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An epidemiological survey on the prevalence of equine peripheral dental caries in the United Kingdom and possible risk factors for its development

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The authors have no competing interests to declare

Ethical Animal Research

This study was approved by the R(D)SVS veterinary ethical review committee. Explicit owner informed consent for inclusion of animals in this study was not stated.

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Authorship

The overall study was designed by P.M. Dixon with contribution of R.J.M. Reardon, S. Smith and G. Maclachlan. Design of the dental chart and questionnaire was done by D. Borkent, P.M. Dixon and R.J.M. Reardon. Statistical data analysis and interpretation was performed by D. Borkent and R.J.M. Reardon. The draft version of the manuscript was made by D. Borkent and revision of the manuscript was done by all authors. All authors approved the final version.

Summary

Background: Equine peripheral caries (PC) is an increasingly recognised disorder that causes premature wear of teeth and dental fractures and thus has major welfare implications. Little information is available on its prevalence or severity in UK horses and there are no proven associations with any risk factors.

Objectives: To document the prevalence of PC over a wide area of the UK, assess its intra-oral distribution and severity in affected horses and examine for potential risk factors for its development.

Study design: cross sectional study

Methods: Experienced personnel were recruited for a UK-wide dental survey on their patients during dental examinations. Established guidelines were used for grading PC. Frequency of PC occurrence was compared between teeth and dental arcades, using McNemar's tests. Potential risk factors for PC were screened using univariable logistic regression prior to building a multivariable model.

Results: A total of 706 horses were examined by 25 participants, showing a 51.7% prevalence of PC (365/706). Some regional differences in prevalence were found. The PC primarily affected the cheek teeth with the 12 caudal being significantly more commonly and more severely affected than the 12

rostral cheek teeth. Most of the hypothesised risk factors including: age, breed, sex, time at pasture, and feeding of silage (haylage) were unproven. A limited association with moderate levels of concentrate feeding was observed. The presence of concurrent dental abnormalities were significantly associated with the likelihood of having PC.

Main limitations: Not all regions in UK were included and there may be inconsistencies between examiners.

Conclusions: Peripheral caries is common in British horses, primarily affecting the caudal cheek teeth. There was limited evidence of an association between feeding and PC. The association between PC and concurrent dental disorders indicates that these should be addressed in affected horses.

Introduction

Equine peripheral dental caries (PC) has been defined as destruction of the calcified dental tissue on non-occlusal aspects of the clinical crowns [1]. Limited numbers of studies have suggested that PC is a common equine disorder in the United Kingdom (UK) [2] and also that it may be increasing in prevalence in Sweden [3]. Alternatively, PC may be increasingly recognised due to improved awareness of this disorder, along with the more thorough oral examinations currently being performed by many equine veterinarians and dental technicians.

The limited gross post-mortem [3] and clinical [2] surveys on equine PC performed to date have both shown that the caudal cheek teeth are more commonly affected with PC, suggesting localisation of the caries-inducing, oral environmental changes in affected horses. Gross examination of equine teeth commonly underestimates the severity of PC in comparison to histological examination, and caries that appears grossly to only affect the cementum often involves the underlying enamel or even dentine on histological examination [4].

In contrast to PC, equine cheek tooth infundibular caries has been a well-recognised disorder for more than a century, with an age-related increase in prevalence [5]. Honma *et al* described grading for equine dental caries [5] and Dacre's modification of this system [6], remains the standard grading tool used to classify the severity of both infundibular and peripheral caries.

Equine PC is believed to be caused by acidogenic bacteria living in dental plaque [7], similar to the very well-studied dental caries of brachydont species. There is anecdotal evidence from Swedish post mortem studies that horses that are fed haylage (silage) as opposed to hay, and those that receive high levels of dietary concentrates are predisposed to PC [3]. Working donkeys in Mexico fed highly refined starch diet have also suffered severe PC of all teeth, including incisors [8].

The aims of this study were to determine the prevalence of equine PC over a wide area of the UK, in horses of different breeds, ages and workloads, and to document the severity and the intraoral

distribution of these lesions by examination of detailed dental records of horses made by experienced operators. The survey also aimed to examine for possible associations between the presence of PC with: diet (in particular with the feeding of haylage and concentrates); with the presence of concurrent dental disorders including infundibular caries and diastemata, and with the frequency of routine dental care.

Materials and methods

Selection of survey participants

Requests to participate in the survey were sent to: local Scottish and Northern England veterinary surgeons who refer to the authors' clinic and who were recognised to have a high level of expertise in equine dentistry; to veterinary surgeons with specialist equine dental knowledge in other parts of the UK and to members of the British Association of Equine Dental Technicians (BAEDT).

Questionnaire and dental chart design

Using the input of five European equine dentistry Diplomates, a dental chart and accompanying questionnaire were designed to record the presence, location and severity of PC and IC on individual teeth (supplementary information S1). Honma's grading system as modified by Dacre [6] was used to grade PC lesions (Fig. 1). The guidelines allowed participants to differentiate between the presence of discolouration of the periphery of the teeth of some horses that is regarded as innocuous and PC. A balance was struck between the length of the questionnaire that would provide maximum information on possible risk factors and the likelihood of decreasing compliance with a very long and complex questionnaire.

A recent UK post-mortem study showed that PC most severely affected the buccal aspects of the mandibular and the palatal aspects of the maxillary cheek teeth [9]. Consequently, participants were asked to grade PC on each cheek tooth at these sites only. Guidelines, including one with images of different grades of PC and IC were distributed to participants, along with a shorter laminated similar guide for field use (supplementary information S2-S4). Details of survey grading and recording systems were presented to BAEDT Annual Congress in 2015.

The questionnaire gathered general information including: breed, age, sex, type of work, postcode; the reason for the current dental examination and the presence of concurrent dental disorders; as well as detailed information on the amount and types of concentrates fed per day, type of forage fed and level of pasture access throughout the year. The survey was performed between 10th of February and 21st of September 2015. The participants were requested to grade consecutive dental examination cases without case selection, to minimise selection bias.

Evaluation of participants' caries grading

After completion of the survey, all participants received an email questionnaire in which they were asked to grade images of different grades of PC-affected and healthy teeth in order to confirm their diagnostic ability.

Potential risk factors

Fourteen potential risk factors were chosen from *a-priori* hypotheses and the literature on dental caries: The presence of concurrent infundibular caries; Concurrent dental disorders other than infundibular caries (including: diastema/periodontal disease, periodontal disease not associated with diastema, dental overgrowths, cheek teeth fractures, "other dental" disorders); Breed (categorised as Warmblood types, Thoroughbred types, Saddlebred horses, Coldbloods, Arabian horses or ponies); Sex; Age; Work type (categorised as recreational, sports/competition or racing use); Location of horse (post code); Fluoridation of drinking water; Type and amounts of concentrates fed; Type of forage fed; Duration of pasture access (mean hours per day over a full year); and Additional treats/supplements fed.

The feeding of concentrates was hypothesised to be an important potential risk factor, so this variable was further scrutinised: Concentrate feeding was divided into two subcategories: pelleted food or nuts (assessed as 1.5 kg per standard 2.5 litre scoop) or loose mix/grain (assessed at 1 kg/scoop). If there was a seasonal difference in concentrate feeding, the number of scoops per day/per season were recorded.

Data analysis

The survey data were collated in Excel. Descriptive analysis, including graphical representation of the data was then performed using R software (version 3.1.2) [10]. For continuous data, a summary of the data was produced containing minimum, maximum, mean and median values. Plots with error bars were created providing an overview of possible association of prevalence of PC with the continuous or categorical variables.

The outcome “presence of peripheral caries” was defined as peripheral caries “yes” (Dacre grade ≥ 1.1 [6]) or “no” (Dacre grade 0[6]). The frequency of presence of peripheral caries was compared between the 12 rostral cheek teeth (Triadan 06-08) and the 12 caudal cheek teeth (Triadan 09-11); and between the mandibular and maxillary arcades, using McNemar’s tests.

The log odds of the outcome versus each continuous variable were examined graphically. If the relationship was nonlinear, categorical or alternative: binary, polytomous categorical (quartiles or quintiles) or quadratic and cubic terms were considered in the univariable and multivariable model to find the ‘best fit’ for the model [11]. Nominal and ordinal categorical variables were numerically coded sequentially, with a 0 being assigned to the reference group. Univariable logistic regression was used to assess the relationships between the potential risk factors and the outcome. Variables with P-values < 0.2 , as well as any considered biologically plausible and any that had been reported as being significant in other studies, were considered for inclusion in the multivariable model. Variables were ordered by Akaike Information Criteria and log likelihood values prior to sequential insertion into a single level multivariable regression model. Variables were retained in the multivariable model if P values were ≤ 0.05 . The Wald test P value was used when comparing categories with the reference category.

Potential confounders were evaluated by resubmitting all of the variables from the univariable analyses that were not included in the final model after the forward stepwise process of model building. The effect of each potential confounder on the estimates for variables in the final model was assessed by adding each one, one at a time into the final model. If addition of the potentially confounding variable altered odds ratios for variables in the final model by more than 20% [11], confounding was considered to be present, the confounder was retained in the final model and adjusted odds ratios were reported for

variables in the final model. Correlation coefficients were produced between all quantitative variables in the final model. Variables with correlation coefficients of >0.4 and <-0.4 were further examined by investigating the effect of removing them individually from the model. The fit of the final multivariable model was assessed using the Hosmer-Lemeshow goodness-of-fit test [12]. The predictive ability of the model was determined by generating a receiver operating characteristic (ROC) curve. All data analyses were performed using R software (version 3.1.2).

Results

Participants and horses

Twenty-five participants took part in the survey including: nine veterinarians who referred to the authors' clinic and 10 veterinarians and six BAEDT members who worked in England and Wales. Completed questionnaires and dental charts from 706 different horses were returned. The caries grading test following completion of the survey showed that all participants could differentiate PC (grade 1.1 or higher) from no caries (grade 0) and also differentiate between normal cheek teeth infundibula and grade 1 or higher infundibular caries.

The mean age of horses in this study was 12.1 years (range 3 - 38 years) that was similar to the mean age of horses with PC (12.0 [range 3-30 years]). Sex was recorded in 673/706 horses and included 35% (236/673) females and 65% (437/673) males (63% geldings [423/673] and 2% [14/673] stallions). Horses were classified as Warmblood types (n=366), ponies (n=183), Thoroughbred types (n=111), Arabian horses (n=14), Coldbloods (n=9) and Saddlebred horses (n=1).

Details of geographical location (postcode) were available for 699 of the 706 horses and their distribution are shown graphically in Fig. 2. No address was recorded for 5 horses and 47 horses lived in areas where mains water was partially fluoridated and so these 52 horses were excluded from this analysis. Of the 654 remaining horses, a prevalence of 51% PC (321/627) was found in horses currently residing in areas where water was not fluoridated whilst 48% of the remaining limited number of cases (13/27) lived in areas with fluoridated water had PC.

Prevalence and oral distribution of peripheral caries

PC was present in 51.7% (365/706) of the horses, 23.2% (164/706) horses had PC only; and 28.5% (201/706) had both infundibular and PC. PC primarily affected the cheek teeth, with only 6 incisors and no 1st premolar ("wolf") or canine teeth affected. PC was bilateral in 88.5% (n=323/365) of affected horses and unilateral in 11.5% (42/365). The median of the maximum grade of PC per horse was 1.1 (range 0- 4). The 12 caudal cheek teeth (Triadan 09-11) were significantly more commonly (odds ratio (OR) 9.38, 95% confidence interval (C.I.) 6.0-15.5, $P<0.001$) affected by PC than the 12 rostral cheek teeth (Triadan 06-08) (Fig. 3). The mandibular cheek teeth were significantly more commonly (OR 3.0, 95% C.I. 2.2-4.1, $P<0.001$) affected by PC than the maxillary cheek teeth.

Risk factors for caries

Of the 14 variables screened at the univariable level, 9 were taken forward for consideration in the multivariable forward stepwise analysis. The results of univariable logistic regression analyses, including some examples of variable categorisation are shown in supplementary information table S5. The final multivariable model of factors which increased likelihood of PC (is presented in Table 1. The presence of infundibular caries increased the risk of PC compared to no concurrent dental disorder.. Horses with diastemata and horses with multiple dental disorders other than infundibular caries (combination of dental fracture / diastema / overgrowths / "other" dental disorder) were also significantly more likely to have PC than horses without any dental disorder. The prevalence of PC varied between regions of the UK, with the highest prevalence observed in South East England and the lowest observed in the Midlands (Table 1). The likelihood of having PC was significantly higher in South East England and South West England than in Scotland (Table 1). The frequency of PC varied with amounts of concentrate fed, but the association was not linear (supplementary information S6). Only the group of horses fed between 2.1 and 3.0 kg concentrates per day had increased risk of PC compared to horses fed no concentrates (Table 1).

The outcome of the Hosmer-Lemeshow goodness-of-fit test was 7.92 (8 degrees of freedom, $P = 0.44$) showing that the model fits the data well. The area under the ROC curve was 0.70, showing that the model has a fair predictive ability.

Discussion

The prevalence of PC (51.7%) found in this survey is much higher than the prevalence of 0.3% found in an Irish abattoir survey [13]; of 0.9% found in two Swedish post-mortem dental surveys [14,15] and of 6.1% in a 2010 Swedish post mortem study [3]. A recent clinical study of PC prevalence in donkeys in Portugal and Spain also reported a low prevalence (5.9 %, 47/800) [16]. The lower prevalences of PC in post mortem studies is unexpected because such examinations allow a more detailed examination of all teeth than clinical examinations. In contrast, a PC prevalence of 69.4% (75/108 horses) was recorded in a recent UK clinical study on referred cases [2], which was higher than in the current study (51.7% PC). This difference could be explained by the use of an oral endoscope in the former study, which allows a more thorough oral examination than the current study, where most participants used a dental mirror and headlight to examine teeth. Additionally, tooth surface palpation can be used to differentiate between discoloration (smooth) and low grade peripheral caries (irregular/rough) and was performed by many of the examiners in this study. Additionally, the horses in the former study were referred dental cases that would be expected to have a higher prevalence of dental disorders than the horses in the current study that were mainly examined during routine dental examinations, even though the examiners were specifically looking for this disorder. Overall, our results suggest that there is a high prevalence of PC in the UK that may be related to the relatively recent recognition and current increased awareness of this disorder making many clinicians very adept at detecting it. For the very opposite reasons, a survey of 400 horses in a UK clinic in the 1990s did not record the presence of PC in any horse [17]. This survey relied on the ability of participants to correctly identify the presence of PC, and a post-survey test confirmed that they could correctly identify PC.

The most common and severely affected teeth, were the 12 caudal cheek teeth (Triadan 09-11) as previously recorded [3], with mandibular being affected more often than maxillary cheek teeth. In the current study the mean age (12 years) of the whole population, and horses with PC was older than the PC affected horses (mean 8.1 years) in a previous study in which it was proposed that high levels of concentrates and haylage were risk factors for PC [3]. The presence, site and grade of infundibular caries was also recorded in this study and although both disorders were concurrently present in 28.5% of horses, these two forms of equine caries appear to be separate disorders. PC preferentially affects the caudal upper and lower cheek teeth indicating an environmental change in the caudal oral cavity that favours the growth of cariogenic bacteria. In contrast, infundibular caries can only affect the maxillary cheek teeth and most evidence would suggest the primary problem to be defects in cemental filling of certain cheek teeth, especially the Triadan 09s [18-22]. Nevertheless, changes in the oral environment also likely play a role and infundibular caries was positively associated with PC. Age was not associated with PC prevalence were found in the current study, which is in contrast to infundibular caries where very distinct age-related increase in prevalence occurs [3, 5, 16, 23-24].

Concurrent dental disorders, other than infundibular caries, were associated with the likelihood of PC, similar to observations in a study of donkeys, in which it was hypothesised that impaired food movement creates an acidogenic bacterial environment in the mouth [8, 16]. A significant positive association between the presence of diastemata and PC was also found in a Swedish post-mortem study [3]. In contrast, Ramzan and Palmer (2011) found no association between presence of diastemata and of PC at either tooth or patient level [2]. By removing interproximal cementum, PC could theoretically create or predispose to diastemata and PC may also play a role in the development of periodontal disease by interrupting the tight connection between cement and junctional epithelium [6]. Conversely, diastemata and periodontal disease, may change the local oral environment, creating a dysbiosis (microbial imbalance) favouring more acidogenic and aciduric micro-organisms which may have a role to the development of PC [7].

We observed regional differences in PC prevalence in the UK which could be due to geological, inter-observer or inter-horse variation in these regions, although the observed associations between regions and likelihood of PC did not vary significantly when additional information, such as horse breed and use

were included in the model. Fluoridation results in significant improvement in oral health in people [25-26], however, in the current study there were insufficient horses from areas with fluoridated water to make the comparison worthwhile. Some relationship between the prevalence of PC and the level of concentrates feeding was shown. There was no linear trend between amount of concentrates fed and likelihood of PC. It is possible that the lack of significance in all groups is related to the sample size, but the association between feeding of concentrates and likelihood of PC was not as strong as expected. The feeding of haylage has been hypothesised to be a risk factor for PC [3], and may be a contributor to the increasing recognition of PC over the past three decades that has coincided with increased use of haylage instead of hay. However, we found no significant difference in prevalence of PC between horses fed haylage and those fed hay or chaff. No relationship was found between PC prevalence and mean time spent on pasture throughout the year. It is not known if natural diet might be protective against caries or alternatively, that there would be an increase in PC prevalence because of the presence of fructans, a fermentable carbohydrate in grass. However, this was not examined here and no horses in this survey were permanently at pasture without supplementary feeding.

The model fit was good and predictive ability was fair. The area under the ROC curve measures the discrimination of the test. With considerable numbers of PC-affected horses in every risk-factor subgroup. Consequently, it is more difficult to predict which horses would or would not have PC than would be the case if there were clear differences in the prevalence of PC between the subgroups. The model shows that certain risk factors are associated with PC but this does not mean that horses without these risk factors cannot have PC.

In conclusion, PC is a common dental disorder in horses the UK, predominantly affecting the caudal cheek teeth. In contrast to expectations, no major link with diet was observed. Positive associations were found between the presence of PC and infundibular caries; cheek teeth diastemata and the presence multiple dental disorders. Some regional variations in PC prevalence were found. Further epidemiological, microbiological and pathological studies are needed to examine the aetiopathogenesis of this common disorder.

Table 1. Multivariable model showing variables significantly associated with the likelihood of having peripheral caries for horses in the UK

Variable	Odd Ratio (95% C.I.)	P-value	Total (n=656)	Prevalence PC (%)	Number with PC
<i>Infundibular Cares</i>		< 0.001			
No	referrent		363	43.8	159
Yes	1.89 (1.32 - 2.71)	<i>< 0.001</i>	293	59.7	175
<i>Concurrent dental disorder other than IC</i>		<0.001			
No	referrent		199	42.2	84
Dental fracture	8 (0.94 - 68.34)	<i>0.06</i>	8	87.5	7
Diastema/Peridontal Disease	2.72 (1.18 - 6.27)	<i>0.02</i>	37	75.7	28
Multiple	2.42 (1.54,3.82)	<i>< 0.001</i>	164	67.1	110
Other	0.97 (0.47,2)	<i>0.9</i>	39	46.2	18
Overgrowths	0.9 (0.58,1.38)	<i>0.6</i>	209	41.6	87
<i>Region</i>		0.004			
Scotland	referrent		158	47.5	75
Midlands	0.62 (0.28,1.41)	<i>0.3</i>	35	34.3	12
North England	1.3 (0.86,1.98)	<i>0.2</i>	296	49.0	145
South East England	4.14 (1.63,10.52)	<i>0.003</i>	36	80.6	29
South West England	2.04 (1.13,3.68)	<i>0.02</i>	90	56.7	51
Wales	1.62 (0.76,3.43)	<i>0.2</i>	41	53.7	22
<i>Concentrates (kg/day)</i>		0.07			
0	referrent		130	43.1	56
0.1 to 2	1.1 (0.72,1.68)	<i>0.7</i>	379	49.1	186
2.1 to 3	1.95 (1.11,3.42)	<i>0.02</i>	114	65.8	75
3+	0.95 (0.41,2.19)	<i>0.9</i>	33	51.5	17

Key: C.I.=confidence interval; PC=Peripheral caries; P-values in bold are from the likelihood ratio test, while those in italics are from the wald test.

Table and figure legends

Fig 1: Examples of different grades of peripheral caries. Grade 0 - no caries; Grade 1.1 - pitting or partial loss of peripheral cementum; Grade 1.2 – total loss of peripheral cementum: Grade 2 - also involves enamel; Grade 3 - also involves dentine; Grade 4 – loss of integrity of tooth.

315

316 Fig. 2: Map of the United Kingdom with red dots marking the location of 699 of the 706 horses examined
 317 in this survey.

318

319 Fig 3: Barplot showing frequency of peripheral caries grades from the buccal and palatal aspects of the
 320 mandibular and maxillary arcades respectively (mean of left and right sided values) (after Dacre 2005),

321 subdivided by tooth (Triadan) and maxillary or mandibular position. max = maxillary cheek tooth; mand
322 = mandibular cheek tooth.

323
324 Table 1. Results of multivariable logistic regression model investigating risk factors for PC in horses in
325 the UK

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Supporting information

Item S1 Dental chart and questionnaire

Item S2 Guidelines for grading caries

Item S3 Grading system peripheral caries: guidelines with images

Item S4 Field guide for grading peripheral caries

Table S5 Univariable logistic regression model investigating risk factors for peripheral caries in horses in the UK

Figure S6 Log odds of peripheral caries (PC) categorised by concentrates (kg/day)