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The aetiopathogenesis of equine periodontal disease – a fresh perspective

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Summary

Periodontal disease is a painful and highly prevalent disorder of horses and thus causes a significant welfare problem. Despite its importance, few scientific studies on its aetiopathogenesis have been performed. Equine periodontitis differs from the plaque-induced periodontitis found in brachydont species where bacteria accumulating in dental plaque induce a destructive inflammatory response in the periodontium. In contrast, equine periodontitis is usually initiated by entrapment of feed between cheek teeth which causes inflammation of periodontal tissue that likely allows bacterial infection of the periodontal tissues that is later exacerbated by the host’s response. Equine oral microbiology is a neglected field of research and identification of the bacteria involved in equine periodontitis by use of molecular bacteriology and examination of the interaction between these bacteria and the equine oral immune response should reveal important information about the pathogenesis of this disease.

1. Introduction

Periodontal disease is increasingly recognised as a common and painful equine oral disorder. With progression of this disease, the tissues surrounding and supporting the tooth are destroyed until eventually the tooth itself may be lost. Earliest recorded observations of equine periodontal disease by Aristotle date back to 333BC (Carmalt 2007) and in the early 1900s, several reports described its clinical features and high prevalence, especially in urban horses (Colyer 1906; Little 1913; Harvey 1920). Colyer (1906) also acknowledged its substantial welfare impact describing periodontal disease as ‘the scourge of the horse.’ More recent studies have shown periodontitis to be present in up to 75% of horses (Baker 1970; Ireland et al. 2012) with its prevalence increasing with advancing age. Other recent equine studies have confirmed this disorder to be very painful, causing quidding and weight loss (Dixon et al. 2008, 2014). Donkeys are also commonly affected with disease noted
in 23.5% of working donkeys (Rodriguez et al. 2013) and 100% of diastemata in one donkey study having associated periodontitis (du Toit et al. 2009). This disorder is not limited to domesticated equids, with Penzhorn (1984) noting periodontitis in free-ranging Cape Mountain Zebra.

Despite the high prevalence of periodontitis in domesticated horses and the significant welfare problem it causes, the disease may initially go unnoticed by owners because associated clinical signs such as quidding and loss of condition can be absent (Dixon et al. 2008; 2014). Although there has been increased recognition of the importance of equine periodontal disease in recent years, very few scientific studies on the aetiopathogenesis of this disorder have been performed. This article aims to critically review current knowledge of this disorder and also outline the potential use of novel methods in investigating its aetiopathogenesis.

2. Equine Periodontal Anatomy

The periodontium is a complex, dynamic structure compromised of four separate tissues, i.e. the gingiva, peripheral cementum, periodontal ligament and alveolar bone that interact together to protect and support the tooth. Staszyk and Gasse (2005) described the equine periodontium as having three major supportive functions, firstly to secure the tooth in the alveolus, secondly; to accept a variety of masticatory forces and thirdly; to restore the tooth to its original position after temporary displacement during mastication. The equine periodontium must also adapt to allow for the prolonged eruption of hypsodont dentition and cope with masticatory forces of over 1550 Newtons exerted on the caudal cheek teeth for up to 18 hours per day (Huthmann et al. 2009).

Although it is intuitive to think of peripheral cementum as part of the tooth itself, especially in hypsodont teeth, where it compromises a substantial part of the clinical crown (Mitchell et al. 2003), it can also be considered as a component of the periodontium due to its distinctive odontogenic development (Staszyk et al. 2015). Throughout the life of the tooth peripheral
cementum is continually produced by cementoblasts and deposited at the apex and around the periphery of the reserve crown, a feature unique to hypsodont periodontium (Staszyk and Gasse 2007). Human cementum has an organic content of around 50% primarily collagen, with small collagen fibrils being produced by cementoblasts and nourished by vessels within the periodontal ligament (Hands 2008). Larger fibrils known as Sharpey’s fibres originate from the periodontal ligament and are incorporated into both the peripheral cementum and alveolar bone, flexibly anchoring the tooth into the alveolus (Grant and Bernick 1972).

In the horse, the periodontal ligament is a highly vascular and cellular structure which largely consists of collagen fibres, fibroblasts and ground substance interspersed with blood and lymphatic vessels (Staszyk and Gasse 2005). The equine periodontal ligament contains unique vasculature which both nourishes and supports the tooth during mastication and prolonged eruption. Periodontal blood vessels are integrated into the equine periodontal ligament in three distinct ways (Staszyk and Gasse 2004). The Type 1 arrangement of periodontal blood vessel groups are protected from masticatory forces by a sheath of ‘veil cells’ and connective tissue which is thought to protect blood flow during mastication. An elaborate collagen network anchors blood vessels in the Type 2 arrangement, resulting in a functional fibro-vascular unit able to resist forces of traction during mastication. In the Type 3 vascular arrangement, dilated ballooned venules running parallel to the tooth surface between collagen fibre bundles have a cushioning effect, absorbing the substantial forces of mastication. Staszyk and Gasse (2004) also noted presence of elastic oxytalan fibres in the equine periodontal ligament, which allow regeneration and remodelling, whilst improving periodontal blood vessel stability.

Matrix metalloproteinase-1 which initiates collagen breakdown to allow remodelling has been detected in the equine periodontal ligament (Warhonowicz et al. 2007). As eruption progresses, equine teeth reduce in size and shape (narrowing towards the apex), and in turn the surrounding alveolar bone must constantly remodel order to provide sufficient support to the tooth. Sharpey’s fibres in the periodontal ligament insert into a thin, compact layer of “cortical” alveolar bone which
lines the alveolus and is radiographically known as the *lamina dura*. The alveolar bone surrounding this superficial compact layer is more porous which may reflect its constant remodelling (Dixon and du Toit 2011).

The gingiva is a firm, keratinised epithelium covering the underlying alveolar bone, periodontal ligament and reserve crown and acts as a physical barrier against oral microbial invasion of the periodontal tissues. It is possible to further classify gingival tissue by its location within the oral cavity and with regards to its position relative to the tooth. Stasyzk et al. (2015) describe two distinct zones of equine gingiva: the interdental gingiva also known as the interdental papilla and the remaining bulk of the gingiva located on the buccal, labial, palatal and lingual aspects of teeth.

The free gingiva is the most occlusal and mobile aspect of the gingiva and acts as an interface with the epithelium of the gingival (crevicular) sulcus which is a shallow pocket between the tooth and adjacent sulcular epithelium. Junctional epithelium at the base of the sulcus adheres tightly to the peripheral cementum on the tooth surface.

Gingival sulcus depth may be between 1-4mm in periodontally healthy horses (Cox et al. 2012) and an increase in sulcar depth indicates the presence of periodontal disease. Gingival crevicular fluid containing antibodies, enzymes and other inflammatory mediators and immune components is secreted into the sulcus, and together with gingival tissue plays an important role in responding to immunological challenges posed by oral bacterial communities. In periodontal health, the gingiva provides a tight seal around the erupted crown and protect underlying structures by forming a mechanical barrier. However in periodontal disease, this barrier is damaged leaving underlying sensitives tissues exposed and open to both mechanical damage and bacterial colonisation. A very complete description of equine periodontal anatomy has recently been published by (Stasyzk et al. 2015).

3. Aetiopathogenesis of periodontal disease

*Plaque induced periodontal disease in brachydont species*
Periodontal disease is also of major importance in humans and brachydont domestic animals and consequently has been extensively studied in many brachydont species, often as a model for human disease (Giannobile et al. 1994). In brachydont dentition, the initiating factor for gingivitis, which is the earliest and often reversible stage of periodontal disease, is the accumulation of dental plaque in the gingival sulcus, which may eventually become calcified (calculus) (Theilade et al. 1966; Mariotti 1999). More specifically, the presence of plaque in the gingival sulcus initiates a bacterial–induced inflammatory reaction (gingivitis) that may or may not proceed to involve the deeper periodontal tissues (Page and Kornman 1997). More severe periodontal disease is most frequently due to the host’s response to the bacterial invasion (Bartold and Dyke 2013).

Entrapped food induced periodontal disease in hypsodont species

Plaque induced periodontitis does not appear to be a common problem in horses, unlike in brachydont species (Dixon et al. 2000), an exception being the canine teeth, where the presence of calculus can cause gingivitis (Fig 1), but rarely, more severe periodontitis.

Fig. 1 Calculus on a canine tooth with local gingivitis (arrows).

In equidae, food trapping in anatomical defects such as diastemata between adjacent cheek teeth, is the usual instigator of periodontitis. In a study of referred equine cases with cheek teeth disorders,
periodontal disease in the absence of intercurrent dental disorders, and without the presence of plaque was identified in just 3/349 (0.9%) cases (Dixon et al. 1999; 2000).

Equine periodontitis is particularly associated with diastemata which can be described as abnormal spaces between adjacent teeth - which should normally be in tight occlusal apposition. Food material becomes impacted into this abnormal space during mastication, often becoming tightly entrapped and initiating inflammation of the underlying gingiva initially, that invariably progresses to the deeper periodontal tissues (Figs 2 & 3). Colyer (1906) noted the high prevalence and importance of equine periodontal disease and attributed it to a coarse diet, but Dixon et al. (2000) noted that the illustrated specimens of cheek teeth periodontal disease from Colyer’s study as illustrated by Miles and Grigson (1990) all had diastemata, and that the reported periodontal disease appears to have been initiated by interproximal food trapping. Little (1913) had previously attributed diastemata as a cause of equine periodontal disease, especially periodontal disease in the interdental (interproximal) spaces adjacent to the mandibular 310 and 410 cheek teeth.

Fig 2. Post-mortem image of left maxillary cheek teeth showing deep periodontal pockets between the mesial 4 cheek teeth with deep periodontal pocketing of feed.
Fig 3. Oral endoscopic view of severe equine cheek teeth periodontal disease caused by a diastema. The teeth adjacent to the diastema have caries of the peripheral cementum and are covered in grey-coloured plaque. There is marked loss of periodontal tissues in the interdental space (yellow arrow) but no loss of gingiva which is markedly hyperplastic (white arrow).

Diastemata may be congenital or acquired. Dixon et al. (2008; 2014) classified equine diastemata as primary diastemata (where the teeth developed too far apart and/or with insufficient angulation to occlusally compress them) (Fig 2) and as secondary diastemata, such as caused by displaced (Fig 4) or rotated cheek teeth (Fig 5), dental overgrowths or supernumerary cheek teeth. In addition senile diastemata may form due to the tapering of equine cheek teeth towards their apices resulting in decreased surface area at the occlusal surface, along with age-related loss of angulation of the 06s and 11s (Fig 6). Diastemata have been documented in up to 50% of horses in a UK equine practice survey, with feed material becoming trapped in 91.4% of diastemata (Walker et al. 2012). In addition, 34% of diastemata had associated gingivitis and 44% were accompanied by periodontal pockets (Walker et al. 2012). Impaction of feed material in periodontal pockets has also been
recorded in 76% of cheek teeth diastemata in donkeys (du Toit et al. 2009), with 71% of these donkeys also having concurrent dental disorders such as displaced cheek teeth, which likely initiated or contributed to diastemata formation. Malerupted (rotated) maxillary cheek teeth have also been described as the primary cause of diastemata formation and associated severe periodontitis (Casey and Tremaine 2010) (Fig 5). Voss (1937) suggested that irregular feeding times interrupted salivary flow in horses, which in turn could contribute to the development of periodontal disease.

**Fig 4.** Secondary diastema. This post-mortem image shows a developmentally displaced (and also curved) cheek tooth with secondary diastemata rostral and caudal distal to it and a deep periodontal pocket on its lingual aspect (arrow). There are also “primary” diastemata between other adjacent teeth.
Fig 5. These post-mortem images (occlusal aspect –top image: buccal aspect –bottom image) show a rotated 208 tooth (yellow star) with a narrow diastema rostrally and wide diastema caudally (yellow arrows).

Fig 6. Caudal cheek teeth of a horse with senile diastemata. Note the 209 (yellow star) worn down to roots with compensatory cementum deposition and loss of some infundibulae and senile excavation in the other two teeth (red stars).
Feed Stasis and Bacterial Proliferation

The above described trapping, stasis and subsequent decomposition and fermentation of food material in equine interdental spaces can as noted, abrade the sensitive gingiva, causing mechanical damage and gingival inflammation (Little 1913; Baker 1979; Cox et al. 2012). Over time, impacted feed, (a porous foreign body) acts as a bacterial nidus, further supporting the proliferation of bacteria which ferment the trapped feed. The Lactobacillales order of bacteria which include Lactobacilli, Streptococci and Enterococci ferment plant material by anaerobically metabolising carbohydrates to produce lactic acid (Gänzle 2015).

Inflammation of the periodontium

The initial insult provokes a substantial inflammatory response within gingival tissue due to both mechanical abrasion of sensitive gingival epithelium and bacterial proliferation, as noted earlier. This is apparent clinically as gingivitis with hyperaemia and bleeding upon gingival probing. In man, a number of different pathogenic bacteria are implicated in the induction of a marked host inflammatory response which in turn leads to destruction of the periodontal ligament and resorption of alveolar bone and cementum which can leads to end stage disease, i.e. loss of the tooth. This exaggerated inflammatory response shown in human periodontitis cases is the result of prolonged cytokine production in gingival tissue leading to increased production of proteases, which although can break down invading microbes, can also damage the host’s periodontal tissues (Teng 2003). This response may show individual variation, including a genetic component in humans (Yoshie et al. 2007).

Apart from the study of Cox et al. (2012) there appears to be no published work on equine periodontal disease histology. These authors showed the mucosal surface of equine periodontal pockets to be hyperplastic, with epithelial disruption and presence of large numbers of
inflammatory cells such as neutrophils in the lamina propria (Fig 7) and adjacent connective tissues (Fig 8) and destruction of the periodontal tissue including peripheral cementum in which the periodontal membranes were once attached (Fig 8).

Fig 7. Histological section of periodontium from a horse with periodontitis. This shows a periodontal pocket circa 5mm deep. The calcified dental tissue (i.e. tooth) on the right side of periodontal pocket has been lost during decalcification. Moderate gingival hyperplasia (red arrows) is present on the gingiva facing the periodontal pocket and at the free gingival margin—(as is grossly seen in another horse with periodontitis in Fig 3). There is also modest infiltration of inflammatory cells into the lamina propria (yellow star). (bar = 2mm). Image courtesy of Dr. A. Cox.
Fig 8. Massive infiltration of inflammatory cells into interdental subgingival connective tissue (CT) of a horse with periodontitis. The separation of this connective tissue from cementum (C) is artefactual during histological preparation. (bar = 50 µm). Image courtesy of Dr. A. Cox.

Accumulations of food material which may be obvious clinically, has also been confirmed on histopathology, alongside large numbers of bacteria and micro abscesses in the submucosa of equine periodontal pockets (Cox et al. 2012). In absence of clinical intervention, the disease progresses and inflammation spreads to the periodontal ligament with infiltration of mononuclear cells (Figs 7 and 8). The ligament is gradually destroyed over time, as is the surrounding alveolar bone and cementum (Fig 9), decreasing tooth support and further deepening periodontal pockets. Teeth may become mobile at this stage. The increasing depth of the equine periodontal pocket provides the ideal environment for further invasion and proliferation of anaerobic bacteria and the cycle of inflammation and tissue degradation continues until tooth loss occurs.
Fig 9. Disintegration of peripheral cementum (C) of the periodontium due to advanced periodontal disease includes the development of cemental clefts (yellow arrow) that become infilled with plaque (P) – as also grossly seen in another horse with periodontitis in Fig. 3. Image courtesy of Dr A Cox.

4. Oral Microbiology

It is well recognised that bacteria play a major role in the aetio-pathogenesis of human (Socransky et al. 1998), canine (Hennet and Harvey 1991a; 1991b) and feline (Harris et al. 2015) periodontal disease and it is easy to appreciate the potential importance of bacteria in equine periodontitis. This role was recently supported by the histopathological finding of spirochetes in the sulcular epithelium (Fig 10) of diseased equine periodontal pockets, which also had cocci on the epithelial surface (Fig 11) (Cox et al. 2012). The ecological community of bacteria both commensal and pathogenic inhabiting the oral cavity is known as the oral microbiome. There are approximately 700 different species identified to date in human healthy and diseased oral cavities, (Dewhirst et al. 2010), even though the human oral microbiome has not yet been fully characterised. This bacterial community is incredibly complex and dynamic, with species interacting with each other and also with the host immune system. In order to survive in an environment being constantly washed with host saliva, in addition to being challenged with mechanical abrasion from masticatory movements, bacteria have found a method of adhering to the surface of oral tissue and so exist in a biofilm.
A biofilm is defined as ‘a biopolymer matrix-enclosed bacterial population, adherent to each other and surfaces’ (Costerton et al. 1999). Multispecies bacterial communities existing within the oral biofilm are supported and protected by the surrounding matrix. The composition of an oral biofilm is very much dependent upon its location within the oral cavity, and thus tooth-associated biofilms have been divided into two categories in the human mouth. Supragingival biofilms adhere to the
surface of the clinical crown, and subgingival biofilms adhere below the gum line, either within the (normal) gingival sulcus or (abnormal) periodontal pocket (Kolenbrander et al. 2010). Early bacterial colonisers which are well adapted to community formation and multispecies growth (Kolenbrander et al. 2010) initially adhere to the salivary pellicle, a layer of proteins and glycoproteins which permanently coats all normal oral surfaces as recently reviewed by Borkent and Dixon (2015). Adhesion and subsequent proliferation of early colonisers is followed by co-adhesion of genetically distinct bacteria to the existing attached population. The salivary pellicle functions as a defensive layer, lubricating and protecting the surface of the tooth itself (the enamel surface in brachydont dentition) and surrounding soft tissue (Gibbins et al. 2014) and is distinct from the oral biofilm which attaches to its surface and the subsequent plaque which develops (Marsh and Bradshaw 1995).

In addition to co-adhesion, distinct bacterial species also interact via cell surface components when both are suspended in fluid, a process that is termed co-aggregation. For example, strains of *Fusobacterium nucleatum* are able to co-aggregate with early and late oral biofilm and may play a bridging role in the development of human dental plaque (Kolenbrander and London 1993; Kolenbrander et al. 2010). Under certain conditions, the biofilm can become increasingly complex and mature into dental plaque. The oral biofilm is highly intricate with dynamic microbial interactions, including complex cell signalling between bacteria of differing genera as well as transfer of DNA between bacteria. Conjugative transposons which facilitate DNA transfer between bacteria have been detected in many genera of human oral bacteria such as *Fusobacterium*, *Streptococcus* and *Veillonella* (Rice 1998). This is a cause for concern as there is potential for transference of antibiotic resistance genes between different species of bacteria in the dental biofilm.

Bacteria within the biofilm matrix can be protected from exposure to host innate and adaptive immune mechanisms as well as to administered antimicrobial compounds. Front-line immune responses such as phagocytosis are ineffective in the biofilm matrix as bacterial cells cannot be
readily engulfed at this site (Kharazmi 1991) and infiltration of neutrophils into the plaque may even provide an additional matrix for bacterial attachment in man (Walker et al. 2005). Although ineffective in removing the bacterial biofilm, the immune response has a significant side effect on surrounding tissue, stimulating inflammation and often destruction of the human periodontium (Teng 2003). Due to the limitations of the host immune system and of antimicrobial therapy in combating potentially periodonto-pathogenic bacteria within the dental biofilm, mechanical removal of dental plaque is necessary to treat brachydont periodontal disease.

**Bacteria in oral health and disease**

Current understanding of the role of bacteria in disease is changing and traditional principles such as Koch’s postulates are becoming increasingly irrelevant in modern microbiology. Due to the difficulties in culturing some bacteria, it cannot be said that an organism is not involved in disease purely because it cannot be grown in culture. Likewise, to suggest that an organism is not involved in a disease because it may be found in healthy individuals or is unable to replicate disease when inoculated into a healthy individual would be to ignore variations in host-pathogen interactions. The human oral microbiome in health is markedly different to that found in periodontitis lesions (Wang et al. 2013). Analysis of the equine oral microbiome, has shown similar findings, with samples from orally healthy horses showing major dissimilarities to samples taken from diseased periodontal pockets (Kennedy et al. 2015). This substantial shift in oral microbiota with periodontitis can be interpreted in several ways. The traditional hypothesis of pathogenic (or certain) bacteria occurring in high numbers or solely detected in periodontitis lesions being the cause of the disease appears to be overly simplistic and it is more likely that many factors with complex interactions are involved.

Significant local environmental changes occur during the development of periodontal pockets (Loesche et al 1983), which may be especially deep around equine cheek teeth (Cox et al. 2012) The anaerobic or partially aerobic environment of deep human periodontal pockets encourages invasion and proliferation of micro-aerophilic organisms, anaerobes and spirochetes, while the
environment of the general oral cavity supports a significantly different microbiota (Loesche et al. 1983). Another hypothesis which can be applied to the diseased oral microbiome is the *keystone pathogen hypothesis* which maintains that certain pathogens existing at low abundance in the oral cavity may modulate their environment, disturbing the normally symbiotic relationship between the oral bacteria, creating a state of *dysbiosis* (dysregulation of commensal oral bacteria) thus contributing to the development of inflammatory disease. *Porphyromonas gingivalis*, is a well-known keystone pathogen in human periodontal disease due to its ability to modulate the host immune system, and thus alter host immune responses to the entire oral biofilm (Hajishengallis et al. 2012).

**The oral microbiome in health and periodontitis**

It has been acknowledged that the equine oral microbiome has been a neglected field of research until recently (Dacre et al. 2008; Sykora et al. 2014) and the microbiome in equine oral health received little attention. Baker (1979) performed traditional bacterial culture and biochemical identification of orally healthy and periodontitis affected horses and recorded a significant bacterial population shift between oral health and periodontitis. High counts of *Streptococi* and *Micrococi* were detected in orally healthy samples with intermediate counts of *Veillonella* and low counts of *Lactobacillus spp.*, *Fusobacteria* spp. and coliforms. In periodontitis, the predominant genera present were *Streptococi*, *Fusobacteria* and coliforms. In addition, *Campylobacter* and spirochetes were detected upon direct smears of diseased samples (Baker 1979). In addition, Sykora et al. (2014), found *Porphyromonas gingivalis*, Tannerella and Treponema species were more commonly isolated from horses with periodontitis secondary to Equine Odontoclastic Tooth Resorption and Hypercementosis (EOTRH) than in orally healthy horses by using PCR assays of gingival crevicular fluid samples.

When analysing any microbiological community, the number and diversity of bacterial species detected is dependent on the method of analysis used (Lozupone and Knight 2008). Studies in other
species have estimated that around 50% of oral bacteria cannot be cultured by conventional means (Socransky et al. 1963). It is therefore most likely that previous culture studies have vastly underestimated the number and variety of bacterial species present in the equine oral cavity because novel species and uncultivable bacteria would have gone undetected. It is now possible to characterise the oral microbiome using methods which do not rely on culture and can detect novel and uncultivable bacteria. One such method involves high-throughput sequencing of the gene encoding the 16s sub-unit of the bacterial ribosome (16s rRNA) that is useful in assessing the composition of complex microbial communities directly from clinical samples (Song et al. 2013). The 16s RNA gene is universal in bacteria but not found in mammalian cells. It is around 1550 base pairs long and consists of nine hypervariable regions (V1-V9) which are between constant regions (Song et al. 2013). These constant regions are highly conserved between phyla, thought to be due to the critical importance of the ribosome to basic cell function (Clarridge 2004). Sequencing of hypervariable regions may allow for differentiation up to species level (Chakravoty et al. 2007). Most hypervariable regions occur within the first 500 bases (Keller et al. 2010) and so sequencing of the whole gene is not required, with read lengths of 500-700 base pairs being sufficient for identification at species level (Clarridge 2004; Paster et al. 2006; Song 2013). Whole 16s rRNA sequencing is desirable if a previously unknown species is identified (Clarridge 2004) and it is highly likely that the equine oral microbiome in both health and disease contains many novel and previously uncharacterised species as no prior studies have been published using this technique.

A recent high throughput 16s rRNA sequencing study performed by the authors (Kennedy et al. 2015) has revealed a population shift towards gram negative organisms as well as increasing numbers of spirochetes in horses with periodontal disease, as would be expected. Bacteria belonging to the Prevotella, Veillonella, Treponema and Tannerella genera were found to significantly increase in equine periodontitis lesions (Kennedy et al. 2015), with some species belonging to these genera being well known human periodontal pathogens (Sykora et al. 2014).
In addition to high throughput 16s rRNA sequencing to uncover the oral microbiome, the 16s rRNA gene can also be used as a target in PCR reactions, to screen samples for specific bacteria as used by Sykora et al. (2014).

5. Conclusion

Periodontal disease is a common and painful equine disease that is usually induced by the mechanical impaction of food between and around teeth. Further progression of the disease is very likely dependent on invasion of the periodontal tissues by the extremely complex oral bacteria along with the host’s immune response to these microorganisms, with a severe host inflammatory response resulting in increased tissue breakdown and progression of the disease. In addition, keystone oral bacterial pathogens may alter the host’s immune response to other components of the biofilm. Whilst it is clear that feed stasis and subsequent bacterial proliferation play an important role in the initiation and progression of periodontitis in the horse, there is a great need for further studies into the aetiopathogenesis of this disorder. Recent work has given an insight into which bacterial species are present in the periodontal pockets of horses with periodontitis however it is crucial to distinguish which species are important in disease pathogenesis and which simply flourish due to the change in oral environment. In particular, the interaction between bacteria of the diseased equine oral microbiome and the local immune system requires further investigation in order to provide additional insights into the aetiopathogenesis of equine periodontal disease.

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**Legends of Figures**

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Fig 10: Spirochaetal bacteria in gingival epithelium of a diseased equine periodontal pocket. Modified Young’s silver stain (bar = 10mcm) Image courtesy of Dr. A. Cox.

Fig 11: Cocci on the gingival epithelial surface of a periodontitis affected horse. Bar = 10mcm. Image courtesy of Dr. A. Cox.

Authors’ declaration of interest

No conflicts of interest have been declared

Ethical animal research

Ethical review not applicable for this review article.

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