 2006). However, severe periodontal disease associated with diastemata may damage the periodontal ligament, possibly causing loosening of the CT and an acquired displacement. Thus this significant association between displaced teeth and diastemata may be as a result displaced teeth causing diastemata, or diastemata leading to periodontal disease causing CT to displace. Worn teeth are typically seen in geriatric equids associated with smooth mouth (complete wearing out of the enamel) and thus may occur in association with senile diastemata (apical narrowing of older teeth creating spaces between adjacent CT) (Dixon 2006) and not necessarily be a risk factor.

As expected diastemata were more common in the older donkeys (mean age with diastemata 31years, without diastemata 27years) in this aged population ($P = 0.001$). Old age can therefore be regarded as a risk factor for the presence of diastemata. Lower body condition scores were also shown to be associated with older age (mean age of CS 1 was

34, of BCS 5 was 28; P = 0.005). The mean body condition score of donkeys given supplemental feed (3.2) was statistically significantly lower than donkeys not receiving supplemental feed (3.5) (P = 0.008). This small difference could be due to the close monitoring that donkeys receive at The Donkey Sanctuary and any donkey that has even mild weight loss will have its diet supplemented, and will be maintained on extra feed until its body condition score improves. There was no significant difference in the percentage of dental disease in donkeys given supplemental feed (96.8%) and donkey not given supplemental feed (92.5%). There was no significant association with extra feeding to either colic-related disease or the presence of diastemata.

This appears to be the first extensive post mortem study examining dental disorders in donkeys. Although this study was performed on a predominantly aged donkey population, the recognised dental disorders are very similar to those described in the horse. In particular the high prevalence of the very painful disorder of diastemata recorded in donkeys highlights the importance of regular dental care to maintain oral health in aged patients. This study also examined the association between specific dental disorders and specific disease syndromes, in particular colic related death. The findings present strong evidence that the presence of CT diastemata is a significant risk factor for the development of colic in geriatric donkeys. There was also a strong association between the presence of missing, displaced and worn CT to the presence of CT diastemata. The identification of risk factors may allow prophylactic dental treatment to be implemented before the onset of colic or progression of CT diastemata.

Chapter 5: Detailed investigation of dental caries and pulpar exposure using CAT, histology and SEM

5.1 Introduction

Infundibular cemental caries has long been recognised in horses (Colyer 1906; Hofmeyr 1960; Honma *et al.* 1962; Baker 1974), and more recently, dental disorders such as dental caries and apical infections with pulpar exposure have been investigated more extensively (Dixon 1997; Kilic *et al.* 1997d; Mitchell *et al.* 2003; Dacre 2005b; Johnson 2006). Infundibular caries has been recognised with increasing frequency and have been confused with areas of cemental hypoplasia on clinical examination (Kilic *et al.* 1997d; Fitzgibbons 2007). Clinical descriptions of severe peripheral and generalised caries in groups of horses in Sweden, Hong Kong and Mexico with dietary aetiologies have also recently been reported (P.M.Dixon and M. Hernandez-Gil, personal communication). No detailed studies on infundibular caries and pulpar exposure have been performed in donkeys. The aim of this study was to perform computed axial tomography (CAT), histology and SEM on cheek teeth with these disorders in donkeys and identify the relevant pathological changes.

5.2 Materials and methods

The disarticulated skulls of 54 donkeys, estimated age 8 – 43 years (median 28 years), that were euthanased on humane grounds for non-dental reasons were examined and all dental abnormalities were recorded on a customised dental chart (Appendix 1), and digitally photographed. The presence or absence of occlusal pulpar exposure for each pulp horn of each tooth was recorded, as was the presence of infundibular caries in maxillary CT. Infundibular caries were defined as infundibula with carious lesions (erosions and dark discoloration) of the cementum with or without extension of caries to the enamel and dentine, as based on visual assessment. The presence of periodontal disease around each tooth was noted and graded using an index based on the percentage of loss of attachments as used by Wiggs and Lobprise (1997c): Stage 0 = no attachment loss; stage 1 = gingivitis; stage 2 = early periodontal disease (up to 25% attachment loss); stage 3 = moderate (25 – 50% attachment loss); stage 4 = severe (greater than 50% loss).

Sixteen CT with pulpar exposure and caries chosen at random were extracted and fixed in 10% formalin for the following examinations. Computed axial tomography (CAT) was performed on 4 teeth from 3 donkeys, decalcified histology on 8 teeth from 5 donkeys and scanning electron microscopy (SEM) on 4 teeth from 3 donkeys, with all of these teeth having infundibular caries and/or pulp exposure or peripheral caries.

5.3 Results

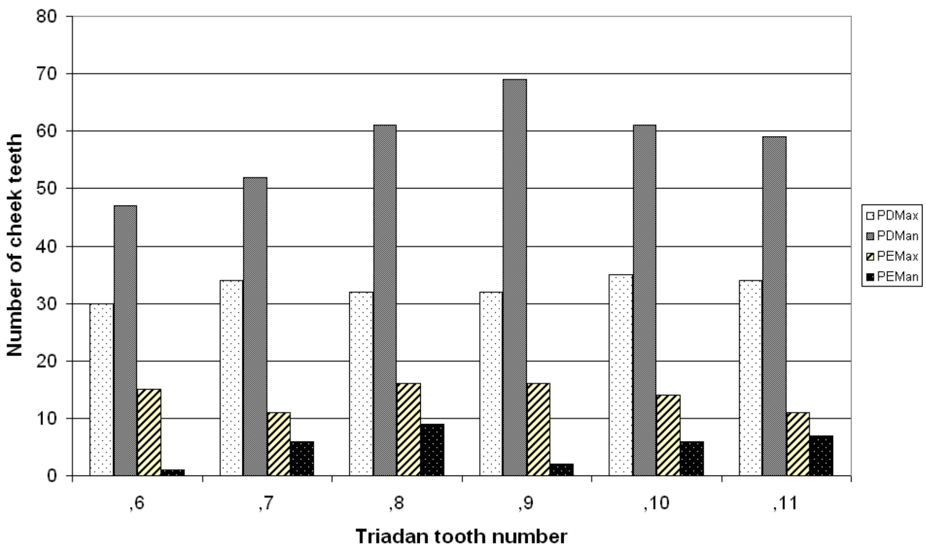
Dental disorders were present in 96% (52) of the skulls examined. Occlusal pulpar exposure was present in some teeth in 51.8% (28) of donkeys, with a total of 114 teeth having one or more pulp horns occlusally exposed. Maxillary cheek teeth were more commonly affected (72.8% of all occlusally exposed teeth) than mandibular cheek teeth (27.2%). The number of teeth with pulp horns affected per donkey ranged from 1 – 15 (median 3). The number of pulp horns affected per tooth ranged from 1 – 4 with 69.3% affecting one pulp horn, 25.4% two pulp horns, 4.4% three pulp horns and 0.9% four pulp horns. No specific Triadan tooth position appeared to be preferentially affected with pulpar exposure (Table 5.3.1; Figure 5.3.1.).

Table 5.3.1: **The number of teeth per Triadan position affected with pulpar exposure in maxillary and mandibular cheek teeth.**

Triadan tooth position	06	07	08	09	10	11	Total
Maxillary cheek teeth	15	11	16	16	14	11	83
Mandibular cheek teeth	1	6	9	2	6	7	31

Infundibular caries were present in 27.8% of the donkeys and the number of teeth with caries per donkey ranged from 1 – 6 (median 2). Periodontal disease was present in 94% of the donkey skulls. A total of 548 cheek teeth had associated periodontal disease which was more prevalent in the mandibular (64.1%) than maxillary (35.9%) cheek teeth. Intercurrent dental disorders were present in 47.6% (261) of cheek teeth with periodontal disease, in particular: diastemata (39.2%), displaced teeth (4.7%) or both (3.6%). Diastemata and displaced teeth were observed in 96.3% and 85.2% respectively in all the donkeys examined.

Figure 5.3.1: **Barplot illustrating the Triadan position distribution of periodontal disease and pulpar exposure in maxillary and mandibular cheek teeth of 54 donkey skulls. PD = periodontal disease; PE = pulpar exposure; Max = maxillary; Man = mandibular.**



Most (79.9%) periodontal disease (in both maxillary and mandibular cheek teeth) was classified as grade 1 or 2. This is reflected in a median periodontal grade of 1 or 2 for cheek teeth in each Triadan position (Figure 5.3.2; Table 5.3.2).

Figure 5.3.2: **Barplot of the number of mandibular and maxillary cheek teeth with periodontal disease in each grade.**

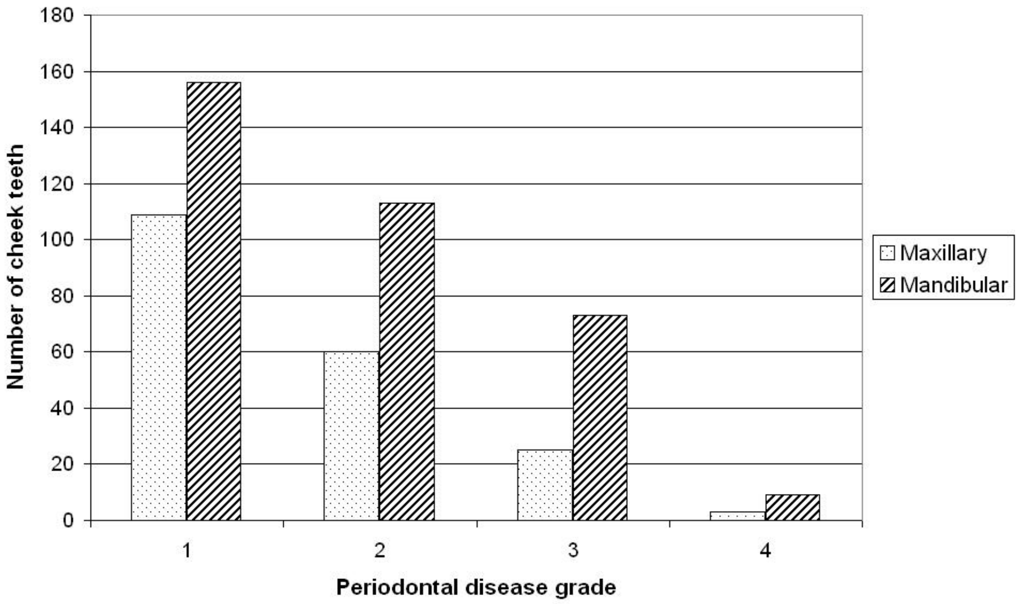


Table 5.3.2: **Total number of teeth with periodontal disease and number of teeth in each periodontal disease grade (1- 4) in each Triadan position.**

Triadan tooth number	Total number with periodontal disease	Grade 1	Grade 2	Grade 3	Grade 4	Median grade
106	13	7	5	1	0	1
107	15	10	3	2	0	1
108	16	9	2	5	0	1
109	17	8	7	2	0	2
110	16	10	3	2	1	1
111	19	13	5	1	0	1
206	17	11	6	0	0	1
207	19	9	10	0	0	2
208	16	9	4	3	0	1
209	15	6	7	2	0	1
210	19	8	4	5	2	2
211	15	9	4	2	0	1
306	21	12	6	3	0	1
307	25	15	7	3	0	1
308	29	10	8	9	2	2
309	37	16	9	12	0	2
310	30	11	10	6	3	2
311	24	13	7	4	0	1
406	26	15	8	3	0	1
407	27	9	15	3	0	2
408	34	10	15	8	1	2
409	32	12	9	10	1	2
410	31	14	8	7	2	2
411	35	19	11	5	0	1

5.3.1 Computed axial tomography

The age of donkeys, Triadan tooth number and pathological changes of each tooth examined by CAT is tabulated in Table 5.3.1.1.

Table 5.3.1.1: **The age of donkey, Triadan position and dental abnormality of each tooth having CAT imaging is tabulated. The identity of affected pulp horn(s) is in brackets.**

Donkey age (years)	**Triadan tooth number**	**Dental pathology**
26	207	Pulpar exposure (pulp 2)
39	211	Pulpar exposure (pulps 1&2)
34	109	Infundibular caries and pulpar exposure (pulp 2)
34	209	Infundibular caries and pulpar exposure (pulps1&2)

The CAT images of the 207 clearly illustrate the absence of secondary dentine within the exposed pulp horn (Figure 5.3.1.1 and 5.3.1.2).

Figure 5.3.1.1: **CAT images of an aged CT 207 with pulp number 2 exposed, that appears as linear areas of hypodensity (solid white arrows) as compared to the other pulp horns. Note the infundibulum on the left image (dashed white arrow).**

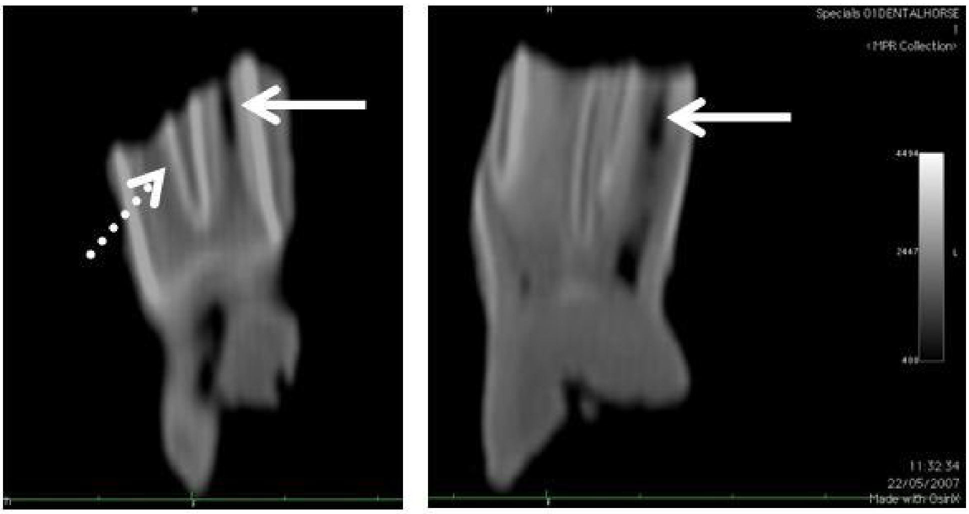
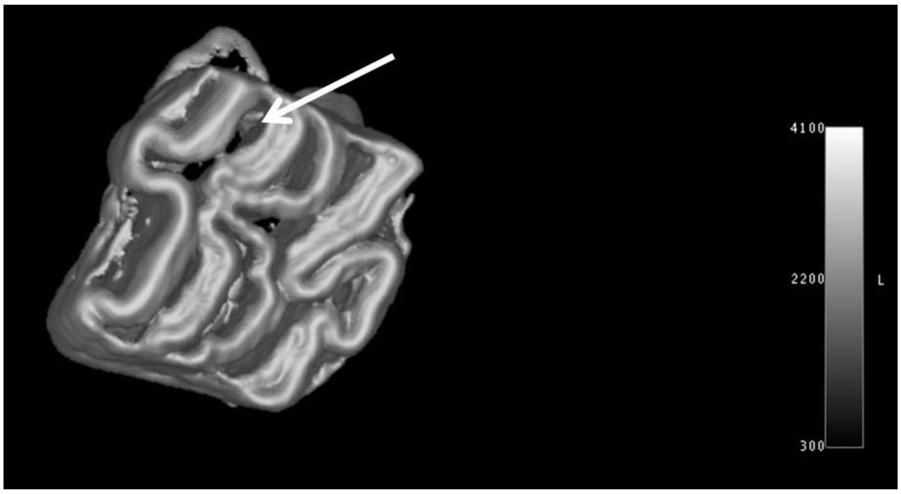
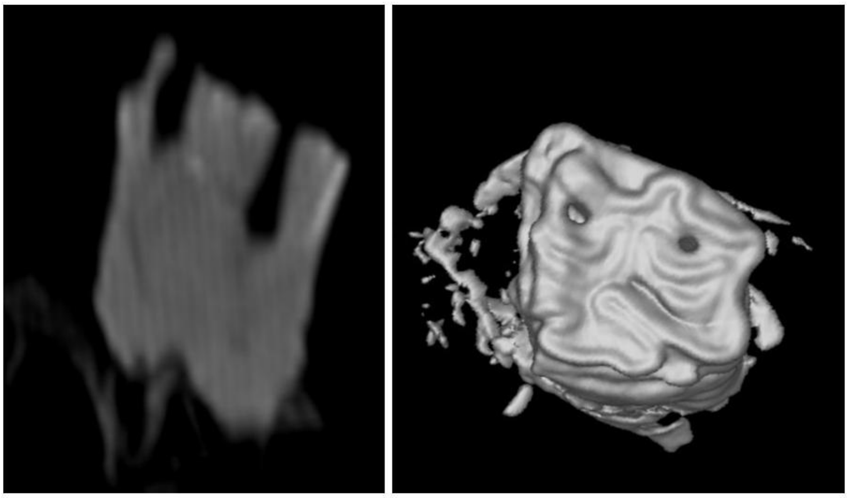


Figure 5.3.1.2: **CAT image of the same 207 with pulpar exposure of pulp horn 2 (Figure 5.3.1.1.) with 3D reconstruction showing defective secondary dentine on the occlusal surface of pulp horn 2 (white arrow).**



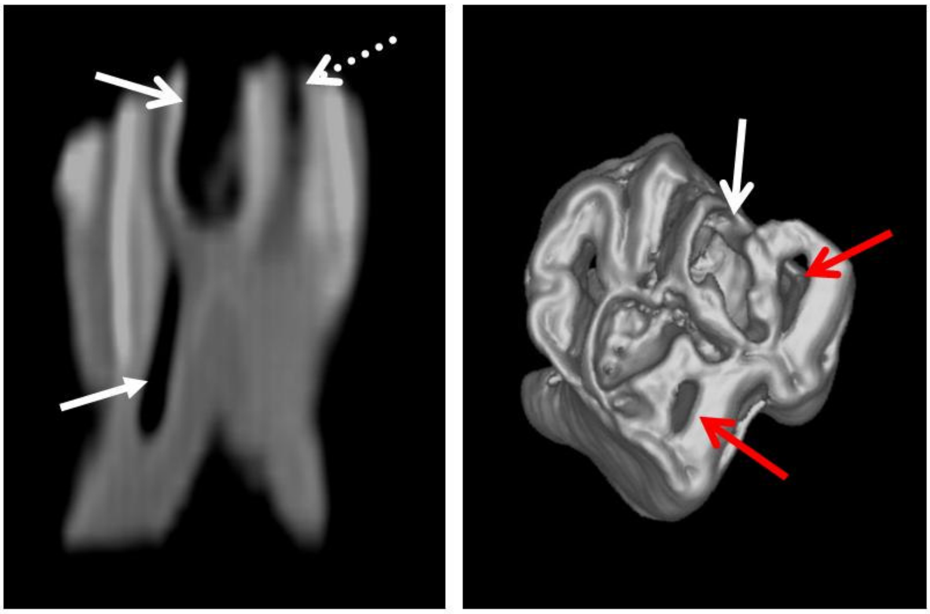
Similarly, occlusal exposure of the pulp horns 1 and 2 is clearly visible in a 211 with secondary dentine absent centrally over these pulps at the occlusal surface (Figure 5.3.1.3)

Figure 5.3.1.3: **CAT images of a 211 with pulpar exposure of pulp horns 1 and 2 in both multiplanar reconstruction (MPR) (left) and 3D (right) reconstructed images.**



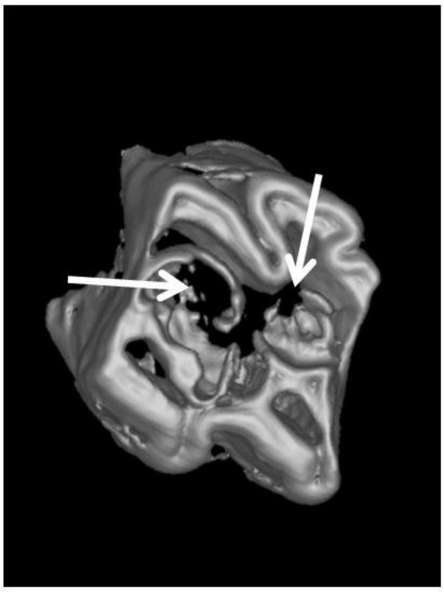
On CAT images, infundibular caries appeared as absence of cementum with the resultant area of hypodensity similar to soft tissue density such as pulp. Sections of the enamel lining of affected infundibula showed some loss of distinct attenuated outline indicating some carious destruction and/or decalcification of enamel (Figure 5.3.1.4).

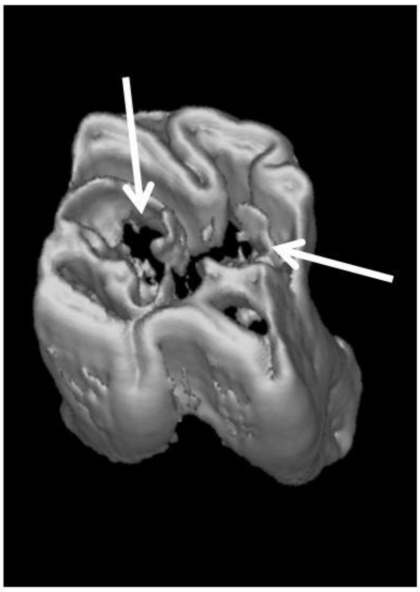
Figure 5.3.1.4: **MPR (left) and 3D reconstructed image with soft tissue density removed (right) of a 109 with caries of both infundibula (open arrow). Also note the exposed pulp horns (red arrows) and viable apical pulp horn (solid arrow).**



The infundibular caries in the 209 also had enamel decalcification/destruction of its infundibular enamel (Figure 5.3.1.5).

Figure 5.3.1.5: **Three dimensional reconstructed images only showing dental tissue of higher density (2500 - 3071 Hounsfield units), thereby predominantly containing enamel structures. Both images show the loss and/or demineralisation of normal infundibular enamel.**





5.3.2 Decalcified histology

Decalcified histology was performed on 8 sections (8 teeth) from 5 donkeys. Due to the old age and subsequent sclerosis (excess mineralisation) of these teeth, decalcification did not soften these specimens enough to allow most histological sections to be cut with a microtome and only 3 sections were successfully processed (Table 5.3.2.1).

Table 5.3.2.1: **Details of teeth examined using decalcified histology; age of the donkey from which the tooth was extracted, Triadan position of affected tooth and pathological changes present.**

Donkey age (years)	Triadan position	Dental pathology
31	107	Pulpar exposure
33	109	Infundibular caries
34	108	Pulpar exposure

Figure 5.3.2.1: **(a) Decalcified H&E longitudinal section of 107 in 31 year old donkey showing occlusal exposure of 2 pulps that are filled with vegetable matter. (b) The apical aspect of the pulp cavity is filled with necrotic pulp and vegetable matter (arrow) but is still surrounded by normal –looking regular secondary dentine (RSD) (x 40).**

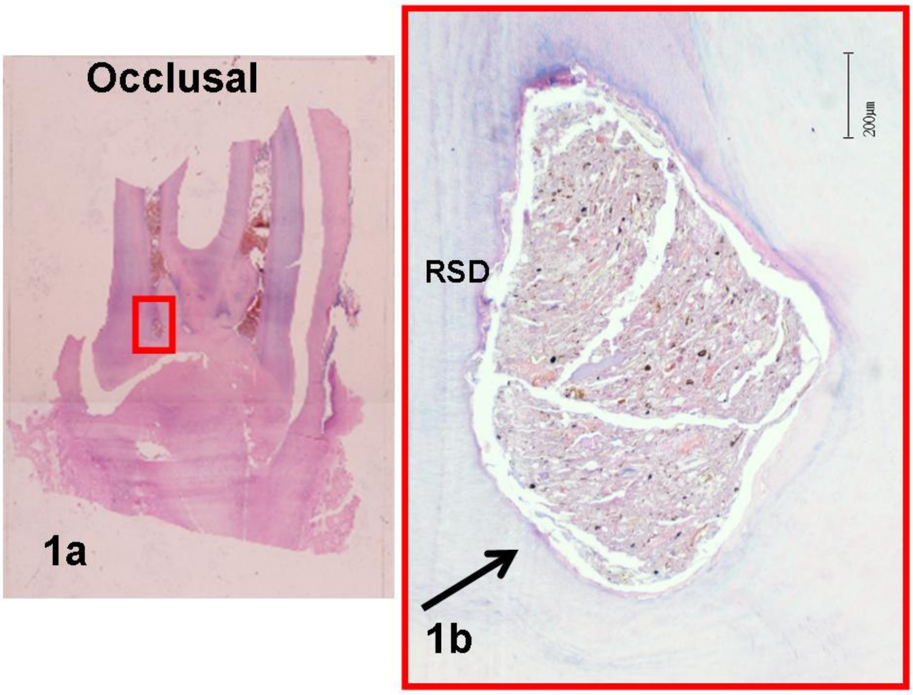


Figure 5.3.2.2: **Higher magnification of Figure 5.3.2.1b at the more occlusal aspect of the pulp horn illustrating the irregular thin layer of predentine (solid arrow) and irregular dark line of mineralization (dashed arrow) that formed prior to necrosis of the pulp. Hyaline like material can be seen within the pulp (open solid arrow), which may be remnants of a blood vessel i.e. collagen or dentine. RSD = regular secondary dentine; P = pulp (x 200).**

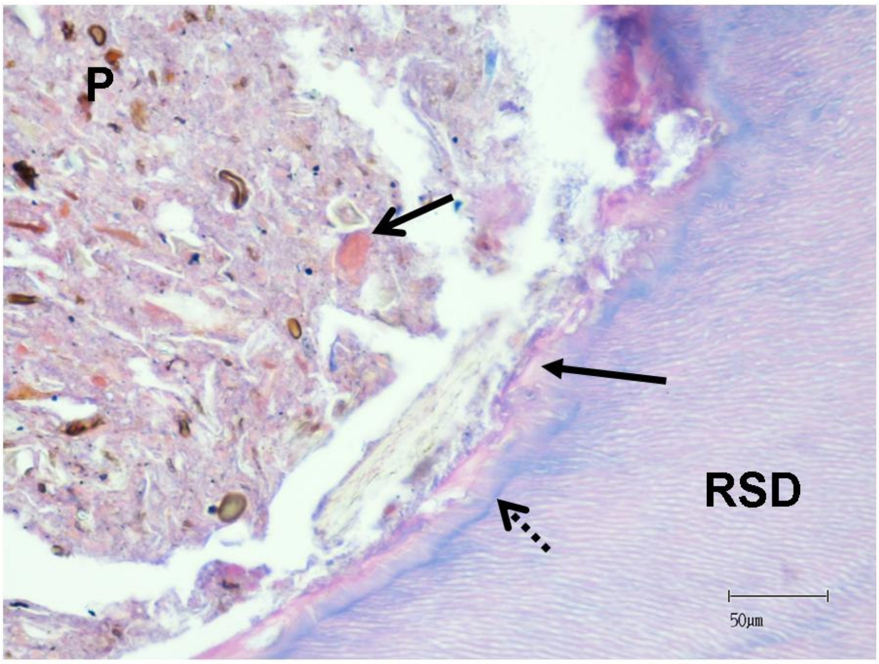


Figure 5.3.2.3: **Longitudinal section of the occlusal aspect of the tooth shown in figures 5.3.2.1 and 5.3.2.2 illustrating pulp exposure with the presence of plant material (arrows) in the remnant of the pulp horn. Note the lack of occlusal secondary dentine within the pulp cavity indicative of pulp disease for a prolonged period. RSD = regular secondary dentine (x 40).**

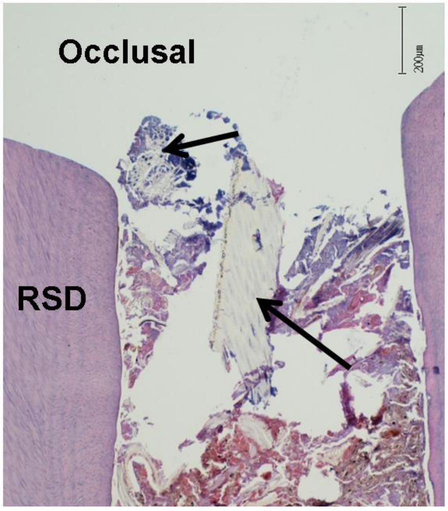


Figure 5.3.2.4: **Higher magnification of the pulp horn of cheek tooth 108 illustrating the poorly defined irregular predentine layer (open arrow). On the pulpar aspect of the predentine layer there is a layer of degenerate cells that are likely the odontoblast cell remnants, which indicates that the pulpar death is recent in contrast to the 107 from the 31 year old donkey. Also note the presence of pioneer organisms (bacteria) in the dentinal tubules (closed arrow). RSD = regular secondary dentine (x 400).**

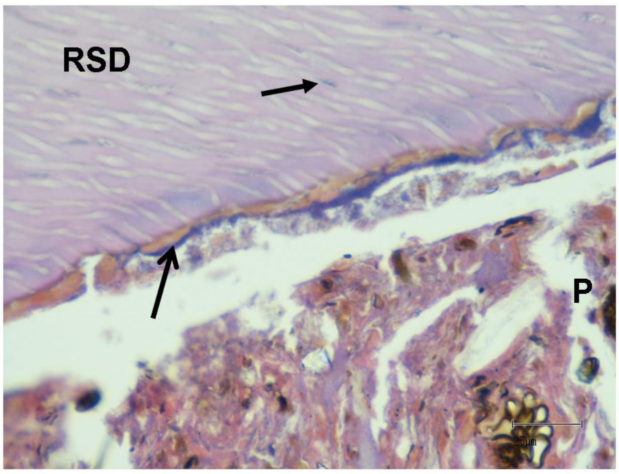


Figure 5.3.2.5: **Longitudinal section of the apical area of the pulp (108 from a 34 year old donkey) with occlusal pulp exposure. Note the mineralization lines (arrows) in the regular secondary dentine (RSD) and the viable pulp (P) despite the occlusal exposure. The arrow heads show separation of the pulp from the predentine ("wheat-sheaving") which is a slide preparation artefact (x 40).**

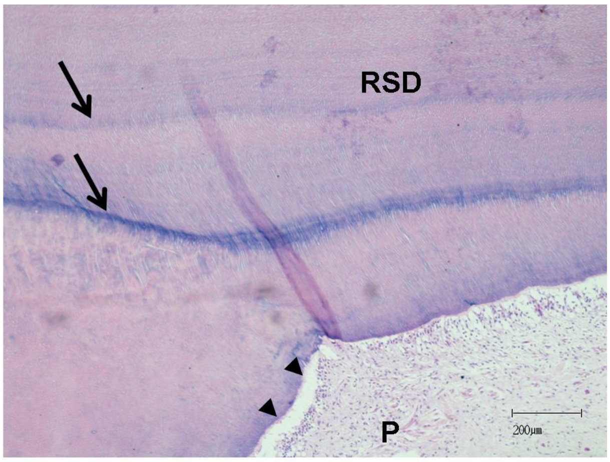


Figure 5.3.2.6: **Higher original magnification of Figure 5.3.2.5 showing a layer of odontoblast-like cells (open head arrow) that are artefactually separated from the predentine. Note that the odontoblast-like cells are not of normal columnar appearance or in well organized rows, which may be a preparation artefact. The more centrally located pulp contains much collagen, fibroblasts, undifferentiated mesenchymal cells and blood vessels (dashed arrow). RSD = regular secondary dentine; P = pulp (x 100).**

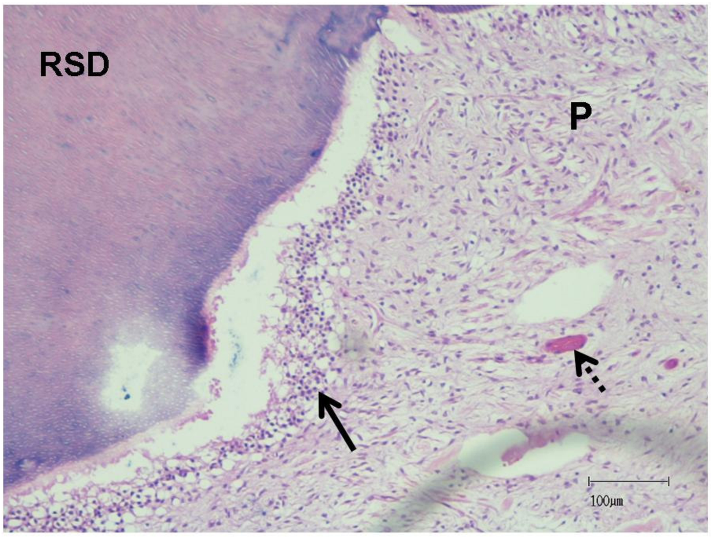


Figure 5.3.2.7: **High magnification of the dentine-pulp interface showing undifferentiated mesenchymal cells (open arrow), fibroblast (dashed arrow) and blood vessel (red arrow). Note the lipid or hydropic degeneration of the odontoblast-like cells indicative of pulp pathology (solid arrow head). ISD = irregular secondary dentine; P = pulp (x 400).**

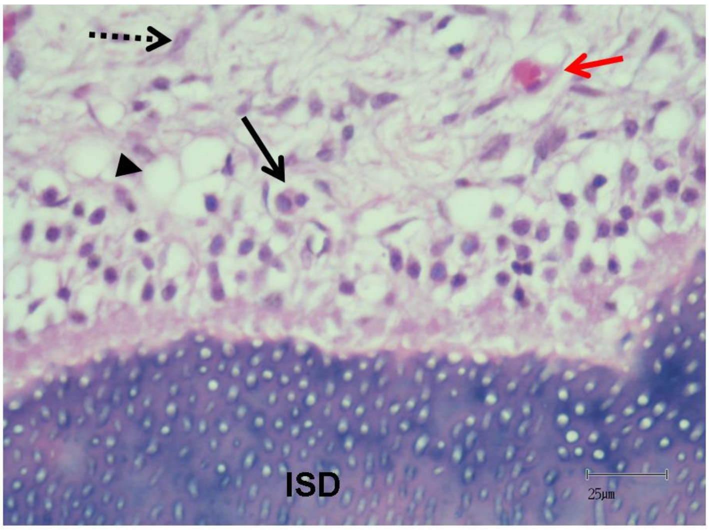


Figure 5.3.2.8: **Longitudinal section of the mid to subocclusal level of a 108 from a 34 year old donkey. The pulp (P) contains an area of old haemorrhage (dashed arrow) with a pulp stone lying apical to it (arrow). The separation (arrow heads) of the pulp from the dentine is artifactual. (x 100). RSD = regular secondary dentine; P = pulp.**

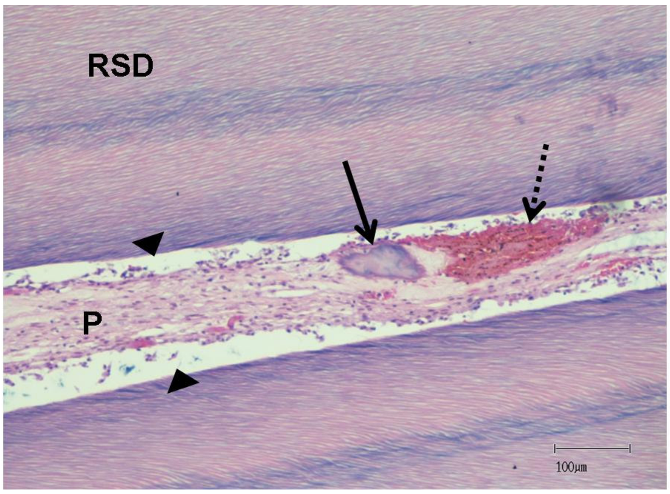


Figure 5.3.2.9: **Higher magnification of haemorrhagic focus (HF) showing linear depositions of haemosiderin (dashed arrow) that indicate chronicity of haemorrhage. Although these brown spicules may also be acid haematin, which is an artefact of formalin pigment. Odontoblast (solid arrow), mesenchymal cells (red arrow) and a pulp stone (PS) are identifiable as is the artefactual separation of the pulp from predentine (double head arrow) (x 400).**

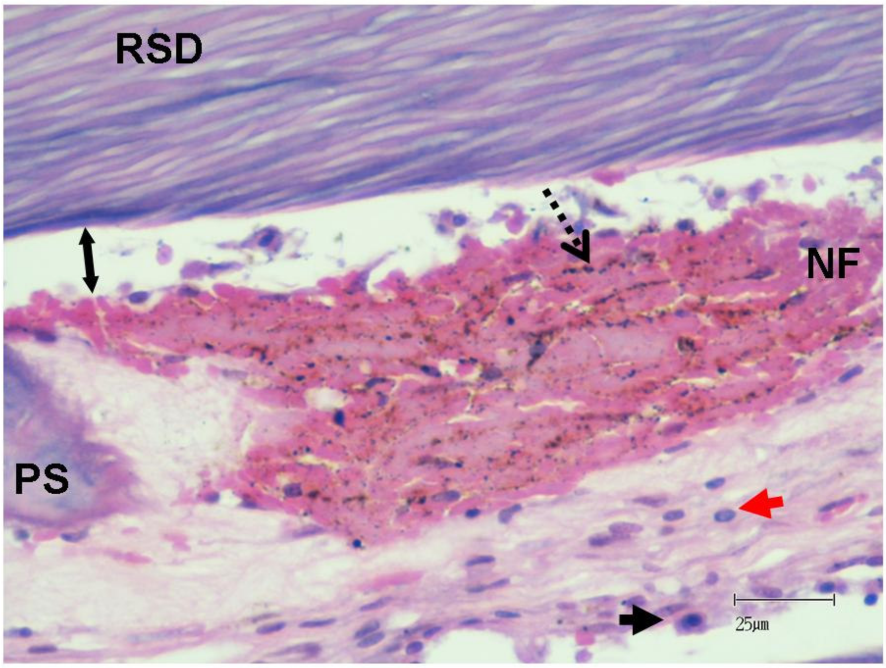
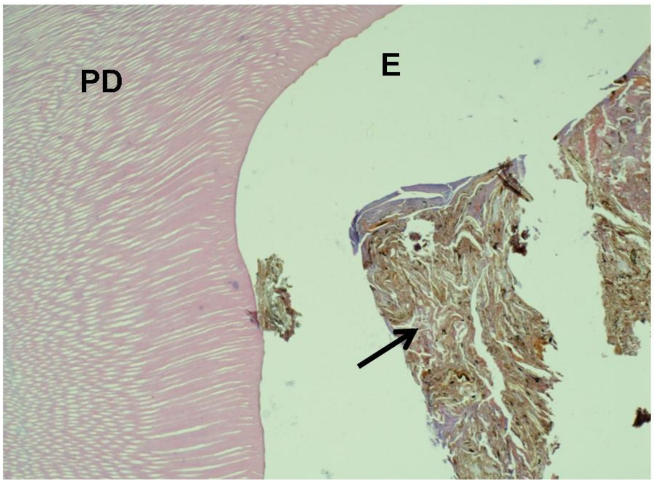
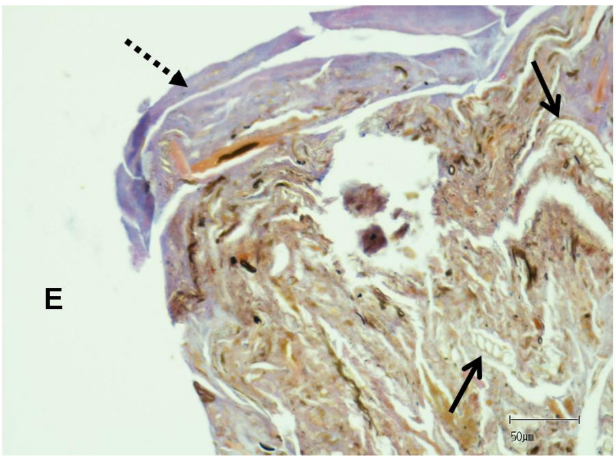


Figure 5.3.2.10: **Longitudinal section of a 109 from a 33 year old donkey with infundibular caries. Decalcification has removed the (E) enamel. Note the necrotic material lying within the infundibulum (arrow) with no recognizable cementum present. Because of the total loss of enamel by decalcification, it is not possible to assess the degree of caries if any it suffered, but is clear the infundibular caries did not extend to involve the primary dentine (PD) (x 100).**



5.3.2.11: **Higher power image of Figure 5.3.2.10 (carious infundibulum of a 109 from 34 year old donkey) showing plant material within its lumen (arrows). A small area of mineralized tissue (dashed arrow) may be infundibular cementum remnants (x 200).**



5.3.3 Undecalcified histology

Undeclacified histology was performed on 4 sections (4 teeth) from 3 donkeys including a 109 with pulpar exposure and a 411 with peripheral caries obtained from a 31 year old donkey; a 209 with infundibular caries from a 33 year old donkey; and a 109 with early stage infundibular caries from a 31 year old donkey.

Figure 5.3.3.1: **An undecalcified longitudinal section of the occlusal aspect of a 411 with peripheral cemental caries (dark area – arrow) involving the cementum (C) and enamel (E). The blue discoloration is from the petroxy blue dye stained resin used to attach the tooth section to the slide which has infiltrated an artefactual separation at the amelo-cemental junction. The arrow head are pointing to air bubbles which are artefacts. Occlusal to the tooth are remnants of the Epi-thin resin (ER) (x 40).**

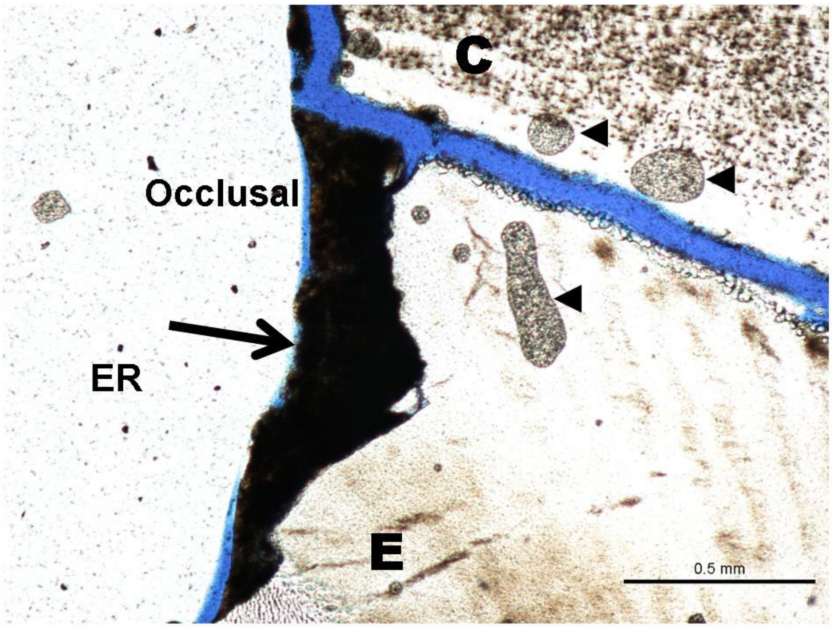


Figure 5.3.3.2: **A higher magnification of the previous Figure (Figure 5.3.3.1) illustrating the extension of the cemental peripheral caries (arrow) to involve the underlying dentine (D). E = enamel (x 100).**

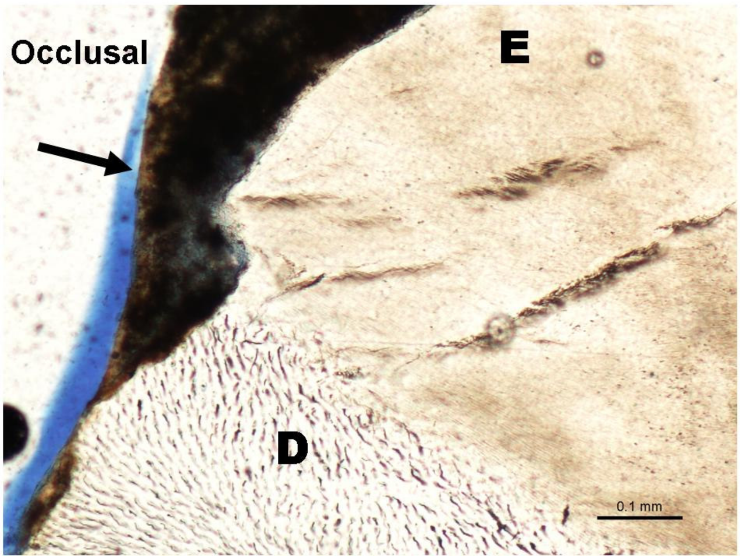


Figure 5.3.3.3: **Early stage infundibular caries in a 109 with infiltration of the dyed resin at the ACJ indicating separation (arrow). The arrow head shows an area of normal ACJ. C = cementum; E = enamel (x 100).**

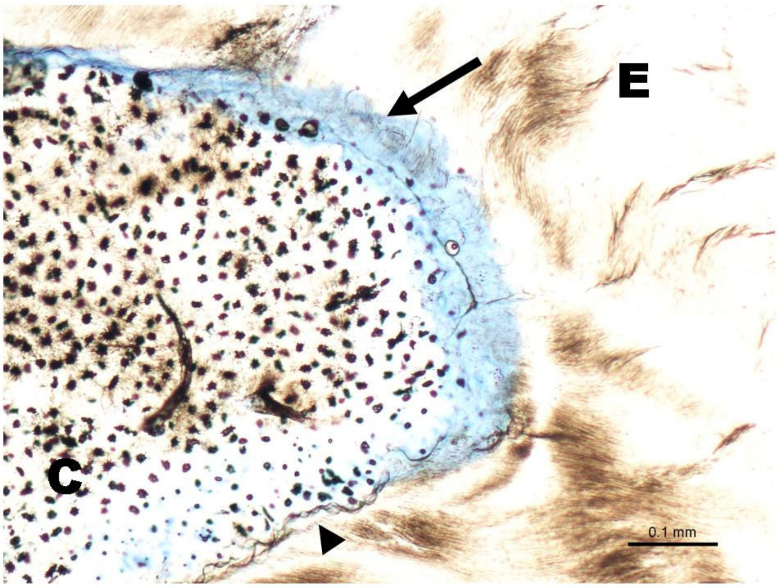


Figure 5.3.3.4: **A longitudinal section of a 109 with pulp exposure showing clear pulpar necrosis (PN), fragments of dentine and vegetable organic matter (arrow). D = dentine (x 100).**

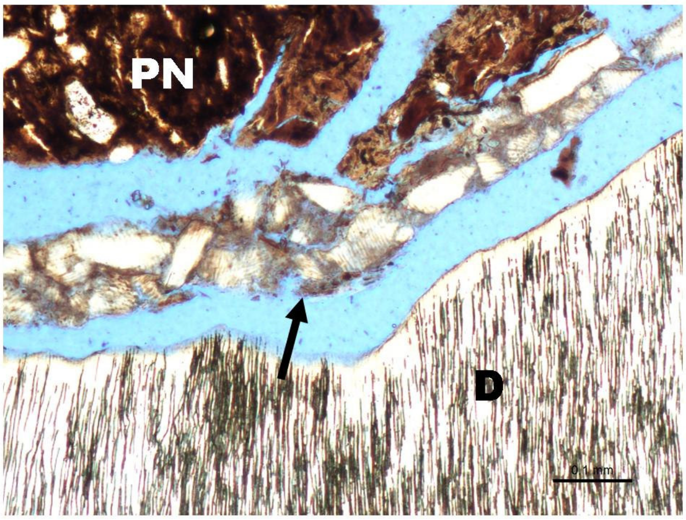


Figure 5.3.3.5: **Infundibular caries in a 209 with some local destruction of the internal aspect of the infundibular enamel (arrow). The blue discoloured area represents where the cementum has been lost as a result of the carious process (replaced by pertoxy dyed resin) and the black circles within the resin are artefactual air bubbles (Original magnification x 40). D = dentine; E = enamel.**

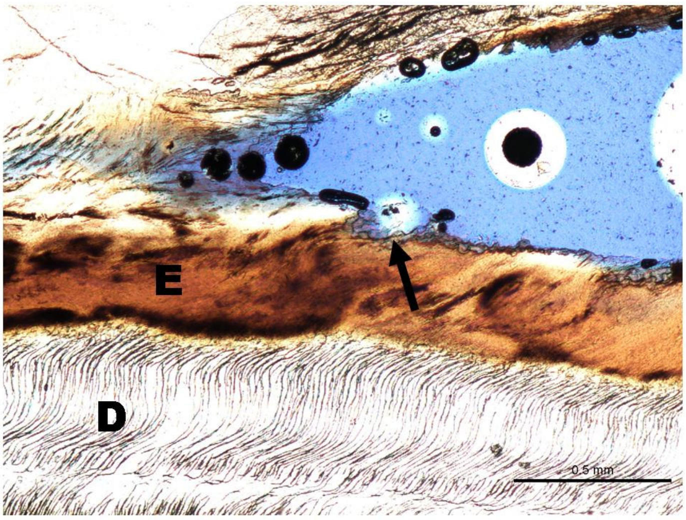


Figure 5.3.3.6: **Higher magnification of previous image (Figure 5.3.3.5) showing disruption of the former site of the infundibular amelo-cemental junction and focal erosion into the adjacent enamel (arrow). The darker spots on the enamel may be clumps of bacteria penetrating enamel (dashed arrow). D = dentine; E = enamel (x 100).**

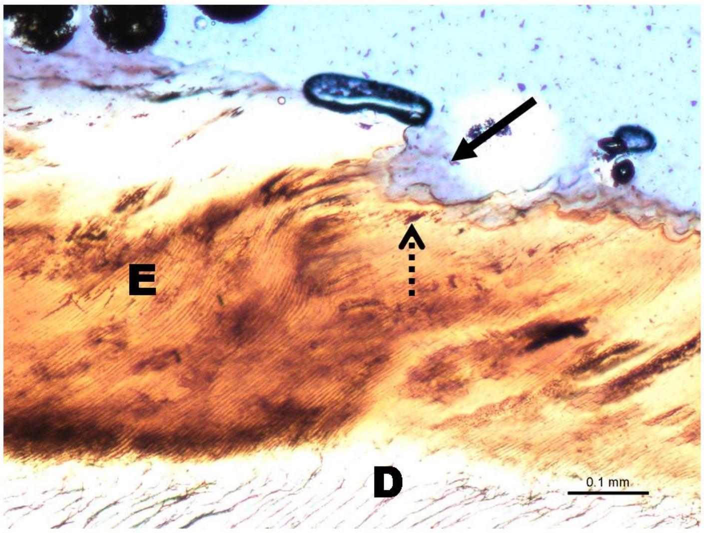
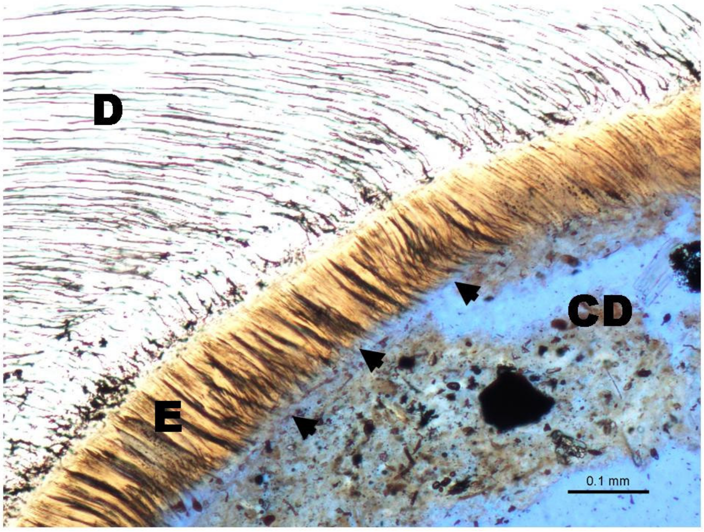


Figure 5.3.3.7: **Infundibular caries causing loss of the normal scalloped appearance of the amelo-cemental junction (arrow heads). D = dentine; E = enamel; CD = cemental and organic debris (x 100).**



5.3.4 Scanning Electron Microscopy

Scanning electron microscopy was performed on 4 teeth from 3 donkeys with caries or pulpar exposure (Table 5.3.4.1).

***Table 5.3.4.1*: Table showing the age of donkey from which the tooth was taken for scanning electron microscopy, its Triadan tooth number and pathological change.**

Donkey age	**Triadan tooth number**	**Dental pathology**
31	411	Peripheral caries
27	108	Pulpar exposure (pulp 2)
33	109	Infundibular caries
33	209	Infundibuar caries

***Figure 5.3.4.1:* Unetched longitudinal section of a 109 from a 33 year old donkey showing the infundibulum to be incompletely filled with carious cementum and some surrounding vegetable matter. Note the plant material embedded between the cementum and adjacent enamel (closed arrow) and thinning of the enamel due to caries (open head arrow). E = enamel; solid line = normal enamel thickness (x 10.2).**

Figure 5.3.4.2: **Higher magnification of above section (Figure 5.3.4.1) illustrating carious damage to enamel, cementum and loss of normal amelo-cemental junction (dashed arrow). Collagen fibres can be seen on the surface of the cementum (arrows) (x 19.5).**

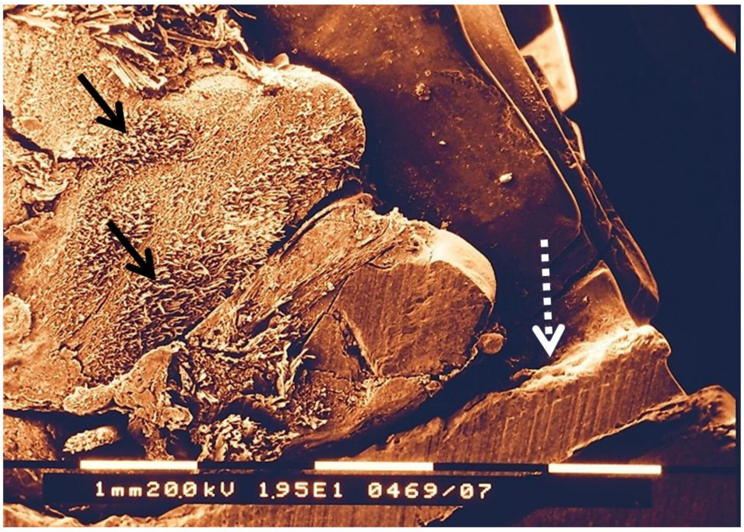


Figure 5.3.4.3: **Higher power image of cememtum (C), plant material (P) and necrotic organic matter (N) within the infundinbulum of Figure 5.3.4.2 (x 156).**

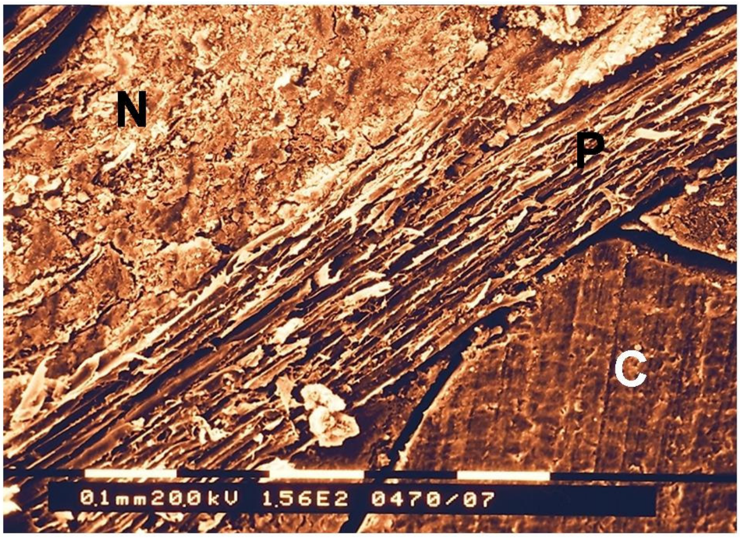


Figure 5.3.4.4: **Unetched longitudinal section of the 109 from a 33 year old donkey shown in Figure 5.3.4.1 and 5.3.4.2 showing the carious process affecting the enamel (E). A glycoprotein matrix is seen on the enamel surface (G) and what appears to be small clumps of bacteria are seen on the glycoprotein matrix (arrow). Original magnification (x 312).**

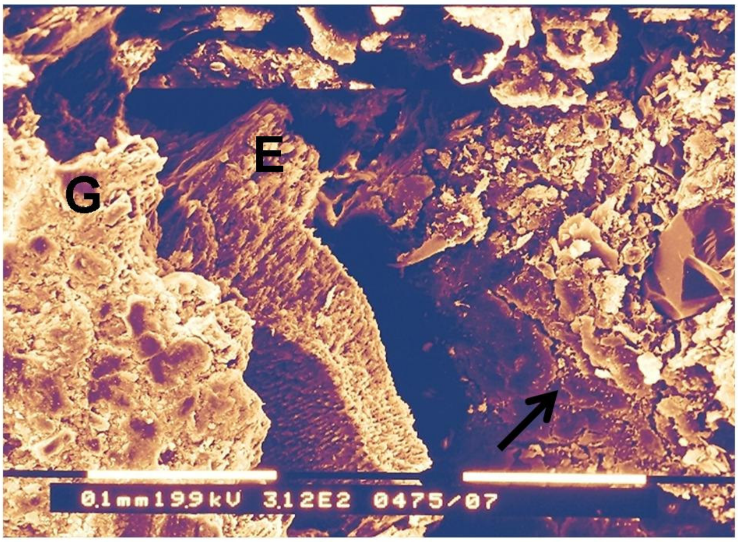


Figure 5.3.4.5: **Close up of Figure 5.3.4.4 showing glycoprotein matrix lying on damaged enamel rods (E) and clumps of bacteria (arrow). Also note the loss of interprismatic enamel with only the rods remaining in the equid type-1 enamel (x 1360).**

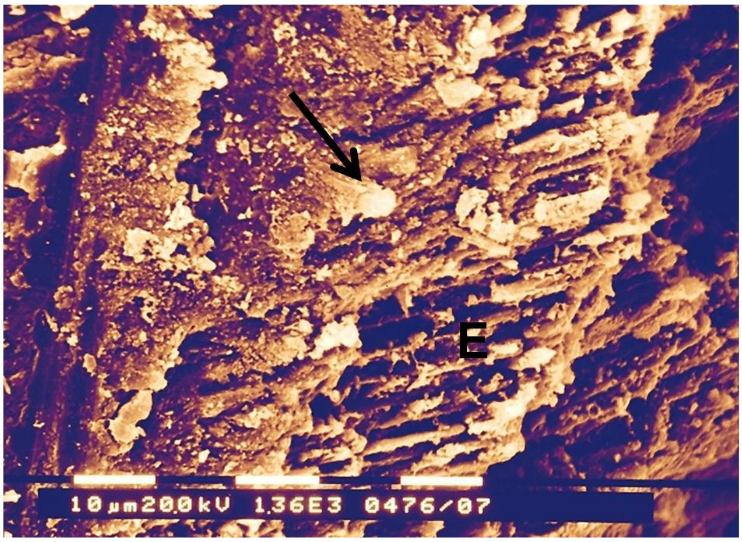


Figure 5.3.4.6: **Unetched longitudinal section of a 109 from a 33 year old donkey showing the presence of plant material within an occlusally exposed pulp horn (open arrow). Irregular secondary dentine fills the more apical aspect of the pulp horn (closed arrow) and the solid line demarcates the thickness of regular secondary dentine (RSD) (x 9.75).**

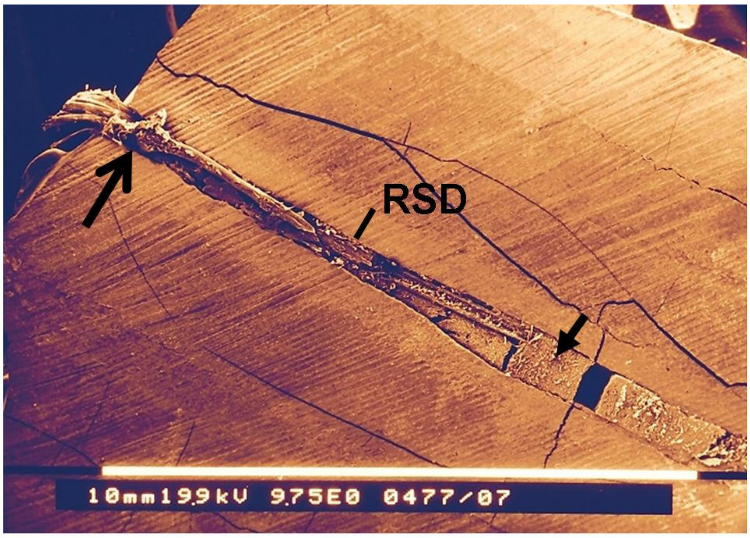


Figure 5.3.4.7: **Higher magnification of above image (Figure 5.3.4.6) showing plant material (P) in the occlusally exposed pulp with some surrounding focal calcified areas (arrows) that may represent areas of tertiary dentine or pulp stone deposition (x 39).**

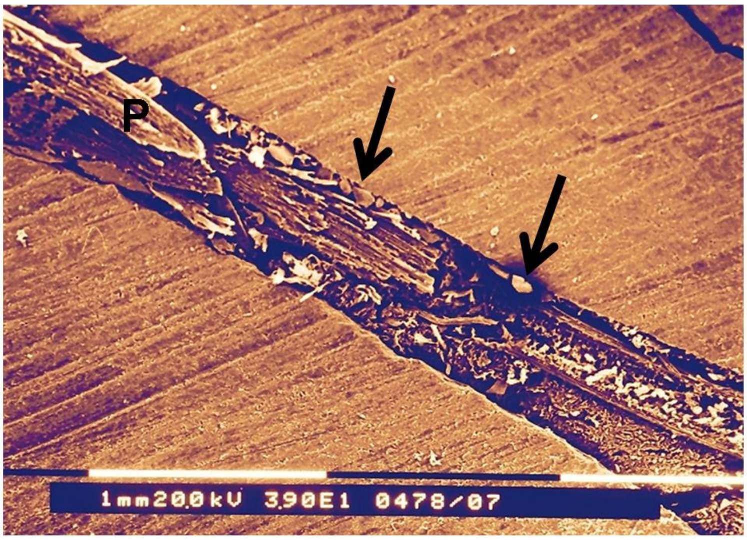


Figure 5.3.4.8: **High power SEM of rounded, focal mineralized tissue (arrow) on the periphery of an occlusally exposed pulp horn, which is possibly tertiary dentine. Because it is not tightly attached to the adjacent secondary dentine (P) it may also be a developing pulp stone (x 150).**

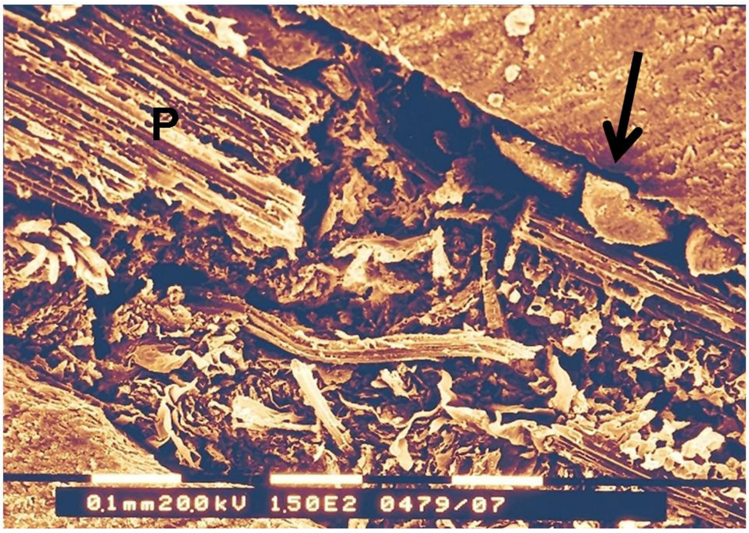


Figure 5.3.4.9: **Unetched longitudinal section of a 109 infundibulum. The infundibular cementum (C) has carious destruction around its periphery (arrow) at the amelo-cemental interface. The site of cemental caries in this infundibulum suggests that initial bacterial penetration occurred along the amelo-cemental junction (x 12.1).**

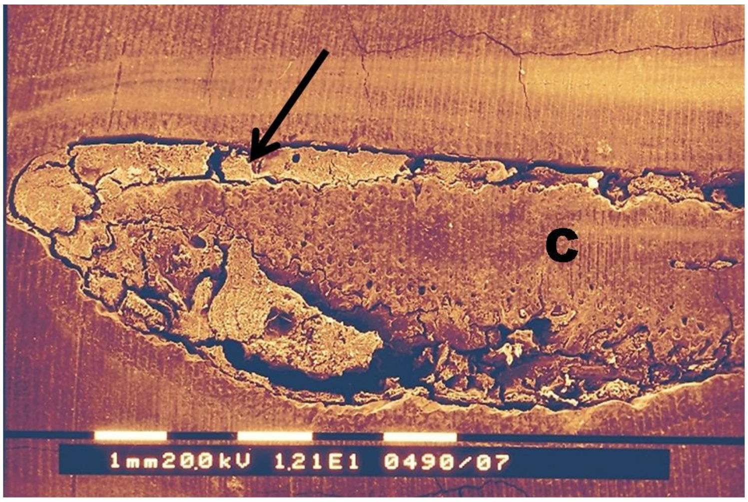


Figure 5.3.4.10: **Higher magnification of the infundibulum shown in Figure 5.3.4.9 showing carious cementum (C). Note the exaggerated scalloped appearance (red arrow head) of the enamel (double headed arrow) where the carious attack has progressed to involve the enamel (E). Also note the cemental lacunae (arrow) and parallel dark lines which are artefacts caused by the tile saw. D = dentine (x 24.2).**

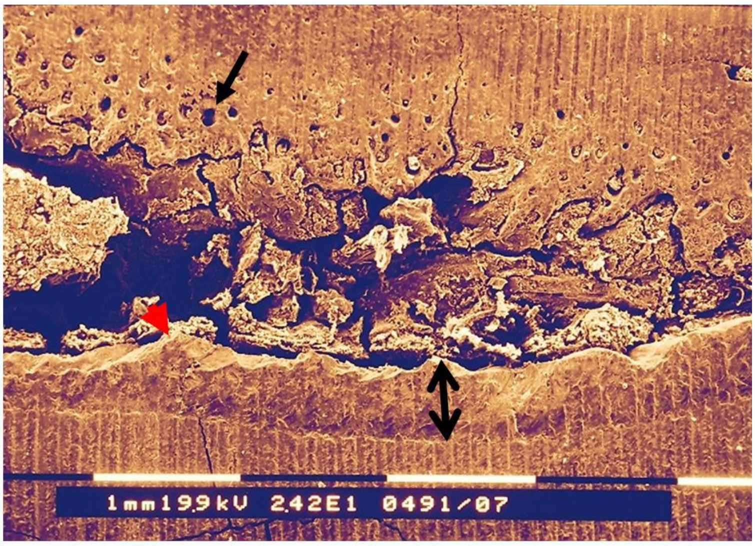


Figure 5.3.4.11: **Apical aspect of a longitudinal section of a 209 infundibulum showing carious changes to the cementum (arrow) with loss of the normal amelo-cemental junction. E = enamel (x 46.4).**

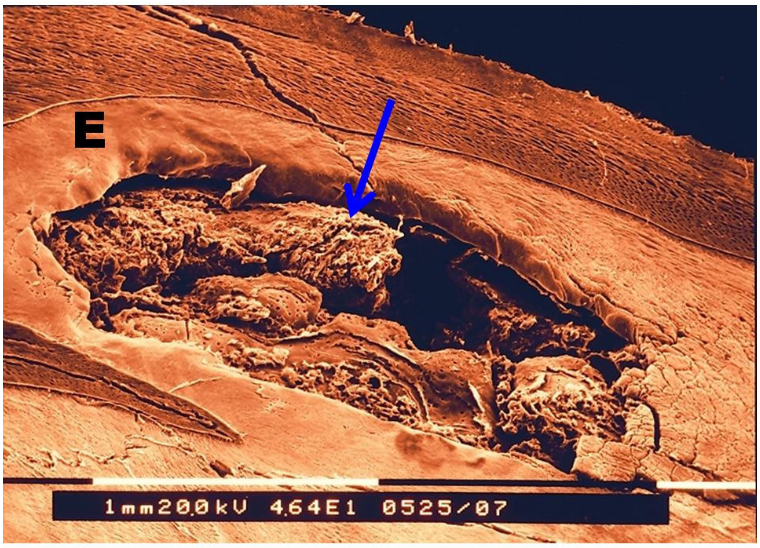


Figure 5.3.4.12: **Higher magnification of the periphery of the carious infundibulum (arrow) of Figure 5.3.4.11. The loss of the normal amelo-cemental junction is due to the carious process.The loss of intratubular dentine within the dentinal tubules in the surrounding dentine (D) is due to the etching process. E = enamel (x 178).**

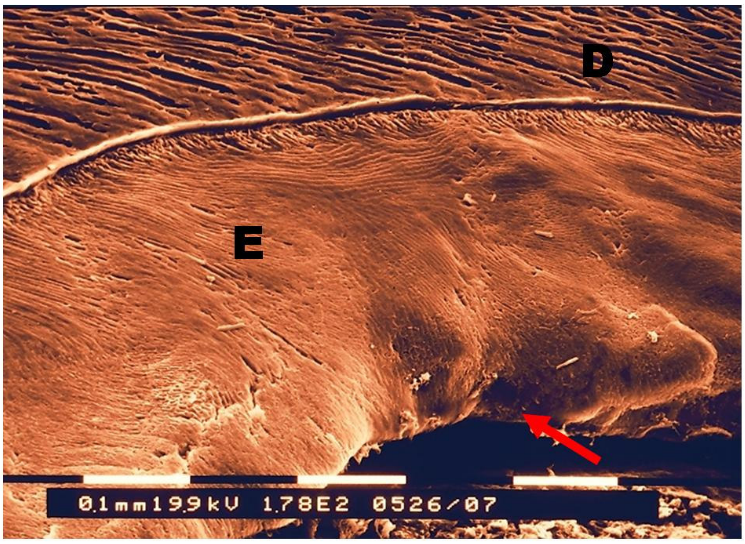


Figure 5.3.4.13: **High power of carious infundibular cementum that clearly shows the remnants of its organic components (collagen fibres) that remains after bacterial acids have removed its inorganic minerals. The empty lacunae (arrows) are prominent in this decalcified cementum (x 326).**

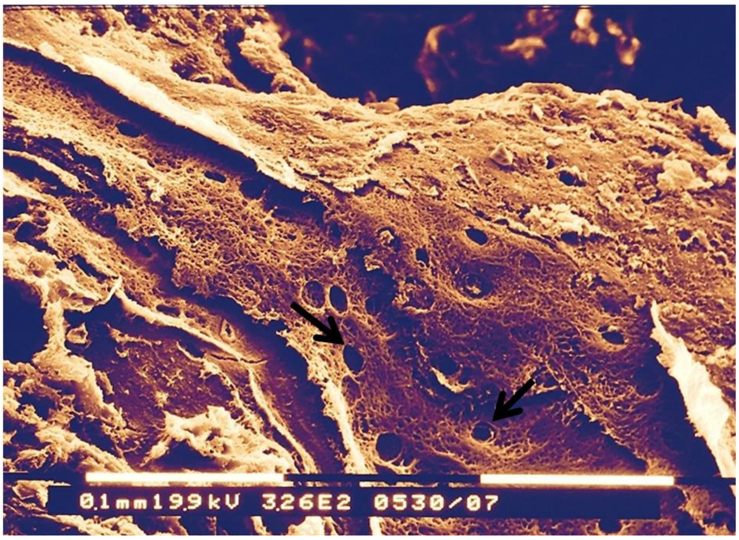


Figure 5.3.4.14: **Peripheral caries (arrow) of a 411 involving all three dental tissues. Top right corner is the occlusal aspect. E = enamel, C = cementum, D = dentine (x 39.0).**

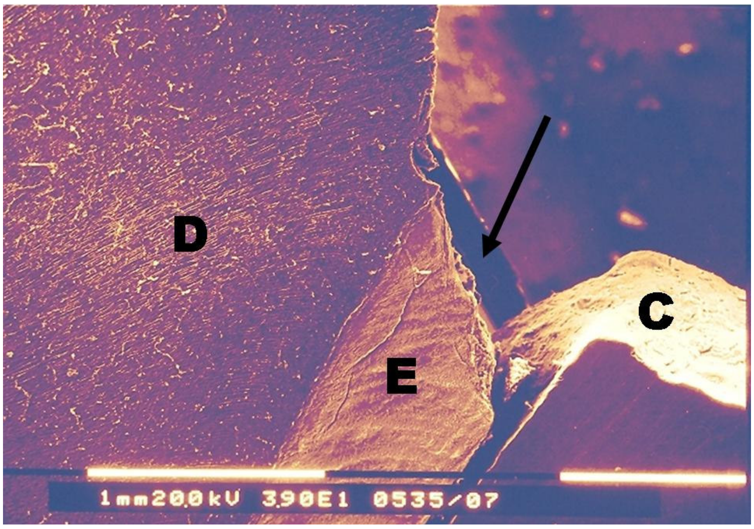


Figure 5.3.4.15: **Higher power SEM of peripheral CT caries showing loss of the normal enamel architecture (arrow). Note the artifactual separation of the enamel (E) and cementum (C) as a result of alcohol dehydration during sample processing. The occlusal aspect is at the top of the picture (x 194).**

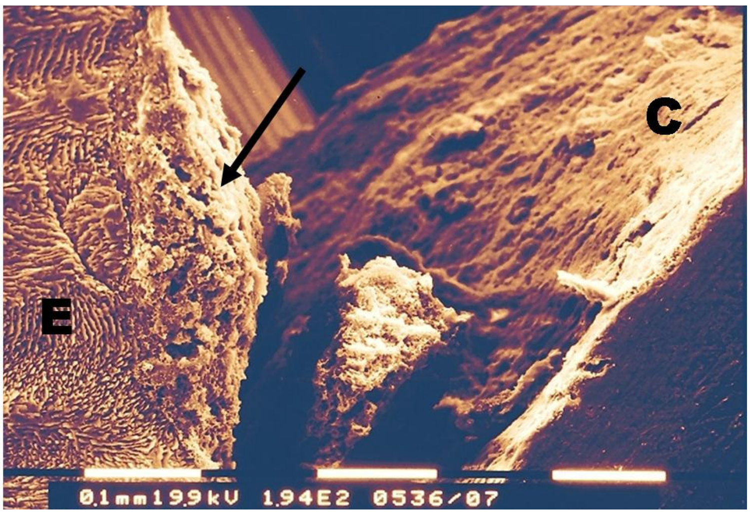


Figure 5.3.4.16: **High magnification of the carious enamel from Figure 5.3.4.15 with possible clumps of bacteria (arrows) lying on the organic pellicle layer (x 573).**

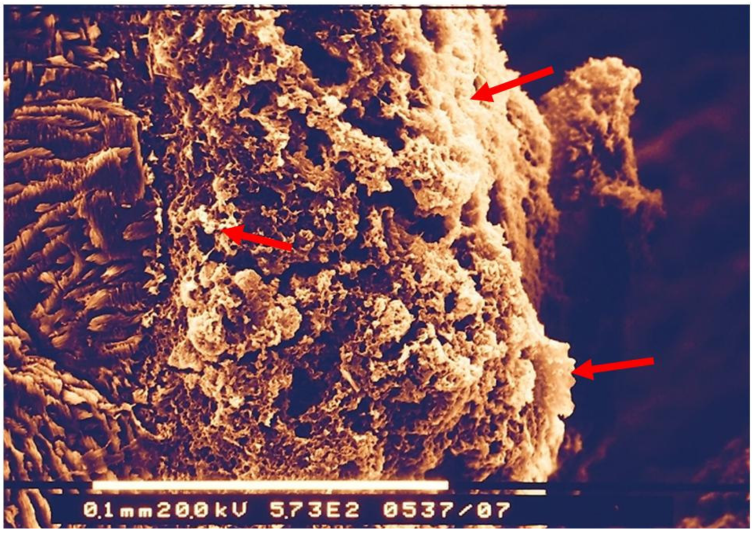
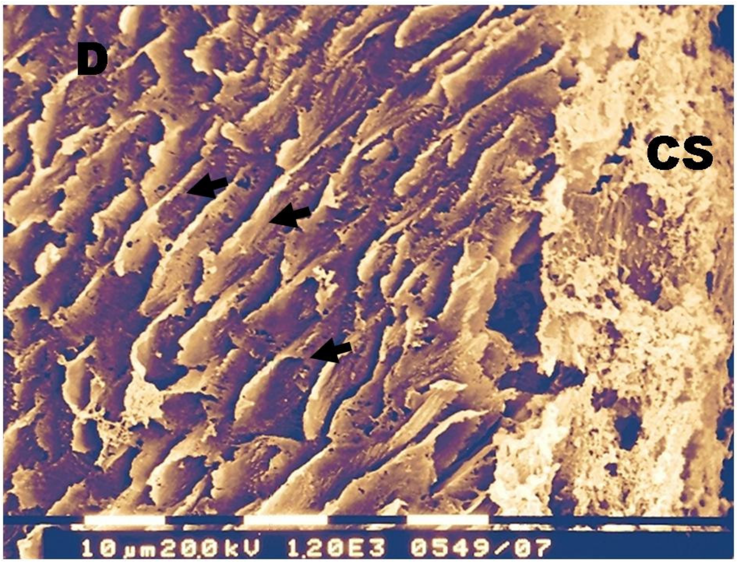


Figure 5.3.4.17: **High magnification of carious dentine (D) illustrating the destruction of the inter- and intra- tubular dentine with only the dentinal tubules remaining (arrows). CS = carious surface (unetched) (x 1200).**



5. 4. Discussion

Radiographic assessment of pulpitis and pulp exposure with or without peri-apical granulomas has been shown to be very inaccurate in a study in monkeys (Cardoso and Mitchell 1971) and does not always correlate well with histological findings (Thoma 1929). Radiographic diagnosis of periapical infections in horses is further complicated by the multiple overlying structures of the equine skull, particularly in early cases of periapical infections where minimal calcified tissue changes are present (Gibbs and Lane 1987; Weller *et al.* 2001; Barakzai and Dixon 2003). Computed tomography appears to allow more accurate identification of apical infections and infundibular caries (Henninger *et al.* 2003; Banner 2006; Simhofer and Boehler 2007).

In this study CAT was able to confirm the presence and extent of pulp exposure and infundibular caries in donkey CT. These lesions were visible on longitudinal planes as areas of hypodensity where secondary dentine and cemental density would normally be observed. Similar images were obtained from 18 clinical cases of infundibular caries and apical infections in horses (Henninger *et al.* 2003). Areas of loss of calcified dental tissue were also observed on the occlusal surfaces of affected CT, which was better visualized with three-dimensional images. The sensitivity of identifying these pathological changes in live equids is yet to be determined, but a small equine study indicated that CAT imaging is an accurate diagnostic tool to determine the presence of pulp disease (Henninger *et al.* 2003).

Decalcified histologicy illustrated the presence of food material and necrotic pulp material within occlusally exposed pulp horns, without surrounding irregular secondary dentine. In severely affected or long standing cases no viable pulps remained. In one case there was even total absence of secondary dentine indicating advanced progression of the caries and complete loss of regenerative capability. This is similar to findings in monkeys where chronic (> 3 months) pulpar exposure resulted in complete pulp necrosis in most such teeth (el-Kafrawy and Mitchell 1970; Cardoso and Mitchell 1971).

Interestingly, one pulp horn with occlusal exposure in this study, had viable pulp present apically and contained a focus of possible haemorrhage with a small pulp stone apical to it (figures 5.3.2.8 and 5.3.2.9). This damaged area of pulp may have caused ischaemia of the more occlusally located pulp causing pulpar exposure, whilst retaining viability in the more apically situated pulp horn. The calcified structure which had a reticular pattern at higher magnifications (x 400) was identified as a false pulp stone i.e. non-tubular calcified deposits (Moss-Salentijn and Hendricks-Klyvert 1988). This is in contrast to a true pulp stone which contains dentinal tubules surrounded by odontoblast-like cells. A histochemical study of false pulp stones showed that they were composed of reticular connective tissue, mucoprotein and acid polysaccharides (Bevelander and Johnson 1956). However, similar structures have also been described as amorphous calcified deposits (and are therefore not true pulp stones) (Bevelander and Johnson 1956; Slootweg 2007).

The presence of viable pulp in the face of pulpitis has been demonstrated in monkeys where tertiary dentine was formed in response to pulp exposure, with resolution of the pulpitis in the pulp apical to the tertiary dentine (Fischer *et al.* 1970). Similarly, in such monkey studies, pulpar exposure in some teeth resulted in pulpitis that was limited to the coronal (occlusal aspect) pulp (el-Kafrawy and Mitchell 1970; Cardoso and Mitchell 1971). The histological inflammatory response in human pulps is indicative of chronic inflammation with mononuclear cells, in particular plasma cell infiltration (Thoma 1929). Unfortunately, most of the histological sections examined in the current study had advanced pulp necrosis and the type of initial inflammatory reaction that occurred could not be assessed.

Other changes noted in the pulp horns with pulpar exposure included areas of lipid or hydropic degeneration of odontoblast-like cells. Odontoblast apoptosis has been shown to occur in rat molars after experimental cavity formation using a burr and was thought to play an important role in reparative dentinogenesis (Kitamura *et al.* 2001). Odontoblast apoptosis was also demonstrated in human teeth with infected but vital pulps and was described as reticular atrophy (Thoma 1929). Apopotosis is typically seen as chromatin condensation (pyknosis) and nuclear fragmentation with a dense cytoplasm, and more histology will be required to confirm this change in donkey pulp tissue. Whether this change observed in a donkey tooth was due to an inflammatory process or was an age-related apoptosis is unknown. As has been demonstrated in human teeth using light microscopy (Soames and Southam 1993) and SEM (Frank 1990), and in horses (Dacre 2005a), pioneer bacteria were seen in the dental tubules surrounding infected pulps.

Histological and ultrastructural examination of CT with infundibular caries illustrated loss of the normal cemental architecture and the presence of necrotic cementum or plant material within the resultant infundibulum. The extent of the carious process could be visualised, extending through the infundibular enamel to involve secondary dentine in some cases. On undecalcified histological section, peripheral caries appeared as focal areas with loss of normal architecture that were sometimes filled with amorphous material. Similarly sections of CT with pulpar exposure merely showed amorphous material within the pulp horn and random small pieces of primary dentine along the pulp horn edge which are most likely preparation artefacts. Infundibular caries with complete loss of cementum and involvement of the enamel showed a 'frayed' pattern to the inner layer of the infundibular enamel with loss of the normal scalloped appearance of the ACJ (or total loss of ACJ), indicating carious demineralisation.

Scanning electron microscopy allowed better visualisation of bacteria within the pulp cavities with pulpar exposure. However, the identification of the inflammatory cells in diseased pulp was difficult, as was also found in a human study (Seltzer *et al.* 1977). Small areas of calcificied tissue (possibly false pulp stones) were identified in an exposed pulp cavity containing plant material. These calcified areas had formed sporadically along the length of the affected pulp horn, and it is unclear if these were pre-existing pulp stones as described or were areas of tertiary dentine induced by pathological stimuli (nidus for calcification) (Bevelander and Johnson 1956).

A longitudinal section of a carious infundibular that contained normal cementum more centrally, showed the carious process infiltrating along the amelo-cemental junction with carious lesions seen in the infundibular enamel and peripheral infundibular cementum. This finding suggests that in some cases of infundibular caries the bacteria penetrate and initiate the carious process at the amelo-cemental junction. This phenomenon of destruction of cementum along calcified junctions, prior to bacterial colonisation along these enlarged junctions has also been described in humans (Frank 1990). One carious infundibulum only contained the fibrillar collagen remnants of cementum, the mineral portion having been dissolved.

Examination of CT affected with peripheral caries by SEM also showed partial demineralisation of all three calcified dental tissues in this destructive process. In particular, the loss of interprismatic enamel and demineralization of the enamel rods was seen in these sections, as also described in carious human teeth (Frank 1990). Carious lesions of donkey dentine appeared as areas of remnant dentinal tubules with loss i.e. demineralization of the inter – and intra-tubular dentine as has been described in human studies (Arends *et al.* 1989; Frank 1990). It is interesting to note that in human studies demineralisation preceded bacterial colonization in enamel and dentine caries, whereas in cemental caries these were shown to occur simultaneously (Frank 1990).

In conclusion, it appears that the CAT, histopathological and ultrastructural appearance of caries and pulpar inflammation and necrosis have a similar pattern to those observed in the teeth of other species. The carious process in donkey teeth appears to have the same pattern for all three calcified dental tissues, with demineralization and colonization by pathogenic bacteria then resulting in destruction of the normal architecture. Pulp stones/areas of amorphous calcified material were identified in donkey teeth pulp in this study, and further research is needed to determine the exact aetiology and significance of these structures.

Chapter 6: An anatomic study of the dimensions of diastemata and associated periodontal food pockets in donkey cheek teeth

6.1 Introduction

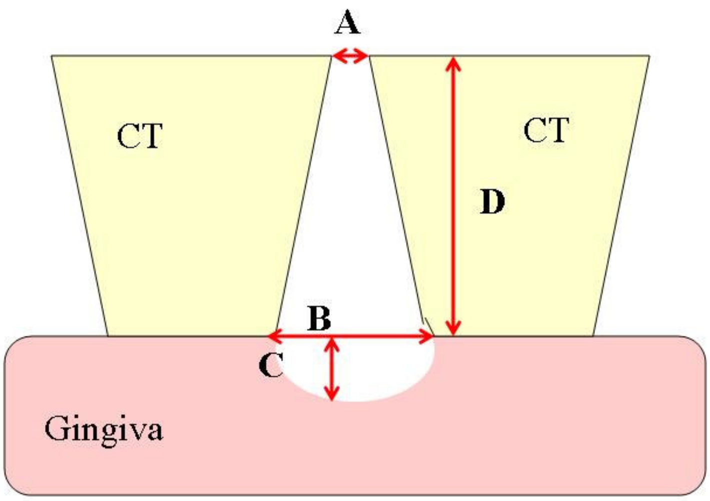
Diastema in equids is defined as the presence of an abnormal interdental space between adjacent incisor or cheek teeth with subsequent periodontal food impaction and CT diastemata are a major cause of quidding in horses (Collins and Dixon 2005; Dixon and Dacre 2005). This is not to be confused with the normal physiological diastema in equids between cheek teeth and incisors ('bars of the mouth'). No detailed investigations of the dimensions of diastemata in equids have been reported to date. The aim of this study was to utilise post mortem examinations of donkey skulls, to define the dimensions of open and valve diastemata (Carmalt 2003), the depth of periodontal pockets and determine the accuracy of defining diastemata as valve or open based on gross observation in donkeys.

6.2 Materials and Methods

The skulls of 16 donkeys (median age 32 years; range 12 – 56) were selected from the post mortem room at The Donkey Sanctuary, Sidmouth, Devon. Skulls were specifically collected if they had diastema(ta). A total of 45 diastemata were present in these 16 skulls, with between 1 – 6 diastema per donkey skull. Diastemata were classed as open (same width throughout the depth of the diastema) or valve (narrower at the occlusal aspect) diastemata based on macroscopic appearance as previously described (Carmalt 2003; Collins and Dixon 2005; Dixon 2006).

The width of each diastema was measured on the lateral (buccal) and medial (lingual/palatal) aspect at the occlusal surface (A) and gingival margin (B). The depth of the periodontal pocket (C) was measured on the lateral and medial aspect where possible (Figure 6.2.1). The erupted crown length was also measured (D). All measurements were obtained using digital callipers (Knighton Tool Supplies, Leicester, UK).

Figure 6.2.1: **Illustration of the three measurements taken per diastema on the lateral and medial aspect. A = diastema width at occlusal level; B = diastema width at gingival margin; C = periodontal pocket depth; D = erupted crown length**



Data were recorded on Excel® and statistics performed using Minitab (Minitab 14 Release) and R (R V2.3.1, R Foundation for Statistical Computing). A one sample t-test was used to determine the difference between lateral and medial aspects of each diastema at occlusal (A), gingival (B) and periodontal pocket level (C). As the data were not normally distributed a Mann-Whitney test was used to determine the ratio of occlusal to gingival margin width in open and valve diastema on the lateral and medial aspects. The depth of periodontal pockets was also compared in open and valve diastemata. The height of erupted cheek tooth crown was correlated with the depth of the periodontal pocket depth using Spearman's rank correlation test. Statistical significance was assumed at $P < 0.05$.

A major difficulty in terms of any statistical analysis was that for 4/16 (25%) of the skulls only one diastema was found/observed, but there were up to 6 diastemata in the other skulls (Figure 6.3.1). This meant for some skulls there was non-independence between diastemata, suggesting a mixed-effect model approach (Pinheiro 2000), but the skulls with a solitary diastema precluded such analysis. The concern was any statistically significant results being unduly influenced by multiple teeth from the same skull, but in the skulls with multiple diastemata it was difficult to select one diastema. Therefore, we employed a "bootstrapping" (Efron and Tibshirani 2000) methodology for the statistical testing. One diastema per skull was selected at random, creating a sample of 16 diastemata, on which the various statistical procedures were performed. This sample creation and subsequent statistical testing was carried out 10,000 times in order to ensure generation of results that were robust in terms of which diastema had been selected. The results are reported as a percentage of the 10,000 iterations in which statistical differences (at the 5%) level were obtained. The greater the percentage, the greater the robustness of any results that indicate statistically significant differences or correlations.

6. 3. Results

A total of 45 diastemata were measured from 16 skulls, with between 1 and 6 diastema per skull (Figure 6.3.1). Twenty diastemata (44.4%) were in the maxillary CT row and 25 (55.6%) were in the mandibular CT row. The diastemata were believed to be secondary to other dental disorders such as displaced teeth (n = 20), absent teeth (n = 7), fractured teeth (n = 2) and focal overgrowths on CT (n = 3) which were seen in 42.2% of cases. Food impaction was found in 40 diastemata (89%) (Figure 6.3.2) with 5 not having any food impaction at the time of our examination, including 3 open and 2 valve diastemata.

Figure 6.3.1: **Number of diastemata per donkey skull.**

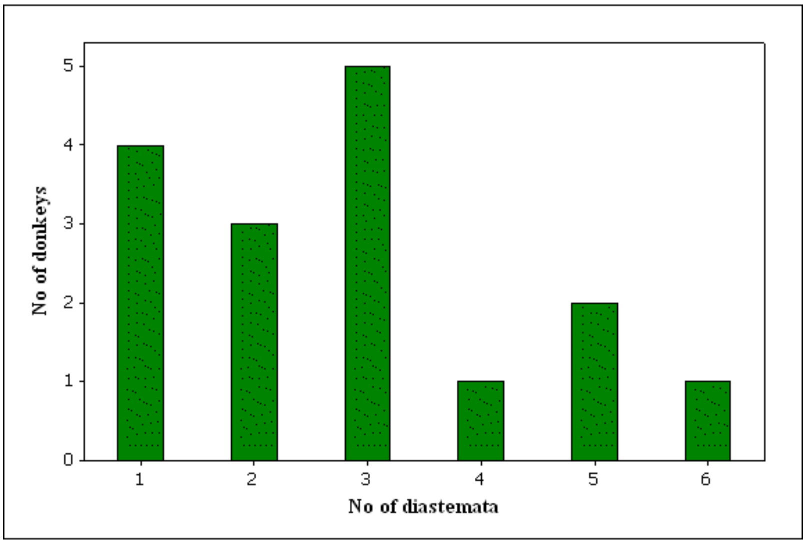
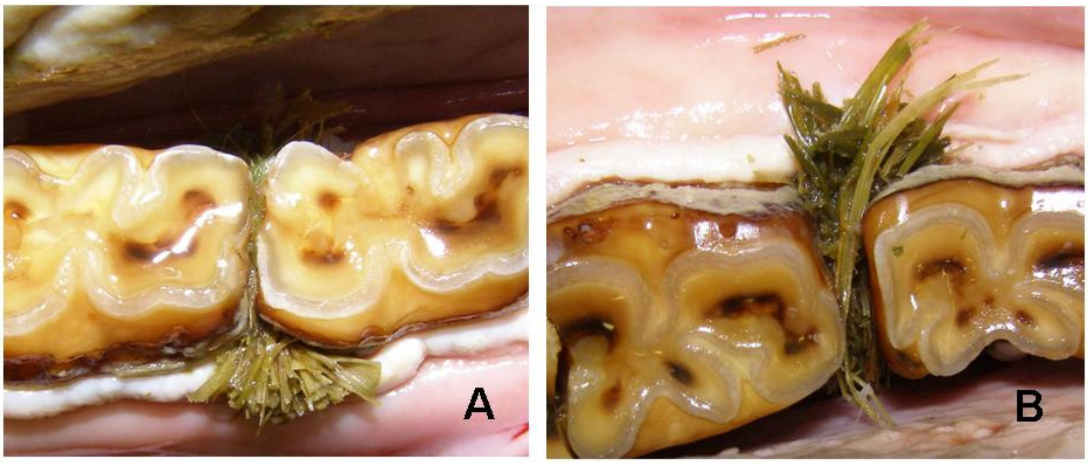


Figure 6.3.2: **Food impacted in a valve (A) and an open (B) mandibular cheek teeth diastemata. Note the excessive central wear (early senile excavation) in the mandibular cheek teeth on the left (A).**



6.3.1 Difference in lateral and medial parameters

The mean, median and ranges of the diastema widths occlusally (A) and at the gingival margin (B) and the periodontal pocket depths (C) at the lateral and medial aspects are tabulated in Table 6.3.1.1.

Table 6.3.1.1: **Mean, median and range of lateral and medial measurements A, B and C and measurement D (mm).**

	Mean	Median	Range
A Lateral	1.98	1.86	0 – 5.74
A Medial	1.99	1.96	0 - 5.24
B Lateral	3.02	2.65	0.73 – 7.44
B Medial	3.20	2.98	0 – 7.41
C Lateral	4.04	3.98	0 – 8.47
C Medial	2.38	2.08	0 – 7.1
D Length	13.15	11.75	3.62 – 25.83

There was no significant difference between the lateral and medial widths of the diastema at either the occlusal surface ($P = 0.939$; Figure 6.3.1.1) or the gingival margin ($P = 0.388$; Figure 6.3.1.2). The bootstrap analyses revealed that there were no significant differences ($P > 0.05$) between the lateral and medial width of the diastema at either the occlusal surface or the gingival margin, with 99.7% and 99.0% of the 10,000 simulated samples, respectively.

Figure 6.3.1.1: **Boxplot of lateral (A-L) and medial (A-M) diastema width at occlusal level (mm).**

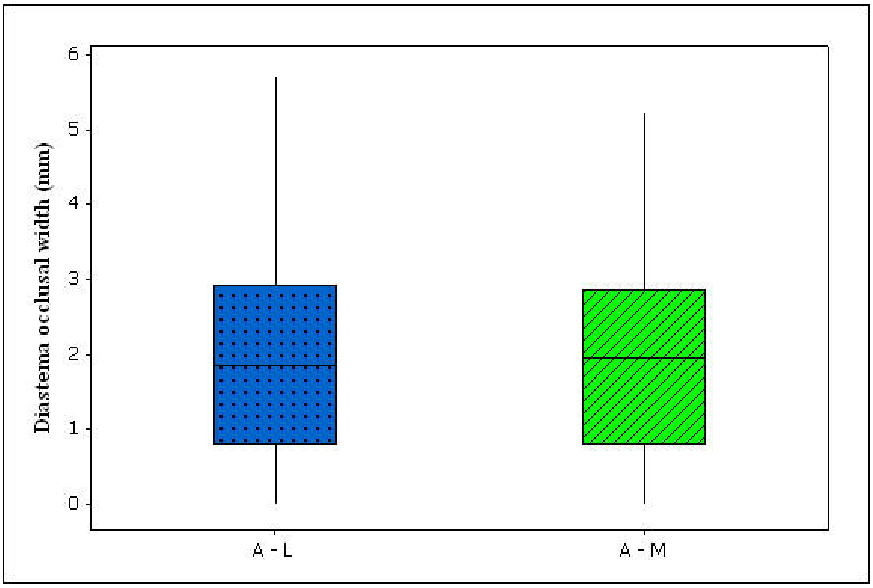
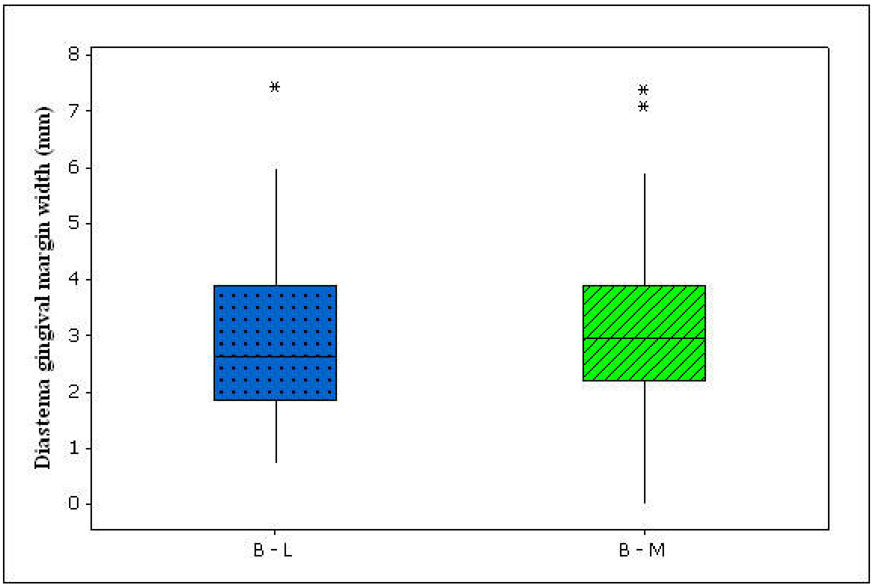


Figure 6.3.1.2: **Boxplot of lateral (B-L) and medial (B-M) diastema width at gingival margin (mm).**

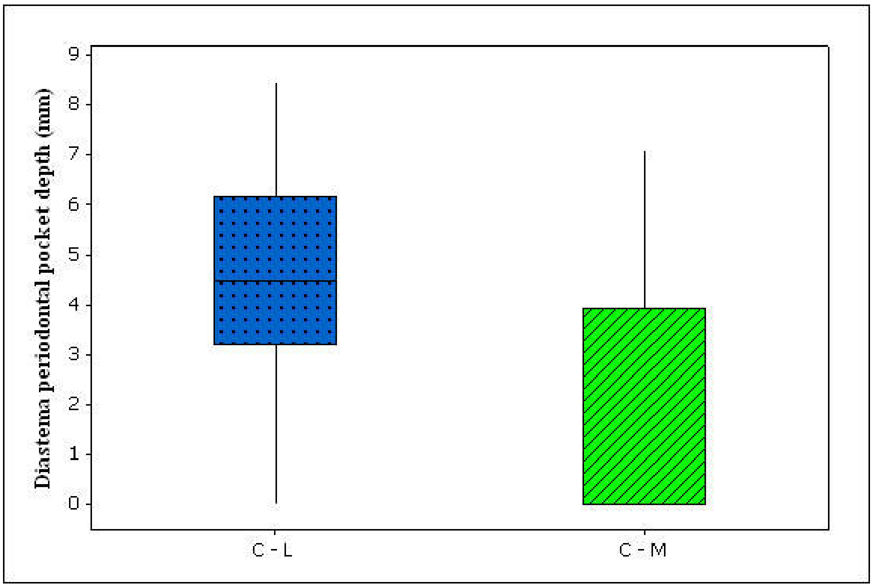


Periodontal pocketing (measurement C) was present in 34/45 diastemata (75.6%) with 33 having pocketing on the lateral aspect and 16 on the medial aspect (Figure 6.3.1.3).

Figure 6.3.1.3: **Extensive periodontal pocket on the medial aspect of a mandibular cheek tooth diastema after removal of impacted food.**

There was a significant difference in the depth of the periodontal pocketing between lateral and medial aspects with a deeper lateral pocketing in all diastemata ($P < 0.001$; Figure 6.3.1.4). From the bootstrap analysis there was a statistically significant difference ($P<0.05$) in the depth of the periodontal pocketing between lateral and medial aspects with a greater depth of pocketing associated with the lateral aspect of maxillary and mandibular cheek teeth diastemata in 92.3% of the simulated samples (Figure 6.3.1.4).

Figure 6.3.1.4: **Boxplot of lateral (C-L) and medial (C-M) periodontal pockets depths (mm).**

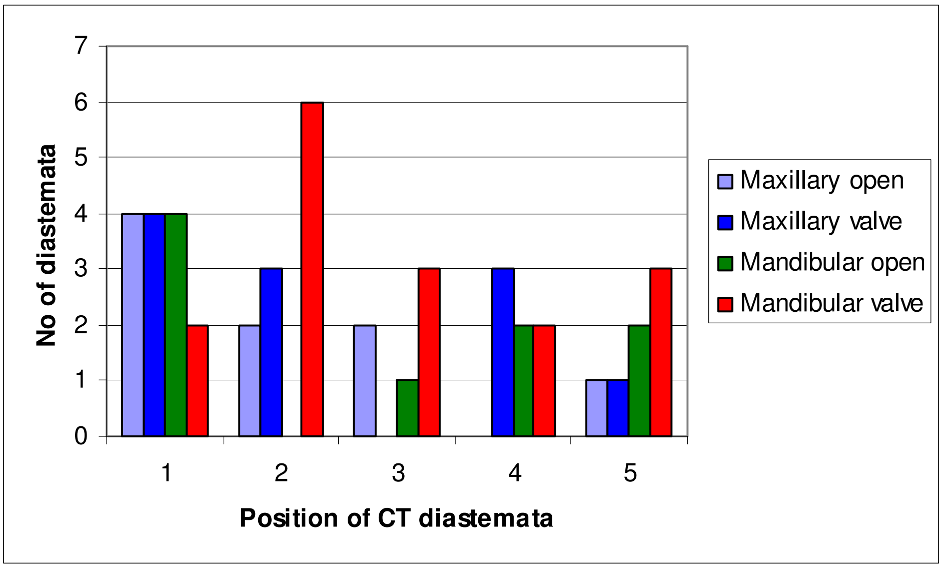


6.3.2 Validating identification of open and valve diastemata

Diastemata were defined as being open or valve based on gross observation (Figure 6.3.1.3). Open diastemata were defined as diastemata where the occlusal width appeared to be the same width or wider than the width at the gingival margin. Valve diastemata were defined as diastemata that appeared to be narrower at the occlusal aspect than the gingival margin. Of these 45 diastemata examined in this study 27 were defined as open and 18 as valve diastemata.

There did not appear to be a particular pattern to the prevalence of open or valve diastemata in the various positions, although overall valve diastemata were more common, with 55% of maxillary diastemata and 64% of mandibular diastemata presenting as valve diastemata (Figure 6.3.2.1).

Figure 6.3.2.1: **The number of open and valve diastemata in each interproximal cheek teeth position. 1 = 06/07; 2 = 07/08; 3 = 08/09; 4 = 09/10; 5 = 10/11.**

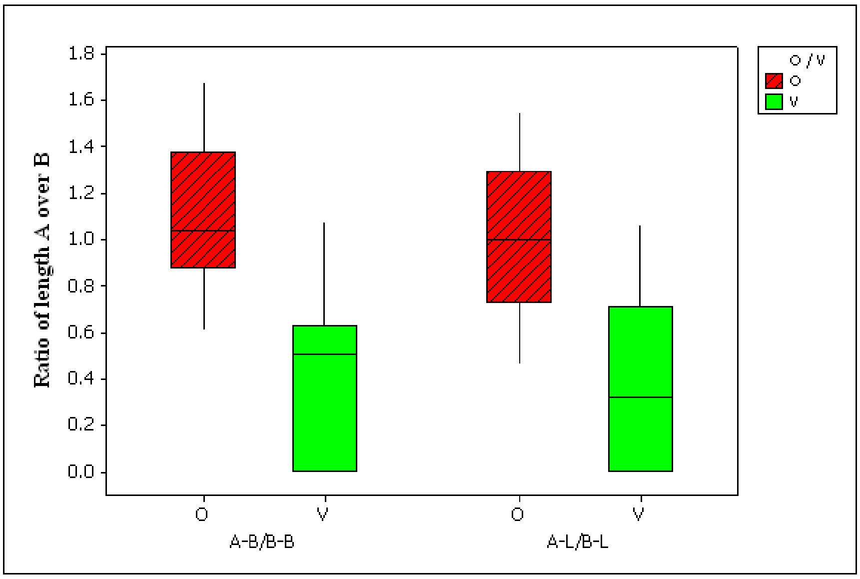


The mean of the ratio (occlusal to gingival margin width) for open and closed diastemata on the lateral and medial aspect is summarised in Table 6.3.2.2. There was a statistically significant difference between open and valve diastemata ratios laterally ($P < 0.001$) and medially ($P < 0.001$; Figure 6.3.2.2). The differences between open and valve diastemata ratios laterally (1.11 and 0.42 respectively) and medially (1.03 and 0.37 respectively) were statistically significantly different ($P < 0.05$) in 99.3% and 86.0% of the simulated samples, respectively.

Table 6.3.2.2: **Summary of mean values (and standard deviations) of ratios of occlusal width (A) to gingival margin width (B) in open and valve diastemata.**

Diastemata type	A/B Laterally	A/B Medially
Open	1.11 (0.31)	1.03 (0.32)
Valve	0.42 (0.33)	0.37 (0.35)

Figure 6.3.2.2: **The differences in ratios of occlusal width (A) to gingival margin (B) widths in open (O) and valve (V) diastemata. Boxes represent interquartile range and horizontal lines represent medians.**



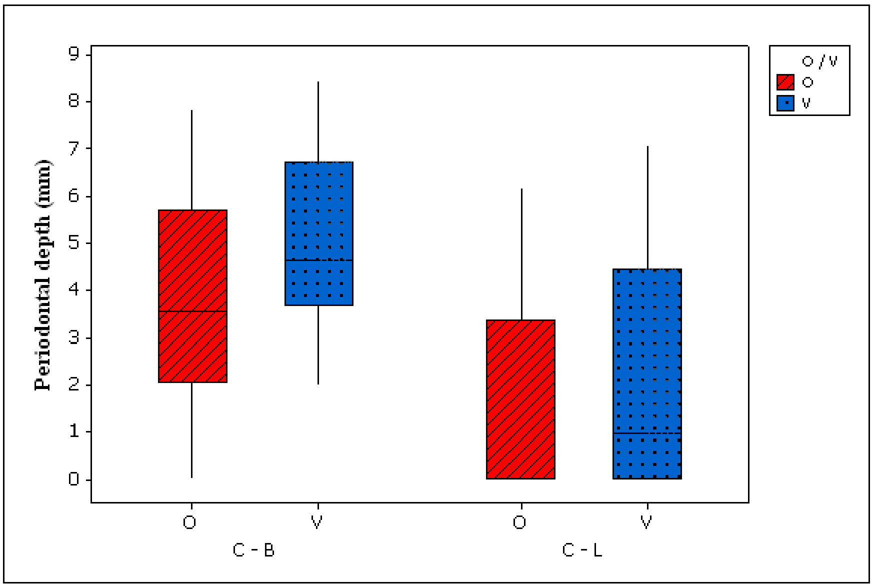
6.3.3 Periodontal pocket depth

The depth of periodontal pockets in open and valve diastema were compared to ascertain if there was a correlation between type of diastema and depth of periodontal pocketing. There did appear to be a tendency towards deeper lateral periodontal pockets in valve diastema, but this was not reflected in the bootstrap analyses, where no statistically significant difference at the 5% level was observed for 99.2% of simulated samples, with a similar lack of difference for the medial pockets (99.6%) (Figure 6.3.3.1).

Table 6.3.3.1: **Means, standard deviation (in brackets) and range of periodontal pocket depths in mm (C) in open and valve diastemata on the lateral and medial aspects and P values of two sample t test.**

	C Lateral Aspect	**C Medial Aspect**
Open	3.78 (2.20); 2.07 – 5.71	1.55 (2.18); 0 – 3.37
Valve	5.09 (1.91); 3.68 – 6.74	2.08 (2.40); 0 – 4.45
P value	0.094	0.532

Figure 6.3.3.1: **Boxplot showing the difference in periodontal pocket depths in open (O) and valve (V) diastemata laterally (C-B) and medially (C-L). Boxes represent interquartile range and horizontal lines represent medians.**



6.3.4. Association of periodontal pocket depth to erupted crown height

There was a significant association between erupted crown height and periodontal pocket depth on the medial aspect of diastemata (P = 0.032; r = -0.32) but not to lateral pocket depth (P = 0.28; r = -0.17) using all the diastemata (Figure 6.3.4.1 and 6.3.4.2). The reason for significance of the Spearman's rank correlation test between crown height and medial periodontal pocket depths using all the diastema could be attributed to the effect of a single donkey with multiple diastemata.

Figure 6.3.4.1: **Scatterplot of erupted crown height to medial periodontal pocket depth (mm).**

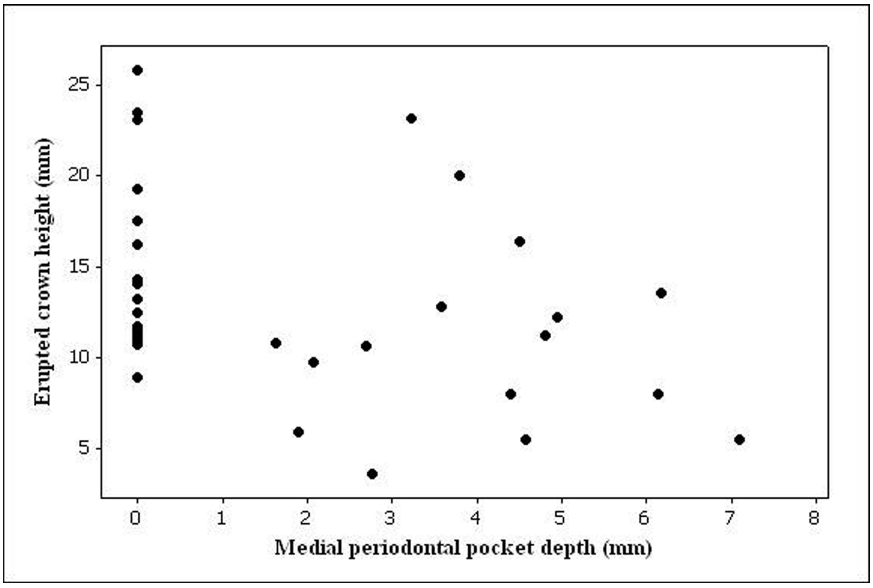
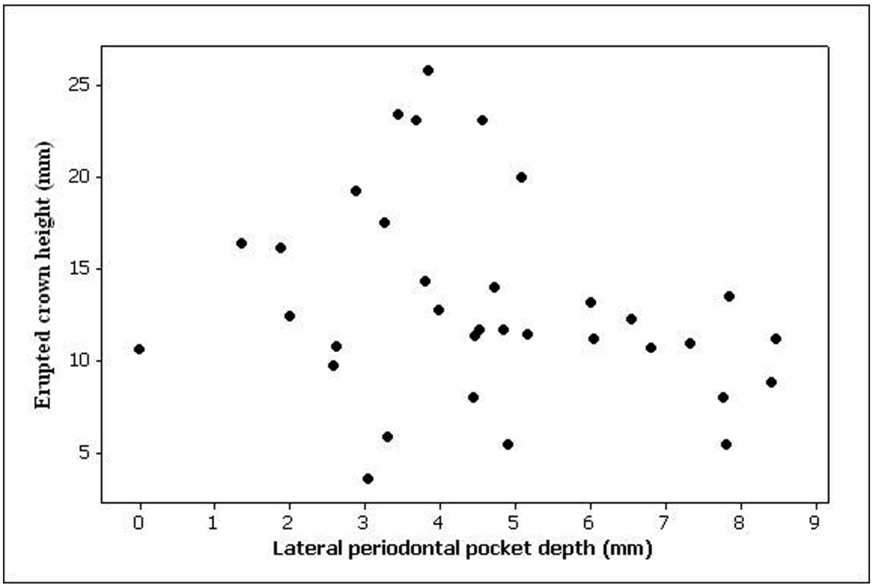


Figure 6.3.4.2: **Scatterplot of erupted crown height to lateral periodontal pocket depth (mm).**



6.4. Discussion

Diastemata were observed with a prevalence of 17.7% in a study examining 90 horses with CT dental disorders (Dixon *et al.* 1999b). A study looking at 355 horse skulls only found a prevalence of 3.7% (Wafa 1988), while a more recent study found a prevalence of 20% (Brigham and Duncanson 2000b). The current study did not examine the prevalence of diastemata in donkeys as skulls were specifically selected for having diastemata at post mortem. Previous equine studies (Dixon *et al.* 1999b; Dixon *et al.* 2008) noted that diastemata occurred with equal frequency in maxillary and mandibular rows and that they were most commonly found between the 09s and 10s. In this study there were slightly more mandibular (55.6%) than maxillary (44.4%) diastemata, as was also demonstrated in the post mortem survey study where 63% of diastemata were in the mandibular and 37% in the maxillary cheek teeth row (chapter 4).

Diastemata formation between equid cheek teeth is normally prevented by the caudal angulation of the rostral CT and rostral angulation of the caudal CT. Diastemata development can be primary or secondary to other dental disorders (Dixon 2006). In this study more than half the cases were not associated with other CT disorders and due to the median age of 32 in this sample can be attributed to senile diastemata.

The classification of diastemata as open or closed was based on previous published definitions with a triangular shaped defect recognised in valve diastema (Hofmeyr 1960; Carmalt 2003). In this study the size of the diastemata were measured at the occlusal surface and gingival margin laterally and medially after visual classification as being open or valve diastemata. The results from this study clearly illustrate a significant difference in the ratios of occlusal to gingival margin diastema width in open and valve diastemata, with no difference between medial and lateral width. However the type of diastema did not affect whether or not food was impacted.

The study by Dixon *et al.* (1999b) showed that food pocketing were commonly associated with periodontal pockets with depths greater than 50mm recorded. Deep periodontal pockets associated with diastemata may progress to the mandibles and maxillae causing osteomyelitis and oro-maxillary fistulas or sinusitis respectively (Dixon *et al.* 1999b). Recently, direct anastomoses between blood vessels of the periodontal ligament and maxillary sinus have been demonstrated in the horse (Masset *et al.* 2006). Thus infection from periodontal pockets may provide a direct route of infection to the sinuses, highlighting the clinical significance of these pockets. Periodontal pockets have also been measured in another equine study where depths up to 35mm were recorded in periodontal disease which was significantly greater than those recorded in non-diseased CT (Stock 1997). In this study depths of up to 8.47mm laterally and 7.1mm medially were recorded. These smaller values are possibly related to the smaller body and hence cheek teeth size of donkeys. Periodontal pockets were more common on the lateral aspect and were predominantly deeper (mean = 4.04mm) than medial pockets (mean = 2.38mm). However, there were no significant differences between the periodontal pocket depths in open or valve diastemata. Periodontal pocket depth was not related to the height of the erupted crown of the adjacent cheek teeth.

Diastemata have been recognised as a very painful oral condition in horses therefore it is important for this disorder to be recognised clinically as a cause of quidding (Dixon *et al.* 1999b; Carmalt 2003; Collins and Dixon 2005; Dixon 2006). This study has aimed to identify the presence of (and define the type of) diastema that may occur in donkeys. Marked periodontal pocketing is commonly associated with donkey CT diastemata and needs to be recognised and treated to prevent progression of periodonitis and even possible loss of cheek teeth.

Chapter 7: Clinical dental examinations of 357 live donkeys in the UK

7.1 Introduction

A limited number of studies have recognised dental disease in donkeys (Rajput *et al.* 1999; Roy 2002; Chabchoub *et al.* 2006), including a larger study looking at working donkeys in Mexico (Fernando-Martinez *et al.* 2006). However, there have been no extensive studies examining donkey dental disease in detail, particularly in different age groups. The aim of this study was to perform a cross-sectional analysis of dental disorders diagnosed in donkeys living at The Donkey Sanctuary, Sidmouth, Devon. We hypothesised that specific dental disorders would be identified in different age groups of donkeys and that this new information would allow a better understanding of the pathogenesis of dental disorders, particularly those occurring in geriatric donkeys. If this aim was achieved it would enable us to implement effective prophylactic dental treatments that may help prevent or reduce the level of debilitating dental disorders present in older donkeys in this donkey population (Chapter 4).

The second part of this study aims to determine if the statistically significant epidemiological factors identified in the post mortem survey (Chapter 4), are also significant in the younger age groups in this live study. In particular, to examine the possible associations between the presence of dental disorders and body contion score, feeding, age, previous colic episodes; between colic episodes and diastemata and age; between body condition score (BCS), age and feed; and between dental disorders.

7.2 Materials and Methods

Dental examinations were performed by the autor at The Donkey Sanctuary between March and April in 2007 in un-sedated donkeys. After flushing out the donkeys' mouths with tap water to remove food, an oral examination was performed with a Hausman's gag, portable head light (Welch Allyn, Aston Abbotts, UK) and dental mirror with the assistance of two experienced donkey handlers. Donkeys that were non-compliant or did not allow complete examination of the oral cavity were not included in the final number of 357. Standard dental charting (Appendix 1) was performed in 357 donkeys of different ages. As in the post mortem study (Chapter 4), accurate ages were not available for all donkeys, and ages were estimated based on owner information and examination of the mandibular incisor occlusal surfaces by the author. Age was then also divided into age groups for repeat analyses to eliminate any inaccuracies from the age estimation: 0-10 years old (n = 52 donkeys), 11-15 years old (n=50), 16-20 years old (n = 50), 21-25 years old (n = 50), 26-30 years old (n = 55), 31-35 years old (n = 50) and 36+ years old (n = 50). Specific dental disorders in CT and incisors were recorded and included; sharp enamel points, focal overgrowths, diastemata, worn teeth, overgrown teeth, displaced teeth, fractured teeth, missing teeth, periodontal disease, infundibular caries, pulpar exposure, wave mouth, smooth mouth, shear mouth, step mouth and oral ulcers.

Dental disease was defined as any dental disorder excluding sharp enamel points as a result of the masticatory action of chewing. Periodontal disease was defined as the presence of periodontal disease ranging from mild gingivitis to severe, deep periodontal pocketing. Pulpar exposure was only noted in cases where there was an obvious defect in the occlusal secondary dentine often surrounded by a dark area of carious dentine. Infundibular caries were defined as infundibula with carious lesions (erosions and dark discoloration) of its cementum with or without extension of caries to the enamel and dentine, as based on visual assessment. Infundibula with cemental hypoplasia were not included in this definition. All other dental disorders have been previously defined (Chapter 4). Epidemiological data recorded included body condition score (BCS), provision of supplemental feed, history of colic episodes, current systemic illness and weight loss.

Body condition score was recorded on a 5 point scale with half points (0.5) awarded (Appendix 1). Supplemental feeding was defined as administration of any extra nutrition in addition to *ad lib* roughage. The history of previous colic episodes was extracted from the veterinary records for each donkey. A colic episode was defined as any clinical signs consistent with colic with or without rectal confirmation i.e. kicking at belly, rolling, lying down, inappetance. Current systemic conditions were divided into 7 related groups: None – no systemic problems; Musculoskeletal – including arthritis and laminitis; Dermatological – skin conditions, sarcoids and eye problems; Respiratory – lung and sinus related disease; Dental – chronic dental disorders; Miscellaneous – all other disorders e.g. cardiovascular, hyperlipaemia.

Statistical analysis

Spearman's rank correlation and Kruskal-Wallis tests were performed to determine possible correlation between BCS, and age and age group respectively. Kruskal-Wallis pair wise comparisons (Dwass-Steel-Chritchlow-Fligner, Stats Direct statistical software version 2.6.5) were performed to compare BCS between different age groups. A non-parametric Mann-Whitney test was used to determine the association between BCS and the presence of dental disease and supplemental feeding. Chi-squared analyses were also performed between age group and presence of colic, supplemental feeding, weight loss, dental disease and specific dental disorders. Tukey contrast tests were performed to determine the significance between prevalence of dental disorders in different age groups. An age group was excluded from the analysis if the prevalence was 0.

General linear models with binomial errors were performed to determine the relationship between the presence of specific dental disorders and epidemiological factors. Univariate analyses were performed as a screening test prior to performing multivariable analyses and the models were selected on statistical significance ($P \leq 0.2$) and biological plausibility. Odds ratios (OR) and their 95% confidence intervals were determined for each explanatory variable to determine their association with response variables. Multivariable analyses were performed to determine if the interaction of explanatory variables altered the main effect on the response variable, as it likely that many of the factors are not mutually independent. Interactions for those variables were included in multivariable models. Any non-statistically significant main effects and interactions were removed and models rerun until the final model was achieved.

General linear models with binomial errors were performed to determine the relationship between the presence of dental disorders in individual teeth per donkey. Multivariable analysis was not performed on any of the univariate analysis results for disorders at tooth level, as the number of affected teeth were so low relative to the total number of teeth examined, such that statistical analyses of these numbers resulted in very large standard errors of residuals indicating poor results. $P < 0.05$ was taken to be statistically significant in the final multivariable analyses. R V2.3.1, R Foundation for Statistical Computing was used for statistical analyses.

7.3 Results

The ages of the 357 donkeys examined ranged from 2 – 53 years (median 23 years). The prevalence of dental disease in these donkeys was 73.1% (261 affected donkeys). Enamel points were present in 96 donkeys (26.9%) of which 44 (12.3% of all donkeys) had other dental disorders ("dental disease") with only 52 (14.6% of total donkeys) having sharp enamel points alone. Thus only 44 donkeys (12.3%) did not have any dental disorders. Oral soft tissue disorders were only noted in 3.4% of donkeys with oral ulcers (2.8%) more common than oral calluses (0.6%).The number and prevalence of each dental disorder in all donkeys (Table 7.3.1) and different age groups (Table 7.3.2) are summarised.

Table 7.3.1: **The number and prevalence (%) of donkeys diagnosed with dental disease, sharp enamel points and other specific dental disorders (diatemata, missing, worn, overgrown, displaced teeth, pulp exposure, periodontal disease, infundibular caries and fractured teeth include any donkey that had 1 or more teeth affected).**

Dental disorder	Number of donkeys	Prevalence (%)
Dental disease	261	73.1
Enamel points	96	26.9
Diastema	188	52.7
Missing teeth	105	29.4
Overgrown teeth	186	52.1
Worn teeth	148	41.5
Displaced medially	92	25.8
Displaced laterally	119	33.3
Periodontal disease	51	14.3
Pulp exposure	28	7.8
Infundibular caries	23	6.4
Fractured tooth	16	4.5
Focal overgrowth	12	3.4
Wave mouth	69	19.3
Step mouth	42	11.8
Smooth mouth	16	4.5
Shear mouth	13	3.9

Table 7.3.2: **The percentage of dental disorders observed in each age group (number of donkeys in brackets). Group A = 0-10 years old (yo); Group B = 11-15 yo; Group C = 16-20 yo; Group D = 21-25 yo; Group E = 26-30 yo; Group F = 31-35 yo; Group G = 36 and older.**

Dental disorder	**Group A (52)**	**Group B (50)**	**Group C (50)**	**Group D (50)**	**Group E (55)**	**Group F (50)**	**Group G (50)**
Dental disease	28% (14)	44% (22)	64% (32)	92% (46)	98% (54)	88% (44)	98% (49)
Enamel points	53.8% (28)	40% (20)	40% (20)	20% (10)	12.7% (7)	16% (8)	6% (3)
Diastema	3.8% (2)	12% (6)	40% (20)	78% (39)	76.4% (42)	72% (36)	86% (43)
Missing	0	2% (1)	12% (6)	30% (15)	41.8% (23)	64% (32)	56% (28)
Worn	7.7% (4)	14% (7)	26% (13)	38% (19)	52.7% (29)	68% (34)	84% (42)
Overgrown	15.4% (8)	22% (11)	36% (18)	60% (30)	67.3% (37)	78% (39)	86% (43)
Displaced medially	0	6% (3)	14% (7)	42% (21)	47.3% (26)	40 % (20)	30% (15)
Displaced laterally	5.8% (3)	8% (4)	26% (13)	48% (24)	56.4% (31)	50% (25)	38% (19)
Periodontal disease	0	2% (1)	2% (1)	28% (14)	20% (10)	28% (14)	18% (9)
Infundibular caries	0	0	0	16% (8)	12.7% (7)	6% (3)	10% (5)
Fracture	3.8% (2)	2% (1)	6% (3)	2% (1)	5.5% (3)	4% (2)	8% (4)
Wave	5.8% (3)	8% (4)	14% (7)	26% (13)	30.9% (17)	30% (15)	22% (11)
Step	0	0	2% (1)	16% (8)	10.9% (6)	22% (11)	32% (16)
Smooth	0	0	0	2% (1)	5.5% (3)	2% (1)	22% (11)
Shear	2% (1)	0	4% (2)	6% (3)	0	8% (4)	6% (3)

There was a significant association between age and the presence of dental disease (P < 0.001; χ^2_1 = 119.88; slope = 0.16 [0.02]; Figure 7.3.1) with the median age of donkeys with dental disease 27 years of age and without dental disease 12 years of age.

***Figure 7.3.1*: Boxplot of age of donkeys with (Y) and without (N) dental disease. Boxes = interquartile range; horizontal line = median; vertical lines = range.**

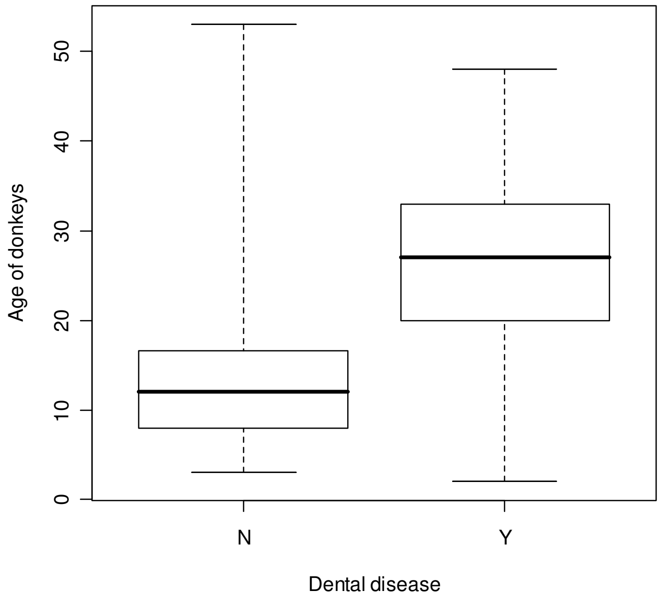
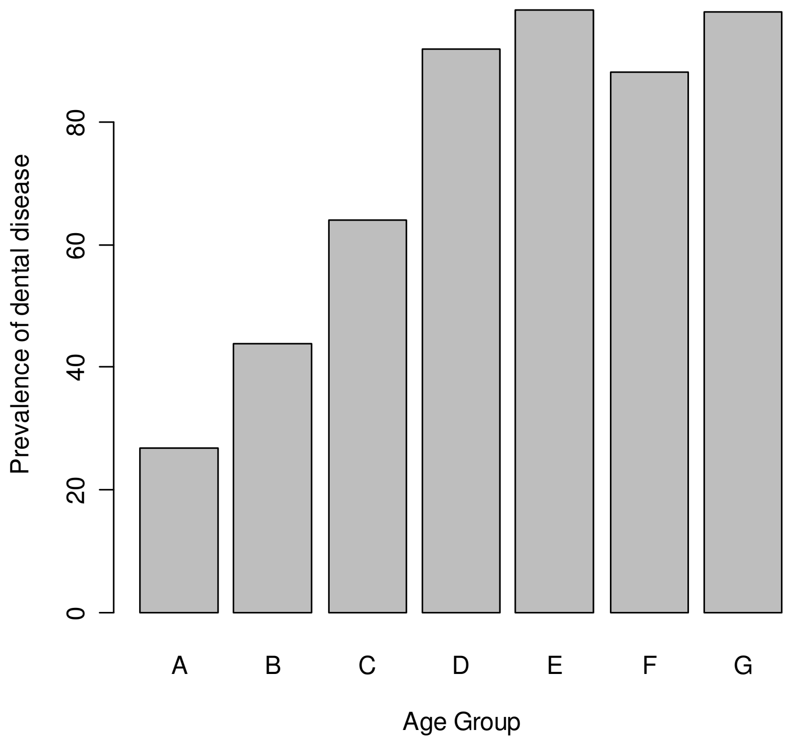


Figure 7.3.2: **Barplot illustrating an increase in the prevalence (%) of dental disease in the older age groups (Groups A - G). Group A = 0-10 years old (yo); Group B = 11-15 yo; Group C = 16-20 yo; Group D = 21-25 yo; Group E = 26-30 yo; Group F = 31-35 yo; Group G = 36+ yo.**



There was also a statistically significant association between the presence of dental disease and age group ($P < 0.001$; $\chi^2_6 = 136.8$). The prevalence of dental disease in group A was significantly less than the prevalence in groups C - G (Table 3.3); group B significantly less than groups D - G ($P < 0.001$); and group C less than that in groups D ($P = 0.02$), E ($P = 0.07$) and G ($P = 0.02$).

***Table 7.3.3:* The P values and odds ratios (and 95% confidence interval) of donkeys having dental disease in age groups B – G relative to donkeys in age group A.**

B to A	C to A	D to A	E to A	F to A	G to A
P = 0.07; 2.1 (0.9 – 4.9)	P = 0.004; 4.8 (2 – 11.2)	P < 0.001; 31.2 (9.4 – 103.2)	P < 0.001; 146.6 (18.4 – 1170.6)	P < 0.001; 19.9 (6.9 – 57.1)	P < 0.001; 133 (16.6 – 1064.2)

The incisor occlusal surface appearance from frontal view was noted in every donkey that was examined. Ventral curvature or 'smile mouth' was observed in 346 of the donkeys examined (97%) (Figure 7.3.5), including 96% of donkeys with dental disease and 99% without dental disease. Other incisor occlusal surface frontal view appearances that were observed were straight horizontal line (4), a left diagonal (down on the left mandibular incisors) (4), mandibular brachygnathia (2) and maxillary brachygnathia (1).

***Figure 7.3.5:* The typical 'smile mouth' appearance of the incisor occlusal Table surface that was observed in most of the donkeys examined.**

7.3.1. Diastemata

Diastemata were present in 52.7% of all donkeys. The 643 diastemata present included 598 CT (93%) and 45 (7%) incisor diastemata (Table 7.3.1.1).

Table 7.3.1.1: **Total number of diastema per CT interdental site and number of diastema with the tooth rostral to the diastema also displaced medially, displaced laterally, periodontal disease, worn, overgrown (OG), or infundibular caries (IC) (maxillary CT only).**

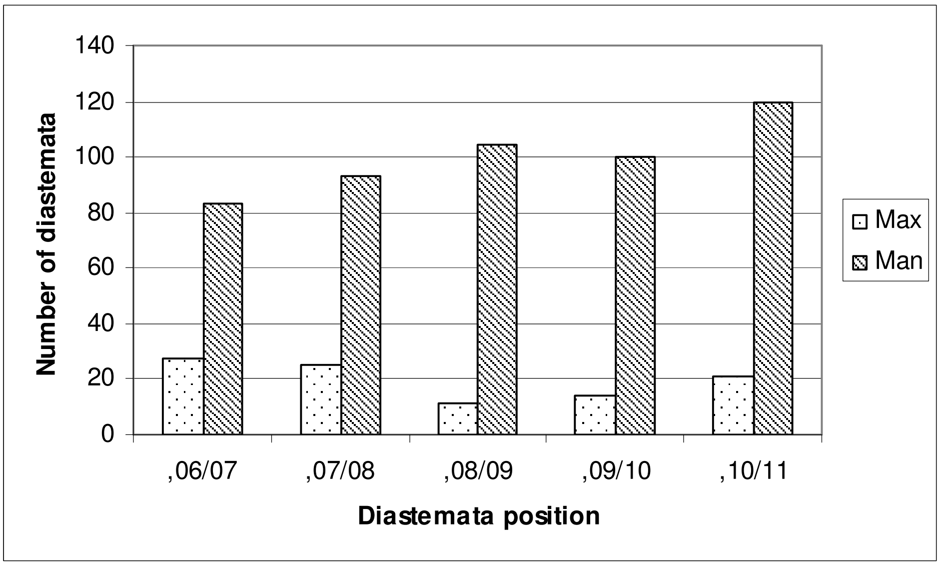
Diastema position	Total diastema	Dis. Medially	Dis. Laterally	Perio. Disease	Worn	OG	IC
Maxillary	98						
106/07	11	0	0	0	1	2	2
107/08	**16**	0	0	1	2	2	1
108/09	9	1	0	1	0	0	0
109/10	7	2	1	0	0	0	0
110/11	11	0	1	1	0	2	0
206/07	**16**	1	0	0	2	4	1
207/08	9	0	0	0	1	0	0
208/09	2	1	0	0	0	0	0
209/10	7	1	0	0	0	2	1
210/11	10	0	3	2	0	0	0
Mandibular	500						
306/07	44	0	2	0	2	1	NA
307/08	54	4	0	3	0	0	NA
308/09	49	1	11	6	1	1	NA
309/10	55	11	9	9	0	0	NA
310/11	**58**	4	19	9	0	3	NA
406/07	39	0	1	2	1	2	NA
407/08	39	3	1	5	1	2	NA
408/09	55	6	3	3	1	2	NA
409/10	45	9	7	1	1	0	NA
410/11	**62**	2	9	1	2	1	NA

CT diastemata were more commonly present between the mandibular CT (500/598 [83.6%] of CT diastemata), with only 98 (16.4%) present between the maxillary CT.

The most common position of mandibular diastemata were the 10/11 position (n = 120 diastemata), followed by the 08/09 (104) and 09/10 positions (100). The 06/07 (27) and 07/08 (25) were the most common positions for maxillary diastemata (Figure 7.3.1.1).

In the maxillary CT, 5.1% of diastemata had concurrent periodontal disease at the site of diastemata, and 23.5% (23/98) were associated with the presence of adjacent displaced, worn or overgrown teeth. In the mandibular CT, 7.8% of the diastemata had periodontal disease and 24.6% (123/500) had displaced, worn or overgrown teeth adjacent to the diastema.

Figure 7.3.1.1: **Barplot showing the number of CT diastemata in the mandibular and maxillary Triadan positions.**



In total there were 16 incisor diastemata of which only 2 involved mandibular incisors. The most common site of incisor diastemata was between the 101 and 201 (6 diastemata).The number of diastemata (incisor and CT) per donkey ranged from 1 – 11 per donkey. The median number of diastemata per donkey in the group of all donkeys that had diastemata was 3 diastemata per donkey (Figure 7.3.1.2).

***Figure 7.3.1.2*: Barplot showing the number of diastemata per affected donkey.**

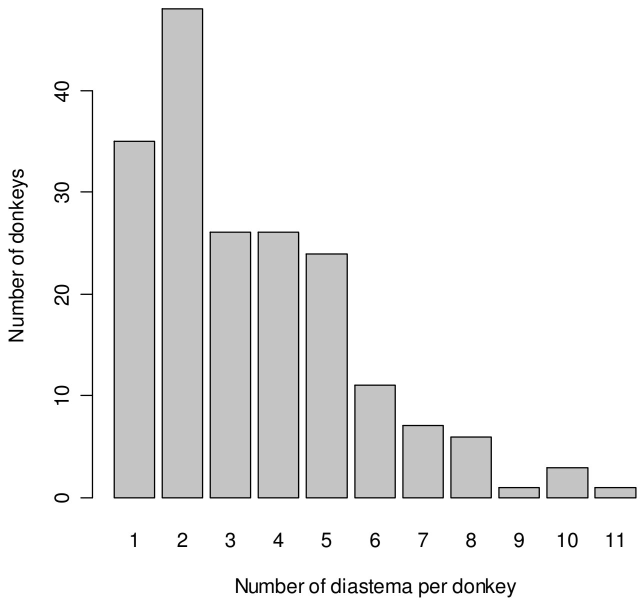
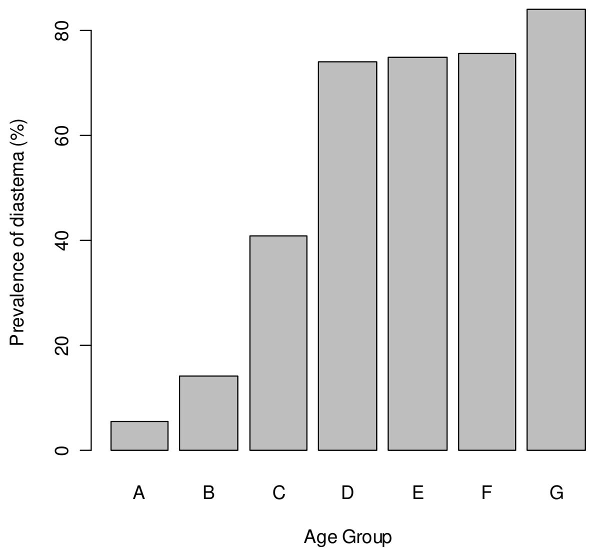


***Figure 7.3.1.3:* Barplot illustrating the increasing prevalence (%) of diastema in the increasing age groups. Group A = 0-10 years old (yo); Group B = 11-15 yo; Group C = 16-20 yo; Group D = 21-25 yo; Group E = 26-30 yo; Group F = 31-35 yo; Group G = 36+ yo.**



There was a statistically significant association between the presence of all (CT and incisor) diastemata and age group ($P < 0.001$; $\chi^2_6 = 160.3$). The prevalence of diastemata in group A was significantly less than groups C - G (Table 7.3.1.2; Figure 7.3.1.3). The prevalence of diastemata in group B was significantly less than group C ($P = 0.04$), D, E, F and G ($P < 0.001$); and group C was significantly less than group D, E, F and G ($P < 0.001$).

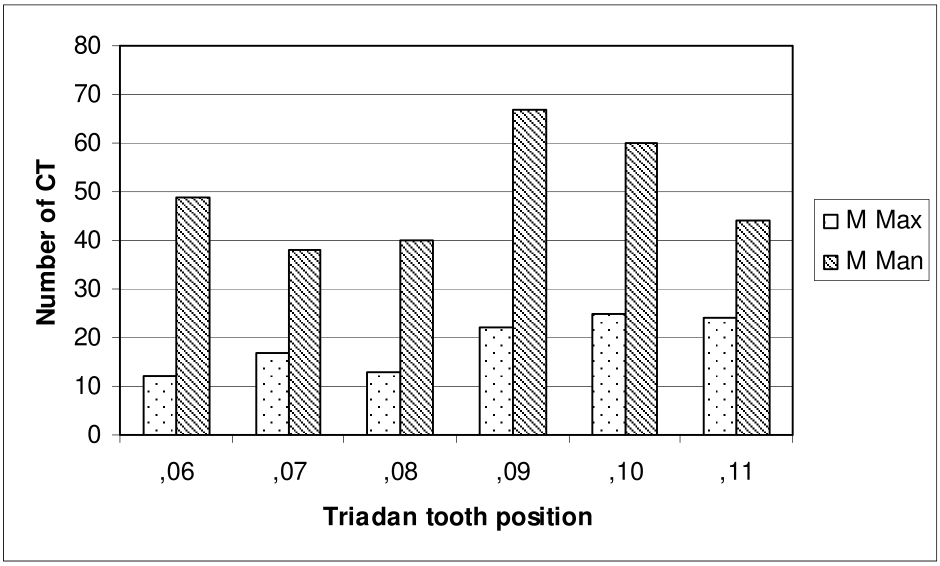
Table 7.3.1.2: **The P values and odds ratios of donkeys in age group B - G having diastemata compared to donkeys in age group A.**

B to A	C to A	D to A	E to A	F to A	G to A
P = 0.14; 3.4 (0.7 – 17.9)	P = 0.004; 16.7 (3.6 – 76.8)	P < 0.001; 88.63 (18.5 – 425.5)	P < 0.001; 80.77 (17.2 – 380.2)	P < 0.001; 64.3 (13.7 – 302)	P < 0.001; 153.6 (30.1 – 782.8)

7.3.2. Missing teeth

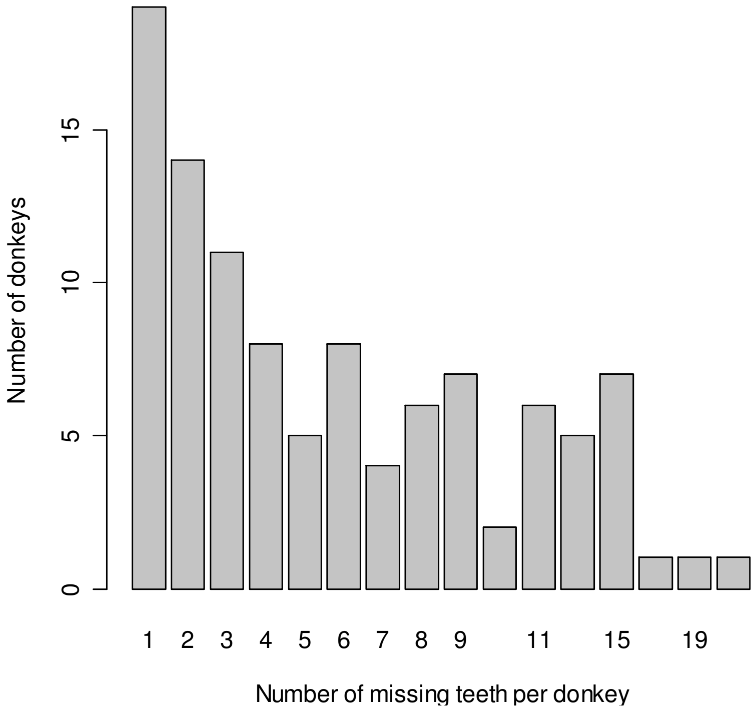
The prevalence of missing teeth in all donkeys was 29.4%. A total of 411 CT (65.4% of missing teeth) and 217 incisors (34.6% of missing teeth) were missing. Mandibular CT were more commonly missing (72.5% of missing CT) than maxillary CT (17.5% of missing CT). The 09s (67) and 10s (60) were also the most common mandibular CT to be lost, whilst the 10s (25) and 11s (24) were the most commonly missing maxillary CT. (Figure 7.3.2.1). Maxillary incisors constituted 68.2% (148) of missing incisors with the 02s most commonly affected position (39% of missing maxillary incisors).

Figure 7.3.2.1: **Barplot showing the number of missing CT in individual mandibular and maxillary Triadan positions.**



Most donkeys with missing teeth only had one tooth missing (range 1 – 26; median 4) (Figure 7.3.2.2).

Figure 7.3.2.2: **Barplot showing the number of missing teeth per affected donkey.**



There was a statistically significant association between missing teeth and age group (P < 0.001; χ^2_6 = 116.3; Figure 7.3.2.3). The prevalence of missing teeth in group B was statistically significantly less than groups D – G (Table 7.3.2.2); C was less than groups E (P = 0.014), F and G (P < 0.001); and group D was also less than group F (P = 0.01).

Figure 7.3.2.3: **Barplot illustrating the increasing prevalence of missing teeth in the increasing age groups. Group A = 0-10; Group B = 11-15; Group C = 16-20; Group D = 21-25; Group E = 26-30; Group F = 31-35; Group G = 36+.**

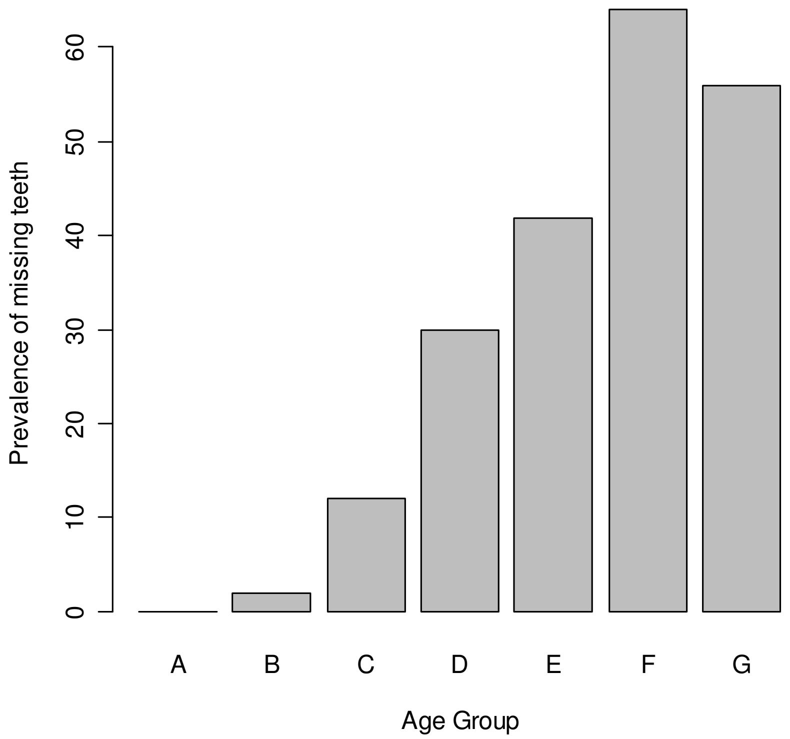


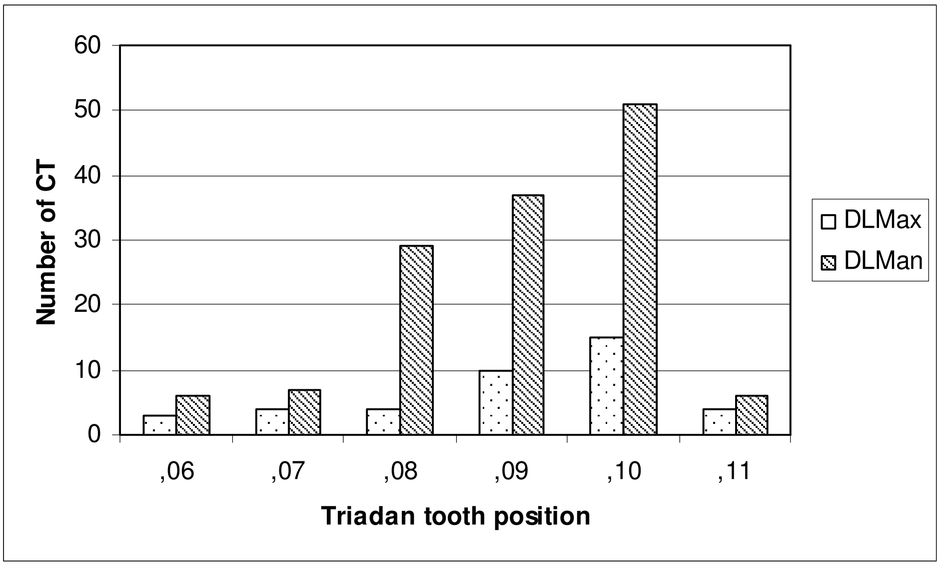
Table 7.3.2.2: **The P values and odds ratios of donkeys in age groups C - G of having missing teeth compared to donkeys in age group B. Group A was excluded as the prevalence was 0.**

C to B	D to B	E to B	F to B	G to B
P = 0.08; 6.7 (0.8 – 58.2)	P = 0.004; (2.6 – 167.8)	P < 0.001; 35.2 (4.5 – 276.1)	P < 0.001; 87.1 (10.9 – 690.8)	P < 0.001; 62.3 (7.9 – 491.9)

7.3.3. Laterally displaced teeth

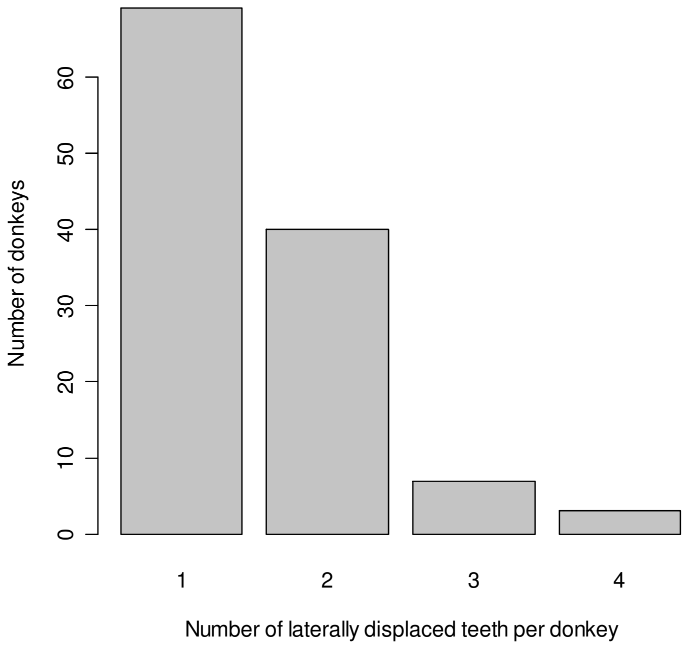
The prevalence of laterally displaced CT in this population was 33.3%. There was a statistically significant association between the presence of laterally displaced teeth and age group ($P < 0.001$; $\chi^2_6 = 66.0$). In total there were 176 laterally displaced CT and only 6 laterally (distally) displaced incisors. Mandibular CT were more commonly laterally displaced (77.3% of laterally displaced CT). In both mandibular and maxillary CT the 10s (51 and 15 respectively) followed by the 09s (37 and 10 respectively) were most commonly laterally displaced Triadan positions (Figure 7.3.3.1). Laterally displaced teeth were significantly associated with age group ($P < 0.001$; $\chi^2_6 = 66.0$). The prevalence in age group A and B was significantly less than the prevalence in E, F and G ($P < 0.02$); and group C than E ($P = 0.03$).

Figure 7.3.3.1: **Barplot showing the number of laterally displaced CT in each mandibular and maxillary Triadan positions.**



Most donkeys with laterally displaced cheek teeth only had one tooth displaced (range 1 – 4; median of 1) (Figure 7.3.3.2).

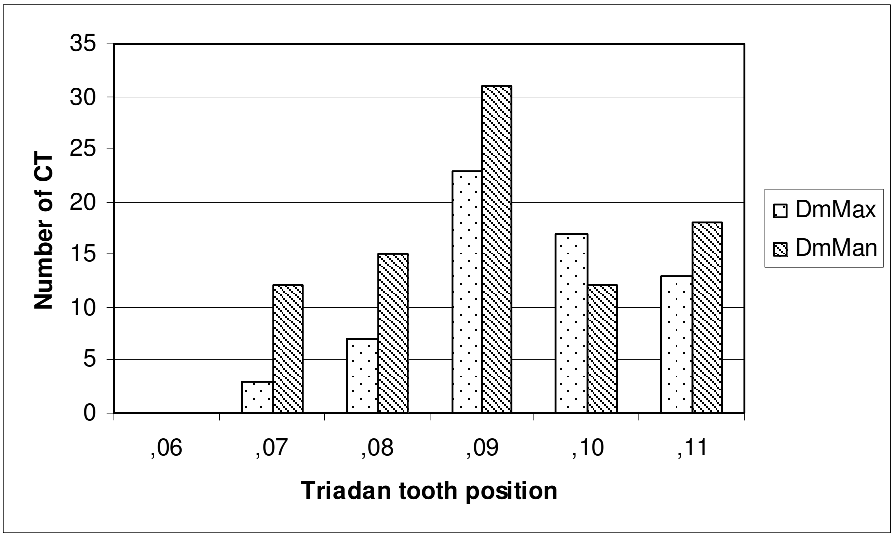
Figure 7.3.3.2: **Barplot showing the number of laterally displaced teeth per affected donkey.**



7.3.4. Medially displaced teeth

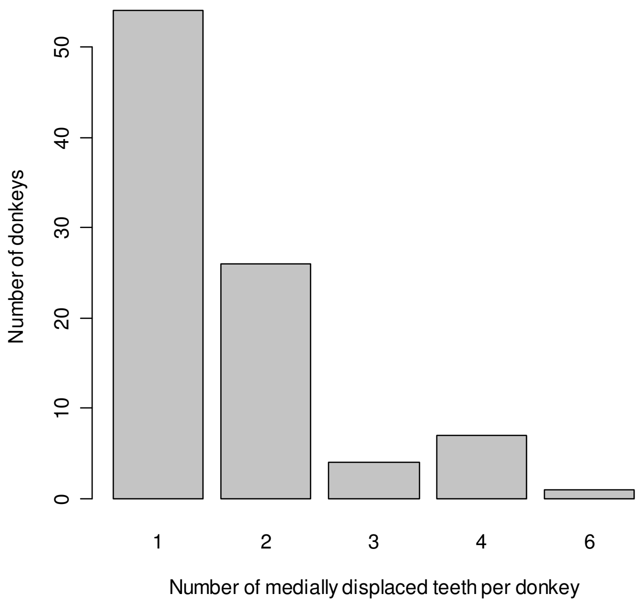
The prevalence of medially displaced CT an all donkeys was 25.8% and included 151 CT and a single medially (mesially) displaced incisor. There was a statistically significant association between the presence of medially displaced teeth and age group ($P < 0.001$; $\chi^2_6 = 71.7$). Mandibular CT were more commonly displaced (58.3% of medially displaced CT) than maxillary CT. The mandibular and maxillary 09s (31 and 23 respectively) were the most commonly medially displaced CT followed by the mandibular 11s (18) and maxillary 10s (17) (Figure 7.3.4.1). Medially displaced teeth were statistically significantly associated with age group ($P < 0.001$; $\chi^2_6 = 71.8$). The prevalence in group B was statistically significantly less than group E and F ($P < 0.005$); and group C from group D and E ($P < 0.03$).

Figure 7.3.4.1: **Barplot showing the number of medially displaced CT in each mandibular and maxillary CT Triadan positions.**



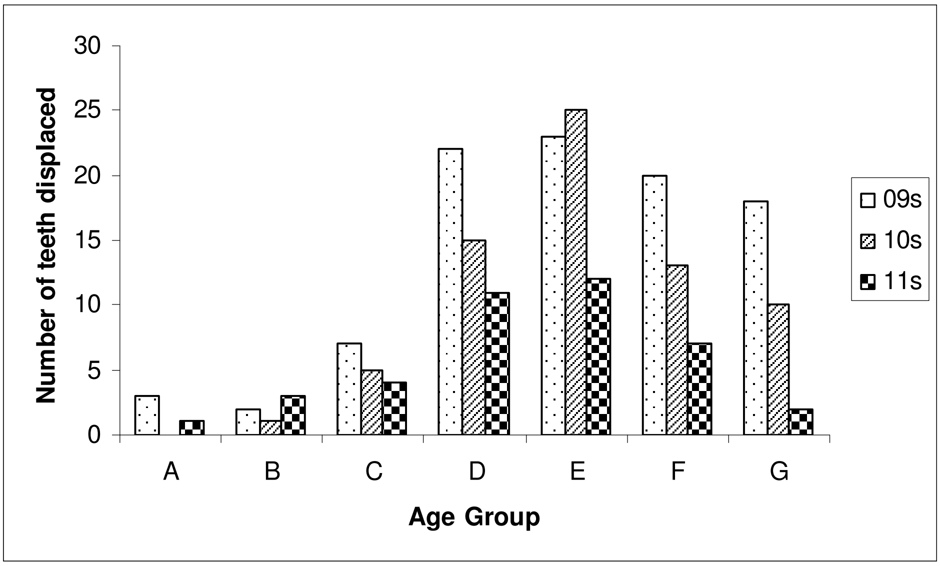
Most donkeys with medially displaced teeth only had one tooth medially displaced, (range 1 – 6; median 1) (Figure 7.3.4.2).

Figure 7.3.4.2: **Barplot showing the number medially displaced cheek teeth per affected donkey.**



The prevalence of all displaced (medially and laterally) Triadan 09 teeth were not statistically significantly associated with age group (P = 0.68; $\chi^2_6 = 3.9$), however Triadan 10 and 11 were (P < 0.007; $\chi^2_6 = 17.8$ and P < 0.001; $\chi^2_6 = 56.8$ respectively) with the prevalence of displaced teeth increasing with increasing age.

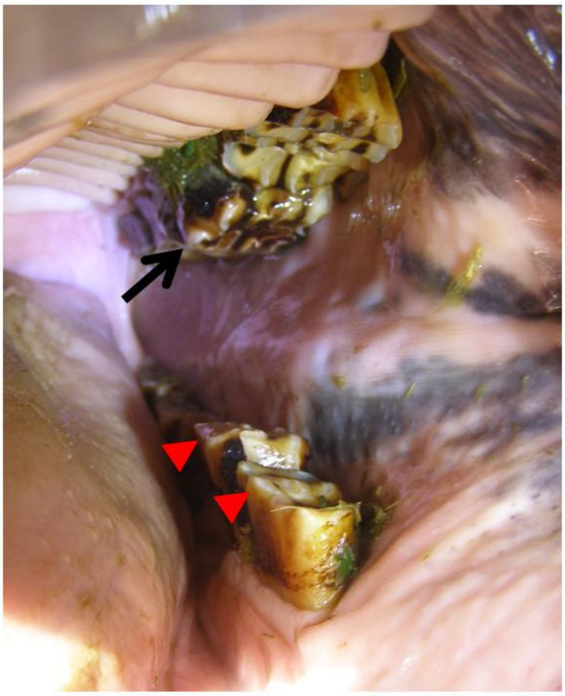
Figure 7.3.4.3: **Barplot showing the number of displaced CT in Triadan positions 09, 10 and 11 in each age group (A – G).**



7.3.5 Overgrown teeth

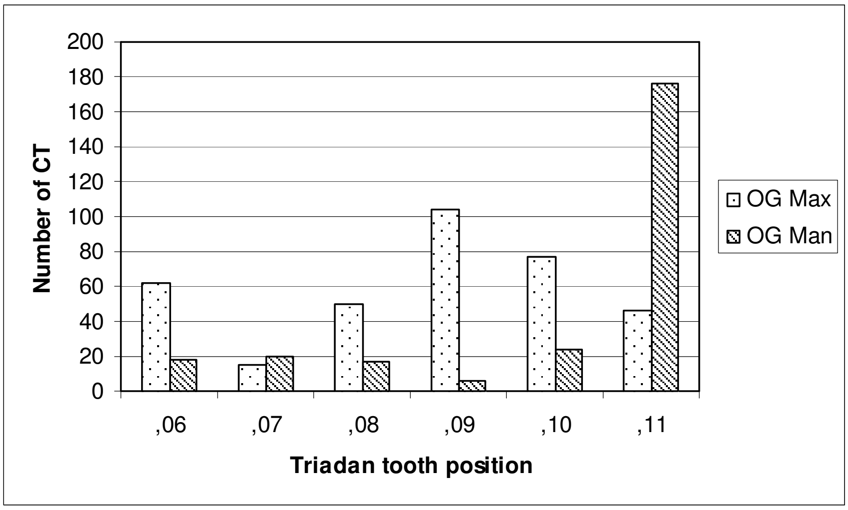
The prevalence of overgrown teeth in all donkeys was 52.1%. There was a total of 615 overgrown CT and only 11 overgrown incisors (Figure 7.3.5.1).

***Figure 7.3.5.1*: Intra oral view of an overgrown 208 (arrow) due to lack of wear due to a corresponding missing mandibular CT. Also note the sharp enamel points on the 306 and 307 (red arrow heads).**



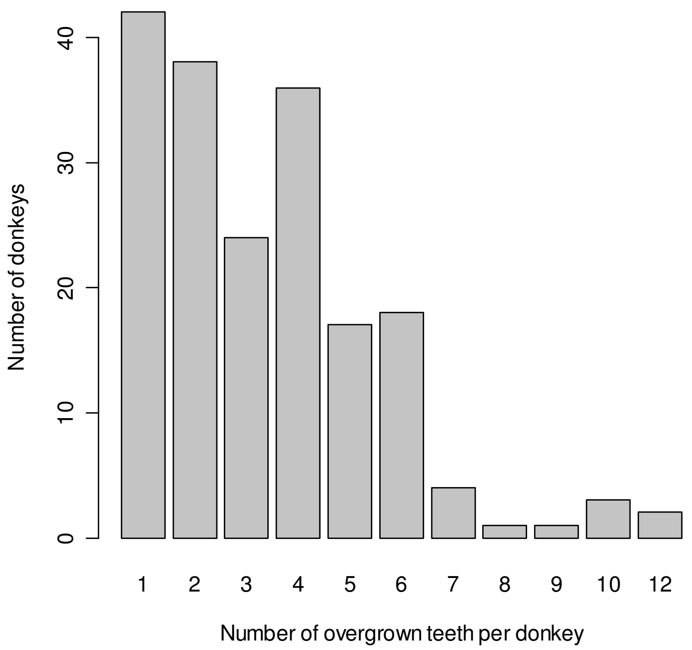
Maxillary CT were more commonly overgrown (57.6% of overgrown CT) than mandibular CT (42.4%). The mandibular 11s were the most common CT with overgrowths (176) followed by the maxillary 09s (104) and 10s (77) (Figure 7.3.5.2). The 11 overgrown incisors were all mandibular incisors.

Figure 7.3.5.2: **Barplot showing the number of overgrown CT in each mandibular and maxillary CT Triadan position.**



The number of overgrown teeth ranged from 1 – 12 per donkey (median 3) (Figure 7.3.5.3).

Figure 7.3.5.3: **Barplot showing the number of overgrown teeth per affected donkey.**



There was a statistically significant association between the presence of overgrown teeth and age group ($P < 0.001$; $\chi^2_6 = 105.2$; Figure 7.3.5.4). There was a significant difference in the prevalence of overgrown teeth in group A to groups D - G (Table 7.3.5.2). The prevalence in group B was less than groups D - G ($P < 0.01$). The prevalence in group C was significantly less than groups E ($P = 0.03$), F and G ($P < 0.01$).

Figure 7.3.5.4: **Barplot illustrating the increasing prevalence of overgrown teeth in the increasing age groups. Group A = 0-10 yo; Group B = 11-15 yo; Group C = 16-20 yo; Group D = 21-25 yo; Group E = 26-30 yo; Group F = 31-35 yo; Group G = 36+ yo.**

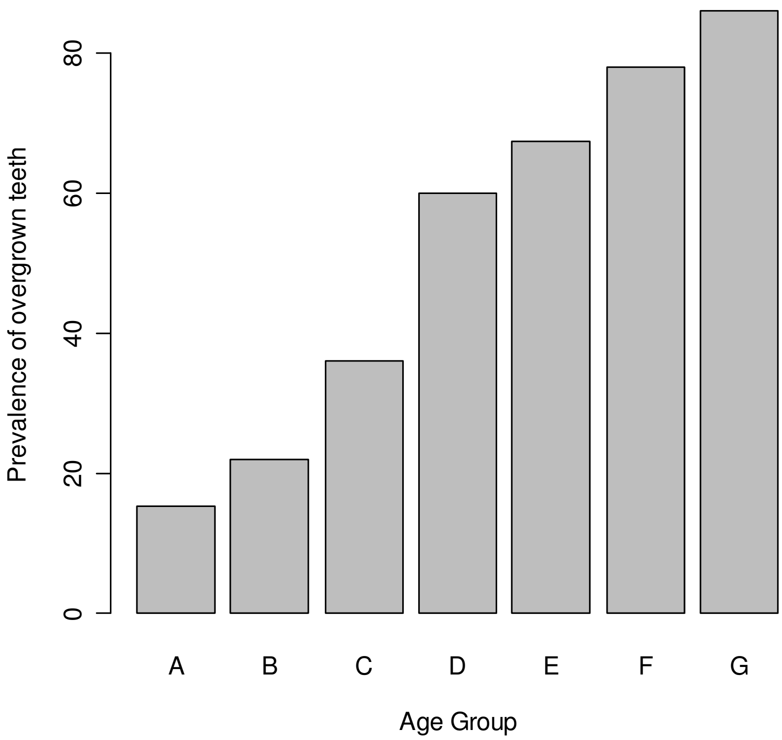


Table 7.3.5.2: **The P values and odds ratios of donkeys in age group B - G having overgrown teeth compared to donkeys in age group A.**

B to A	C to A	D to A	E to A	F to A	G to A
P = 0.4; 1.6 (0.6 – 4.3)	P = 0.02; 3.1 (1.2 – 8.0)	P < 0.001; 8.3 (3.2 – 21.2)	P < 0.001; 11.3 (4.4 – 29.1)	P < 0.001; 19.5 (7.1 – 53.6)	P < 0.001; 40.3 (12.9 – 126.4)

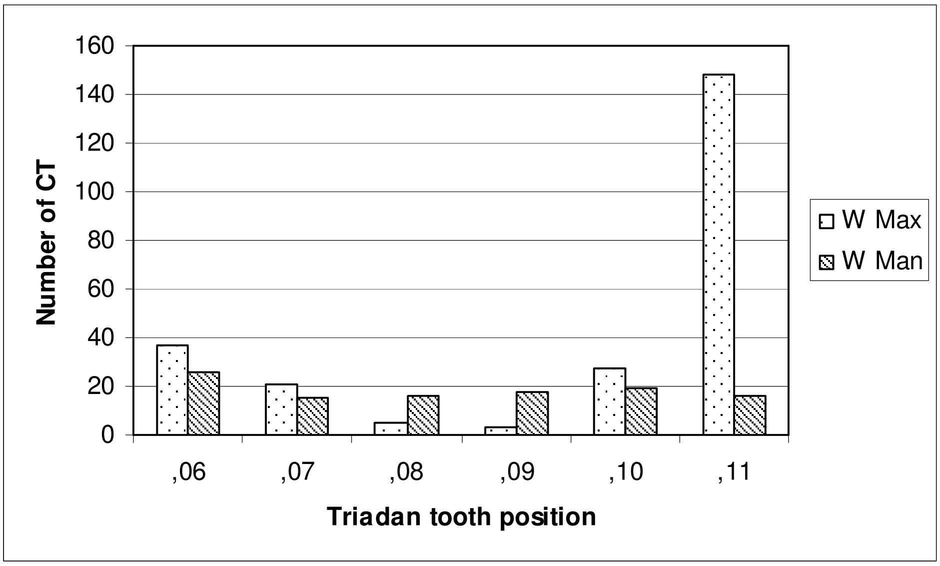
7.3.6. Worn teeth

The prevalence of worn teeth in all donkeys was 41.5% and included 351 worn CT and 96 worn incisors (Figure 7.3.6.1).

Figure 7.3.6.1: **All the maxillary incisors in this aged donkey were worn down to the gingival margin, resulting in overgrowth of the mandibular incisors.**

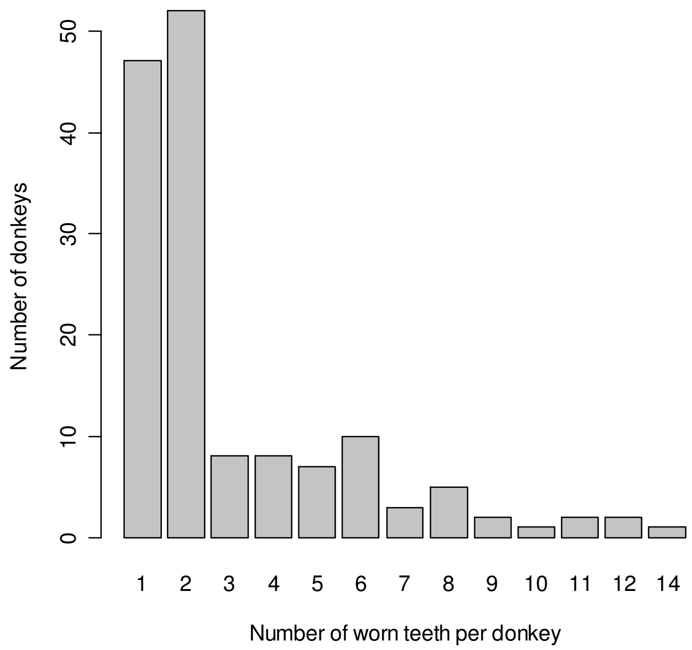
Maxillary CT were more commonly worn (68.7% of the worn CT) compared to mandibular CT (31.3%). The maxillary 11s (n = 148 worn CT) were the most commonly affected Triadan position, followed by the maxillary 06s (n = 37) and 10s (n = 27). The 06s were the most common worn mandibular CT (n = 26) (Figure 7.3.6.1).

Figure 7.3.6.1: **Barplot showing the number of worn CT in each mandibular and maxillary Triadan position.**



The number of worn teeth ranged from 1- 14 per donkey (median 2) Figure 7.3.6.2).

Figure 7.3.6.2: **Barplot showing the number of worn teeth per affected donkey.**



There was a statistically significant association between the presence of worn teeth and age group ($P < 0.001$; $\chi^2_6 = 109.3$; Figure 7.3.6.3). The prevalence of worn teeth in group A was significantly less than groups C - G (Table 7.3.6.2). The prevalence of worn teeth in group B was less than group E, F, G ($P < 0.001$); group C was less than groups F and G ($P < 0.001$); group D less than group F ($P = 0.04$); and group G was greater than groups D ($P < 0.001$) and E ($P = 0.017$).

Figure 7.3.6.3: **Barplot illustrating the increasing prevalence of worn teeth with age. Group A = 0-10; Group B = 11-15; Group C = 16-20; Group D = 21-25; Group E = 26-30; Group F = 31-35; Group G = 36+.**

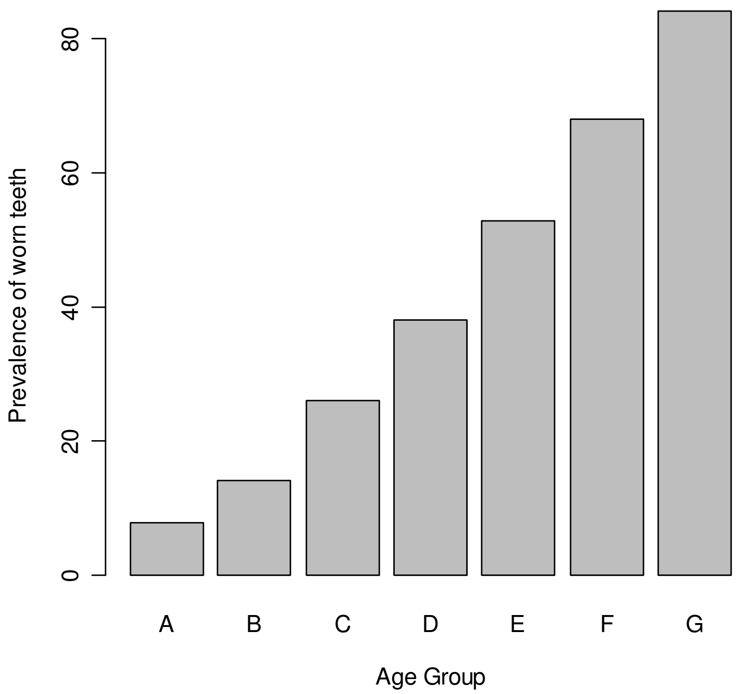


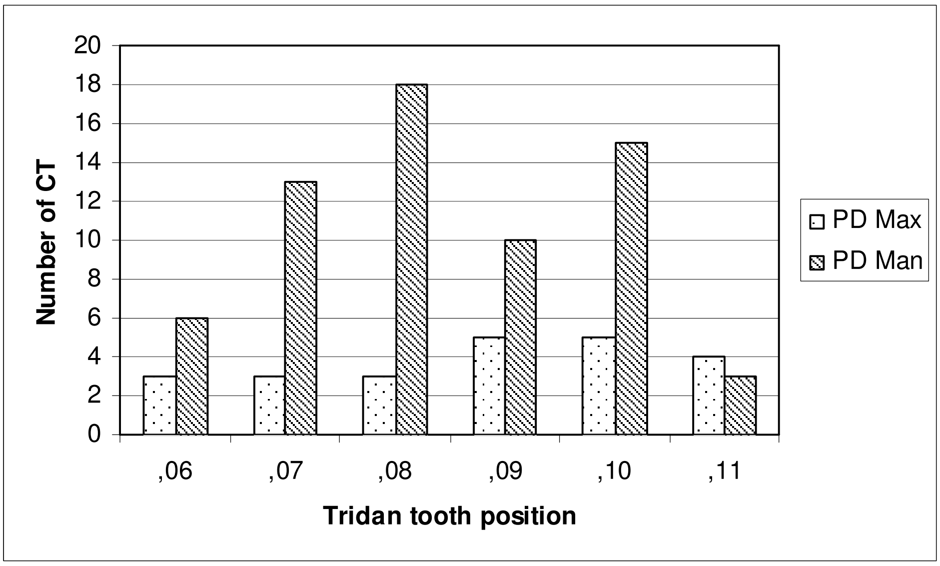
Table 7.3.6.2: **The odds ratios of donkeys in age group B - G having worn teeth compared to donkeys in age group A.**

B to A	C to A	D to A	E to A	F to A	G to A
P = 0.3; 1.9 (0.5 – 7.2)	P = 0.02; 4.2 (1.3 – 14.1)	P < 0.001; 7.4 (2.3 – 23.8)	P < 0.001; 13.4 (4.2 – 42.4)	P < 0.001; 25.5 (7.8 – 83.4)	P < 0.001; 63 (17.6 – 225.3)

7.3.7. Periodontal disease

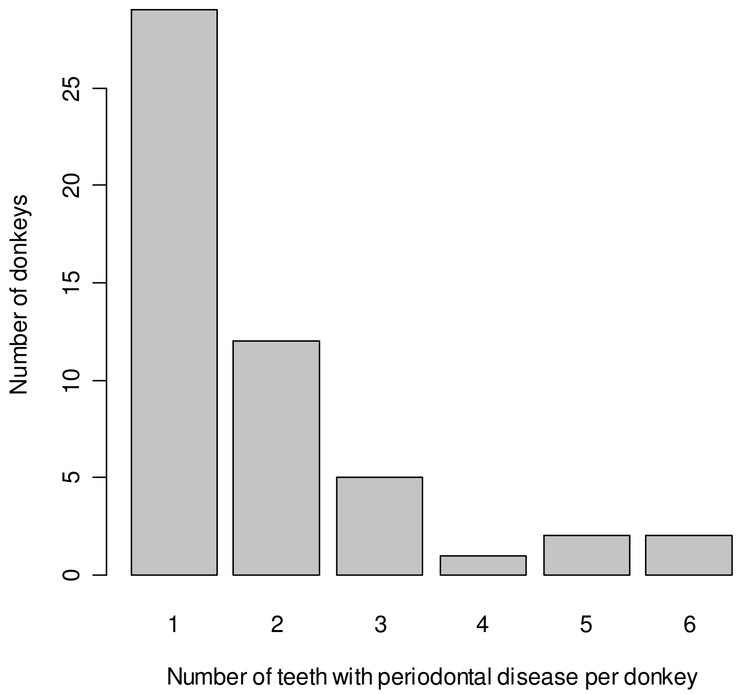
The prevalence of periodontal disease was 14.3%. There was a statistically significant association between the presence of periodontal disease and age group ($P < 0.001$; $\chi^2_6 = 48.8$). Periodontal disease was observed adjacent to 88 CT and 6 incisors. Mandibular CT more commonly had adjacent periodontal disease (73.9% of CT periodontal disease) than maxillary CT (26.1% of affected CT).The mandibular 08s (n = 18 affected), 10s (15) and 07s (13) were the most commonly affected CT. The maxillary 09s (n = 5 CT) and 10s (5) were most commonly involved maxillary CT (Figure 7.3.7.1). Incisor periodontal disease was observed in just one donkey that had all 6 maxillary incisors affected.

Figure 7.3.7.1: **Barplot showing the number of CT with periodontal disease in each mandibular and maxillary Triadan position.**



Most donkeys with periodontal disease had only 1 tooth affected (range 1 – 6; median 1) (Figure 7.3.7.2).

Figure 7.3.7.2: **Barplot showing the number of teeth with adjacent periodontal disease per affected donkey.**



Diastemata were present beside 48.9% of the teeth with periodontal disease. Medially and laterally displaced teeth were associated with 10.6% and 13.5% of teeth with periodontal disease, respectively. Enamel points, overgrown and worn teeth were present in 9.5%, 10.6% and 2.1% of teeth with periodontal disease respectively.

7.3.8. Enamel points

The prevalence of sharp enamel points in all donkeys was 26.9%. Maxillary CT (54.9%) enamel points were more common than mandibular CT (45.1%) sharp enamel points. The 11s were the most commonly affected CT in both the maxillary and mandibular CT rows (Figure 7.3.8.1). The number of teeth with sharp enamel points ranged from 1 – 27 per donkey (Figure 7.3.8.2).

Figure 7.3.8.1: **Barplot showing the number of CT with enamel points in individual mandibular and maxillary Triadan positions.**

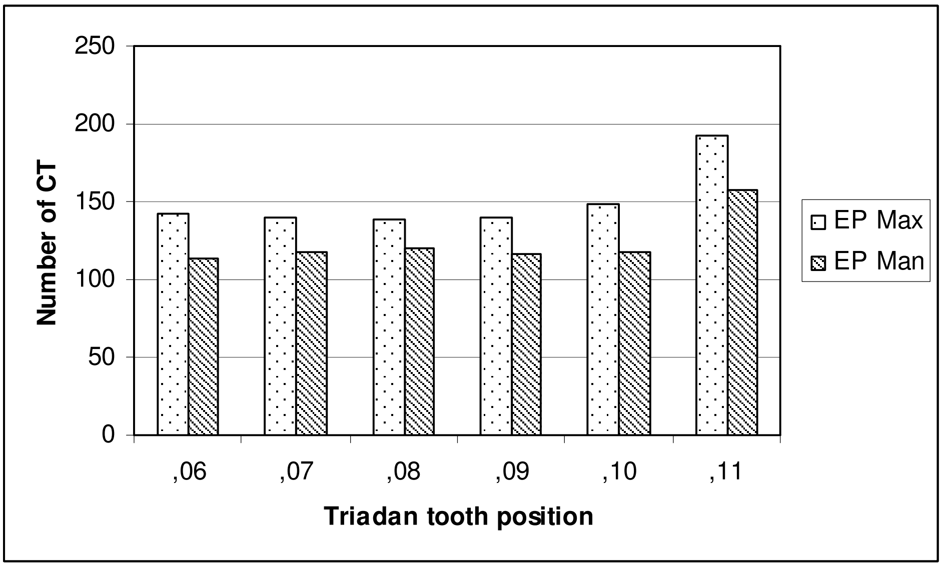
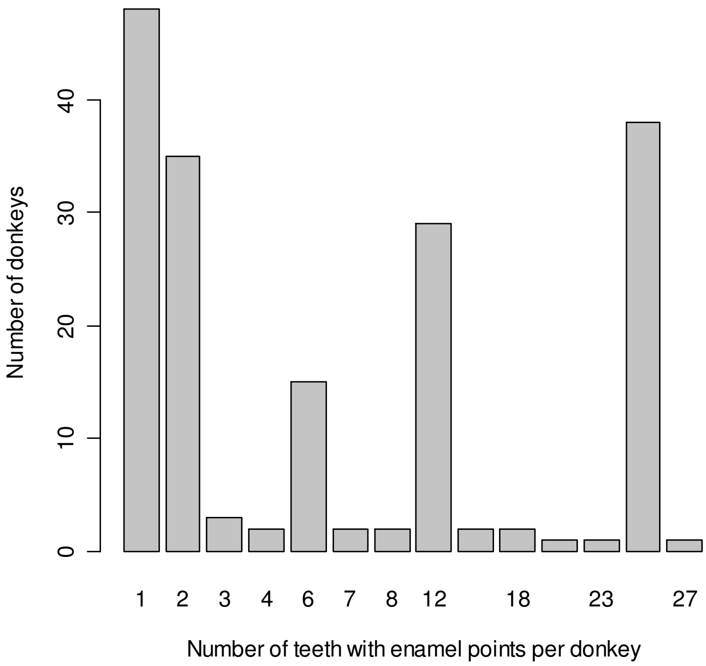
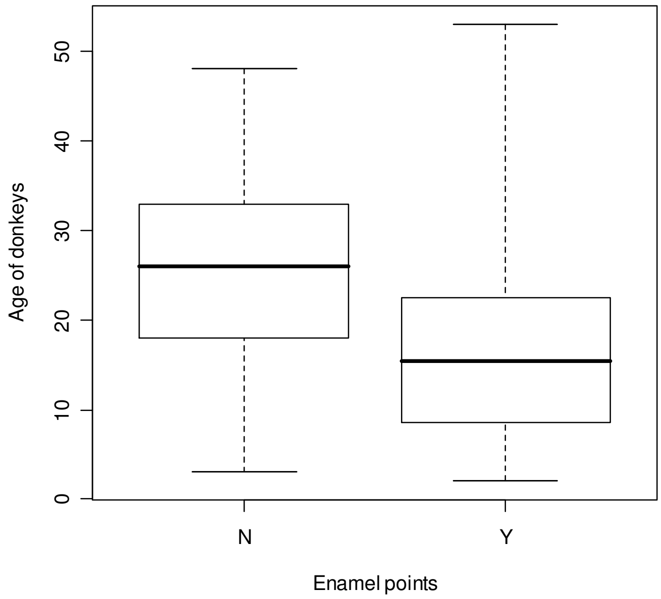


Figure 7.3.8.2: **The number of teeth with enamel points per affected donkey.**



There was a significant association between age and the presence of sharp enamel points with enamel points more likely to be present in younger donkeys (median age 15.5) and older donkeys (median age 26) less likely to have sharp enamel points ($P < 0.001$; $\chi^2_1 = 50.42$; slope = - 0.09; Figure 7.3.8.3).

***Figure 7.3.8.3*: Boxplot showing the age of donkeys with (Y) and without (N) sharp enamel points. Boxes = interquartile range; horizontal line = median; vertical lines = range.**



There was a statistically significant association between the presence of sharp enamel points and age group ($P < 0.001$; $\chi^2_6 = 50.7$; Figure 7.3.8.4). The prevalence of enamel points in group A was significantly greater than the prevalence in groups D – G (Table 7.3.8.1). The prevalence of groups B and C was greater than groups E ($P = 0.03$) and G ($P = 0.007$).

Figure 7.3.8.4: **Barplot illustrating the decreasing prevalence of enamel points in the increasing age groups.**

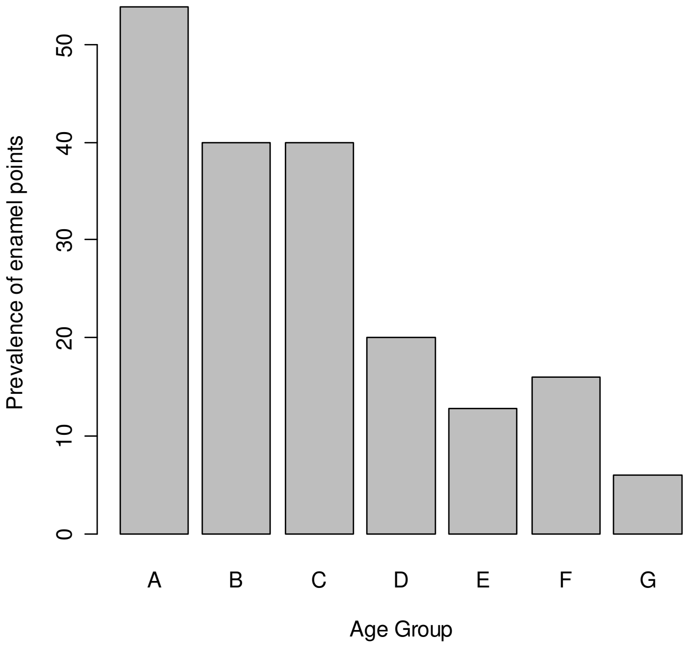


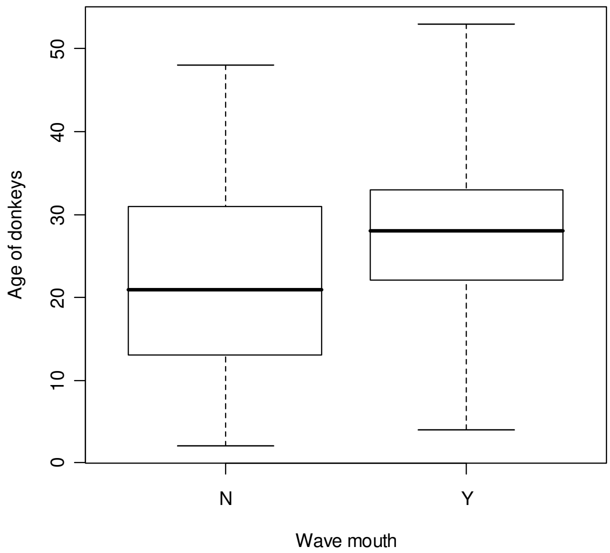
Table 7.3.8.1: **The odds ratios of donkeys in age groups B – G having enamel point compared to donkeys in age group A.**

B to A	C to A	D to A	E to A	F to A	G to A
P = 0.17; 0.57 (0.26 – 1.3)	P = 0.17; 0.57 (0.3 – 1.3)	P = 0.01; 0.2 (0.09 – 0.5)	P < 0.001: 0.13 (0.05 – 0.33)	P = 0.003; 0.16 (0.06 – 0.4)	P < 0.001; 0.06 (0.002 – 0.2)

7.3.9. Wave mouth

A total of 19.3% (69) of donkeys had wave mouth and its presence was significantly associated with estimated age (P < 0.001; $\chi^2_1 = 13.84$; slope = 0.05; Figure 7.3.9.1). Donkeys with wave mouth had a median age of 28 years compared to a median age of 21 years in donkeys without wave mouth.

***Figure 7.3.9.1*: Boxplot of age of donkeys with (Y) and without (N) wavemouth.**



There was a statistically significant association between the presence of wave mouth and age group (P = 0.002; $\chi^2_6 = 23.0$; Figure 7.3.9.2). There was a statistically significant difference between the prevalence in age group A - G (Table 7.3.9.1).

***Figure 7.3.9.2:* Barplot illustrating the increasing prevalence of wave mouth in the increasing age groups.**

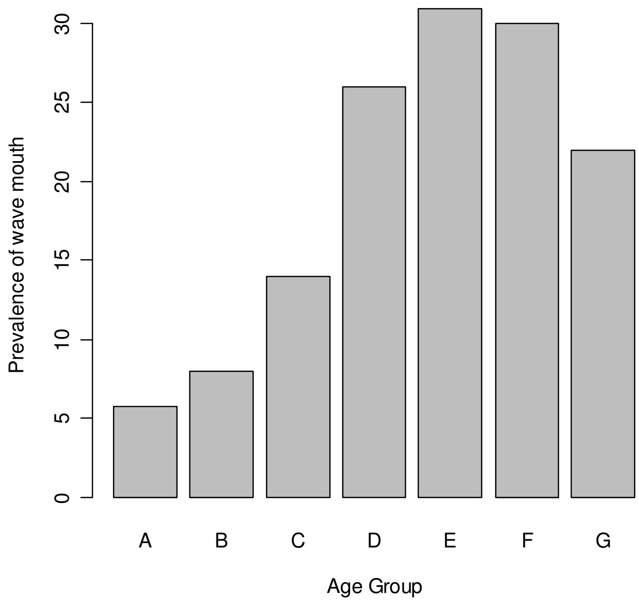


Table 7.3.9.1: **The odds ratios of donkeys in age groups B – G having wave mouth compared to donkeys in age group A.**

B to A	**C to A**	**D to A**	**E to A**	**F to A**	**G to A**
P = 0.66; 1.42 (0.3 – 0.67)	P = 0.18; 2.7 (0.64 – 10.9)	P = 0.01; 5.7 (1.5 – 21.7)	P = 0.003; 7.3 (1.9 – 26.9)	P = 0.003; 7 (1.9 – 26.2)	P = 0.03; 4.6 (1.2 – 17.7)

7.3.10. Step mouth

Step mouth was present in 11.8% of the donkeys examined and its presence was significantly associated with age (P < 0.001); $\chi^2_1 = 35.18$; slope = 0.10; Figure 7.3.10.1). The median age of donkeys with step mouth was 32 years and without step mouth was 21 years.

Figure 7.3.10.1: **Boxplot of age in donkeys with (Y) and without (N) step mouth.**

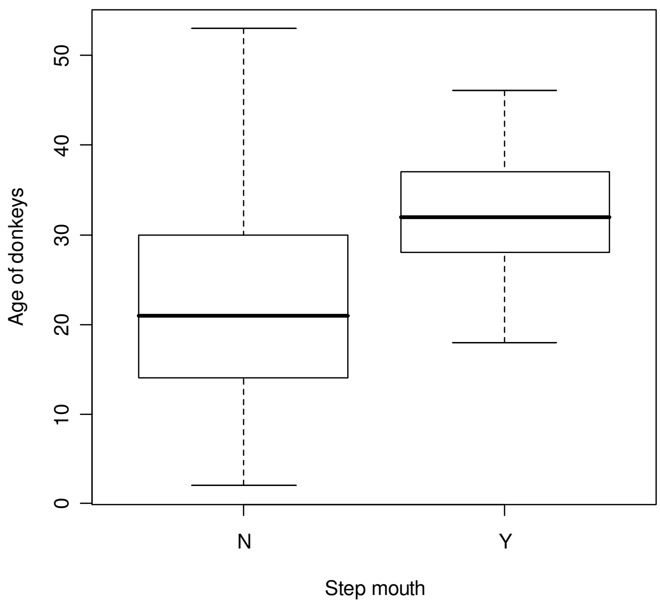


Figure 7.3.10.2: **Intra-oral view of an overgrown maxillary CT (209) (arrow) corresponding to a missing mandibular CT and such overgrowths of all or part of CT are classified as a step mouth. Also note the small diastemata between 306 and 307 (white arrow).**

There was a statistically significant association between the presence of step mouth and age group ($P < 0.001$; $\chi^2_6 = 51.6$; Figure 7.3.10.3). The prevalence of step mouth in group C was statistically significantly less than age groups D, F and G ($P \leq 0.01$) (Table 7.3.10.1).

Figure 7.3.10.3: **Barplot illustrating the increasing prevalence of step mouth in the increasing age groups.**

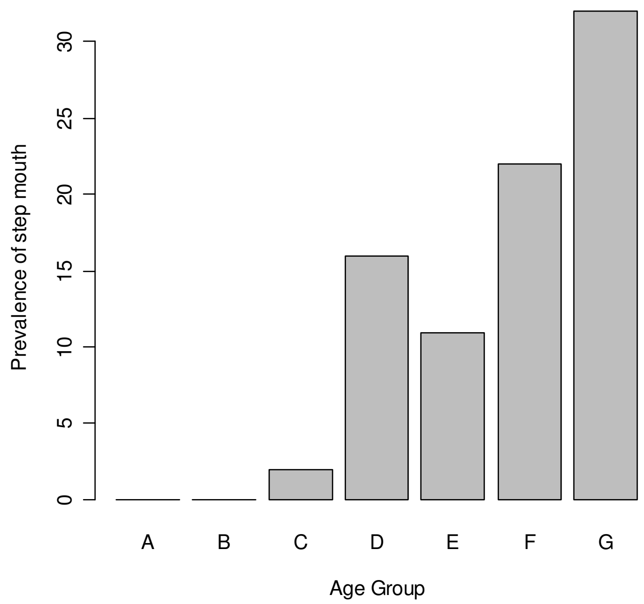


Table 7.3.10.1: **The P values and odds ratios of donkeys in age groups C-G having step mouth compared to donkeys in age group C. Groups A and B were excluded as the prevalence was 0.**

C to D	C to E	C to F	C to G
P = 0.04; 9.3 (1.1-78.5)	P = 0.1; 6 (0.7 – 52.2)	P = 0.01; 13.8 (1.7 – 112.9)	P = 0.002; 23.1 (2.9 – 184)

Most donkeys with step mouth had more than one CT overgrown (92.5%), with only one overgrown CT in 3 donkeys. The most common CT overgrown in the donkeys observed to have step mouth were the 411 (60%) and 311 (55%), followed by the 209 (42.5%) and 109 (40%).

Table 7.3.10.2: **The number of cheek teeth overgrown in each quadrant in donkeys observed to have step mouth (40 donkeys).**

Triadan no.	**06**	**07**	**08**	**09**	**10**	**11**
Quadrant 1	3	0	12	16	15	6
Quadrant 2	7	2	6	7	17	3
Quadrant 3	3	1	3	2	8	22
Quadrant 4	2	3	2	1	6	24

7.3.11. Smooth mouth

Smooth mouth was present in 4.5% of donkeys examined in this study and its presence was associated with age ($P < 0.001$; $\chi^2_1 = 32.9$ slope = 0.17; Figure 3.11.1). The median age of donkeys with smooth mouth was 37 years and without smooth mouth was 22 years.

Figure 7.3.11.1**: Boxplot of age in donkeys with (Y) and without (N) smooth mouth.**

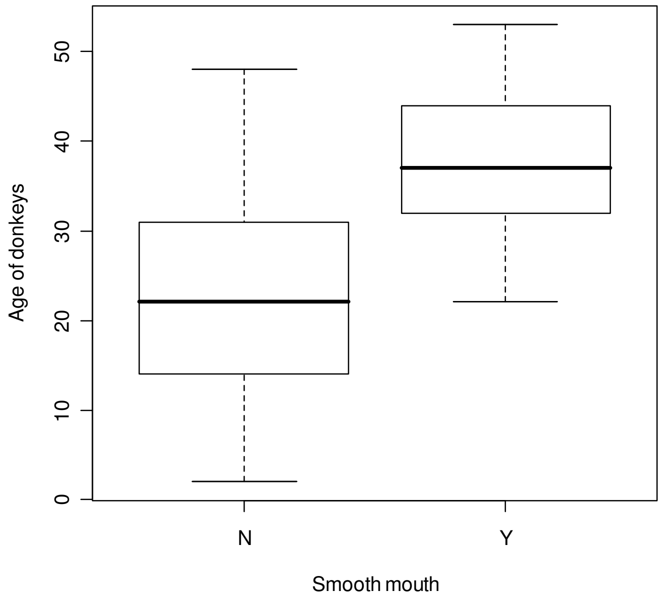
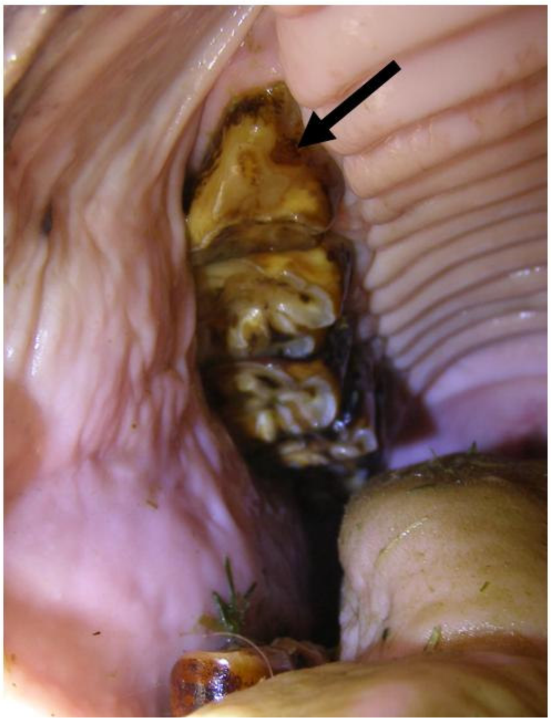


Figure 7.3.11.2: **Intra-oral view of worn maxillary CT (206) (arrow). Also note the decreased enamel infolding in the 207, indicating the beginning of smooth mouth in this row.**



There was a statistically significant association between the presence of smooth mouth and age group ($P < 0.001$; $\chi^2_6 = 35.1$; Figure 7.3.11.2). The prevalence of smooth mouth in group D was significantly less than from group G (Table 7.3.11.1).

Figure 7.3.11.2: **Bar plot showing prevalence of smooth mouth in each age group (A – G).**

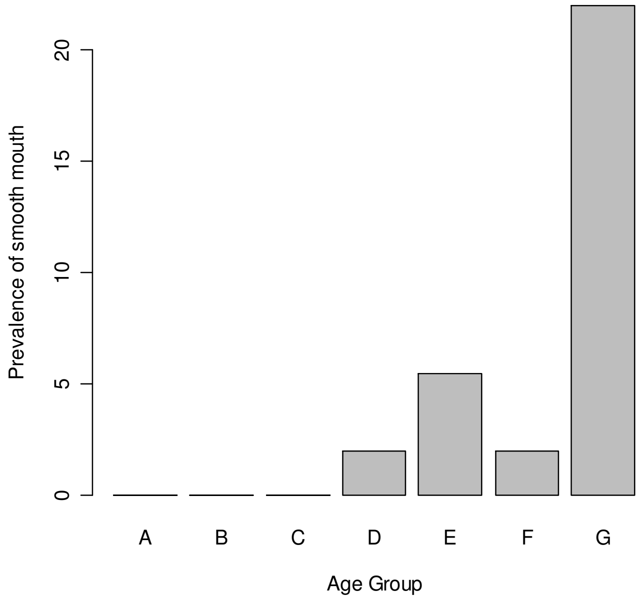


Table 7.3.11.1: **The odds ratios of donkeys in age groups A - F having smooth mouth compared to donkeys in age group G. Groups A, B and C were excluded as the prevalence was 0.**

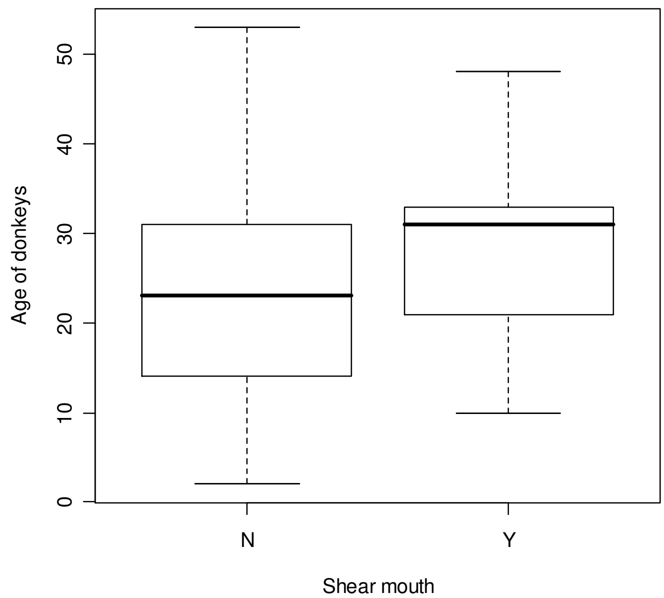
E to D	**F to D**	**G to D**
P = 0.4; 2.8 (0.3 – 28.5)	P = 1; 1 (0.06 – 16.7)	P = 0.01; 13.8 – 1.7 – 113.1

Most donkeys affected with smooth mouth had more than one row affected (75%). The mandibular CT rows (3rd and 4th quadrant) were more commonly affected (68.8% and 43.8% respectively) with smooth mouth than the maxillary CT rows (1st quadrant – 37.5% and 2nd quadrant – 18.8%).

7.3.12. Shear mouth

Shear mouth was present in 3.9% of the donkeys and donkeys with shear mouth had a median age of 31years and those without this disorder had a median age of 23 years, but this was not statistically significant (P = 0.11; χ^2_1 = 2.56; slope = 0.04; Figure 7.3.12.1).

Figure 7.3.12.1: **Boxplot of age in donkeys with (Y) and without (N) shear mouth.**



There was no statistically significant association between shear mouth and age group (P = 0.19; χ^2_6 = 11.7; Figure 7.3.12.2) and there was no statistically significant difference in the prevalence of shear mouth in the different age groups (Table 7.3.12.1).

Figure 7.3.12.2: **Bar plot showing prevalence of shear mouth prevalence in each age group (A – G).**

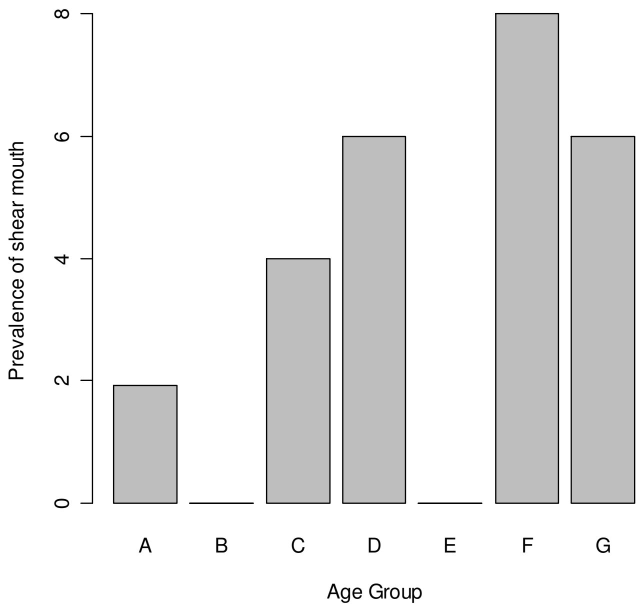


Table 7.3.12.1: **The P values and odds ratios of donkeys in age groups A - F having shear mouth compared to donkeys in age group G. No comparisons were made to group B and E as the prevalence was 0 (NA).**

B to A	C to A	D to A	E to A	F to A	G to A
NA	P = 0.5; 2.1 (0.2 – 24.4)	P = 0.3; 3.3 (0.3 – 32.7)	NA	P = 0.19; 4.4 (0.5 – 41.5)	P = 0.3; 3.3 (0.3 – 32.7)

More than one CT row was involved in 64% of the donkeys with shear mouth, with the maxillary CT rows more commonly involved (1st quadrant – 43% and 2nd quadrant- 57%) than the mandibular CT rows (3rd quadrant – 29% and 4th quadrant – 36%).

7.3.13 Infundibular caries, pulpal exposure and focal overgrowths

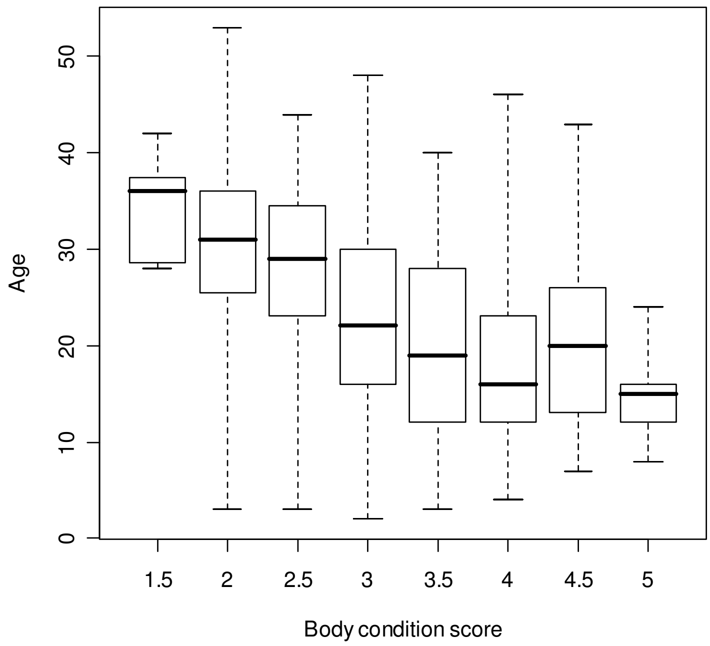
Infundibular caries were present in 39 maxillary CT with the 06s (20 i.e. 55.6%) most commonly affected. Pulpal exposure was observed in 57 CT - all maxillary CT. The 07s (17) and 08s (15) were most commonly affected positions. Pulpar exposure was noted in one mandibular incisor (401). Focal overgrowths ('hooks') were observed in 3.4% of donkeys with the maxillary 06s (9 out of 23 teeth with hooks) and mandibular 11s (12 teeth) most commonly affected.

7.4 Investigation of associated variables

7.4.1 Body condition score and age

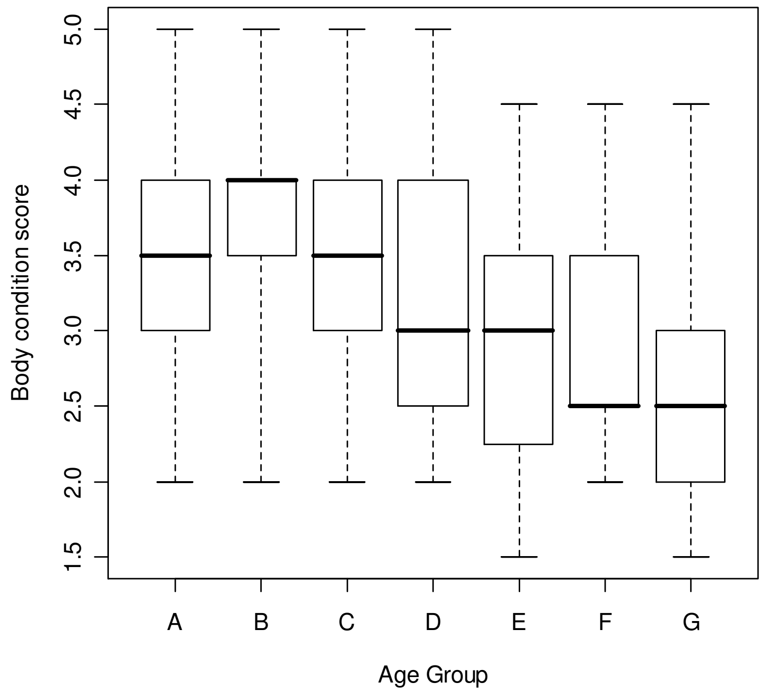
BCS varied from 1.5 – 5 out of 5 with a median value of 3. Weight loss was reported in 9.5% of donkeys (34) and quidding was noted in just 2 donkeys (0.6 %). There was a statistically significant correlation between age and BCS ($P < 0.001$; $r = -0.41$) with older donkeys having a lower BCS (Figure 7.4.1.1).

Figure 7.4.1.1: **Boxplot illustrating the association with BCS and age. Boxes = interquartile range; horizontal line = median; vertical lines = range.**



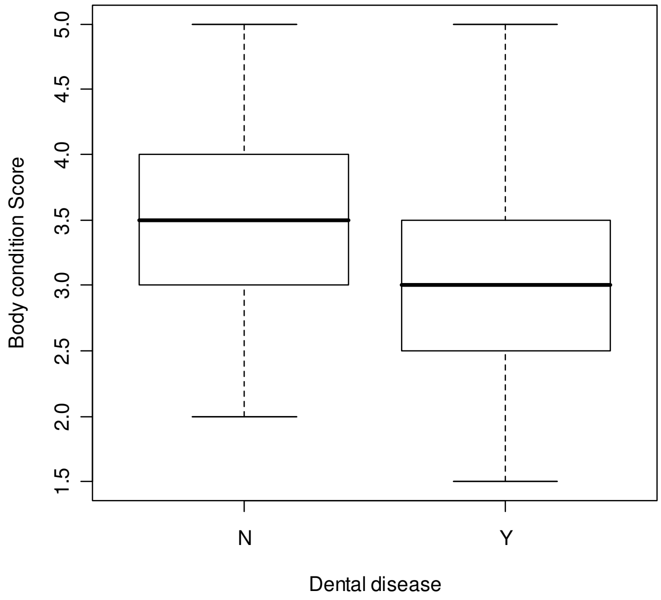
Similarly, there was a statistically significant association between age group and BCS ($P < 0.001$; $\chi^2_6 = 79.4$). The median body condition score of donkeys in age group A was: 3.5(2-5), B: 4(2-5), C: 3.5(2-5), D: 3(2-5), E: 3(1.5-4.5), F: 2.5(2-4.5), G: 2.5(1.5-3) (Figure 7.4.1.2). There was a significant difference of BCS between age groups A and E, F and G ($P < 0.01$), groups B and D, E, F and G ($P < 0.01$), groups C and E, F and G ($P < 0.01$) and group D and G ($P < 0.01$).

Figure 7.4.1.2: **Boxplot illustrating body condition scores for donkeys in each age group (A-G).**



Donkeys' suffering from dental disease were statistically more likely to have a lower BCS ($P < 0.001$; $\chi^2_1 = 28.4$; Figure 7.4.1.3). The median BCS of donkeys with dental disease was 3.0 and without dental disease was 3.5.

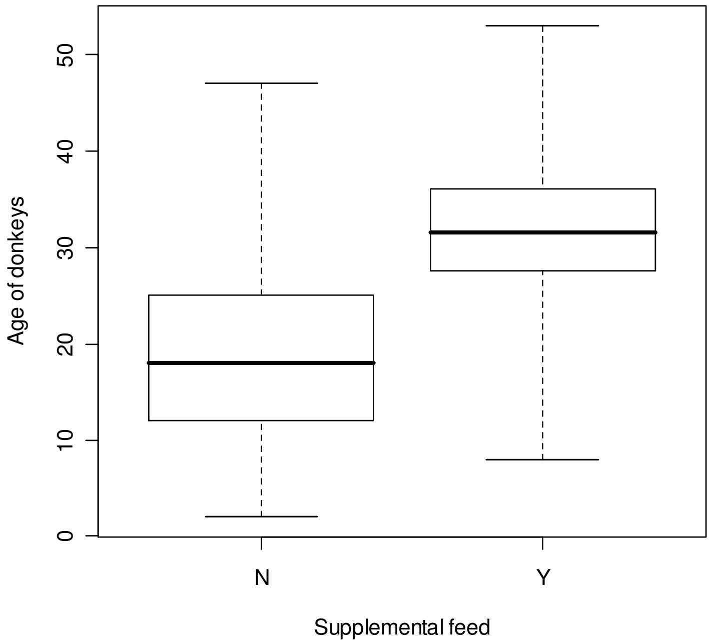
Figure 7.4.1.3: **Boxplot of BCS of donkeys with (Y) and without dental disease (N).**



7.4.2 Supplemental feed

Only 31.4 % (112) of the examined donkeys received supplemental feed in addition to *ad lib* roughage. Donkeys suffering from dental disease were statistically more likely to have supplemental feeding ($P < 0.001$; $\chi^2_1 = 51.55$), as was also indicated by the very high odds ratio (OR and CI) (12.7; 5.0 - 32.3). There was a statistically significant association between age and supplemental feeding of donkeys, with older donkeys (median age 31.5) more likely to receive supplemental feed then donkeys not receiving supplemental feed (median age 18) ($P < 0.001$; $\chi^2_1 = 122.47$; slope = 0.15; Figure 7.4.2.1).

***Figure 7.4.2.1*: Boxplot of age of donkeys receiving supplemental feed (Y) and not receiving any supplemental feed (N).**



There was a statistically significant association between age group and the presence of supplemental feeding ($P < 0.001$; $\chi^2_6 = 137.2$). There was a statistically significant difference in the percentage of donkeys given supplemental feed between age groups A and E, F, G ($P < 0.001$), B and E, F, G ($P < 0.001$), C and E, F, G ($P < 0.001$), and D and F, G ($P < 0.001$) (Figure 7.4.2.2). The odds ratios reflected the increased risk of receiving supplemental feed compared to donkeys in group A (Table 7.4.2.1).

Figure 7.4.2.2: **Boxplot of the prevalence of donkeys given supplemental feed in each age group (A – G).**

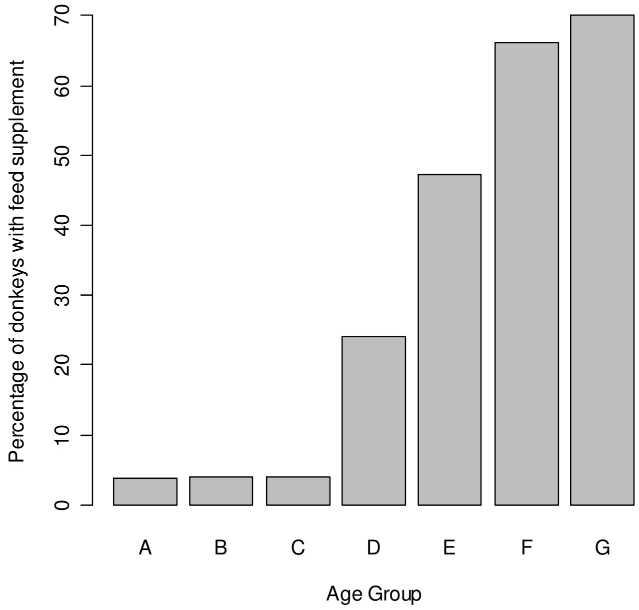
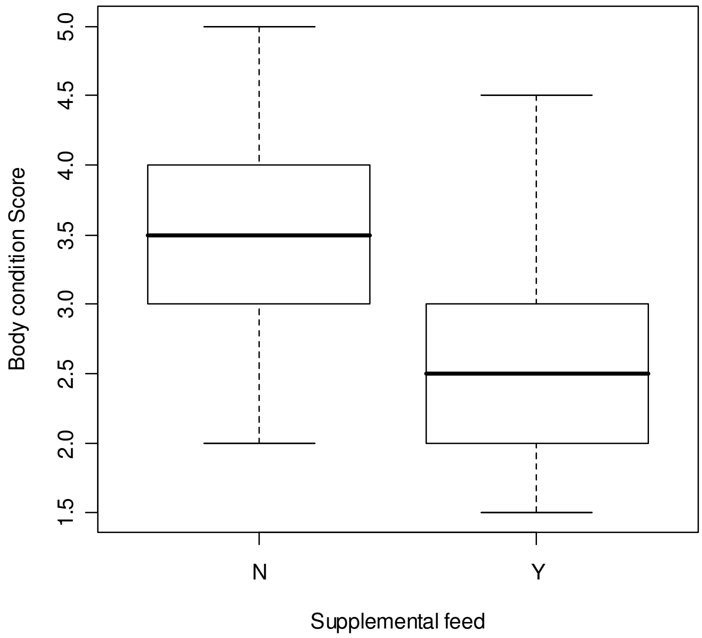


Table 7.4.2.1: **The odds ratios of donkeys in age groups B – G having supplemental feed compared to donkeys in age group A.**

B to A	C to A	D to A	E to A	F to A	G to A
1.04 (0.14 – 7.7)	1.04 (0.14 – 7.7)	7.9 (1.7 – 37.6)	22.4 (4.9 – 101.9)	48.5 (10.5 – 225.1)	58.3 (12.5 – 272.7)

There was a statistically significant association between the BCS of donkeys and supplemental feeding ($P < 0.001$; $\chi^2_1 = 69.21$), with donkeys with lower BCS more likely to be receiving supplemental feed (median BCS 2.5 compared with 3.5) (Figure 7.4.2.3).

Figure 7.4.2.3: **Boxplot of BCS in donkeys receiving supplemental feed (Y) and donkeys not receiving supplemental feed (N).**



Administration of supplemental feed was associated with diastema, missing teeth, overgrown teeth, worn teeth, medially and laterally displaced teeth, periodontal disease and age in the initial univariate model (P < 0.001; Table 7.4.2.2a, Appendix 2). Laterally and medially displaced teeth and periodontal disease were no longer statistically significantly associated with the presence of supplemental feeding in the multivariable analysis. As there was initially a significant interaction between medially and laterally displaced teeth (P = 0.04), and worn teeth with enamel points (P = 0.01; Table 7.4.2.2b, Appendix 2), these explanatory variables subsequently had to be included in the next multivariable model. However in the final model, only the interaction with worn teeth and enamel points remained significant (P = 0.03), and the association of laterally and medially displaced teeth with supplemental feed remained statistically non-significant (P ≥ 0.15; Table 7.4.2.2a, Appendix 2).

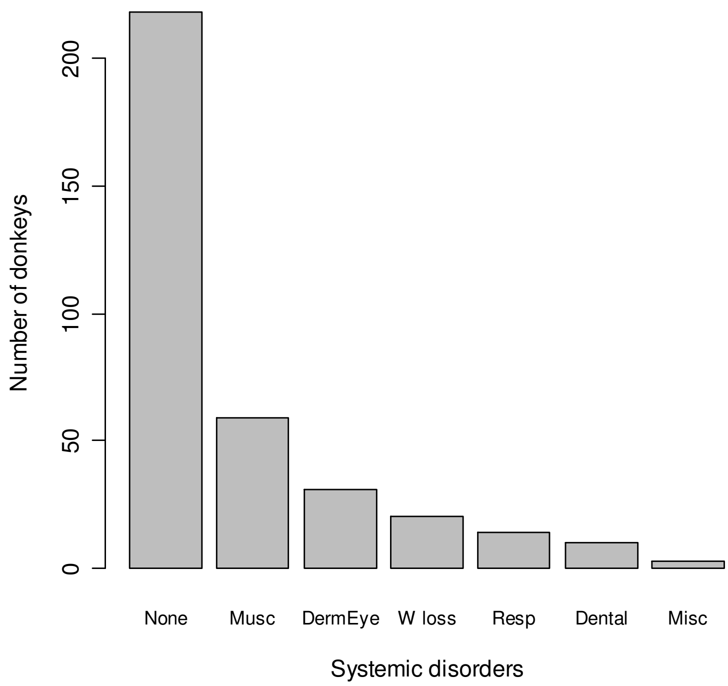
The presence of diastemata, missing, overgrown, worn teeth and the donkey’s age remained statistically significantly associated with supplemental feed in the final model

(P < 0.001; Table 7.4.4.2c, Appendix 2). There was a significant interaction between worn teeth and enamel points (P = 0.03; Table 7.4.4.2c, Appendix 2). Supplemental feed was given to 35.4% of donkeys with neither disorder compared to only 2.3% of donkeys with enamel points, but no worn teeth. This implicated that enamel points are not such an important associated factor in the absence of worn teeth.

7.4.3 Systemic disorders

Most (61.1%) donkeys did not suffer from any recognised clinical disorder at the time of dental examination. Musculoskeletal disease represented the largest (16.5%) type of systemic problem, followed by dermatological and eye problems (9%), weight loss (5.6%), respiratory (4.2%), miscellaneous disorders (0.8%), and chronic dental disease (2.8%), (Figure 7.4.3.1).

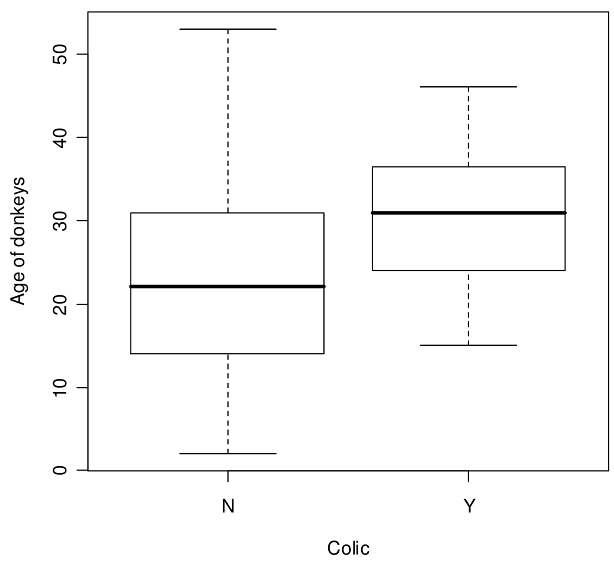
***Figure 7.4.3.1*: Bar chart illustrating number of donkeys in each systemic disorder group.**



Fourteen donkeys suffering from other systemic conditions (7 musculoskeletal, 4 dental, 1 dermatological, 1 respiratory, 1 miscellaneous disorder) also had concurrent weight loss and consequently, a total of 9.5% of donkeys suffered from weight loss.

The medical records showed that 10.1% of the 357 donkeys examined in this study suffered from at least one episode of colic. The time between our dental examination and the last colic episode ranged from < 4 weeks to 62 months previously (median 6 months; interquartile range 2.5-13 months). Of the 36 donkeys with a history of colic, only 7 had colic more than 12 months prior to dental examination, with only 4 cases recorded more than 15 months previously. There was a significant association of the occurrence of a previous colic episode to the presence of dental disease in donkeys ($P = 0.001$; $\chi^2_1 = 12.02$; OR = 7.0 (1.6 - 30.0)). There was also a significant association between occurrence of previous colic episodes and age ($P < 0.001$; $\chi^2_1 = 19.77$; slope = 0.08; Figure 7.4.3.2). Donkeys with a history of previous colic episodes had a median age of 31 years compared to a median age of 22 years for donkeys with no history of colic episodes.

Figure 7.4.3.2: **Boxplot of age of donkeys with (Y) and without (N) history of previous colic episodes.**



There was a statistically significant association between the history of recorded colic episodes and age group (P =0.004; χ^2_6 = 22.8; Figure 7.4.3.3), although only group G had a statistically significantly greater prevalence than group B (Table 7.4.3.1).

Figure 7.4.3.3: **Histogram showing the prevalence of recorded episodes of colic in each age group.**

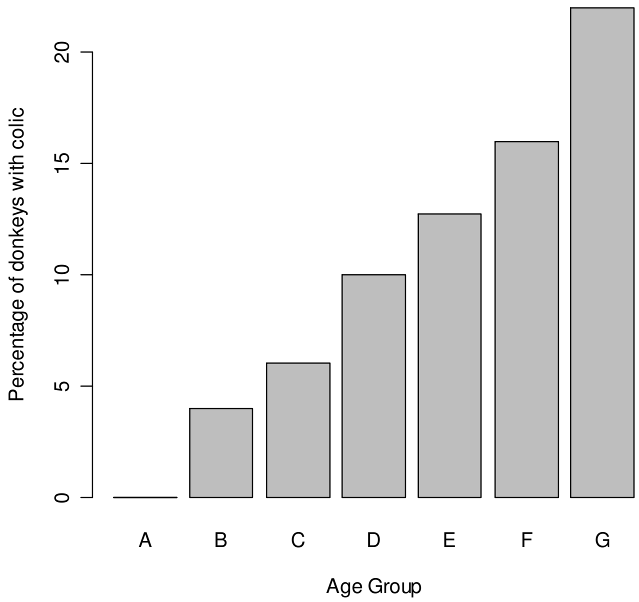


Table 7.4.3.1: **The odds ratios of donkeys in age groups C - G with a history of colic episodes compared to donkeys in age group B. A was removed from the comparison as the prevalence in group A was 0.**

C to B	D to B	E to B	E to B	G to B
P = 0.6; 1.5 (0.2 – 9.7)	P = 0.3; 2.6 (0.5 – 14.5)	P = 0.13; 3.5 (0.7 – 17.8)	P = 0.06 4.6 (0.9 – 22.9)	P = 0.02; 6.8 (1.4 – 32.6)

In the initial univariate analysis colic was associated with diastemata, missing, overgrown, worn teeth, age and supplemental food ($P \leq 0.04$; Table 7.4.3.2a, Appendix

2). Only diastemata and age remained statistically significantly associated with history of colic in the multivariable model with a significant interaction between worn and overgrown teeth (P = 0.04; Table 7.4.3.2b; Appendix 2). In the final model only the presence of diastemata (CT and incisors) (P = 0.012) and age (P < 0.001) were statistically significantly associated with history of colic, with no significant interactions.

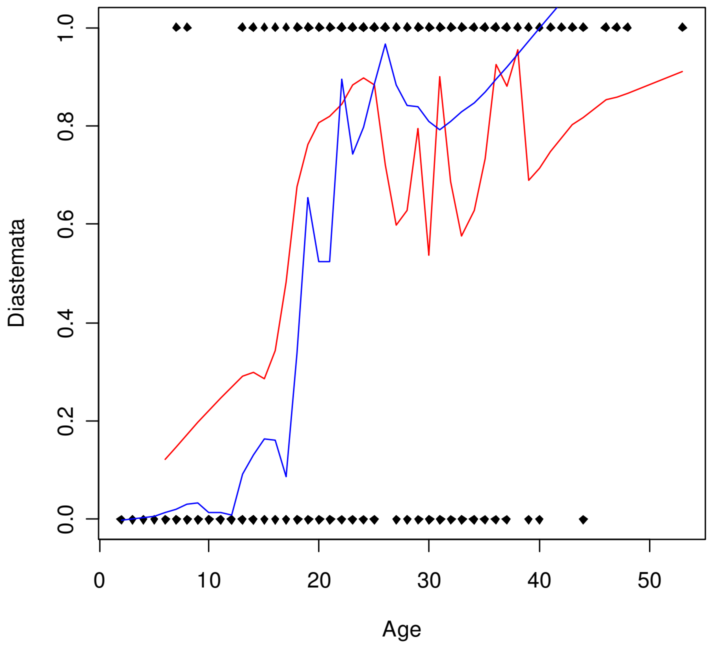
7.4.4 Diastemata

Missing, medially displaced, laterally displaced, worn and overgrown teeth, periodontal disease and age were statistically significantly associated with the presence of diastemata (P < 0.001) in the initial univariate model (Table 7.4.4.1a; Appendix 2).

Periodontal disease had to be removed from the multivariable model as there were insufficient numbers to investigate the interactions. All the binary variables were still significantly associated with the presence of diastema when included in the multivariable analysis (P < 0.001) and there were significant interactions between worn and medially displaced teeth (P = 0.01), and worn teeth and age (P < 0.001; Table 7.4.4.1b). These variables (P < 0.001) and interactions (P ≤ 0.02) remained statistically significant in the final model (Table 7.4.4.1.c).

Diastemata were present in 23.8% of donkeys with no worn and no medially displaced teeth, compared to 66.7% of donkeys with worn teeth and no medially displaced teeth. There were also significant interactions between worn teeth and age. The prevalence of diastemata in older donkeys with worn teeth was less than older donkeys without worn teeth (Figure 7.4.4.1).

***Figure 7.4.4.1:* Plot illustrating the increase in prevalence of diastemata in donkeys with worn teeth (red line) and without worn teeth (blue line).**



7.4.5 Wave mouth

The presence of wave mouth was significantly associated with the presence of overgrown teeth, worn teeth, diastemata and age in the univariate analysis ($P < 0.03$; Table 7.4.5.1a, Appendix 2). Only diastemata and overgrown teeth remained significantly associated with wave mouth in the multivariable analysis ($P < 0.03$; Table 7.4.5.1a), with a significant interaction between worn and overgrown teeth ($P = 0.02$; Table 7.4.5.1b).

In the final model diastemata ($P = 0.03$) and overgrown teeth ($P < 0.001$) remained statistically significantly associated with wave mouth (Table 7.4.5.1c, Appendix 2). Wave mouth was observed in 5% of the donkeys without worn teeth or overgrown teeth but no wave mouth seen in donkeys with overgrown teeth and no worn teeth.

7.4.6 Smooth mouth

The presence of worn and overgrown teeth, and age were significantly associated with the presence of smooth mouth in the univariate analysis ($P < 0.013$; Table 7.4.6.1a, Appendix 2).

Laterally displaced teeth were excluded from the multivariable model as there were no donkeys with smooth mouth and laterally displaced teeth in the absence of diastemata, and only 2 donkeys with smooth mouth, laterally displaced teeth and diastemata (i.e. insufficient numbers for statistical analysis). Worn teeth, overgrown teeth, diastemata and age were included in the multivariable analysis to determine an association with the presence of smooth mouth ($P < 0.2$). Only worn teeth and age were statistically significantly associated with smooth mouth ($P \leq 0.02$; Table 7.4.6.1a, Appendix 2) and there were no significant interactions ($P \geq 0.08$; Table 7.4.6.1b, Appendix 2). In the final model only worn teeth and age were statistically significant ($P \leq 0.001$; Table 7.4.6.1c, Appendix 2).

7.4.7 Step mouth

The presence of diastema, missing teeth, overgrown teeth, worn teeth, and medially and laterally displaced teeth were associated with the presence of step mouth in the univariate analysis ($P < 0.04$; Table 7.4.7.1a, Appendix 2). Time since previous dental treatment and age were included in the multivariable analysis ($P < 0.2$), but overgrown teeth were excluded in the multivariable model as all the donkeys with step mouth had one or more overgrown teeth as it was part of the definition of step mouth i.e. the value of donkeys with step mouth not having overgrown teeth was zero. Diastema, missing teeth, worn teeth, time since previous dental and age were statistically significantly associated with step mouth in the multivariable analysis ($P < 0.046$; Table 7.4.7.1a, Appendix 2) and there were no significant interactions ($P > 0.05$; Table 7.4.7.1b, Appendix 2). In the final model diastemata, missing teeth, worn teeth, time since previous dental and age were statistically significantly associated with step mouth ($P < 0.034$; Table 7.4.7.1c, Appendix 2).

7.4.8 Shear mouth

The only statistically significant dental disorder associated with shear mouth was overgrown teeth ($P = 0.013$; $\chi^2_1 = 6.24$; OR = 5.3 (1.1 – 24.2). Although missing, worn, medially displaced teeth and supplemental feed was included in the multivariable model ($P \leq 0.14$), only overgrown teeth remained significantly associated with shear mouth.

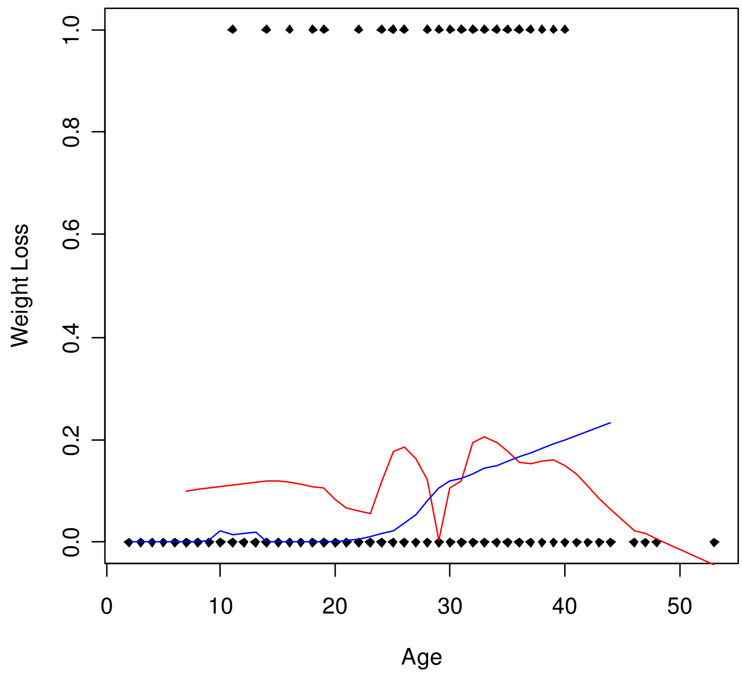
7.4.9 Periodontal disease

The presence of diastema, medially and laterally displaced teeth, overgrown teeth, sharp enamel points and age were associated with the presence of periodontal disease in the univariate analysis ($P < 0.002$; Table 7.4.9.1a, Appendix 2). However, there was no periodontal disease in donkeys with medially or laterally displaced teeth without diastemata, and no periodontal disease in donkeys without diastemata or overgrown teeth i.e. too few numbers. Therefore medially displaced, laterally displaced and overgrown teeth had to be excluded from the multivariable analysis. When performing multivariable analysis diastemata remained statistically significantly associated with periodontal disease ($P < 0.001$), but age and enamel points were not associated with periodontal disease ($P = 0.25$ and $P = 0.24$ respectively) and there were no significant interactions ($P > 0.24$; Table 7.4.9.1; Appendix 2). In the final analysis periodontal disease was associated with diastemata ($P < 0.001$).

7.4.10 Weight loss

There were significant associations between weight loss and dental disease, diastemata and age in the univariate analysis ($P < 0.01$; Table 7.4.10.1a, Appendix 2). In the multivariable analyses the association with dental disease and diastemata remained significant (P ≤ 0.04; Table 7.4.10.1a, Appendix 2) and there was a significant interaction between age and diastemata (P = 0.018). These variables and interaction remained statistically significant in the final model (P = 0.047; Table 7.4.10.1b, Appendix 2). The increased prevalence of weight loss with increasing age was greater in donkeys without diastemata than in donkeys with diastemata. (Figure 7.4.10.1)

Figure 7.4.10.1: **Plot illustrating the increase in prevalence of weight loss with age in donkeys without diastemata (blue line) and with diastemata (red line).**



7.4.11 Musculoskeletal disorders

In the initial univariate analysis there were statistically significant associations with dental disease and age (P ≤ 0.03; Table 7.4.11.1a, Appendix 2). In the multivariable model there were significant associations between the presence of musculoskeletal disorders and dental disease and age (Table 7.4.11.1b, Appendix 2), with no significant interactions between explanatory variables (P > 0.36).

7.4.12 Associations of dental disorders in individual teeth

The association between dental disorders (periodontal disease, pulpal exposure, medially and laterally displaced teeth) within individual teeth and teeth adjacent to diastemata was assessed with univariate analysis (Table 7.4.12.1, Appendix 2).

There was a statistically significant positive association between the presence of diastemata in the interdental space adjacent to a tooth with the presence of medially and laterally displaced teeth and enamel points ($P < 0.001$; Table 7.4.12.1, Appendix 2). The presence of periodontal disease beside a specific tooth was positively associated with the tooth having medial or lateral displacement, or being overgrown ($P < 0.001$). Pulpal exposure was positively associated with the presence of medially and laterally displaced, overgrown teeth and worn teeth ($P < 0.014$). Medially displaced teeth were positively associated with overgrown teeth and teeth with enamel points in the same tooth ($P < 0.001$). Laterally displaced teeth were associated with worn teeth, overgrown teeth and teeth with sharp enamel points in the same tooth ($P = 0.001$).

7.4.13 Multivariable age group analyses

The univariate and multivariable analysis of supplemental feeding, colic, diastemata, wave mouth, smooth mouth, step mouth and periodontal disease was repeated using age group instead of age to exclude any inaccurate results from data based on estimated donkey ages (Table 7.4.13.1; Appendix 2). Most of the statistically significant results were still significant with exceptions of the interactions between worn teeth and teeth with enamel points in the model with supplemental feeding, and age group in the models with step and smooth mouth. In contrast there was a significant association between periodontal disease and age group, but not in the initial model with age. Furthermore there was a significant interaction between diastemata and age group ($P = 0.03$) in the colic model indicating that the increase in colic with age group is greater in the presence of diastemata. There was also a significant interaction between diastemata and overgrown teeth in the model with smooth mouth, which indicated that the presence of overgrown teeth were more important than diastemata in the association with smooth mouth.

7.5 Discussion

The prevalence of dental disease in this population was 73%, which is within the prevalence of 10 - 80% that has previously been reported in horses (Anon 1965; Baker 1979; Uhlinger 1987; Wafa 1988; Kirkland *et al.* 1994). The large range of dental disease prevalence observed in different horse studies may have been affected by differences in the ages of horses in each study. The age of the 500 horses examined by Kirkland *et al.* (1994) ranged from 0.5 to 30 years and over 80% of these had oral (soft tissue) or dental (teeth related) pathology. The age of the 233 horses examined by Uhlinger (1987) ranged from 6 – 11 years, with one or more dental abnormalities observed in 24% of the horses not exhibiting any clinical signs associated with dental disease, and in 65% of the horses with clinical signs. In the current study, the median age of all 357 donkeys examined was 23 years (range 2 – 53 years). As expected, this is lower than the median age of the 349 donkeys examined at post mortem (31 years) that had a dental disease prevalence of 93% (chapter 4). The effect of age can be illustrated by the statistically significant difference in the prevalence of dental disease in donkeys 20 years and under (≤64%) and over 20 years of age (≥ 88%). This study has shown a definite increase in the prevalence of dental disease in increasing age groups of equids, with an increased risk (odds ratio) of having dental disease in donkeys older than 15 years, ranging from 4.8 – 133 compared to donkeys 10 years and under.

Ventral curvature of the incisor occlusal surface table (‘smile mouth’) was observed in 97% donkeys, with a prevalence of 96% in donkeys with dental disease and 99% without dental disease. Therefore, ‘smile mouth’ of the incisors can be regarded as a normal appearance of donkey incisor occlusal surface table and should not be straightened unless it is extreme and interfering with ingestion of food.

Sharp enamel points were observed in 27% of the donkeys, with the maxillary and mandibular 11s the most commonly affected position. Despite the high prevalence of enamel points, ulcers were only observed in 2.2% of the donkeys. There was also a high prevalence of donkeys with 6, 12, and 24 teeth affected by enamel points which illustrates the involvement of entire CT rows during examination. In a study of 233 horses sharp enamel point were observed in 8% of horses without any dental related clinical signs and in 28% of horses exhibiting clinical signs (Uhlinger 1987). Unfortunately, as these donkeys are managed in large groups, individual dental related clinical signs are rarely observed, and enamel sharp points could not be correlated with clinical signs in this study.

Large clinical studies of 1000 and 32,000 horses found prevalences of sharp enamel points to be 99.2 and 91.7%, respectively (Becker 1942, 1945). In a post mortem study of 355 horses, unusually sharp enamel points were observed in only 9 horses (2.5%), but the prevalence of all sharp enamel points was not recorded (Wafa 1988). In the current donkey study, maxillary CT were more commonly affected with enamel points (55%). Enamel points were also shown more likely to be observed in younger donkeys (median age 15.5) than older donkeys (median age of 26 without enamel points). Donkeys 10 years and less had a prevalence of 53% which was significantly higher than donkeys older than 20 years (≤ 20%). Donkeys over 20 years of age were less likely to have sharp enamel points compared to donkeys less than 10 years (odds ratio 0.06 – 0.2).

Diastemata were observed in 53% of all donkeys examined. In a study of 349 referred horses with CT disorders, diastemata were observed in 16 horses (4.6%) (Dixon *et al.* 1999b). Wafa's (1988) study found diastemata in 13 horses (3.6%). Similar to the donkey post mortem study (chapter 4), the presence of diastemata in this clinical study was significantly associated with age and the prevalence in the younger age groups (≤

12% in donkeys under 15) was much lower than in older age groups (≥ 72% in donkeys ≥ 20 years). The significant OR (and CI) of donkeys over 20 years of age having diastemata compared to donkeys under 10 years varied from 16.7 (3.6 – 7.8) – 153.6 (30.1 – 782.8). The prevalence of diastemata in this clinical study is lower than the prevalence of 85% observed in the post mortem study of 349 donkeys which is most likely due to the fact that the donkeys at post mortem were older and additionally small caudally-located diastemata might have been missed on oral examination of live donkeys.

Diastemata were more prevalent between the mandibular CT (84% of all diastemata) with the caudal mandibular interdental spaces more commonly affected (10/11, 08/09 and 09/10 interdental spaces). The rostral CT interdental spaces (06/07 and 07/08 interdental spaces) were more commonly affected in the maxillary row. Similarly, a recent study of 60 referred horses with 273 diastemata, found the disorder to be more prevalent between mandibular CT (85%) compared to maxillary CT (15%), with the caudal CT most commonly involved (Dixon *et al.* 2008). In this donkey study, there was a significant association of diastemata with missing, displaced, worn and overgrown teeth indicating that most of the diastemata observed in this study were secondary to other dental disorders. The above-noted referred study of 60 horses classified 29 of the cases of diastemata to have primary (inadequate rostro-caudal angulation or CT too far apart) and 31 cases to have diastemata secondary to other dental abnormalities (Dixon *et al.* 2008).

Missing teeth were observed in 29% of the donkeys, with 65% involving CT and 35% involving incisors. Mandibular CT (73%) were more commonly affected than maxillary CT (27%). The 09s and 10s were the most common mandibular CT to be missing, whereas the 10s and 11s were the most common maxillary CT missing. Maxillary incisors (68% of missing incisors) were more often missing than mandibular incisors (32%). The presence of missing teeth was significantly associated with age, with a prevalence of ≤ 2% in donkeys 15 years and under and a prevalence of ≥ 41% in donkeys over 20 years of age. Missing teeth were only noted in 9 horses (2.6%) (median age 10) of 349 horses referred for cheek teeth disorders (Dixon *et al.* 2000a) and in 2% of 355 horses examined in a post mortem survey (Uhlinger 1987). This is similar to the prevalence observed in the lower age group (≤ 15) of donkeys in this study, as the median age of the two horse studies was less than 15 years.

The prevalence of overgrown teeth was 52%, the majority of which were CT (98%). Maxillary CT (53%) were overgrown slightly more commonly than mandibular CT (47%), with the maxillary 09s and 10s, and mandibular 11s most commonly overgrown. Overgrown teeth were more likely to be present in older donkeys with a prevalence of ≥60% in donkeys > 20 years of age, compared to ≤ 22% in donkeys ≤ 15 years of age. The 9.3% prevalence of overgrown teeth identified in 355 horses (age 6 - 11 years) (Uhlinger 1987) was lower than the prevalence in the current donkey study even when just examining the age group < 10 years (15.4%). Most of the overgrowths observed by Uhlinger (1987) were rostral overgrowths of the maxillary 06s and caudal overgrowths of the mandibular 11s.

Worn teeth were observed in 42% of donkeys of which most were CT (79%). The maxillary CT (69%) were more commonly worn than mandibular CT, with the upper 10 and 11 positions most commonly affected. This could be due to the prominent curve of Spee observed in donkeys that result in excessive wear on the caudal maxillary CT. The 06s were the most common worn mandibular CT. Donkeys over 25 years of age had a significantly higher prevalence (> 53%) of worn teeth than donkeys less than 15 years (<14%), as expected confirming that old age was associated with an increase in the number of worn teeth. Not all donkeys with worn teeth were classified as having smooth mouth, therefore there is a much higher prevalence of worn teeth compared to smooth mouth (4.5%). The study by Wafa (1988) showed a 35% prevalence of smooth mouth in horses over 20 years of age, with some CT having complete absence of enamel.

Medially and laterally displaced teeth were present in 26% and 33% respectively, of donkeys. Mandibular CT were most commonly displaced (58% of medially and 77.3% of laterally displaced CT). Once again, age was significantly associated with the presence of displaced teeth. Donkeys ≤ 15 years of age had a prevalence of medially and laterally displaced CT of ≤ 6% and 8%, respectively. In contrast, donkeys > 20 years of age had a prevalence of medially and laterally displaced CT of ≥ 30% and 35%, respectively. Cheek teeth displacement was found in 7.2% of 209 horses with full permanent dentition (Wafa 1988) with the maxillary and mandibular 08s most commonly displaced, compared to this donkey study where the 10s and 09s were most commonly laterally displaced and the 09s and 08s medially displaced. A prevalence of 6.6% displaced teeth was observed in the study examining 349 referred cases with cheek teeth disorders (median age 7) of which mandibular CT were most commonly displaced (77.3% of displaced teeth) (Dixon *et al.* 1999b). This is therefore similar to the prevalence observed in the younger age groups (< 15 years) in this population of donkeys. Interestingly a relative decline in the prevalence of displaced CT was seen in donkeys greater than 35 years of age (30% medially and 38% laterally) and this could be attributed to loss of previously displaced teeth.

The prevalence of periodontal disease in all the donkeys was 14% with a prevalence of ≥18% in donkeys older than 20 years and very little (≤ 2%) seen in donkeys ≤ 20 yo. The study by Wafa (1988) observed a high prevalence of periodontal disease (36.6%), with a higher prevalence (> 52%) in the older age groups (>15 years) and the 2-5 year age group. The periodontal disease observed in the 2-5 year age group by Wafa (1988) was associated with eruption of teeth and was no greater than grade 1 (out of grade 4)(hyperaemia and oedema). Periodontal disease was observed in 166 in 484 horses (34.3%) by (Colyer 1906) most of which was associated with periodontal food impaction and the presence of spaces between teeth i.e. diastemata.

In the current study, periodontal disease was predominantly observed in the mandibular CT and in particular around the 08s, 10s and 07s. In maxillary CT periodontal disease was most commonly present around the 09s and 10s. Diastemata were present in 48% of these cases of periodontal disease. The low prevalence observed is this study compared to the study by Wafa (1988) might be because examination of live, unsedated donkeys makes meticulous examination of the gingiva difficult. Unfortunately due to the relative low numbers of donkeys with periodontal disease, statistical analyses to determine the association of periodontal disease to diastemata could not be performed. Wafa (1988) found periodontal disease in 85% of the cases with diastemata. Similarly in a recent study, periodontal disease was seen adjacent to 75.8% of 273 CT diastemata in 60 horses (Dixon *et al.* 2008). In this donkey study, periodontal disease was only observed in 7.7% of all individual teeth affected with diastemata. It is likely that lower grades of periodontal disease, particularly surrounding the caudal cheek, would have been missed on clinical examinations in unsedated donkeys, which accounts for the lower associated periodontal disease prevalence. Other conditions commonly associated with periodontal disease in horses include abnormalities of wear such as step mouth (Wafa 1988). Similarly, in the study by Dixon *et al.* (2000a) periodontal disease was observed in 72.7% of cases with abnormalities of CT wear, i.e. overgrowths, step, wave and shear mouth.

There was a significant association between age and the presence of wave mouth, step mouth and smooth mouth, all which had a relatively low prevalence (19.3, 11.8 and 4.5% respectively). The presence of shear mouth was not associated with age and had a very low prevalence (3.9%). Wafa (1988) found wave mouth, step mouth, shear mouth and smooth mouth in 2%, 3.7%, 0.6% and 4.8% of 355 horses with an age range of 0 – 20 years However, the prevalence of smooth mouth in horses over 20 years of age was 35% (Wafa 1988), highlighting the expected increase in prevalence with age. Uhlinger (1987) noted a prevalence of 4.6% and 13.2% wave mouth, 4.0% and 8.4% step mouth, and 2.6% and 12.0% shear mouth in horses without and with clinical signs of dental disease. A study of 349 referred horses found the presence of step mouth in 4.3% and wave and shear mouth in 8.3% of cases aged 3-34 (median 10 years) (Dixon *et al.* 2000a), which was much lower than the median ages of 28, 32 and 31 observed for wave, step and shear mouth respectively, in the donkeys in this study. The median age of smooth mouth in the current study was 37 years implicating it to be more prevalent in older donkeys, as was observed in the study by Wafa (1988) where more than 80% of the smooth mouth observed was in horses in older than 20 years of age. Thus it can be concluded that the prevalence of wave, step, shear and smooth mouth is relatively low in most equid populations examined.

Infundibular caries was identified with a much lower prevalence (6.4%) than the 13-100% reported in various studies in horses (Colyer 1906; Honma *et al.* 1962; Baker 1970). This could be due to the fact that infundibular cemental hypoplasia was not included in the definition of infundibular caries in this study. Dental caries of the maxillary CT, including infundibular caries, was observed in 30.7% of 355 horses (Wafa 1988). In a recent study examining maxillary CT from 33 horses, true caries were identified in 7.8% of the infundibulae, cemental hypoplasia in 21.8% of infundibulae and infundibular cemental discoloration or other defects in 55.2% of infundibulae (Fitzgibbons 2007). This supports the belief that the previous studies with higher prevalences of infundibular caries included other infundibular cemental defects and not only true carious lesions. In the current study infundibular caries had a higher prevalence in the older age groups (57% of 15-20 year old; 75% of > 20 years), with the 09s (35%) and 10s (18%) most commonly affected (Wafa 1988). Fractured teeth were observed in 5% of the donkeys here which is greater then the 0.4% observed in horses (Taylor and Dixon 2007). Unfortunately the precise fracture patterns of these teeth were not observed.

When evaluating individual tooth data, diastemata were associated with displacement of and presence of enamel points of the CT rostral to the diastema. Acquired displacement of teeth have previously been proposed to be associated with diastemata (Collins and Dixon 2005; Dixon *et al.* 2008). Displaced teeth allow for the impaction of food particles in the interdental space, gingival recession and thus the creation of a larger diastema. Similarly, dental overgrowths, especially at the periphery of CT rows, have been proposed to selectively push individual CT apart during the masticatory action, causing diastemata (Collins and Dixon 2005; Dixon *et al.* 2008). This study found a definite association between the presence of diastemata and displaced teeth. Similarly, a statistically significant association between diastemata and teeth with enamel points were observed. However it is possible that the enamel points could develop secondary to decreased normal masticatory movement over an area of painful diastemata.

Similarly teeth with periodontal disease and pulpal exposure were statistically associated with teeth that were medially or laterally displaced. Medially and laterally displaced teeth were associated the presence of overgrown teeth and teeth with enamel points at individual tooth level. Teeth with pulpal exposure were associated with medially or laterally displaced or overgrown teeth. It would seem that teeth will be more likely to have pulpal exposure if they are wearing incorrectly i.e. displaced, and this study supports this theory. Unfortunately, these statistically significant findings from univariate models could not be confirmed by multivariable models due to the relatively low numbers of teeth involved compared to all teeth examined (> 11 000 teeth in 357 donkeys), and this needs to be considered when interpreting these results.

Quidding was only observed in 2 donkeys despite the high prevalence of dental disease in this population. Quidding has been shown to be an important clinical sign associated with dental disease and particularly the presence of diastemata (Dixon *et al.* 1999b; Dixon *et al.* 2008). It is proposed that the low prevalence of quidding seen in this population is due to herd management of these donkeys, thereby potentially missing individual donkeys exhibiting quidding. The lack of observed clinical signs in horses in extensive surroundings had also previously been noted (Dixon *et al.* 2008). Similarly, it has been shown that weight loss or low body condition scores are associated with severe dental disease and diastemata (Dixon *et al.* 1999b; Dixon *et al.* 2008). Although a there was a significant association between BCS and dental disease in this study, the actual BCS difference was very small (0.5). The supplemental feeding was highly associated with the presence of dental disease with an odds ratio of 12.7. There is also an association between supplemental feed and low BCS and older age. Therefore it is more likely that older donkeys have dental disease, a low BCS and are fed supplemental feed.

Sixty one percent of the donkeys examined had no recorded systemic disorders. Musculoskeletal disorders were the most commonly recorded disease (17% prevalence) and its presence was significantly associated with dental disease and increasing age. Weight loss (9.5% of donkeys) was significantly associated with dental disease and disastemata. However, there was a significant interaction between age and diastemata, where the increased prevalence of weight loss with increasing age was greater in donkeys without diastemata. Previous colic episodes were observed in 10% of the donkeys, 1 – 62 months prior to the current dental examination and dental disease was a high risk factor (OR = 12) for the occurrence of these colic episodes. Although it cannot be confirmed that the donkeys had dental disease at the time of their colic episodes, it is likely that dental disease was at least developing at that time. Dental disorders have been reported to be associated with colic (White 1990; Cox *et al.* 2007), and in particular the donkey post mortem survey (chapter 4) showed a definite association of diastemata to colic related death. There was a significant association between previous colic episodes and increasing age. No other current systemic disorders were associated with any dental related factors.

At a donkey level there was an association between donkeys with diastemata and with displaced (medially and laterally), worn, overgrown teeth, and increasing age. Unfortunately, no multivariable analyses with periodontal disease could be performed due to the relatively low numbers of donkeys with periodontal disease. There was also a significant interaction between medially displaced and worn teeth, with a higher prevalence of diastemata (66.7%) in donkeys with worn teeth and without medially displaced teeth as compared to donkeys with neither (23.8%) of these dental disorders. Worn teeth are a more important variable associated with diastemata in the absence of medially displaced teeth. There was also a significant interaction between worn teeth and age, with a greater increase in prevalence of diastemata in donkeys with worn teeth compared to donkeys without worn teeth. So despite the increasing prevalence of diastemata with increasing age, worn teeth make the effect of this association greater.

There was also a significant association between donkeys with wave mouth, and the presence of overgrown teeth and diastemata. As overgrown teeth are part of the definition of wave mouth this result is not surprising. Similarly the presence of diastemata are likely to be associated with painful periodontal disease (Dixon *et al.* 2008), and will the be associated with abnormal masticatory movements that may lead to abnormal wear. There was an interaction between overgrown and worn teeth in donkeys with wave mouth, as no donkeys had wave mouth with overgrown teeth and no worn teeth, but wave mouth was observed in 5% of the donkeys with neither overgrown nor worn teeth.

Smooth mouth was associated with worn teeth and age. The association of worn teeth and smooth mouth is expected as the presence of worn teeth is a characteristic of smooth mouth. Likewise, with old age the reserve crown and peripheral enamel is depleted, and result in the appearance of worn teeth. Step mouth was significantly associated with the presence of diastemata, missing, worn and time since previous dental and age. Once again, worn teeth and missing teeth would lead to the condition of step mouth. The association with age implicates the increased prevalence of worn teeth with increasing age. The increased time since previous dental treatment observed in donkeys with step mouth highlights the importance of regular dental care to prevent development of step mouth.

Periodontal disease was significantly associated with the presence of diastemata. Diastemata with food pocketing has been shown to be a major cause of periodontal disease (Collins and Dixon 2005; Dixon *et al.* 2008), and the current result supports this proposal. The addition of supplemental feed was associated with the presence of diastemata, missing teeth, worn teeth and age. As all of these dental disorders reduce efficient mastication, these donkeys are likely to need supplemental feeding. There was a significant interaction between donkeys with sharp enamel points and worn teeth being fed supplemental feed, which implicated that enamel points were a less important associated variable with supplemental feed in the absence of worn teeth.

Repeating the multivariable models using age groups instead of age confirmed that the significant results found in the models using age were accurate. The few differences encountered, particularly the lack of age group association in donkeys with step and smooth mouth are due to the fact that these were excluded from the analysis as the prevalence of step and smooth mouth was 0 in groups A-B and A-C respectively.

In conclusion, dental disorders are a significant problem in the population of donkeys examined in this study with an overall prevalence of 73%. A positive age effect was observed with most dental disorders, particularly in the 15 – 20 year age group, which highlights the importance of managing dental disorders from a young age to prevent progression of the recorded dental disorders.

Chapter 8: Clinical dental examinations of 203 working donkeys in Mexico

8.1 Introduction

There are about 3.3 million donkeys in Mexico, most of which are working animals mainly used for transporting water and firewood. These animals provide a good work source and play important economical and socio-cultural functions in poorer communities (Starkey and Starkey 2004; Oseguera Montiel *et al.* 2006). Consequently, the welfare of these working animals is closely interlinked with the welfare of the human population in developing countries. Dental disease can have detrimental effects on the welfare of equids as it can cause decreased feed efficiency and severe oral discomfort (Lowder 1988; Dixon *et al.* 2000a). In turn, dental disease can lead to weight loss and subsequent sores from poorly fitting harnesses, and furthermore may contribute to injuries from beatings, because the donkeys are too weak to work normally. A large study examining working equids (horses, mules and donkeys) including 2,314 donkeys in Mexico found the prevalence of severe dental disease to be 14% in donkeys, with about 80% of these cases having a body condition score below 2.5 (Fernando-Martinez *et al.* 2006).

The aim of this study was to determine the prevalence of dental disease (excluding sharp enamel points) and of specific dental disorders in selected populations of working donkeys in Mexico. Furthermore, this study aimed to determine the clinical significance of these dental disorders by examining possible associations with body condition score and diet composition as similarly performed in the UK donkey study (Chapter 7). In addition faecal worm egg counts were performed to determine the parasite burden in this population.

8.2 Materials and methods

Ten villages, in 8 geographical areas, didvided into 2 climatic areas (temperate and tropical) in Mexico were visited by mobile veterinary clinic teams from the Donkey Sanctuary-World Horse Welfare-Universidad Nacional Autonoma de Mexico (DS-ILPH-UNAM) programme over a 9 day period in May 2008. Following presentation to the mobile clinic for a general clinical examination and routine anthelmintic administration, a clinical oral examination using a full mouth speculum and light source (Welch, Allyn, Aston Abbotts, UK) was performed by the author without sedation. Donkeys that were unable to be fully examined were excluded from this study. Body condition score was assessed on a 1 – 5 grade scale utilising half grades (Appendix 1), and details of diet composition were recorded. Faeces was collected to perform faecal egg counts (FEC) using a FECPAK F100 system (FECPAK international). For this procedure, 10 grams of faeces were collected from each donkey and mixed with 40 ml water, saturated saline solution was added to 45 ml of this mixture to make a total volume of 230 ml and this was poured through a 1mm aperture sieve; 1 ml of this solution was placed in the FECPAK perspex slide and the number of eggs was microscopically counted and the findings were calculated to obtain the number of eggs per gram of faeces (epg).

Age was estimated from mandibular incisor occlusal table appearance, overall body appearance and owner information. All donkeys with significant dental disorders were treated, and owners of donkeys having a poor condition score given feeding advice.

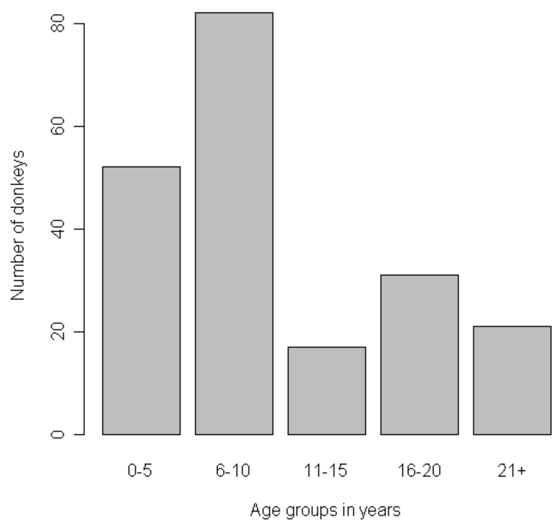
All dental abnormalities including small focal overgrowths (usually on upper 06s and lower 11s) and soft tissue injuries such as dental-related buccal calluses and ulcers were regarded as dental disease. Donkeys having only sharp enamel points (overgrowths) on the buccal aspect of the maxillary arcade and lingual aspect of the mandibular arcade were not classified as having dental disease. Enamel points were graded 0 – 3, with 0 indicating absence of enamel points and 3 indicating very large/sharp enamel points. The definitions of all other dental disorders were as described in chapters 4 and 7.

Kruskal-Wallis chi-squared analyses were performed to determine the associations between BCS and age group, FEC, food groups, geographical and climatic areas. Post hoc analysis was performed (Dwass-Steel-Chritchlow-Fligner, Stats Direct statistical software version 2.6.5) to compare BCS between different age groups. Chi-squared analysis was performed to determine the association between age groups and climatic area. For FEC analysis general linear models with negative binomial errors were used, with the diverse measure of aggregation, k, associated with the FEC data being 0.55. General linear mixed effect models with binomial errors were performed to determine the relationships between supplemental feed and age group, dental disease and climatic areas; between dental disease, FEC, age groups and area, and between dental disorders. Models for multivariable analysis were selected on univariate statistical significance ($P \leq 0.2$) and biological plausibility. Odds ratios (OR) and their 95% confidence intervals were determined for each explanatory variable to determine their association with response variables. R V2.3.1, R Foundation for Statistical Computing was used for statistical analyses. $P < 0.05$ was taken as statistical significance.

8.3 Results

A total of 203 donkeys were examined of which 69% were male and the most common age group was the 6 – 10 year age group (Figure 8.3.1). The youngest donkeys examined were 2 years old and the oldest was estimated to be approximately 25 years old. As more accurate ageing was usually not possible, age was classified into 5 groups of 5 year intervals: 0-5 (n = 53); 6-10 (n = 81); 11-15 (n = 15); 16 – 20 (n = 33); ≥ 21 years (n = 21). Due to the low number of donkeys in some age groups, age was evaluated as young (0 – 10 years) and older (>10 years) donkeys for some of the statistical analysis.

Figure 8.3.1: **Histogram showing the number of donkeys in each age group.**



The 8 areas where examinations were performed were categorised into two climatic areas with A – D in temperate conditions and E – H in tropical areas (Table 8.3.1; Figure 8.3.2).

Table 8.3.1: **The categories of geographical areas visited in Mexico**

Area categories	Area	Climate
A	Morelos	Temperate
B	Puebla	Temperate
C	Caolocaco – Mexico DF	Temperate
D	Querétaro	Temperate – savannah
E	Cerro Colorado – Veracruz	Tropical
F	Paso de Limone – Veracruz	Tropical
G	Martinica – Veracruz	Tropical
H	Plan de Arroyos – Veracruz	Tropical

Figure 8.3.2: **A map of Mexico illustrating the areas where donkeys were clinically examined. Areas A – C (blue circle) and D (black circle) are temperate climatic areas, whereas E – F (red circle) are tropical climatic areas (http://www.map-of-mexico.co.uk/large-political-mexican-map.htm).**

The greatest number of donkeys examined in one day was in area F (42 donkeys) and the least (11 donkeys) in area H (Figure 8.3.3). The age distribution of donkeys per area is illustrated in Figure 8.3.4.

Figure 8.3.3: **A histogram illustrating the number of donkeys examined in each geographical area. Dark grey = temperate areas; light grey = tropical areas.**

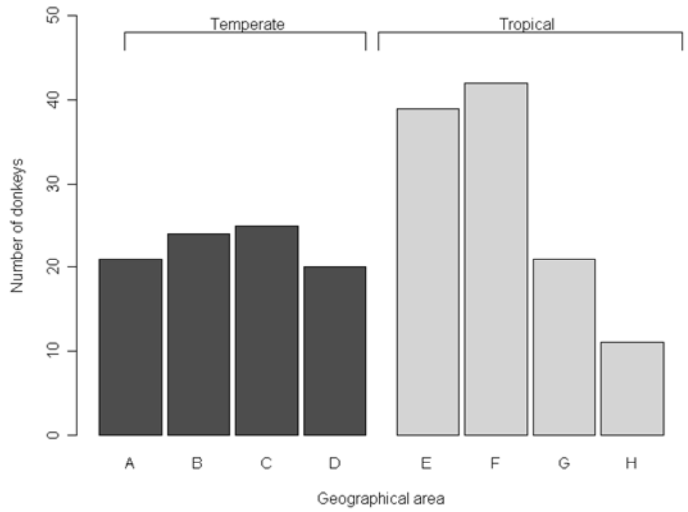
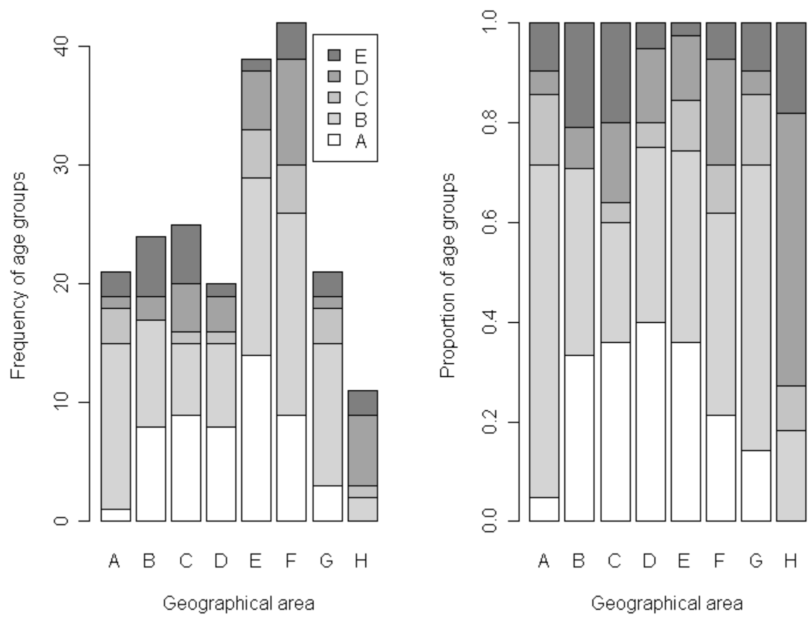


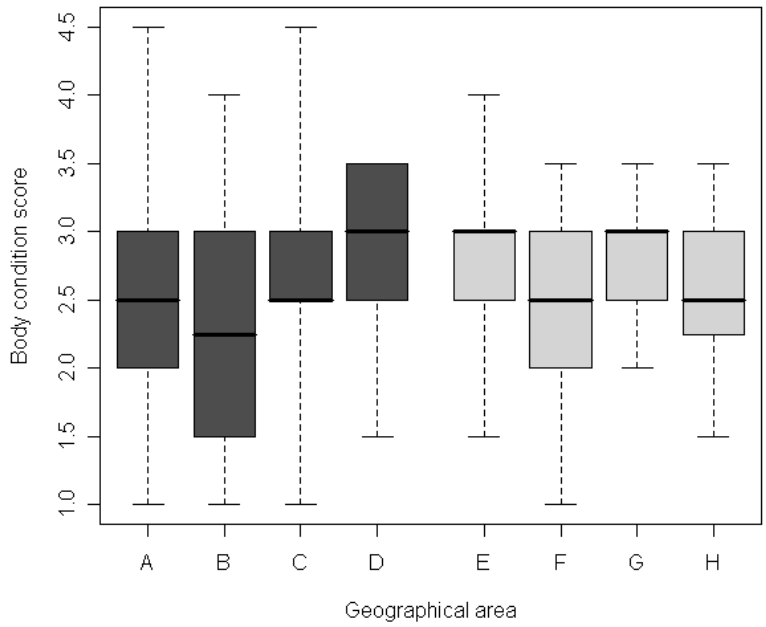
Figure 8.3.4: **Two boxplots illustrating the number of donkeys per age group per geographical area (left); and the proportion of donkeys per age group per geographical area. Age groups: A = 0-5; B = 6-10; C = 11-15; D = 16 – 20; E > 20 years; geographical areas: A – D = temperate; E – H = tropical.**



Age groups were not statistically significantly associated with climatic areas (P = 0.15; χ^2_6 = 6.7), indicating that the general age ranges were similar in the tropical and temperate areas investigated in this study.

BCS ranged from 1 – 4.5 with a median of 2.5. BCS was not statistically significantly associated with geographical area (P = 0.17; χ^2_6 = 10.4; Figure 8.3.5), neither was there a significant difference in BCS in temperate and tropical climatic areas (P = 0.88; χ^2_1 = 0.02), with a median BCS of 2.5 in both climatic areas.

Figure 8.3.5: **Boxplot illustrating the body condition score in each geographical area. Dark horizontal line = median; vertical boxes = interquartile range; vertical lines = range. Dark grey = temperate areas; light grey = tropical areas.**

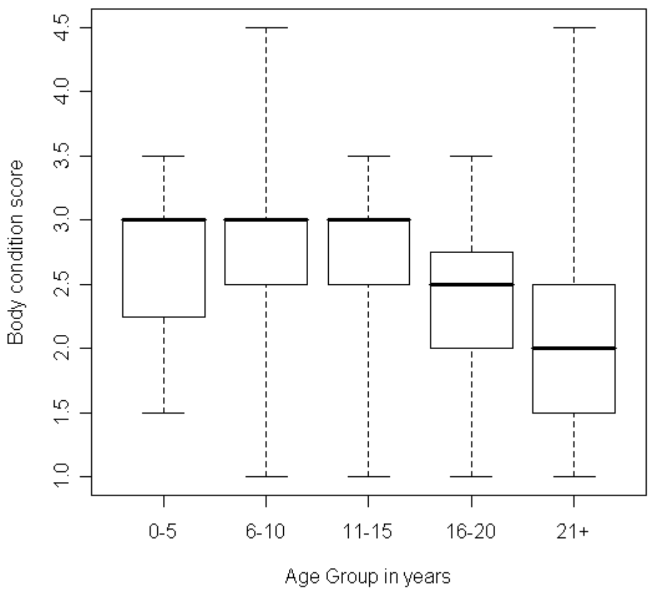


There was significant association of BCS with age group (P < 0.001; Figure 8.3.6). As expected older donkeys had a lower BCS (≤ 2.5 for >15 years of age) compared to younger donkeys (BCS 3 for ≤ 15 years of age). BCS of donkeys ≥ 20 years was significantly less than donkeys ≤ 15 years of age, as was BCS of donkeys 16 – 20 years of age less than 6 – 10 years (P < 0.03; Table 8.3.2).

***Table 8.3.2*: P values of pair wise comparison of BCS in different age groups. Bold values are significant.**

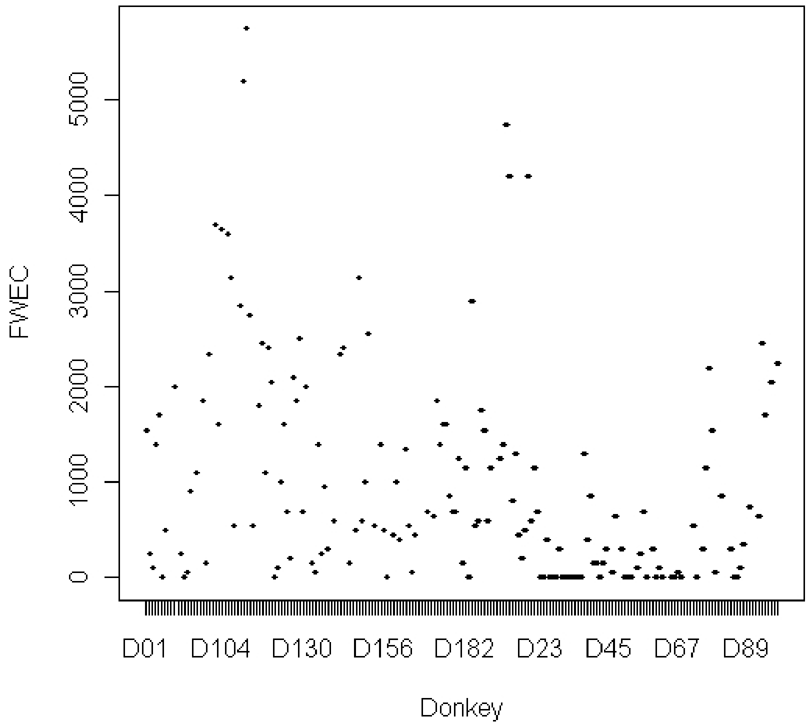
Age Groups (in years)	0 – 10	11-15	16-20	21-25	>25
0–10	-	-	-	-	-
11-15	0.47	-	-	-	-
16-20	0.89	0.99	-	-	-
21-25	0.31	**0.008**	0.23	-	-
> 25	**0.003**	**<0.001**	**0.03**	0.09	-

***Figure 8.3.6:* Boxplot of donkey body condition score (0 – 5) in each age group.**



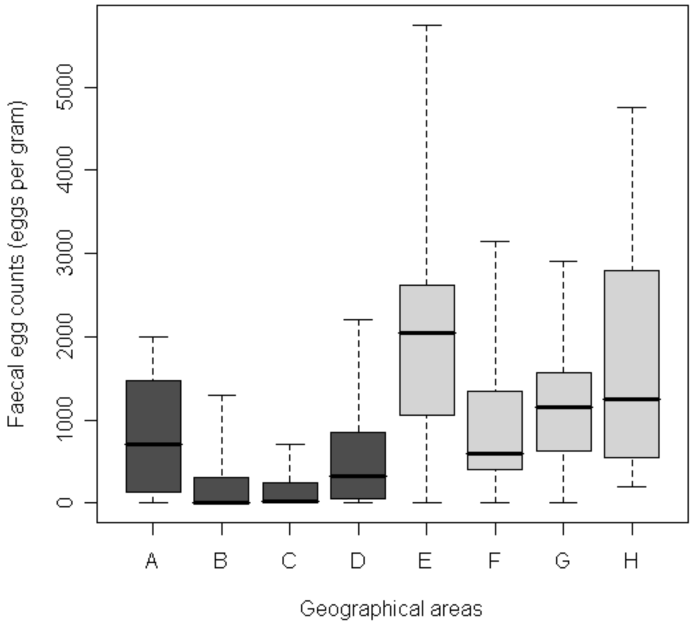
Due to practical difficulties in acquiring faecal samples, FEC were only performed on 161 donkeys (79%). The FEC ranged from 0 – 5750 epg with a median of 600 (100 – 1550) (Figure 8.3.7).

Figure 8.3.7: **Scatterplot showing the FEC distribution in the donkeys that had FEC performed.**



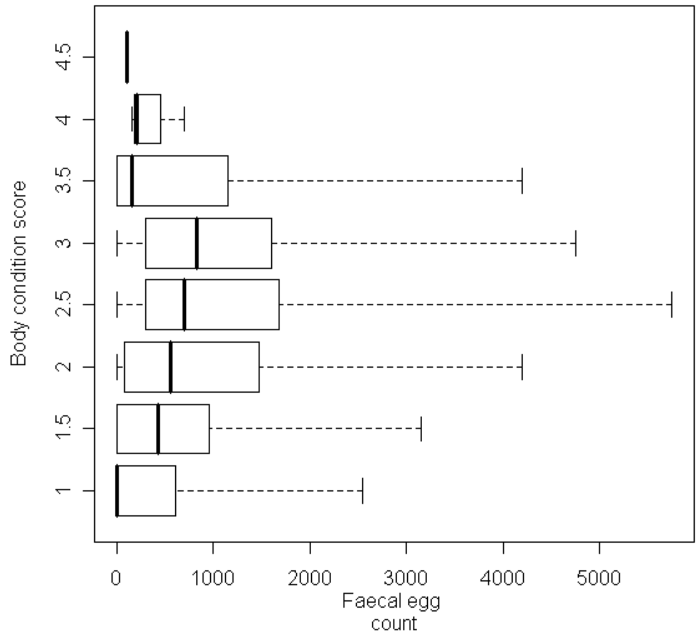
The distribution of FEC in different geographical areas is illustrated in Figure 8.3.8. There was a statistically significant difference in the FEC in different climatic areas ($P < 0.001$) with higher FEC in donkeys from tropical areas (Figure 8.3.8).

Figure 8.3.8: **Boxplot illustrating the distribution of FEC in different geographical areas. The dark grey shaded columns (A-D) represent the temperate areas and the light grey (E-H) the tropical areas.**



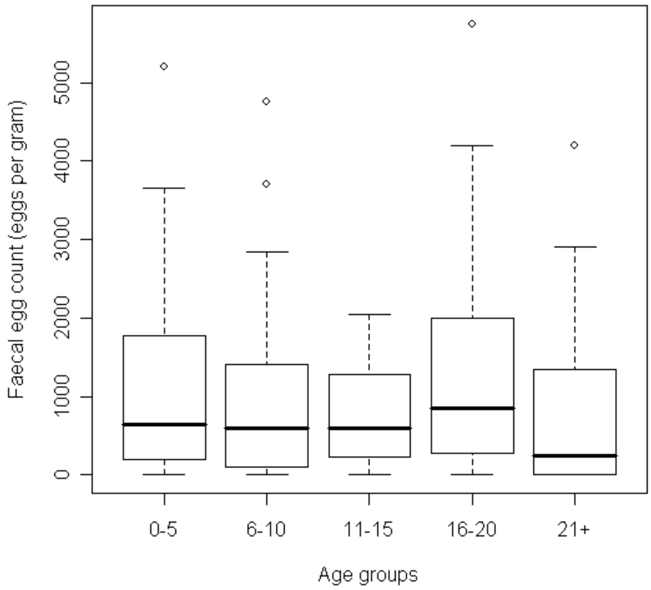
BCS (P = 0.4; χ^2_4 = 3.8; Figure 8.3.9) was not associated with FEC (P = 0.73), although donkeys with BCS in the higher and lower ranges had the lower FEC.

Figure 8.3.9: **Boxplot of body condition score (BCS) to faecal egg count (FEC); dark line vertical = median; horizontal boxes = interquartile range; horizontal lines = range.**



FEC was not statistically significantly associated with age groups (P = 0.55), or younger (0 – 10 years) or older (>10 years) age groups (P = 0.71) (Figure 8.3.10).

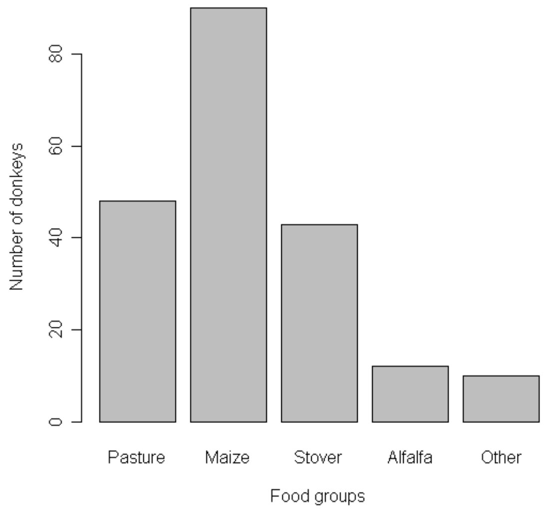
Figure 8.3.10: **Boxplot illustrating the faecal egg counts (eggs per gram) for donkeys in each age group.**



Pasture was the main food source for donkeys in all areas, but many donkeys were given additional feed which consisted of one or more of the following: maize (whole kernels or cracked), maize stover (dried stalks and leaves), alfalfa (fresh), bran, commercial feed, sugar cane, sorghum, oats, tortillas, vegetables, cactus and one donkey received oranges. The different climatic areas affected the type of pasture that was available to the donkeys at pasture, with lush grass more readily available in the tropical areas. The diet of donkeys was divided into 5 groups for statistical analyses: A = pasture only; B = maize as the main supplement with or without other supplements; C = maize stover (dried stalks and leaves) as the main supplement with or without other supplements; D = alfalfa as the main supplement with or without other supplements; E = all other feedstuffs on their own or in combination. Unfortunately, no accurate quantification of supplementary feeding could be determined from information obtained from owners.

Pasture and maize (whole and cracked kernels) was the most common diet (44.3%), followed by pasture alone (23.6%), and pasture and maize stover (21.2%) (Figure 8.3.11).

Figure 8.3.11: **Plot illustrating the number of donkeys fed pasture, pasture and maize, pasture and stover, pasture and alfalfa and pasture with any other supplement.**



Due to low numbers in the alfafa and other food groups, no analysis could be performed on food groups. However, general linear models were performed on pasture alone (no supplement) and supplemented feed (all feeds). Supplemental feed was not statistically significantly associated with age groups (P = 0.58; $\chi^2_4 = 0.29$) or younger (0 – 10 years) or older (> 10 years) ages (P = 0.65; $\chi^2_1 = 21$). Supplemental feed was not associated with dental disease (P = 0.45; $\chi^2_1 = 0.57$), however it was significantly associated with climatic area (P = 0.004; $\chi^2_1 = 12.3$). Body condition score was not statistically significantly associated with food groups (P = 0.3). The median body condition score of donkeys receiving supplemental feed in addition to pasture was 3 and donkeys on pasture alone was 2.5, although this difference was not statistically significant (P = 0.34).

8.3.1 Dental disease

Dental disease was observed in 62.1% (126) of all donkeys, with sharp enamel points observed in 98% of donkeys (Table 9.3.3). The 4 (2%) donkeys that did not have sharp enamel points were over 20 years of age and had advanced dental disease. Serious dental disease that required urgent dental treatment (any disorders other than ulcers, calluses [mucosal hyperkeratinisation in response to repeated trauma by dental overgrowths]) and focal overgrowths was detected in 18.2% (37) of the donkeys examined. Maxillary cheek teeth enamel points were very common (95.8%) and often very severe (60.3% were grade 3) compared to mandibular cheek teeth (68.7% sharp; 38.4% grade 1). Individual enamel points other than the buccal and lingual aspect of maxillary and mandibular cheek teeth were also observed in 70 teeth, with the 207 and 311 most commonly affected. Focal overgrowths were observed in 32.5% of donkeys, with maxillary 06s having 79% of these lesions (Table 8.3.1.1; Figure 8.3.1.1 and 8.3.1.2). Mandibular 06s were the most common overgrown tooth (31%) with 18.7% of donkeys having one or more overgrown teeth. Maxillary 06s were the most commonly worn tooth overall (37.6%) followed by the mandibular 11s (14.9%), which was observed in 16.3% of donkeys (Table 8.3.1.1). Sloping overgrowths involving most of the occlusal surface ("ramps"), were present in 8.4% of donkeys and were most commonly affected the mandibular 06s (91.7%; Table 8.3.1.1).

Table 8.3.1.1: **Table with prevalence of enamel overgrowths and dental disorders in 203 working donkeys examined in Mexico.**

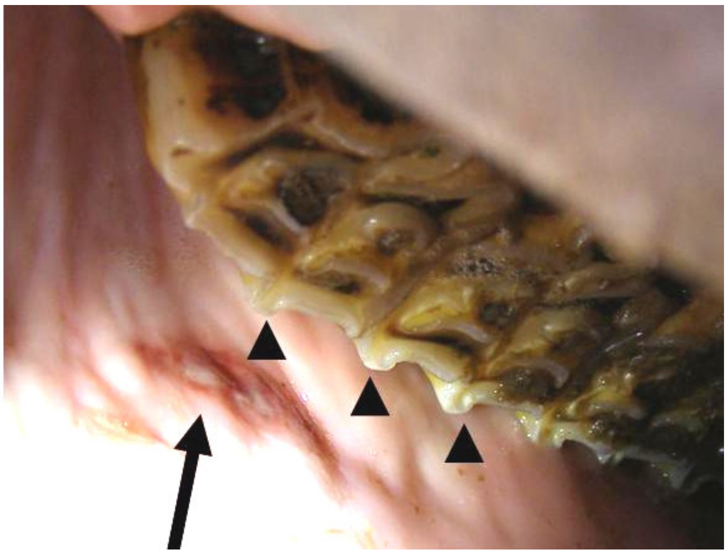
Dental disorder	Total prevalence (%)
Dental disease	62
Sharp enamel points	98
Diastemata	3.9
Missing teeth	0.5
Overgrown teeth	18.7
Worn teeth	16.3
Displaced teeth	1.5
Focal overgrowths	32.5
Ramps	8.4
Buccal calluses	13.3
Buccal ulcers	14.3
Pulpar exposure	0.5
Fractured teeth	2
Wave mouth	5.9
Step mouth	1.5
Smooth mouth	3.9
Shear mouth	0.5

Figure 8.3.1.1: **Rostral focal overgrowth on 106 (arrow) in a 5 – 10 year old donkey. Such overgrowths were observed on the maxillary 06s and mandibular 11s of many donkeys.**

Figure 8.3.1.2: **A large focal overgrowth (arrow) on a 106 of a 16 – 20 year old donkey that had never had any dental treatment. The 105 ("wolf tooth") is still present.**

Buccal calluses and ulcers were observed in 13.3% and 14.3% of donkeys, respectively (Figure 8.3.1.3). Calluses were mostly associated with enamel overgrowths on the maxillary 07s (32.6%), whereas ulcers were more commonly associated with maxillary 06 overgrowths (48.4%; Table 8.3.1.1). Smooth mouth (3.9%), wave mouth (5.9%), step mouth (1.5%) and shear mouth (0.5%) were observed with a low prevalence. Other abnormalities noted were missing tooth (1), exaggerated transverse ridges (2 teeth), laterally displaced teeth (3), supernumerary tooth (1), dysplastic tooth (1), pulpar exposure (1) and fractures (4). The number of teeth affected with different dental disorders in each Triadan position is summarised in Table 8.3.1.2 (Appendix 2).

Figure 8.3.1.3: **A large buccal ulcer (arrow) with adjacent bruising is associated with sharp enamel points at the cingulae (lateral vertical ridges) as indicated by arrow heads and use of a tight permanent head collar in a 6 year old donkey.**



Dental disease was not statistically significantly associated with BCS (P = 0.74; χ^2_1 = 0.11; Figure 8.3.1.4), geographical area (P = 0.07; χ^2_7 = 13.14) or climatic area (P = 0.12; χ^2_1 = 2.4), but was significantly associated with age group (P < 0.001; χ^2_4 = 31.4; odds ratio 10.4[2.7 – 40.4]; Figure 8.3.1.5). The prevalence of dental disease in donkeys

0 – 5 years of age was significantly less than donkeys 6 – 10, 16 - 20, and > 20 years of age ($P \leq 0.009$), and significantly less dental disease was present in donkeys 11 – 15 years of age less than in donkeys > 20 years of age (P = 0.04) (Figure 8.3.1.5). Gender was also significantly associated with dental disease (P = 0.002; $\chi^2_1 = 9.8$; odds ratio = 2.6 [1.4 – 4.9]) with males more likely to have dental disease (69%) than females (46%).

Figure 8.3.1.4: **Boxplot of BCS and dental disease. Vertical boxes = interquartile range, vertical lines = range; bold horizontal line = median.**

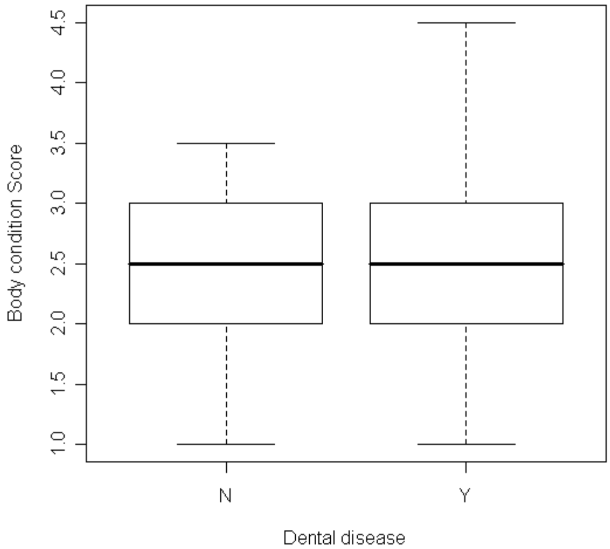
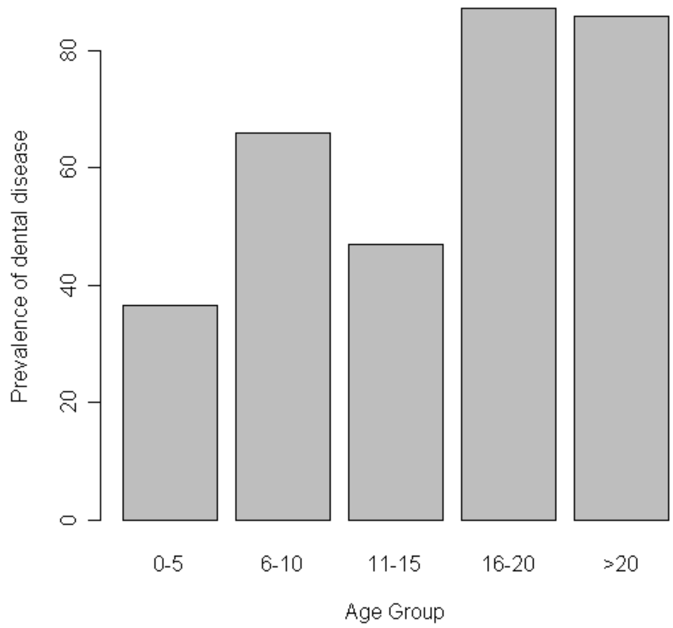


Figure 8.3.1.5: **The prevalence (%) of dental disease in each age group. A = 0-5 years; B = 6 – 10; C = 11- 15; D = 16 – 20; E = > 20years.**



Specific dental disorders

The prevalence of each specific dental disorder in different age groups (Table 8.3.1.3) and geographical areas (Table 8.3.1.4) are tabulated below.

Table 8.3.1.3: **The prevalence (%) of dental disease and specific dental disorders in each age group (number of donkeys examined in brackets). Age Group A = 0 – 5; B = 6 – 10; C = 11 – 15; D = 16 – 20; E = >21 years old.**

Dental disorder	Age Group A (52)	Age Group B (82)	Age Group C (17)	Age Group D (31)	Age Group E (21)
Dental disease	36.5% (19)	65.9% (54)	47% (8)	87% (27)	85.7% (18)
Diastema	0%	1.2% (1)	5.9% (1)	12.9% (4)	9.5% (2)
Overgrown teeth	3.8% (2)	13.4% (11)	11.8% (2)	38.7% (12)	52.4% (11)
Worn teeth	1.9% (1)	10.9% (9)	17.6% (3)	29.0% (9)	52.4% (11)
Missing teeth	0%	0%	0%	0%	4.8% (1)
Focal overgrowths	9.6% (5)	38.1% (32)	23.5% (4)	51.6%(16)	42.9% (9)
Ulcers	11.5% (6)	17.1% (14)	5.9% (1)	19.4% (6)	9.5% (2)
Calluses	15.4% (8)	14.6% (12)	11.8% (2)	12.9% (4)	4.8% (1)
Wave mouth	0%	3.7% (3)	11.8% (2)	12.9% (4)	14.3% (3)
Step mouth	0%	0%	0%	3.2% (1)	9.5% (2)
Smooth mouth	0%	0%	0%	6.5% (2)	28.6% (6)
Shear mouth	0%	1.2% (1)	0%	0%	0%

Table 8.3.1.4: **The prevalence (%) of dental disease and specific dental disorders in each geographical area (total number of donkeys examined in each area in brackets).**

Dental disorder	Area A (21)	Area B (24)	Area C (25)	Area D (20)	Area E (39)	Area F (42)	Area G (21)	Area H (21)
Dental disease	76%	50%	48%	50%	59%	71.4%	61.9%	90.9%
Diastema	4.8%	8.3%	8%	0%	0%	0%	0%	27.3%
Overgrown teeth	52.4%	25%	24%	10%	0%	11.9%	9.5%	54.5%
Worn teeth	28.6%	20.8%	20%	20%	0%	16.7%	9.5%	19%
Missing teeth	0%	4.2%	0%	0%	0%	0%	0%	0%
Focal overgrowths	47.6%	20.8%	16%	30%	20.5%	38.1%	47.6%	33.3%
Ulcers	14.3%	16.7%	4%	10%	17.9%	21.4%	9.5%	9.1%
Calluses	0%	0%	0%	0%	33.3%	23.8%	14.3%	9.1%
Wave mouth	23.8%	4.2%	0%	0%	0%	9.5%	0%	18.2%
Step mouth	0%	0%	8%	0%	0%	0%	0%	9.1%
Smooth mouth	4.8%	8.3%	8%	5%	0%	2.4%	0%	9.1%
Shear mouth	0%	0%	0%	0%	0%	2.4%	0%	0%

As only 8 donkeys were diagnosed with diastemata, no univariate analyses with diastemata as a dental disorder could be performed. The presence of overgrown teeth was statistically significantly associated with worn teeth, old/ young age groups and climatic areas in the univariate analysis ($P \leq 0.003$; Table 8.3.1.5). Overgrown teeth were present in 7% of donkeys without worn teeth and in 79% of donkeys with worn teeth; in 10% of younger donkeys and 36% of older donkeys; and 28% of donkeys in the temperate climatic area and only 12% of donkeys in tropical climatic area.

Worn teeth were significantly associated with focal overgrowths, young/old age groups and climatic area ($P \leq 0.04$; Table 8.3.1.5). Worn teeth were seen in 27% of donkeys with hooks and 11% without hooks; 33% of older donkeys and 7% of younger donkeys; and 22% of donkeys in the temperate climate and 12% of donkeys in the tropical area.

The presence of focal overgrowths was significantly associated with worn teeth, young/old age group and climatic area ($P \leq 0.04$; Table 8.3.1.5). Focal overgrowths were seen in 55% of donkeys with worn teeth and 28% of donkeys without worn teeth; in 28% of younger donkeys and 42% of older donkeys; and in 28% of donkeys in temperate climate and 36% of donkeys in tropical climate.

Univariate analyses of ulcers with overgrown teeth, young/old age group and climate, or calluses with hooks or young/old age group were not statistically significant ($P \geq 0.2$; Table 8.3.1.5). All other statistical analyses with binary variables could not be performed as inadequate numbers of observations were present. Similarly, multivariable analyses could not be performed due to inadequate numbers of obsevations.

Table 8.3.1.5: **Univariate analyses of dental disorders (P values, chi-squared values and odds ratio with 95% confidence interval).**

Binary variables	**Univariate P values**	**Chi-squared value**	**Odds ratio (95% confidence interval)**
Overgrown teeth			
Focal overgrowths	P = 0.17	$\chi^2_1 = 1.9$	1.6 (0.8 – 3.5)
Worn	**P < 0.001**	$\chi^2_1 = 74.9$	48.9 (17.5 – 136.5)
Young or old age	**P < 0.001**	$\chi^2_1 = 20.0$	0.2 (0.1 – 0.4)
Climatic area	**P = 0.003**	$\chi^2_1 = 8.7$	0.4 (0.2 – 0.7)
Worn teeth			
Focal overgrowths	**P = 0.004**	$\chi^2_1 = 8.3$	3.1 (1.4 – 6.6)
Young or old age	**P < 0.001**	$\chi^2_1 = 21.2$	0.2 (0.1 – 0.4)
Climatic area	**P = 0.043**	$\chi^2_1 = 4.2$	0.5 (0.2 – 0.98)
Focal overgrowths			
Overgrown	P = 0.17	$\chi^2_1 = 1.9$	1.7 (0.8 – 3.5)
Worn	**P = 0.004**	$\chi^2_1 = 8.2$	3.1 (1.4 – 6.6)
Young or old age	**P = 0.039**	$\chi^2_1 = 4.2$	0.5 (0.3 – 0.97)
Climatic area	**P = 0.020**	$\chi^2_1 = 1.7$	1.5 (0.8 – 2.7)
Ulcers			
Overgrown	P = 0.82	$\chi^2_1 = 0.05$	0.9 (0.3 – 2.5)
Focal overgrowths	P = 0.33	$\chi^2_1 = 1.1$	0.6 (0.3 – 1.5)
Young or old age	P = 0.72	$\chi^2_1 = 0.13$	1.2 (0.5 – 2.7)
Climatic area	P = 0.24	$\chi^2_1 = 1.4$	1.6 (0.7 – 3.7)
Calluses			
Hooks	P = 0.73	$\chi^2_1 = 0.12$	0.9 (0.4 – 2.1)
Young or old age	P = 0.35	$\chi^2_1 = 0.9$	1.6 (0.6 – 3.9)

8.4 Discussion

The age distribution of the 203 working donkeys examined in Mexico was much younger than that of the British donkeys examined in the post mortem study (median age

31; Chapter 4) (du Toit *et al.* 2008a; du Toit *et al.* 2008b) and the live UK study (median age 23, Chapter 7). The prevalence of dental disease in the Mexican donkeys was 62% which is only slightly less than the 73% observed in the UK clinical examination study (total 357 donkeys). As the most common age group in the Mexican working donkeys was the 6 – 10 years of age group, the results of this study is more comparable to the 0–10 year age group donkeys in the U.K. clinical examinations study (chapter 7). However, the prevalence of dental disease in the UK 0 – 10 year age group was only 28% (Chapter 4). It is most likely that the overall higher prevalence of dental disease seen in the Mexican donkeys (62%) and in particular the 6 – 10 year age group (66%), compared to the 0 - 10 year age group in the UK study, is due to the higher prevalence of focal dental overgrowths (33%), ulcers (14.3%) and calluses (13.3%) observed in the working donkeys compared to the UK donkey data (3.4%, 2.2% and 0% respectively). The low prevalence of focal overgrowths and ulcers seen in UK donkeys could be due to regular routine dental treatment that these donkeys receive. Furthermore, it is likely that the tight nose bands and head collars that many of the Mexican donkeys permanently wear contribute to the development of the ulcers and calluses observed, particularly considering that the majority of these soft tissue lesions are opposite the maxillary 06s and 07s. The higher prevalence of dental disease in male animals could be due to the fact that many female donkeys are used for breeding and therefore less likely to wear tight nosebands which appears to contribute to soft tissue buccal injuries.

The prevalence of serious dental disorders in this study (18.2%) was comparable to a previous study on working donkeys in Mexico that found a prevalence of serious dental abnormalities of 14% (Fernando-Martinez *et al.* 2006). The prevalence of sharp enamel points in this study (98%) was much greater than the prevalence observed in UK donkeys (27%; chapter 7). However this was very similar to the 99% prevalence observed in the above-noted Mexican study (Fernando-Martinez *et al.* 2006). As forage is the main constituent of Mexican donkeys' diet, this result is surprising as eating roughage is associated with greater lateral masticatory movement (Leue 1941; Bonin *et al.* 2007) that should restrict the formation of enamel points on the buccal maxillary and lingual mandibular aspect of cheek teeth. However, as these donkeys never had any dental treatments, any enamel points that have formed over a prolonged period of time would not have been removed and would therefore be expected to be present. Alternatively, this finding may question the current belief that sharp enamel edges are in fact an abnormality, but alternatively are a normal feature of equidae cheek teeth.

Focal overgrowths were very common (33%) in Mexican donkey cheek teeth, and predominantly affected the maxillary 06s or mandibular 11s as small, rostral and caudal overgrowths, respectively. This 33% prevalence is lower than the 50% prevalence observed by Fernando-Martinez *et al.* (2006) in a study of Mexican working donkeys. Other common dental disorders observed in this population were overgrown teeth (19%), worn teeth (16%), and diastemata (3.9%). All these disorders have a much lower prevalence than was observed in the post mortem and live studies of UK donkeys (chapters 4 and 7), which could be attributed to the lower median age in the Mexican donkeys. In particular, the prevalence of wave mouth (5.9%), smooth mouth (3.9%), step mouth (1.5%) and shear mouth (0.5%) were more similar to the prevalence of these disorders observed in British donkeys under 10 years old (0 – 5.8%; chapter 7).

Although there was a statistically significant association in the Mexican donkeys of dental disease to age group, there was no definite increase in dental disease with increasing age. In particular donkeys in age group B (i.e. 6 - 10 years of age) had a high prevalence of dental disease (66%), which could be attributed to the high prevalence of ulcers (17%) and calluses (15%) observed in this age group. The significant association of young/old age group to worn teeth, overgrown teeth and focal overgrowths was due to the increase in prevalence of these three disorders with increasing age group. Similarly, step mouth and smooth were only observed in donkeys $\geq$ 15 years of age.

The lack of a statistically significant association of dental disease to body condition score in this study indicated that most of the dental disorders present, did not affect the energy intake of affected donkeys. The median BCS of donkeys with and without dental disease was 2.5. This is in contrast to a previous study examining the prevalence of serious dental disease in working donkeys in Mexico that indicated the 80% of donkeys with serious dental disorders had a BCS of < 2.5. (Fernando-Martinez *et al.* 2006). A study on working donkeys in India found a very weak correlation between dental disease and body condition score, using a dental disease scoring system (Roy 2006). However, it has to be remembered that the study by Fernando-Martinez (2006) only examined the prevalence of serious dental disease and an association with lower BCS was not statistically shown in affected donkeys. In this study, dental disease was not statistically significantly associated with feed groups and therefore the presence of dental disease was not a determining factor in the decision of feed types. From our observations, we conclude that owners tend to feed whatever local feed that is available and also owners very rarely observed their animals whilst eating. Therefore, even if animals had dental disease that caused quidding (Dixon *et al.* 2000a), owners were unlikely to recognise this and so be aware of the dental disease and consequently of the need to feed more edible feedstuffs.

In this study 81% of working donkeys had a gastrointestinal (GIT) parasitic infection at the time of examination as determined by FEC. This is similar to the GIT parasite burden of donkeys living on the eastern coast (tropical) of Mexico (90%) in a study performed during the dry season (Valdez-Cruz *et al.* 2006), as was the present study. Furthermore, a South African study also demonstrated GIT parasite burdens in more than 90% of donkeys examined (Wells *et al.* 1998). However, it has to be remembered that donkeys might still have had a parasite infection at the time of examination which was not necessarily detectable at the time of faecal examination. Furthermore, it has been demonstrated that some individual equids tend to be consistent high nematode egg shedders, whereas other individual equids with inherent immunity will have little to no egg shedding (Duncan and Love 1991). The median FEC in this group of donkeys was 600 indicating that the level of infection on these donkeys was mild – moderate (Soulsby 1982). This median FEC was less than that determined in donkeys in South Africa (>

2000 epg) (Wells *et al.* 1998), but similar to the level determined in the previous study in Mexico (588 epg) (Valdez-Cruz *et al.* 2006).

Although parasitic egg identification or larval culture was not performed in this study, the predominant parasite likely to have been detected by the strongyle FEC are large strongyles, as a study of Mexican donkeys showed >90% of larvae to be large strongyles (Valdez-Cruz *et al.* 2006). This is in contrast to a study on donkeys in South Africa where almost 65% of the larval cultures had more than 90% cyathostomes, i.e. smaller strongyles (Wells *et al.* 1998). In this study, there was no correlation of FEC groups to body condition score and this was also demonstrated in the previous study on working donkeys in Mexico (Valdez-Cruz *et al.* 2006). This would indicate that there are other important factors that affect the body condition score in donkeys even in the presence of high faecal worm egg counts and dental disease. There was no association with FEC and food groups or supplemental feeding – although the latter was not quantified. This is in contrast to a study in South Africa where working donkeys consistently had a lower FEC if they had access to better feeds (Wells *et al.* 1998). The significant association of higher FEC in the tropical area agrees with a previous study in cattle (Kaufmann and Pfister 1990).

In conclusion, this study has shown that dental disease is present in 62% of working donkeys in the specific areas of Mexico that were investigated and that there is no statistically significant association between BCS and dental disease. This is believed to be due to the widespread feeding of supplemental feed sources to most donkeys in Mexico. Furthermore, body condition score was not significantly associated with high parasite burdens as assessed by FEC. Despite the absence of weight loss, 18% of the donkeys examined had serious dental disorders leading to direct welfare implications and required urgent dental treatment to alleviate any associated suffering. Additional to welfare reasons, treatments of these dental disorders are likely to improve the feed efficiency and increase the work rate in affected donkeys.

Chapter 9: Summary and Conclusion

Dental disease has been recognised in equids worldwide and has been shown to adversely affect the welfare of animals with dental disorders (Colyer 1906; Becker 1945; Roy 2002; Dixon and Dacre 2005; Chabchoub *et al.* 2006; Fernando-Martinez *et al.* 2006; Roy 2006). Most of the world's population of donkeys (estimated 44 million) are working animals and have been increasing in number for the last 30 years. As it has been shown that their numbers are unlikely to decline in the foreseeable future (Starkey and Starkey 2004), the importance of maintaining the optimum welfare of these animals cannot be over emphasised. In addition to the welfare of working donkeys, we must also consider the close links of these animals to the welfare of the human population for which they are often the only source of work power, particularly in rural populations in sub-Saharan Africa, Asia and Latin America (Starkey and Starkey 2004). To date, very little work had been published on the normal dental anatomy of donkeys (Bunger and Hertsch 1981; Misk and Seilem 1999; Muylle *et al.* 1999a) and only a few studies had recognised dental disease in donkeys (Rajput *et al.* 1999; Roy 2002; Chabchoub *et al.* 2006; Fernando-Martinez *et al.* 2006; Roy 2006).

The first part of this study was to examine donkey dental anatomy in detail to determine any similarities or differences to what has been reported in horses (Dixon and Copeland 1993; Kilic 1995; Dixon 2002, 2005). Dental anatomy of donkeys appeared to be largely similar to that which had been described in horses. Gross examinations have confirmed that donkeys have the same number of teeth (36-44) as horses, but a small increased prevalence of canine teeth in female donkeys compared to horses. On gross measurements, donkeys' cheek teeth appear to be smaller than horse teeth in length and width, although no direct comparison can be made as there is great variation in size between different breeds of donkeys, horses and ponies, with donkeys generally smaller than horses.

Similar to horses, there are two infundibula in the maxillary cheek teeth and none in the mandibular cheek teeth. The degree of peripheral enamel infolding in mandibular and maxillary cheek teeth was measured. The ratio of mean peripheral enamel perimeter length to tooth perimeter length on sub-occlusal transverse section was 1.47 in mandibular cheek teeth and 1.17 in maxillary cheek teeth without infundibula, confirming that there is a greater degree of enamel infolding in mandibular cheek teeth. However, the ratio of total enamel perimeter to tooth perimeter of maxillary CT 1.87 when infundibular enamel was included.

Donkey peripheral cementum has been shown to be a viable tissue with continued deposition of cementum at the gingival margin, as has been demonstrated in equine teeth. There was no difference in the increase of cementum occlusally between mandibular and maxillary cheek teeth, although mandibular cheek teeth did have a greater amount of total peripheral cementum. Peripheral cementum therefore has an important structural function on the clinical crown donkey cheek teeth. Equids also have various degrees of anisognathia (wider maxillary to mandibular jaw). In horses this has been shown to be 23% on average. In donkeys the mean degree of anisognathia has been demonstrated to be greater than horses (27%). This could contribute to the development of disorders such as shear mouth, which has been identified in donkeys.

The normal ranges of donkey enamel, dentine and cementum densities as assessed by CT and quantified in Hounsfield units have been established and appear to be similar to those found in equine teeth. Enamel was the most dense dental tissue (mean 3040 Hounsfield units), with primary (mean 2194) dentine also more dense than peripheral cementum (mean 1941). Infundibular cementum was only slightly less dense than peripheral cementum (mean 1806) and secondary dentine was even less dense (mean 1632). The density was not significantly different between maxillary and mandibular cheek teeth or younger and older donkeys.

Endodontic anatomy images from CAT were comparable to that seen on gross anatomy, with 5 pulp cavities in mandibular and maxillary cheek teeth 07s – 10s, 6 pulp cavities in 06s and 11s, with an occasional 7th pulp cavity present in the maxillary 11s. A modification of the endodontic numbering system as developed by Dacre (2005) has been proposed. The increased recognition of disorders involving the endodontic system such as pulpar exposure and fractures have emphasised the need for a numbering system to record and correlate disorders of pulp horns identified at clinical examination. It is hoped that the modification of the mandibular cheek teeth pulp horn numbering system to correlate with the maxillary cheek teeth will ease the use of this system.

The depth of pulp cavities and thickness of secondary dentine have been determined, although the values obtained are more representative of older donkeys, with only 4 out of 74 teeth being from a donkey under 10 years of age. The depth of secondary dentine above the pulp cavities in maxillary cheek teeth ranged from 1.20 – 1.40 cm and mandibular secondary dentine thickness ranged from 1.18 -1.61 cm. Knowledge of these results is clinically relevant when performing corrective dental procedures on donkeys, particularly when treating larger overgrowths. The widths of pulp horns were measured at different level of the teeth and as expected, pulpar widths increased apically. There was a significant decrease in tooth length in older donkeys, however a significant decrease in pulp width could not be demonstrated. This was once again believed to be due to the poor representation of truly young donkeys in the samples.

Maxillary cheek teeth showed a 50% prevalence of pulp horn communication close to the apex whilst in the mandibular cheek teeth 88.9% of pulps communicated at this site. These values are slightly higher than those demonstrated in equine teeth, but these included many communications in the apical third which could have been the merging of pulp horns into the common pulp chambers. Furthermore these communications were not histologically confirmed and therefore separation by undecalcified secondary dentine would have been missed. Ideally, this study should be repeated in more teeth using CAT followed by histological confirmation. Endodontic treatment has recently gained increased interest in equid dentistry and knowledge of the endodontic anatomy, including the prevalence of, and sites of pulp horn communication are important factors to consider before performing any endodontic procedure. As in horses, there was a greater degree of pulp horn communication in mandibular cheek teeth, which indicates that endodontic treatment is less likely to be successful in mandibular compared to maxillary cheek teeth.

Decalcified histology of donkey teeth has allowed us to define the normal histological structure of donkey dentine and cementum. The recently introduced equine dentine classification system (Dacre 2005a) that subdivides dentine into primary, regular secondary, irregular secondary and tertiary dentine was found to be equally applicable to donkeys. In particular the presence of irregular secondary dentine in all the teeth sections with the absence of tertiary dentine observed in these grossly normal teeth support this classification system. Normal values of primary and secondary dentine thickness around the pulp horns in cheek teeth on transverse sections of different age ranges have been established. These normal values will be of particular use as reference values when examining donkey teeth with pathology of the pulps, as these measurements reflect pulpar health and subsequent dentinal deposition. Primary dentine was significantly thicker palatally compared to buccally in maxillary cheek teeth, but not in mandibular cheek teeth. There was no difference in the secondary dentine thickness in either maxillary or mandibular cheek teeth. It is believed that this confirms that these teeth were not exposed to abnormal attrition forces or pathological stimulus that may have resulted in differences in secondary dentine thickness.

Undecalcified histology has been useful in examining enamel under light microscopy and we have identified the presence of enamel spindles which has not been previously described in equid dental anatomy. Enamel spindles are incidental findings of odontoblast processes that become trapped within the enamel at the early stages of tooth development. This has shown that embryology of equid teeth is similar to what has been decribed in other species. This understanding and classification of normal donkey dental anatomy is essential to allow accurate identification of pathological changes in diseased teeth on similar sections.

Scanning electron microscopy has demonstrated the presence of a smear layer (organic pellicle) on the clinical crown including the occlusal surface, which is believed to have an important protective role in protecting the underlying dentinal tubules. The ultrastructural anatomy of donkey dental enamel has been defined and is similar to findings in domestic horse enamel (Kilic *et al.* 1997b). Equid type-1, type-2 and type-3 enamel have been identified in donkey cheek teeth. The proportions of type-1 to type-2 enamel (the predominant enamel types) in donkey maxillary and mandibular cheek teeth have been found to be almost 1:1 compared to equid enamel in horses which had a greater proportion of type-1 to type-2 in maxillary cheek teeth. It is perceived that there is also a greater proportion of type 3 enamel in donkeys, but this has not been quantitatively measured in this study. Incisors were composed of predominantly Equid enamel type-2 to be able to withstand the shearing forces that incisors are exposed to during grazing.

Enamel thickness as measured on transverse section showed a consistent increase in enamel thickness when parallel to the long axis of the jaw, and on the buccal aspect compared to the palatal or lingual aspect in maxillary and mandibular cheek teeth respectively. In other herbivore species where the enamel was thicker lingually in the mandibular cheek teeth, the greater enamel thickness has been proposed to correspond to the side of greater attrition/force. It is possible that the greater degree of anisognathia may contribute to greater attrition forces on the buccal aspect of donkey mandibular cheek teeth and hence the thicker enamel on the buccal aspect. Recent studies have examined the degree of force applied during equine mastication and further studies will be required to determine where the attrition forces are greatest within a cheek teeth row and within each cheek tooth to confirm this theory.

On an ultrastructural level, we have established normal values of enamel prisms widths and enamel interprismatic distances for the different enamel types. The prisms were bigger in Equid enamel type-2 than type -1, with a greater interprismatic distance in type-1. Likewise, normal values for dentinal tubule width and density have been established for primary and secondary dentine. Primary dentine has a greater dentinal tubule diameter, with less intertubular dentine and a lower tubular density than secondary dentine. This study also agreed with the work by Muyelle *et al.* (2001, 2002) that used the term intratubular dentine (not peritubular) to describe the more mineralised dentine that lines the inside of primary dentine tubules.

Direct comparison of enamel, primary and secondary dentine hardness in donkey and horse incisors using a Knoop Hardness indenter failed to demonstrate a significant difference in dental tissue hardness between donkeys and horses. However, it is still speculated that donkey dental attrition is slower to ensure a greater longevity compared to horses. Ideally, microhardness determination should be performed on the cheek teeth of donkeys and horses to determine if there is a difference in the masticatory teeth. If there is no difference in cheek teeth microhardness, the perceived difference in rates of tooth attrition between donkeys and horses may be attributed to other external factors such as diet, masticatory cycle and duration of mastication.

Dental disease in donkeys was first investigated at post mortem examination of 349 donkey skulls (median age of 31 years). Dental disease was defined as any dental disorder excluding sharp enamel points on the buccal aspect of maxillary CT and lingual aspect of mandibular CT. Dental disease was observed in 93.4% of these donkey skulls examined with a particularly high prevalence of diastemata (85%). Diastemata were more common in the mandibular CT and in particular the caudal CT interdental spaces (09/10 and 10/11), compared to maxillary CT diastemata that were more prevalent in the rostral CT interdental spaces (06/07). Donkeys with diastemata commonly also had displaced and missing CT.

Displaced teeth were observed in 42.7% and were more commonly observed in the maxillary CT. The 09s and 10s were the most commonly displaced CT with bilateral displacements only observed in 13.2% of the donkeys with displaced teeth. A total of 55.6% of the donkeys had missing teeth with the maxillary 10s and mandibular 09s the most common CT missing. Worn teeth were observed in 33.3% of the donkeys with mandibular CT (64.1%) more commonly affected than maxillary CT (35.9%). Focal overgrowths were seen in 13.5% of the donkeys with maxillary CT more commonly affected. Interestingly calculus was observed in 19.5% of donkeys which was almost exclusively seen on the maxillary CT. Buccal ulceration was observed in 8.3% of donkeys often associated with sharp, overgrown and displaced CT. Step mouth (5.2%) and wave mouth (4.3%) was also observed with a low prevalence. Ultimately most dental disorders (apart from sharp enamel points) were observed with a higher prevalence than what has been observed in horse studies. This could be attributed to the older median age of the donkeys and the fact that this was a post mortem study, as live surveys can restrict detailed examination of CT, in particular the caudal CT.

This post mortem study also investigated some epidemiological factors that could be associated with dental disease such as body condition score, supplemental feeding, time since previous dental examination and nature of intercurrent disease that necessitated euthanasia. Dental disease was significantly associated with increasing age group, and older age groups were a high risk factor for the presence of diastemata. The presence of diastemata were significantly associated with the presence of concurrent displaced, missing and worn CT. Diastemata were found to be a common dental disorder in these donkeys and were significantly associated with colic as an intercurrent disease requiring euthanasia.

The presence of periodontal disease, pulpar exposure and caries were not noted in the initial post mortem study and it was for this reason that another 54 donkey skulls were examined at post mortem for detailed investigation of these disorders. As there were only a few skulls the actual prevalence of these disorders are not likely to be accurate for the whole population. However, the fact that pulp exposure and periodontal disease were seen more commonly in the maxillary and mandibular cheek teeth respectively is likely to accurately reflect the distribution of these disorders. Furthermore, CAT, histological and SEM images of cheek teeth affected with pulp exposure and caries was able to demonstrate that the basic pathological processes to be very similar to what has been described in other species. More specifically, the demonstration of a tooth with pulp exposure and viable pulp apically indicate some reparative capacity of equine cheek teeth in some situations, which has been previously proposed in equine teeth (Dacre 2005b).

As CT diastemata were the most prevalent dental disorder and clinically significant disorder recorded in the post mortem study, a small study was undertaken to examine diastemata and associated periodontal pockets. There was a significant difference in the ratio of diastemata occlusal to gingival margin width between valve and open diastemata, thus validating the definition of the type of diastemata. More importantly it was found that periodontal pockets were often deeper buccally. This has important clinical implications as it is technically difficult to get good access to the buccal aspect of cheek teeth to ensure proper assessment and cleaning out of periodontal pockets. It therefore seems likely that periodontal pocket depths associated with diastemta may often be underestimated when performing oral examinations.

A cross-sectional study of clinical dental examinations of 357 donkeys at The Donkey Sanctuary was performed to enable determination of the prevalence of dental disorders in different age groups and verify the findings of the post mortem study. In particular it was hoped that identification of dental disorders in the younger age groups might help to elucidate the pathophysiology of these disorders. A total of 357 donkeys were examined within 7 age groups ranging from 2 – 53 years of age. Dental disease was noted in 73% of donkeys with prevalence ranging from 28% in the youngest group (0-10 years) to 98% in the oldest age group (> 35 years). Similarly, specific dental disorders such as diastemata, missing teeth, overgrown teeth, worn teeth, displaced teeth and periodontal disease increased in prevalence with increasing age groups. The only exception was sharp enamel points that were most prevalent in the youngest age group (53%) with a decrease in the older age groups (< 20%). The largest significant increases for most dental disorders were observed in the 15 – 20 year age group.

As in the post mortem study diastemata were more commonly observed in the mandibular CT and in particular the caudal CT. The presence of diastemata were also significantly associated with other dental disorders such as missing, displaced, worn and overgrown cheek teeth indicating that diastemata either occur secondary to other dental disorders, or by causing periodontal disease and abnormal masticatory action, contribute to the development of other dental disorders. Once again CT overgrowths were more commonly seen maxillary CT, most commonly involving the 09s and 10s. Worn teeth were more commonly seen in the maxillary CT (particularly 10s and 11s) which is in contrast to the post mortem study where the mandibular CT were more commonly affected. This is believed to be due to the older age of the donkeys in the post mortem study where worn mandibular CT were often seen as the first teeth affected with smooth mouth. In contrast, the clinical study often showed individual worn maxillary CT were opposing overgrown mandibular teeth. Mandibular CT were also more often affected with medially and laterally displaced teeth which only had a slightly lower overall prevalence (26 and 33% respectively) than the post mortem study (42%). This is most likely due to the lower median age of the donkeys in the clinical examinations study. Periodontal disease was also observed with an increasing prevalence in older donkeys with an overall prevalence of 14%.

Wave mouth, step mouth, smooth mouth and shear mouth was observed with a low prevalence as in the post mortem and other horse studies (Uhlinger 1987; Dixon *et al.* 2000a). Infundibular caries was another condition that was not noted in the larger post mortem study and was only noted with a relatively low prevalence in the live donkeys. Once again lack of good visualisation could contribute to this low prevalence, but more importantly the recognition of a high prevalence of infundibular hypoplasia, defects and discoloration (Fitzgibbons 2007) that were not assessed as caries, might explain the difference between this and other studies.

In the second part of the clinical examinations study it was clearly shown that donkeys in older age groups were significantly associated with dental disease, poor body condition score and previous colic episodes. The presence of dental disease was associated with weight loss, poor body condition score, supplemental feeding and previous colic episodes. The presence of most dental disorders in donkeys was significantly associated with one or more other dental disorders. In particular diastemata were associated with missing, overgrown, worn and displaced teeth, as well as periodontal disease. This supports the theory that dental disease does not have a single aetiology and that as the cheek teeth have to work as a single functional unit, the presence of one disorder will inevitably lead to the development of other disorders due to disturbance in tooth positions and altered masticatory action.

Apical infections of CT which are a major problem in horses, were not identified in the clinical or post mortem study. The herd management of these donkeys would allow individual subtle clinical signs to be missed. During post mortem and clinical examinations no donkeys were observed to have possible related clinical signs (quidding, nasal discharge, facial swelling) and due to practical reasons donkeys with deep periodontal pockets or fractured teeth could not be radiographed. Interestingly, during extraction of a tooth (109) from a donkey at post mortem for normal anatomy i.e. grossly normal dentition and tooth, the apex was found to be grossly infected and surrounded by purulent exudate. So it does seem likely that subclinical apical infections were missed in both the post mortem and clinical studies.

A similar dental clinical examination study was performed on 203 working donkeys in Mexico. As the age distribution was very young, donkeys were categorised more broadly into young (0 - 10 years of age) and older (>10 years) age groups, to enable statistical analysis of associations. Dental disease was observed in 62% of these donkeys and enamel points (that were not included in the definition of dental disease) in 98%. Serious dental disease that required urgent dental treatment was observed in 18% of all the donkeys examined. Focal overgrowths ('hooks') were more commonly seen in this population (32.5%) than the U.K. donkeys. This higher prevalence in the working donkeys was believed to be due to the absence of routine dental treatment that the donkeys in the U.K. receive. FEC were determined from 79% of the donkeys to take parasite burden into account when evaluating the effect of dental disease on body condition score. However, no statistically significant association of body condition score to dental disease, FEC, feed supplement or climatic area could be demonstrated. It can be said that generally the donkey owners in Mexico looked after their animals well and did feed them local available resources when possible. So although dental disease did not appear to affect body condition score clinically, it is still believed that donkeys affected by dental disease will be less efficient energy converters. Furthermore, severe dental disease seen in at least 18% of these donkeys was a significant welfare problem that could be managed and treated with appropriate training of local veterinary surgeons.

In conclusion, donkey dental anatomy has been investigated in detail and found to be very similar to that which has been described in the horse. The significance of the anatomical differences observed (increased anisognathia, greater amount of Equid type-2 enamel and differences in dentine thickness in a buccal and lingual direction around pulp horns) is yet to be determined. Dental disease and specific dental disorders in donkeys have been well defined in this study and a definite increase in the prevalence of most dental disorders with age has been demonstrated. The importance of diastemata in the U.K. donkey population has been highlighted and in particular an association with colic has been demonstrated. In addition the clinical significance of dental disease has been demonstrated by an association with decreased body condition score and need for supplemental feeding in some populations. Ultimately, this study has emphasised that the presence of dental disorders needs to be considered when assessing the welfare of donkeys.

Future work should investigate the prevalence and clinical significance of apical infections in donkeys in the U.K., which was not investigated in this study. Furthermore, studies investigating the prevalence and clinical significance of dental disease in working donkeys in other developing countries will enable an assessment of the importance of the provision of dental treatment for these animals. Ideally, long term studies evaluating the effect of dental treatment on working donkeys will determine the true clinical significance of dental disease on the welfare of working donkeys.

Appendix 1

1. Post mortem dental chart

Donkey: **Age:**

1. Missing, worn, overgrown teeth

100s									200s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11
400s									**300s**								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11

M = missing; W = worn; OG = overgrown

2. Displaced teeth

100s									200s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11
400s									**300s**								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11

DM = displaced medially; DL = displaced laterally

3. Diastema

106/7	107/8	108/9	109/10	110/11	206/7	207/8	208/9	209/10	210/11
406/7	**407/8**	**408/9**	**409/10**	**410/11**	**306/7**	**307/8**	**308/9**	**309/10**	**310/11**

O = open; C = closed/valve

4. Periodontal disease

100s									200s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11
400s									**300s**								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11

0 = none; 1 = gingivitis; 2 = <25%; 3 = 25-50%; 4 = >50%

5. Pulpar exposure

100s									200s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11
400s									300s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11

IC = Infundibular caries

6. Soft tissue injury

100s									200s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11
400s									300s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11

U = ulcer; C = callus

7. Tartar/ Sharp

100s									200s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11
400s									300s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11

8. Fractures

100s									200s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11
400s									300s								
11	10	09	08	07	06	03	02	01	01	02	03	06	07	08	09	10	11

9. Comments

2. Live survey dental chart

ANIMAL DETAILS

Village:		Owner:			**Spec**	Ho		Do	Mu
Age:	BCS:	**Sex**	M	F	**Feed**	Grs		Fo	Grn
Reason for examination:	Routine	Sick	Dental related	**Previous treatment:**	Colic	Weight loss	Dewormed	Dental Treatment	Other

PHYSICAL EXAMINATION

Facial swellings or tracts/uni nasal discharge		Cheek Pain on palpation		Facial asymmetry from food packing		Quidding		Handling	
R	L	R	L	Yes	No	Yes	No	Yes	No

INCISORS

Incisor line					Jaw Alignment				Occlusal Surface Angle
Ven Cur	Dor Cur	R Diag	L Diag	Irregular	Overjet	Underjet	Twstd R	Twstd L	

Rostral-Caudal movement (mm)						Excursion											
3	4	5	6	7	8	> 1	1	¾	½	¼	N	N	¼	½	¾	1	> 1
						1 ½	1 ¼	1	¾	½	N	N	½	¾	1	1 ¼	1 ½

	100			200			300			400		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Retained												
Supernumerary												
Displaced												
Fractured												
Missing												
Carious												
Overgrowth												
Apical Infection												
Pulp exposure												
Diastema												

CANINES

	4				
100	C	L	D	Ca	F
200	C	L	D	Ca	F
300	C	L	D	Ca	F
400	C	L	D	Ca	F

C, calculus; L, enlarged; D displaced; Ca caries; F fracture

WOLF TOOTH

	5					
100	N	B	RD	RLD	LD	MD
200	N	B	RD	RLD	LD	MD
300	N	B	RD	RLD	LD	MD
400	N	B	RD	RLD	LD	MD

N, normal; B, blind; RD, rostrally disp; RLD, rostro-laterally disp; LD, laterally disp; MD, medially disp.

CHEEK TEETH

Position of upper CT rows		Disparity in length of CT rows			
Rostral	Caudal	Up Lef	Up Rig	Lo Lef	Lo Rig

Enamel Points

	1	2	3
100			
200			
300			
400			

Disorders in whole arcade

	Wave	Smooth	Shear	Step Mouth
100				
200				
300				
400				

	100						200						300						400					
	6	7	8	9	10	11	6	7	8	9	10	11	6	7	8	9	10	11	6	7	8	9	10	11
Missing																								
Diastema																								
Medially displaced																								
Laterally displaced																								
Step																								
Periodontal Dis.																								
Fracture																								
Fracture type S slab; M mid; O other																								
Peripheral Caries																								
Infundibular caries																								
Pulpar exposure																								
Supernumerary																								
Retained deciduous																								
Apical infection																								
Enamel points																								
Overgrown																								
Hooks																								
Worn to gum																								
Ulcers/callus																								

	100						200						300						400					
	6			11			6			11			6			11			6			11		
Hooks (tall overgrowths)	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Ramps (sloping overgrowths)	P	L	G	P	L	G	P	L	G	P	L	G	P	L	G	P	L	G	P	L	G	P	L	G

P, just present; L, damage to lips; G, damage to gums

Body Condition Score Chart (adapted from The Professional Handbook of the Donkey 4th Edition)

Condition score	Neck and shoulders	Withers	Ribs and belly	Back and loins	Hindquarters
1. Poor	Neck thin, bones easily felt. Neck meets shoulder abruptly, scapular spine palpable.	Dorsal spine of withers prominent and easily felt.	Ribs easily visualised and palpated.	Prominent dorsal and transverse processes.	Hip bones visible and palpable with little muscle cover. May be cavity under tail.
2. Moderate	Some muscle development over bones. Slight step where neck meets shoulders.	Some cover over dorsal withers. Spinous processes palpable but not prominent.	Ribs not visible but still palpable.	Dorsal and transverse processes palpable with poor muscle development.	Hip bones palpable with poor muscle cover on hindquarters.
3.Ideal	Good muscle development, bones palpable with moderate pressure. Neck flows into rounded shoulder.	Good cover of muscle/fat over dorsal spinous processes, withers flow smoothly into back.	Ribs just covered by mascle/fat, ribs palpable with light pressure. Belly firm with good muscle, flat outline.	Cannot palpate individual spinous or transverse processes. Muscle development good.	Good hindquarter muscle development. Hip bones rounded and palpable with light pressure.
4. Fat	Thick neck with hard crest. Even fat layer over shoulder.	Withers broad, bones palpable with firm pressure.	Ribs palpable with firm pressure dorsally, more easily ventrally. Belly overdeveloped	Dorsal spinous process palpable with firm pressure. Slight crease along midline.	Hindquarters rounded. Hip bones palpable with firm pressure. Fat deposits evenly placed.
5. Obese	Thick neck, bulging crest, may fall to one side. Shoulder rounded with bulging fat.	Withers broad, unable to palpate bones.	Large, uneven fat deposits covering ribs which are not palpable. Belly pendulous.	Back broad, spinous and transverse processes not palpable. Deep crease along midline with bulging fat on either side.	Hip bones not palpable. Fat may overhang tail head. Fat often bulging and uneven.

Appendix 2

***Table 2.3.3.3a*: The P values of comparisons between younger (≤ 15 years) and older (> 15 years) donkeys gross measurements in maxillary cheek teeth.**

Gross measurements	P value	F number
Tooth Length	**0.041**	$F_{1,12} = 5.24$
Gross LM width	0.63	$F_{1,12} = 0.25$
Gross RC width	0.28	$F_{1,12} = 7.38$
Gross medial root	0.20	$F_{1,11} = 1.82$
Gross lateral rostral root	0.09	$F_{1,11} = 3.42$
Gross lateral caudal root	**0.014**	$F_{1,11} = 8.4$
Rostral infundibulum	**0.034**	$F_{1,12} = 5.58$
Caudal infundibulum	**0.017**	$F_{1,12} = 0.034$

***Table 2.3.3.3b*: The P values of comparisons between younger (≤ 15 years) and older (> 15 years) donkeys gross measurements in mandibular cheek teeth.**

Gross measurements	P value	F number
Tooth Length	**0.001**	$F_{1,13} = 3.13$
Gross LM width	0.98	$F_{1,13} = 0.001$
Gross RC width	**0.018**	$F_{1,13} = 7.38$
Gross rostral root	0.46	$F_{1,12} = 0.572$
Gross caudal root	0.96	$F_{1,12} = 0.0024$

***Table 2.3.3.4a*: P values of comparison between younger (≤ 15 years) and older (> 15 years) donkeys pulp horn and secondary dentine measurements in maxillary cheek teeth.**

Pulp horn and Secondary dentine depth	P value	F number
PH1	0.069	$F_{1,13} = 3.99$
Secondary dentine PC 1	0.086	$F_{1,13} = 3.49$
PH2	**0.033**	$F_{1,10} = 5.81$
Secondary dentine PC2	0.093	$F_{1,13} = 3.32$
PH3	**0.041**	$F_{1,13} = 5.25$
Secondary dentine PC3	0.14	$F_{1,13} = 2.46$
PH4	**0.0073**	$F_{1,13} = 10.34$
Secondary dentine PC4	**0.036**	$F_{1,13} = 5.61$
PH5	**0.03**	$F_{1,13} = 6.31$
Secondary dentine PC5	0.061	$F_{1,13} = 4.29$

***Table 2.3.3.4b*: P values of comparison between younger (≤ 15 years) and older (> 15 years) donkeys pulp horn and secondary dentine measurements in mandibular cheek teeth.**

Pulp horn and Secondary dentine depth	P value	F number
PH1	**0.047**	$F_{1,13} = 4.81$
Secondary dentine PC 1	0.56	$F_{1,13} = 0.35$
PH2	0.077	$F_{1,10} = 9.09$
Secondary dentine PC2	**0.031**	$F_{1,13} = 5.86$
PH3	**0.006**	$F_{1,13} = 10.91$
Secondary dentine PC3	0.91	$F_{1,13} = 0.015$
PH4	**0.019**	$F_{1,13} = 7.23$
Secondary dentine PC4	0.31	$F_{1,13} = 1.14$
PH5	0.18	$F_{1,13} = 2.03$
Secondary dentine PC5	0.69	$F_{1,13} = 0.65$

Table 2.3.3.5a: **P values of comparison between younger (≤ 15 years) and older (> 15 years) donkeys pulp horn widths at sub-occlusal (a), mid (b) and pre-apical (c) level in maxillary cheek teeth.**

Pulp cavity width	**P value**	**F number**
PC1a	0.78	$F_{1,12} = 1.85$
PC1b	0.98	$F_{1,12} = 0.92$
PC1c	0.51	$F_{1,12} = 0.85$
PC2a	**0.45**	$F_{1,12} = 1.62$
PC2b	0.89	$F_{1,12} = 4.12$
PC2c	0.69	$F_{1,12} = 1.05$
PC3a	0.64	$F_{1,12} = 2.53$
PC3b	0.59	$F_{1,12} = 6.14$
PC3c	**0.37**	$F_{1,12} = 9.62$
PC4a	0.93	$F_{1,12} = 2.75$
PC4b	**0.11**	$F_{1,12} = 3.51$
PC4c	0.63	$F_{1,12} = 3.01$
PC5a	0.89	$F_{112} = 3.61$
PC5b	**0.46**	$F_{1,12} = 4.07$
PC5c	0.75	$F_{1,12} = 3.08$

Table 2.3.3.5b: **P values of comparison between younger (≤ 15 years) and older (> 15 years) donkeys pulp horn widths at sub-occlusal (a), mid (b) and pre-apical (c) level in mandibular cheek teeth.**

Pulp cavity width	**P value**	**F number**
PC1a	0.78	$F_{1,13} = 0.085$
PC1b	0.98	$F_{1,13} = 0.0005$
PC1c	0.51	$F_{1,13} = 0.45$
PC2a	**0.45**	$F_{1,13} = 0.62$
PC2b	0.89	$F_{1,13} = 0.02$
PC2c	0.69	$F_{1,13} = 0.16$
PC3a	0.64	$F_{1,13} = 0.23$
PC3b	0.59	$F_{1,13} = 0.29$
PC3c	**0.37**	$F_{1,13} = 0.85$
PC4a	0.93	$F_{1,13} = 0.008$
PC4b	**0.11**	$F_{1,13} = 3.03$
PC4c	0.63	$F_{1,13} = 0.24$
PC5a	0.89	$F_{1,13} = 0.02$
PC5b	**0.46**	$F_{1,13} = 0.58$
PC5c	0.75	$F_{1,13} = 0.1$

Table 2.3.3.6a: **P values of comparison between younger (≤ 15 years) and older (> 15 years) donkeys dental tissue radio density in maxillary cheek teeth.**

Dental tissue density	**P value**	**F number**
Pulp	0.45	$F_{1,13} = 0.60$
Primary Dentine	0.61	$F_{1,13} = 0.27$
Secondary Dentine	0.74	$F_{1,13} = 0.12$
Peripheral Cementum	0.27	$F_{1,13} = 1.31$
Infundibular cementum	0.104	$F_{1,13} = 3.10$
Enamel	0.82	$F_{1,13} = 3.61$

Table 2.3.3.6b: **P values of comparison between younger (≤ 15 years) and older (> 15 years) donkeys dental tissue density (in Hounsfield units) in mandibular cheek teeth.**

Dental tissue density	**P value**	**F number**
Pulp	0.53	$F_{1,13} = 0.41$
Primary Dentine	0.32	$F_{1,13} = 1.07$
Secondary Dentine	0.47	$F_{1,13} = 0.56$
Peripheral Cementum	0.38	$F_{1,13} = 0.84$
Enamel	0.52	$F_{1,13} = 0.4$

Table 7.3.4: **Number of affected teeth per Triadan position for each recorded dental disorder. DL = displaced laterally; DM = displaced medially; FO = focal overgrowths; IC = infundibular caries; M = missing; OG = overgrown; PD = periodontal disease; PE = pulpal exposure; S = sharp enamel points; Sm = Smooth; St = Step; U = ulcers; W = worn. Numbers in bold indicate the higher values per dental disorder.**

Tooth	DL	DM	FO	IC	M	OG	PD	PE	EP	U	W
106	3	0	**4**	**13**	7	22	1	5	75	2	24
107	2	2	0	4	11	9	2	9	73	0	13
108	2	4	0	3	7	30	2	9	74	0	3
109	1	**12**	0	2	12	**52**	2	**7**	73	0	1
110	**7**	11	0	3	13	42	3	1	78	0	17
111	2	7	0	0	**15**	22	2	0	**99**	1	**80**
206	0	0	**5**	**7**	5	40	2	5	67	2	13
207	2	1	0	1	6	6	1	8	67	1	8
208	2	3	0	0	6	20	1	6	65	0	2
209	**9**	**11**	0	4	10	**52**	3	**7**	67	0	2
210	8	6	1	2	**12**	35	2	0	71	0	10
211	2	6	0	0	9	24	2	0	**94**	1	**68**
306	3	0	0	NA	23	9	2	0	57	0	**13**
307	3	5	0	NA	16	7	5	0	58	0	10
308	15	5	0	NA	16	9	**8**	0	59	0	8
309	18	**18**	0	NA	**32**	3	**6**	0	59	0	**13**
310	**31**	8	0	NA	**29**	12	**11**	0	60	0	12
311	3	11	**6**	NA	25	**85**	2	0	**79**	0	9
406	3	0	0	NA	26	9	4	0	57	0	**13**
407	4	7	0	NA	22	13	8	0	59	1	5
408	14	10	0	NA	24	8	**10**	0	61	0	8
409	19	**13**	0	NA	**35**	3	4	0	57	0	5
410	**20**	4	1	NA	**31**	12	4	0	58	0	7
411	3	7	**6**	NA	19	**91**	0	0	**79**	0	7

Table 7.4.2.2a: **A summary of the relationship between numbers of donkeys receiving supplemental feed, the presence of dental disorders, time since last dental treatment and age, the percentage of total donkeys this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope for numerical variables.**

	Total	**Donkeys with supplemental feed**	**%**	**χ^2 & P value**	**Odds ratio/Slope**	**Multi-variate P values**
Number of donkeys	357	112	31.4			
Binary variables						
Diastema	188	86	45.7	**P < 0.001**; χ^2_1 = 39.77	4.6 (2.8 – 7.7)	**P < 0.001**
No diastema	169	26	15.4			
Missing teeth	105	76	72.4	**P < 0.001**; χ^2_1 = 113.69	15.7 (9.0 – 27.4)	**P < 0.001**
No missing teeth	252	36	14.3			
Displaced laterally	119	59	49.6	**P < 0.001**; χ^2_1 = 26.76	3.4 (2.1 – 5.5)	P = 0.15
No teeth displaced laterally	238	53	22.2			
Displaced medially	92	42	45.7	**P < 0.001**; χ^2_1 = 11.30	2.3 (1.4 – 3.8)	P = 0.77
No teeth displaced medially	265	70	26.4			
Worn teeth	148	74	52.7	**P < 0.001**; χ^2_1 = 40.78	4.5 (2.8 – 7.3)	**P = 0.01**
No Worn teeth	209	38	18.2			
Overgrown teeth	186	91	48.9	**P < 0.001**; χ^2_1 = 58.99	6.8 (3.9 – 11.8)	**P = 0.01**
No overgrown teeth	171	21	12.3			
Periodontal disease	51	28	54.9	**P < 0.001**; χ^2_1 = 15.24	3.4 (1.8 – 6.2)	P = 0.98
No periodontal disease	306	84	27.5			
Enamel points	96	13	13.5	**P < 0.001**; χ^2_1 = 21.54	0.3 (0.14 – 0.5)	P = 0.11
No enamel points	261	99	37.9			
Numerical variables						
Time since previous dental				P = 0.69; χ^2_1 = 0.16	0.015 (0.04)	-
Age				**P < 0.001**; χ^2_1 = 122.47	0.15 (0.02)	**P < 0.001**

Table 7.4.2.2b: **P values of multivariable model of interactions of explanatory variables with supplemental feeding as the response variable; DL = displace laterally; DM = displaced medially; OG = overgrown; PD = periodontal disease; EP = enamel points.**

	Diastemata	**Missing**	**DL**	**DM**	**OG**	**Worn**	**PD**	**EP**	**Age**
Diastemata	-	-	-	-	-	-	-	-	-
Missing	0.91	-	-	-	-	-	-	-	-
DL	0.16	0.07	-	-	-	-	-	-	-
DM	0.25	0.52	**0.04**	-	-	-	-	-	-
OG	0.32	0.06	0.42	0.08	-	-	-	-	-
Worn	0.28	0.32	0.09	0.52	0.81	-	-	-	-
PD	0.17	0.73	0.47	0.07	0.81	0.37	-	-	-
EP	0.84	012	0.79	0.19	0.42	**0.01**	0.69	-	-
Age	0.48	0.46	0.20	0.25	0.42	0.07	0.47	0.50	-

Table 7.4.2.2c: **P values of final multivariable model with supplemental feed as the response variable (DM=displaced medially; OG= overgrown teeth; PD=periodontal disease)**

Diastemata	**Missing**	**Worn**	**EP**	**Age**	**Worn:EP**
< 0.001	< 0.001	<0.001	0.09	< 0.001	0.03

Table 7.4.3.2a: **A summary of the relationship between numbers of donkeys with a history of colic, and the presence of dental disorders, time since last dental treatment and age, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope (+ standard error) for numerical variables.**

	Total	**Donkeys with colic**	**%**	**χ^2 & P value**	**Odds ratio/Slope**	**Multi-variate P value**
Number of donkeys	357	36	10.1			
Binary variables						
Diastemata	188	25	13.3	**P = 0.03**; χ^2_1= 4.7	2.2 (1.1 -4.6)	**P = 0.02**
No diastemata	169	11	6.5			
Missing teeth	105	16	15.2	**P = 0.04**; χ^2_1= 4.1	2.1 (1.0 -4.2)	P = 0.33
No missing teeth	252	20	7.9			
Displaced laterally	119	16	13.4	P = 0.14; χ^2_1 = 2.14	1.7 (0.8 – 3.4)	P = 0.68
No teeth displaced laterally	238	20	8.4			
Displaced medially	92	8	8.7	P = 0.60; χ^2_1 = 0.27	0.8 (0.4 – 1.8)	NA
No teeth displaced medially	265	28	10.6			
Overgrown teeth	186	27	14.5	**P = 0.003**; χ^2_1 = 8.61	3.0 (1.4 – 6.6)	P = 0.22
No overgrown teeth	171	9	5.3			
Worn teeth	148	22	14.8	**P = 0.012**; χ^2_1 = 6.27	2.4 (1.2 – 4.9)	P = 0.07
No Worn teeth	209	14	6.7			
Periodontal disease	51	7	13.7	P = 0.34; χ^2_1 = 0.90	0.9 (0.8 – 1.04)	NA
No periodontal disease	306	29	9.5			
Supplemental food	112	22	19.6	**P < 0.001**; χ^2_1 = 15.13	4.0 (2.0 – 8.2)	P = 0.08
No supplemental food	245	14	5.7			
Numerical variables						
Time since previous dental				P = 0.15; χ^2_1 = 2.1	- 0.09 (0.06)	P = 0.10
Age				**P < 0.001**; χ^2_1 = 19.7	0.007 (0.02)	**P = 0.002**

Table 7.4.3.3b: **P values of multivariable model of interactions of explanatory variables with the previous colic episode as the response variable.**

	Diastemata	Missing	DL	OG	Worn	Previous dental	Age
Diastemata	-	-	-	-	-	-	
Missing	0.72	-	-	-	-	-	
DL	0.82	0.51	-	-	-	-	
OG	0.07	0.14	0.39	-	-	-	
Worn	0.2	0.79	0.60	**0.04**	-	-	
Previous Dental	0.68	0.27	0.74	0.24	0.82	-	
Age	0.48	0.86	0.20	0.59	0.19	0.39	-
Supplemental food	0.29	0.33	0.98	0.12	0.66	0.89	0.18

Table 7.4.4.1a: **A summary of the relationship between numbers of donkeys with all diastemata, and other dental disorders, time since last dental treatment and age, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope (+ standard error) for numerical variables.**

	Total	Donkeys with diastema	%	χ^2 & P value	Odds ratio/Slope	Multi-variate P value
Number of donkeys	357	188	52.7			
Binary variables						
Missing teeth	105	83	79.0	**P < 0.001**; χ^2_1= 43.78	5.3 (3.1 – 9.0)	**P < 0.001**
No missing teeth	252	105	41.7			
Displaced laterally	119	102	85.7	**P < 0.001**; χ^2_1 = 84.89	10.6 (5.9 – 18.9)	**P < 0.001**
No teeth displaced laterally	238	86	36.1			
Displaced medially	92	80	87.0	**P < 0.001**; χ^2_1 = 64.39	9.7 (5.0 – 18.7)	**P < 0.001**
No teeth displaced medially	265	108	40.8			
Overgrown teeth	186	135	72.6	**P < 0.001**; χ^2_1 = 60.18	5.6 (3.5 – 8.8)	**P < 0.001**
No overgrown teeth	171	53	31.0			
Worn teeth	148	107	72.3	**P < 0.001**; χ^2_1 = 37.36	3.9 (2.5 – 6.2)	**P < 0.001**
No Worn teeth	209	81	38.8			
Periodontal disease	51	49	96.1	**P < 0.001**; χ^2_1 = 53.9	28.6 (6.8 – 120.4)	NA
No periodontal disease	306	139	45.4			

Numerical variables			
Time since previous dental	P = 0.36; χ^2_1 = 0.84	- 0.03 (0.03)	NA
Age	**P < 0.001**; χ^2_1 = 135.66	0.15 (0.016)	**P < 0.001**

Table 7.4.4.1b: **P values of multivariable model of interactions of explanatory variables with the presence of diastemata as the response variable**

	Missing	DL	DM	OG	Worn	Age
Missing	-	-	-	-	-	-
DL	0.54	-	-	-	-	-
DM	1.00	0.55	-	-	-	-
OG	0.46	0.37	0.15	-	-	-
Worn	0.97	0.25	**0.01**	0.89	-	-
Age	0.07	0.29	0.42	0.08	**< 0.001**	-

Table 7.4.4.1c: **P values of final multivariable model with diastemata as the response variable**

Missing	DL	DM	OG	Worn	Age	DM:W	W:Age
<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.02	<0.001

Table 7.4.5.1a: **A summary of the relationship between numbers of donkeys with wave mouth, and other dental disorders, time since last dental treatment and age, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope (+ standard error) for numerical variables.**

	Total	**Donkeys with wave mouth**	**%**	**χ^2 & P value**	**Odds ratio/Slope**	**Multivariable P value**
Number of donkeys	357	69	19.3			
Binary variables						
Diastema	188	46	24.5	**P = 0.03**; χ^2_1 = 4.79	1.8 (1.1 – 3.1)	**P = 0.03**
No diastema	169	23	13.6			
Missing teeth	105	23	21.9	P = 0.48; χ^2_1 =0.20	1.2 (0.7 – 2.1)	-
No missing teeth	252	47	22.9			
Displaced laterally	119	28	23.5	P = 0.06; χ^2_1 = 3.45	1.7 (0.9 – 2.9)	P = 0.39
No teeth displaced laterally	238	41	17.2			
Displaced medially	92	24	26.1	P = 0.14; χ^2_1= 2.2	1.5 (0.9 – 2.7)	P = 0.51
No teeth displaced medially	265	45	17.0			
Overgrown teeth	186	63	33.9	**P < 0.001**; χ^2_1 = 56.78	12 (5.3 – 27.2)	**P < 0.001**
No overgrown teeth	171	6	3.5			
Worn teeth	148	47	31.8	**P < 0.001**; χ^2_1 = 20.91	3.5 (2.0 – 6.0)	P = 0.10
No worn teeth	209	22	10.5			
Numerical variables						
Time since previous dental				P = 0.23; χ^2_1= 1.45	0.05 (0.04)	-
Age				**P < 0.001**; χ^2_1= 13.84	0.05 (0.013)	P = 0.89

Table 7.4.5.1b: **P values of multivariable model of interactions of explanatory variables with the presence of wave mouth as the response variable.**

	Diastemata	**DM**	**DL**	**OG**	**Worn**	**Age**
Diastemata	-	-	-	-	-	-
DM	0.19	-	-	-	-	-
DL	0.39	0.46	-	-	-	-
OG	0.39	0.78	0.25	-	-	-
Worn	0.32	0.56	0.39	**0.02**	-	-
Age	0.13	0.21	0.92	0.10	0.92	-

Table 7.4.5.1c: **P values of final multivariable model with wave mouth as the response variable.**

Diastemata	**OG**	**Worn**	**OG:Worn**
0.03	<0.001	0.11	0.04

Table 7.4.6.1a: **A summary of the relationship between numbers of donkeys with smooth mouth, and other dental disorders, time since last dental treatment and age, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope (+ standard error) for numerical variables.**

	Total	**Donkeys with Smooth mouth**	**%**	**χ^2 & P value**	**Odds ratio/Slope**	**Multi-variate P value**
Number of donkeys	357	16				
Binary variables						
Diastema	188	12	6.4	P = 0.06; χ^2_1 = 3.53	2.8 (0.9 – 8.9)	P = 0.06
No diastema	169	4	2.4			
Displaced laterally	119	2	1.7	P = 0.05; χ^2_1 = 3.84	0.3 (0.06 – 1.23)	**NA**
No teeth displaced laterally	238	14	5.9			
Displaced medially	92	4	4.3	P = 0.94; χ^2_1 = 0.005	0.9 (0.3 – 3.1)	-
No teeth displaced medially	265	12	4.5			
Overgrown teeth	186	13	7.0	**P = 0.013**; χ^2_1 = 6.18	4.2 (1.2 – 15.1)	P = 0.06
No overgrown teeth	171	3	1.8			
Worn teeth	148	13	8.8	**P = 0.001**; χ^2_1 = 11.60	6.6 (1.8 – 23.7)	**P = 0.02**
No Worn teeth	209	3	1.4			
Numerical variables						
Time since previous dental				P = 0.55; χ^2_1 = -0.05	-0.05 (0.09)	-
Age				**P < 0.001**; χ^2_1 = 32.9	0.17 (0.03)	**P < 0.001**

Table 7.4.6.1b: **P values of multivariable model of interactions of explanatory variables with the presence of smooth mouth as the response variable**

	Diastemata	**OG**	**Worn**	**Age**
Diastemata	-	-	-	-
OG	0.08	-	-	-
Worn	0.17	0.50	-	-
Age	0.31	0.05	0.58	-

Table 7.4.5.1c: **P values of final multivariable models with smooth mouth as the response variable**

Worn	Age
0.001	< 0.001

Table 7.4.7.1a: **A summary of the relationship between numbers of donkeys with step mouth, and other dental disorders, time since last dental treatment and age, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope (+ standard error) for numerical variables.**

	Total	Donkeys with step mouth	%	χ^2 & P value	Odds ratio/Slope	Multi-variate P values
Number of donkeys	357	42	11.8			
Binary variables						
Diastema	188	31	16.5	**P = 0.003**; χ^2_1 = 8.91	2.8 (1.4 – 5.9)	**P = 0.003**
No diastema	169	11	6.5			
Missing teeth	105	27	25.7	**P < 0.001**; χ^2_1 = 25.18	5.5 (2.8 – 10.8)	**P < 0.001**
No missing teeth	252	15	6.0			
Worn teeth	148	37	25.0	**P < 0.001**; χ^2_1 = 44.96	13.6 (5.2 – 35.7)	**P < 0.001**
No Worn teeth	209	5	2.4			
Overgrown teeth	186	42	22.6	**P < 0.001**; χ^2_1 = 50.13	48.07 (6.5 – 355.9)	NA
No overgrown teeth	171	0	0			
Displaced laterally	119	20	16.8	**P = 0.04**; χ^2_1 = 4.18	2.0 (1 – 3.8)	P = 0.91
No teeth displaced laterally	238	22	9.2			
Displaced medially	92	18	19.6	**P = 0.01**; χ^2_1 = 6.63	2.4 (1.3 – 4.8)	P = 0.21
No teeth displaced medially	265	24	9.1			
Numerical variables						
Time since previous dental				P = 0.05; χ^2_1 = 7.88	0.15 (0.05)	**P = 0.046**
Age				**P < 0.001**; χ^2_1 = 35.18	0.10 (0.02)	**P = 0.015**

Table 7.4.7.1b: **P values of multivariable model of interactions of explanatory variables with the presence of step mouth as the response variable**

	Diastemata	**Missing**	**DL**	**DM**	**Worn**	**Prev Dental Rx**	**Age**
Diastemata	-	-	-	-	-	-	-
Missing	0.05	-	-	-	-	-	-
DL	0.12	0.72	-	-	-	-	-
DM	0.26	0.61	0.55	-	-	-	-
Worn	0.63	0.20	0.39	0.49	-	-	-
Prev Dental Rx	0.83	0.84	0.24	0.73	0.39	-	-
Age	0.51	0.82	0.98	0.61	0.81	0.58	-

Table 7.4.7.1c: **P values of final multivariable model with step mouth as the response variable.**

Diastemata	**Missing**	**Worn**	**Time since previous dental**	**Age**
0.003	< 0.001	< 0.001	0.034	0.012

Table 7.4.9.1: **A summary of the relationship between numbers of donkeys with periodontal disease, and other dental disorders, time since last dental treatment and age, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope (+ standard error) for numerical variables.**

	Total	**Donkeys with periodontal disease**	**%**	**χ^2 & P value**	**Odds ratio/Slope**	**Multivariable P value**
Number of donkeys	357	51	14.3			
Binary variables						
Diastema	188	49	26.1	**P < 0.001**; χ^2_1 = 53.89	28.6 (6.8 – 120.4)	**P < 0.001**
No diastema	169	2	1.2			
Displaced laterally	119	33	27.7	**P < 0.001**; χ^2_1 = 20.21	4.1 (2.2 – 7.6)	-
No teeth displaced laterally	238	18	7.6			
Displaced medially	92	28	30.4	**P < 0.001**; χ^2_1 = 24.53	4.8 (2.6 – 9.0)	**-**
No teeth displaced medially	265	23	8.7			
Overgrown teeth	187	42	22.5	**P < 0.001**; χ^2_1 = 24.48	5.9 (2.7 – 12.9)	-
No overgrown teeth	170	8	4.7			
Worn teeth	148	26	17.6	P = 0.11; χ^2_1 = 2.63	1.6 (0.9 – 3)	-
No worn teeth	209	24	11.5			
Enamel points	96	5	5.2	**P = 0.002**; χ^2_1 = 9.97	0.3 (0.1 – 0.7)	P = 0.24
No enamel points	261	45	17.2			
Numerical variables						
Time since previous dental				P = 0.39; χ^2_1 = 0.74	- 0.04 (0.05)	-
Age				**P < 0.001**; χ^2_1 = 22.94	0.07 (0.016)	P = 0.25

Table 7.4.10.1a: **A summary of the relationship between numbers of donkeys with weight loss and other dental disease, diastemata and age, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope (+ standard error) for numerical variables.**

	Total	Donkeys with weight loss	%	χ^2 & P value	Odds ratio/Slope	Multi-variate P value
	357	34	9.5			
Dental disease	261	33	12.6	**P < 0.01**; χ^2_1 = 2.6	13.8 (1.8 – 102.7)	**P < 0.001**
No dental disease	96	1	1			
Diastemata	188	28	14.9	**P < 0.001** ; χ^2_1 = 210.1	4.8 (1.9 – 11.8)	**P = 0.04**
No diastemata	169	6	3.5			
Age				**P < 0.001**	0.06 (0.02)	P = 0.17

Table 7.4.10.1b: **P values of final multivariable model with weight loss as the response variable.**

Dental disease	**Diastemata**	**Age**	**Diastemata: Age**
< 0.001	0.036	0.17	0.047

Table 7.4.11.1a: **A summary of the relationship between numbers of donkeys with locomotory disorders, and other dental disease, diastemata and age, the percentage this represents, the univariate statistical results (χ^2 test statistic and statistical significance) and the univariate odds ratios (+ 95% confidence interval) for categorical variables and slope (+ standard error) for numerical variables.**

		Donkeys with musculoskeletal disorders				
	357	59	16.5			
Dental disease	261	54	20.7	**P < 0.001** ; χ^2_1 = 305.4	4.7 (1.8 – 12.3)	**P < 0.001**
No dental disease	96	5	5.2			
Diastemata	188	43	22.8	**P < 0.001** ; χ^2_1 = 308.1	2.8 (1.5 – 5.3)	P = 0.09
No diastemata	169	16	9.5			
Age				**P < 0.001**	0.06 (0.014)	**P = 0.03**

Table 7.4.11.1b: **P values of final multivariable models with locomotory disorders as the response variables.**

Dental disease	**Age**
< 0.001	0.01

Table 7.4.12.1: **A summary of the results of the univariate analysis of the association between dental disorders at tooth level.**

	Diastemata	**Periodontal disease**	**Pulpal exposure**	**Displaced laterally**	**Displaced medially**
Explanatory variable					
Diastemata	NA	NA	NA	NA	NA
Displaced medially	**P < 0.001**; t = 13.01	**P < 0.001**; t = 8.29	**P < 0.001**; t = 9.97	NA	NA
Displaced laterally	**P < 0.001**; t = 23.75	**P < 0.001**; t = 31.27	**P < 0.001**; t = 9.97	NA	NA
Worn tooth	P = 0.96; t = 0.05	NA	**P < 0.001**; t = 6.55	**P = 0.004**; t = - 2.90	P = 0.48; t = - 0.70
Overgrown tooth	P = 0.8; t = 0.25	**P < 0.001**; t = 1032.59	**P < 0.001**; t = 18.10	**P = 0.002**; t = 3.03	**P < 0.001**; t = 13.63
Enamel points	**P < 0.001**; t = 4.73		NA	**P = 0.001**; t = - 3.27	**P = 0.014**; t = - 2.47

Table 7.4.13.1: **A summary of the relationship between response (italics) and explanatory variables in: a) final multivariable models of donkey data (P values and univariate odds ratio with 95% confidence interval); and b) univariate models of individual tooth data (P values). M = missing teeth; OG = overgrown teeth; W = worn teeth; DM = displaced medially; DL = displaced laterally; PD = periodontal disease; PE = pulp exposure; NA = not applicable; NA^{+} = univariate odds ratio not applicable as all the cases were associated with a factor; * = final model had significant interactions.**

a) Factor	**Final P value**	**Univariate odds ratio**	**Factor**	**Final P value**	**Univariate odds ratio**
Supplemental feed			*Wave mouth**		
Diastemata	< 0.001	4.6 (2.8 – 7.7)	Diastemata	0.03	1.8 (1.1 – 3.1)
Missing	< 0.001	15.7 (9 – 27.4)	OG	< 0.001	11.8 (5.2 – 26.8)
OG	< 0.001	6.7 (3.9 – 11.6)	W	0.11	1.2 (0.7 – 2.1)
W	< 0.001	4.5 (2.8 – 7.3)	*Smooth mouth**		
Age group	< 0.001; χ^2_6 = 39.7	NA	Diastemata	0.06	2.8 (0.9 – 8.9)
*Colic**			OG	0.06	4.2 (1.2 – 14.9)
Diastemata	0.03	2.2 (1.0 – 4.6)	W	0.02	6.6 (1.8 – 23.7
Age Group	0.005; χ^2_6 = 22.8	NA	*Step mouth*		
Diastemata			Diastemata	0.003	2.8 (1.4 – 5.9)
Missing	< 0.001	5.2 (3.1 – 9.0)	Missing	< 0.001	5.5 (2.8 – 10.8)
OG	< 0.001	5.7 (3.6 – 9.1)	OG	< 0.001	NA^{+}
W	0.01	3.9 (2.4 – 6.2)	W	< 0.001	13.6 (5.2 – 35.8)
DM	< 0.001	9.7 (5.0 – 18.7)	*Periodontal disease*		
DL	< 0.001	10.6 (5.9 – 8.9)	Diastemata	0.001	28.6 (6.8 – 120.5)
Age group	< 0.001; χ^2_6 = 51.6	NA	Age Group	0.014; χ^2_6 = 15.9	NA

b) Factor	Final P value	Factor	Final P value	Factor	Final P value
Diastemata		W	< 0.001; t = 6.55	*Displaced medially*	
OG	0.8; t = 0.25	DM	< 0.001; t = 9.97	OG	< 0.001; t = 13.63
W	0.96; t = 0.05	DL	< 0.001; t = 9.97	W	P = 0.48; t = - 0.70
DM	< 0.001; t = 13.01	*Periodontal disease*		EP	0.014; t = - 2.47
DL	< 0.001; t = 23.75	OG	< 0.001; t = 1032.59	*Displaced laterally*	
EP	< 0.001; t = 4.73	DM	< 0.001; ; t = 8.29	OG	0.002; t = 3.03
Pulpar exposure		DL	< 0.001; t = 31.27	W	0.004; t = - 2.90
OG	< 0.001; t = 18.10			EP	0.001; t = - 3.27

Table 8.3.1.2: **Table illustrating the number of teeth in each Triadan position, affected by different dental disorders. The numbers in bold indicate the Triadan position having the highest prevalence of each disorder. Dias = diastema; OG = overgrown; W = worn; FO = focal overgrowths; EP = enamel points; cal = calusses; Fract = fractures.**

Tooth	Dias	OG	W	FO	EP	Ulcer	Cal	Fract	Ramp
106	1	3	**20**	**46**	**5**	**7**	10	0	1
107	0	3	7	0	2	0	**14**	0	0
108	0	3	2	0	3	0	8	0	0
109	1	1	3	0	2	2	2	0	0
110	0	2	3	0	5	0	2	0	0
111	0	2	5	0	1	4	11	0	0
206	2	7	**18**	**45**	6	**8**	10	0	1
207	1	2	8	0	**10**	2	**17**	2	0
208	0	5	1	0	3	2	8	0	0
209	1	2	1	0	3	2	2	0	0
210	0	2	4	0	6	0	2	0	0
211	0	0	7	0	0	1	8	0	0
306	3	**18**	3	3	2	0	0	1	**12**
307	1	3	0	1	0	0	0	0	0
308	4	2	0	0	1	0	0	0	0
309	0	2	3	0	2	0	0	0	0
310	1	2	5	0	2	1	0	0	0
311	0	7	2	**9**	**7**	2	1	0	0
406	1	**13**	2	5	0	0	0	0	**10**
407	1	4	0	0	2	0	0	0	0
408	2	2	2	0	3	0	0	0	0
409	1	2	1	0	1	0	0	0	0
410	0	2	2	0	2	0	0	0	0
411	0	11	2	**6**	2	0	0	0	0

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