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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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30

31 **Authorship**

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

37 **Summary**

38

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

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

45 **Study design:** cross sectional study

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

50 **Results:** A total of 706 horses were examined by 25 participants, showing a 51.7% prevalence of PC
51 (365/706). Some regional differences in prevalence were found. The PC primarily affected the cheek
52 teeth with the 12 caudal being significantly more commonly and more severely affected than the 12

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

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

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

62

63 **Introduction**

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

70

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

77

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

82

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

88

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

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

96

97 **Materials and methods**

98 *Selection of survey participants*

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

103 *Questionnaire and dental chart design*

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

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

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

124 **Evaluation of participants' caries grading**

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

128

129 **Potential risk factors**

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

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

143 **Data analysis**

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

149 The outcome “presence of peripheral caries” was defined as peripheral caries “yes” (Dacre grade ≥ 1.1
150 [6]) or “no” (Dacre grade 0[6]). The frequency of presence of peripheral caries was compared between
151 the 12 rostral cheek teeth (Triadan 06-08) and the 12 caudal cheek teeth (Triadan 09-11); and between
152 the mandibular and maxillary arcades, using McNemar’s tests.

153

154 The log odds of the outcome versus each continuous variable were examined graphically. If the
155 relationship was nonlinear, categorical or alternative: binary, polytomous categorical (quartiles or
156 quintiles) or quadratic and cubic terms were considered in the univariable and multivariable model to
157 find the ‘best fit’ for the model [11]. Nominal and ordinal categorical variables were numerically coded
158 sequentially, with a 0 being assigned to the reference group. Univariable logistic regression was used
159 to assess the relationships between the potential risk factors and the outcome. Variables with P-values
160 < 0.2 , as well as any considered biologically plausible and any that had been reported as being
161 significant in other studies, were considered for inclusion in the multivariable model. Variables were
162 ordered by Akaike Information Criteria and log likelihood values prior to sequential insertion into a single
163 level multivariable regression model. Variables were retained in the multivariable model if P values were
164 ≤ 0.05 . The Wald test P value was used when comparing categories with the reference category.

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

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

177 **Results**

178 Participants and horses

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

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

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

196

197 *Prevalence and oral distribution of peripheral caries*

198 PC was present in 51.7% (365/706) of the horses, 23.2% (164/706) horses had PC only; and 28.5%
199 (201/706) had both infundibular and PC. PC primarily affected the cheek teeth, with only 6 incisors and
200 no 1st premolar (“wolf”) or canine teeth affected. PC was bilateral in 88.5% (n=323/365) of affected
201 horses and unilateral in 11.5% (42/365). The median of the maximum grade of PC per horse was 1.1
202 (range 0- 4). The 12 caudal cheek teeth (Triadan 09-11) were significantly more commonly (odds ratio
203 (OR) 9.38, 95% confidence interval (C.I.) 6.0-15.5, P<0.001) affected by PC than the 12 rostral cheek
204 teeth (Triadan 06-08) (Fig. 3). The mandibular cheek teeth were significantly more commonly (OR 3.0,
205 95% C.I. 2.2-4.1, P<0.001) affected by PC than the maxillary cheek teeth.

206

207 *Risk factors for caries*

208 Of the 14 variables screened at the univariable level, 9 were taken forward for consideration in the
209 multivariable forward stepwise analysis. The results of univariable logistic regression analyses,
210 including some examples of variable categorisation are shown in supplementary information table S5.
211 The final multivariable model of factors which increased likelihood of PC (is presented in Table 1. The
212 presence of infundibular caries increased the risk of PC compared to no concurrent dental disorder..
213 Horses with diastemata and horses with multiple dental disorders other than infundibular caries
214 (combination of dental fracture / diastema / overgrowths / “other” dental disorder) were also significantly
215 more likely to have PC than horses without any dental disorder. The prevalence of PC varied between
216 regions of the UK, with the highest prevalence observed in South East England and the lowest observed
217 in the Midlands (Table 1). The likelihood of having PC was significantly higher in South East England
218 and South West England than in Scotland (Table 1).

219 The frequency of PC varied with amounts of concentrate fed, but the association was not linear
220 (supplementary information S6). Only the group of horses fed between 2.1 and 3.0 kg concentrates per
221 day had increased risk of PC compared to horses fed no concentrates (Table 1).

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

225

226 **Discussion**

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

248

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

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

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

277 were included in the model. Fluoridation results in significant improvement in oral health in people [25-
278 26], however, in the current study there were insufficient horses from areas with fluoridated water to
279 make the comparison worthwhile. Some relationship between the prevalence of PC and the level of
280 concentrates feeding was shown. There was no linear trend between amount of concentrates fed and
281 likelihood of PC. It is possible that the lack of significance in all groups is related to the sample size, but
282 the association between feeding of concentrates and likelihood of PC was not as strong as expected.
283 The feeding of haylage has been hypothesised to be a risk factor for PC [3], and may be a contributor
284 to the increasing recognition of PC over the past three decades that has coincided with increased use
285 of haylage instead of hay. However, we found no significant difference in prevalence of PC between
286 horses fed haylage and those fed hay or chaff. No relationship was found between PC prevalence and
287 mean time spent on pasture throughout the year. It is not known if natural diet might be protective
288 against caries or alternatively, that there would be an increase in PC prevalence because of the
289 presence of fructans, a fermentable carbohydrate in grass. However, this was not examined here and
290 no horses in this survey were permanently at pasture without supplementary feeding.

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

297

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

304

305 Table 1. Multivariable model showing variables significantly associated with the likelihood of having
 306 peripheral caries for horses in the UK
 307

Variable	Odd Ratio (95% C.I.)	P-value	Total (n=656)	Prevalence PC (%)	Number with PC
Infundibular Cares		< 0.001			
No	referrent		363	43.8	159
Yes	1.89 (1.32 - 2.71)	<i>< 0.001</i>	293	59.7	175
Concurrent dental disorder other than IC		<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
Region		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
Concentrates (kg/day)		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

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

310

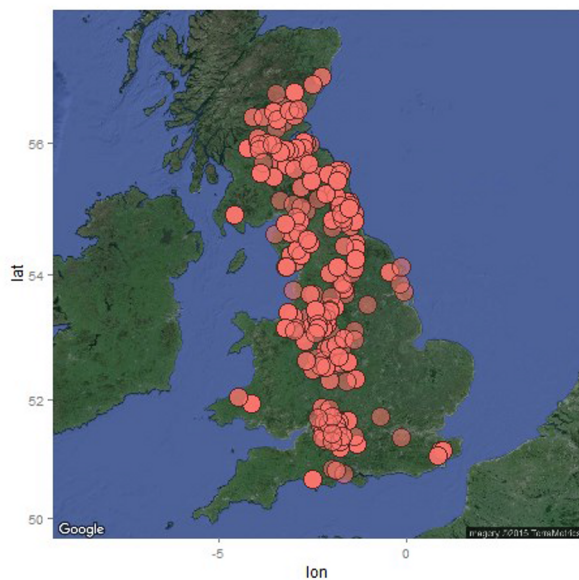
311 **Table and figure legends**

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



315

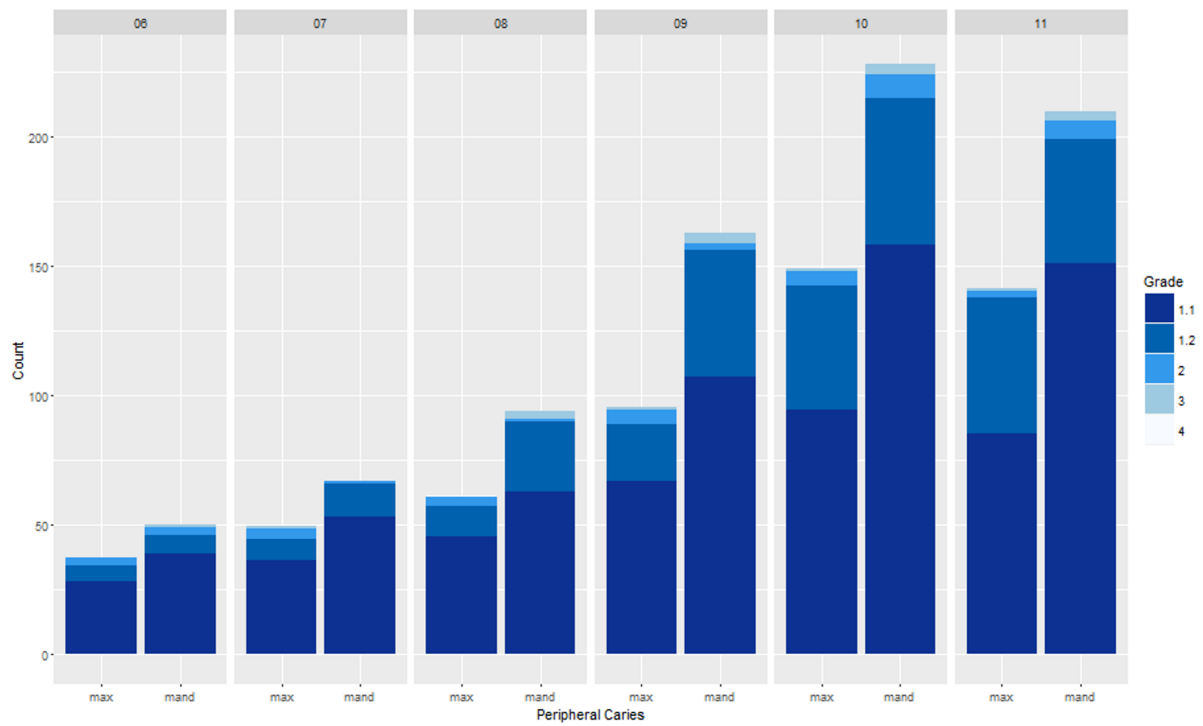
316 Fig. 2: Map of the United Kingdom with red dots marking the location of 699 of the 706 horses examined
 317 in this survey.



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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391 **Supporting information**

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393 **Item S1** Dental chart and questionnaire

394 **Item S2** Guidelines for grading caries

395 **Item S3** Grading system peripheral caries: guidelines with images

396 **Item S4** Field guide for grading peripheral caries

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

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

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