Research Article

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DOI: 10.4172/2574-2868.100013

Journal of Veterinary Medicine and Surgery
ISSN 2574-2868

2017
Vol. 1 No. 3: 13

Lack of Association between Exposure to Natural Sunlight and Dental Disease in French Companion Rabbits

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Citation: Thilliez N, Larrat S, Vergneau-Grosset C. (2017) Lack of Association between Exposure to Natural Sunlight and Dental Disease in French Companion Rabbits. J Vet Med Surg. Vol. 1 No. 3: 13.

Abstract

Objective: Dental disease is common in rabbits, with a reported prevalence of 12-38%. Hypovitaminosis D has been suggested as a possible causal factor. This case-control study aimed at determining whether companion rabbits with access to natural sunlight, and thus UVB, had a lower risk of developing dental disease.

Methods: A web-based questionnaire was sent to owners to gather data regarding dental health and husbandry of their companion rabbits. Three dependent variables were examined: presence of any dental disease, current or previous incisors malocclusion, and presence of cheek teeth malocclusion. The variable of interest was the frequency of access to an outdoor enclosure as reported by the owner. A causal diagram was used to select potential confounding variables. Variables were checked for collinearity and data was analysed through logistic regressions.

Results: The owners of 527 rabbits fully answered the survey. The presence of dental disease, incisor and cheek teeth malocclusions were not significantly associated with the frequency of access to an outdoor enclosure (all P-values>0.05). There was a statistically significant association between the presence of dental disease or cheek teeth malocclusion and age (P-values<0.001). The weight was not significantly associated with the presence of dental disease or incisors malocclusion (P-value>0.05).

Conclusion: These results suggest that exposure to natural sunlight, as currently performed in rabbits under French latitudes, does not decrease the odds of dental disease. Further studies are warranted to document the role of vitamin D in dental disease and, if indicated, to determine adapted schedules of UVB exposure.

Keywords: Rabbit; *Oryctolagus cuniculus*; Dental disease; Incisive malocclusion; Cheek teeth malocclusion; UVB; Hypovitaminosis D

Received: July 05, 2017; **Accepted:** July 12, 2017; **Published:** August 17, 2017

Introduction

Companion rabbits are very common in the United Kingdom and in the United States of America with an estimated population of 1.2 million and 3.2 million rabbits respectively [1-3]. Knowledge of their specific physiology and diseases is thus becoming relevant for the small animal veterinarians. In contrast to domestic carnivores, rabbits display aradicular hypsodont teeth [4,5]. Cheek teeth and incisors are continuously erupting [5,6]. Teeth length is balanced by attrition and abrasion during mastication [5,7]. The precise mechanisms regulating this balanced state are still debated [7]. However dental disease is undoubtedly among the most common conditions of companion rabbits, with a reported prevalence ranging from 12.2% to 38.1% [8,9]. Possible causes of dental disease include metabolic bone disease associated with calcium and/or vitamin D deficiencies, vitamin D excess, genetic or congenital factors, trauma, iatrogenic malocclusion and insufficient dental wear because of a lack of abrasive food [5,7,8,10-14].

Rickets has been documented in rabbits [15,16] supporting a contribution of vitamin D. Moreover, rickets is also known to cause dental problems in humans [17]. In support with the hypovitaminosis D hypothesis, advanced dental diseases have been documented in rabbits kept in hutch but not in those having access to natural sunlight [18]. In laboratory rabbits kept indoors, there is some controversy about the occurrence of dental disease [19-21]. Hypovitaminosis D can be prevented in rabbits by exposure to UVB radiations [16,22] including exposure to sunlight [23,24]. Thus, exposure to UVB could potentially have a beneficial effect on dental health in companion rabbits. The objective of this study was to determine whether companion rabbits with an access to outdoor enclosures, and exposure to UVB were less likely to develop dental disease.

Materials and Methods

A causal diagram listing possible causes of dental disease was designed based on the method described by Dohoo [25]. A web-based questionnaire (Google Form) designed to be answered by rabbit owners was created to investigate potential contributing factors to dental disease. The questionnaire was sent to rabbit owners through the rabbit-hobbyists association Marguerite et cie The questionnaire contained sections regarding patient signalment (including age, sex, and weight), husbandry, feeding habits and potential dental disease. Rabbits with dental disease were defined as animals that needed dental trimming at least once or that had undergone incisor extraction. Three dependent variables were examined: 1) the presence of any dental disease, 2) current or previous incisors malocclusion, and 3) the presence of cheek teeth malocclusion. The variable of interest was the frequency of access to an outdoor enclosure recorded as: no outdoor access, access less than once a week on average, access more than once a week on average.

The causal diagram was used to select potential confounding variables [25]. Collinearity between variables was assessed. In case of severe collinearity, one of the two collinear variables was selected for the model. The final logistic regression models included the dependent variable, independent variables and the confounding factors. The presence of outliers and influential values was evaluated by examination of the deviance and Pearson residuals, and dfBetas. Data points modifying the estimates by 20% or more were considered for removal. Linearity between continuous variables and predicted log-odds was assessed visually. Goodness of fit was assessed with the Hosmer and Lesmeshow test. The predictive value of the model was evaluated with a ROC curve. P-values under 0.05 were considered statistically significant.

Results

Data regarding 535 rabbits was collected with the web-based questionnaire. Among these, 527 had precise information about incisors and cheek teeth diseases. The median age in the study population was 4 (range: 0-10). The median weight was 1.8 kg (range: 0.54-5.9 kg). Females represented 42.9% of the rabbits. The studied population was composed of 81.4% sterilized rabbits; females were neutered significantly more often (87.2% of females versus 77% of males, P=0.002 [Chi^2]). Rabbits without access to outdoor enclosures, with access to outdoor enclosures less than once a week on average, and with access to outdoor enclosures more than once a week on average represented 24.2%, 36.9% and 38.8% of the sample, respectively. The prevalence of dental disease was 33.8% (95% confidence interval: 30-37.9%). The prevalence of incisor and cheek teeth diseases were 11% (8.6-14%) and 27.1% (23.5-31.1%) respectively.

The age, weight and acceptance of hay were identified as confounding factors based on the causal diagram. Incisive and cheek teeth disorders were also included as confounding factors for each other. Acceptance of hay was strongly associated with age in our sample (Mann-Whitney-Wilcoxon test, P<0.001), therefore the factor "acceptance of hay" was excluded from the final models. The presence of cheek teeth disease was strongly associated with age, and age was removed from the model investigating incisors disorders.

The presence of dental disease was not significantly associated with the frequency of access to an outdoor enclosure (**Table 1,** logistic regression, access to outdoor less than once a week: P-value=0.61; access to outdoor more than once a week: P-value=0.09). There was a statistically significant association between the presence of dental disease and age (Odds ratio: 1.29; 95% Confidence Interval: 1.19-1.41; P-value<0.001). Rabbit weight was not significantly linked with the presence of the dental disease (P-value=0.16). The goodness of fit of the model was acceptable (Hosmer and Lemeshow test, P=0.2). The area under the curve of the ROC curve was 0.68, indicating a poor to moderate predictive value of the model.

The presence of incisors disease was not significantly associated with the frequency of access to an outdoor enclosure (**Table 2,** logistic regression, access to outdoor less than once a week: P-value=0.73; access to outdoor more than once a week: P-value=0.99). There was a statistically significant association between the presence of cheek teeth disease and incisors disease (Odds ratio=2.71; 95% Confidence Interval: 1.52-4.81; P-value<0.001). The weight was not statistically associated to the presence of incisors disease (P-value=0.14). The goodness of fit of the model was acceptable (Hosmer and Lemeshow test, P=0.37). The area under the curve of the ROC curve was 0.62, indicating a poor to moderate predictive value of the model.

The presence of cheek teeth disease was not significantly associated with the frequency of access to an outdoor enclosure (**Table 3,** logistic regression, access to outdoor less than once a week: P-value=0.78; access to outdoor more than once a week: P-value=0.13). There was a statistically significant association between the presence of cheek teeth disease and age (Odds

Table 1 Results of logistic regression of the presence of dental disease in relation to outdoor access, age and weight of rabbits (n=535). no outliers were removed from the analysis, goodness of fit: Hosmer and Lemeshow test: P=0.2, area under the curve of the ROC curve: 0.68=poor to moderate predictive value.

	Odds ratio (95 % Confidence Interval)	P-value
No access to outdoor	Reference	-
Access to outdoor less than once a week	0.88 (0.54-1.45)	0.61
Access to outdoor more than once a week	0.65 (0.39-1.07)	0.09
Age	1.29 (1.19-1.41)	<0.001
Weight	0.83 (0.64-1.07)	0.16

Table 2 Results of logistic regression of the presence of incisors disease in relation to outdoor access, presence of cheek teeth disease, and weight of rabbits (n=527). No outliers were removed from the analysis, goodness of fit: Hosmer and Lemeshow test: P=0.37, area under the curve of the ROC curve: 0.62=poor to moderate predictive value.

	Odds ratio (95 % Confidence Interval)	P-value
No access to outdoor	Reference	-
Access to outdoor less than once a week	1.14 (0.55-2.44)	0.73
Access to outdoor more than once a week	1 (0.48-2.17)	0.99
Presence of cheek teeth disease	2.71 (1.52-4.81)	<0.001
Weight	1.28 (0.91-1.77)	0.14

Table 3 Results of logistic regression of the presence of cheek teeth disease in relation to the outdoor access, presence of incisors disease and age of rabbits (n=527). Overdispersion was detected (Hosmer and Lemeshow test, P=0.04) and corrected with the removal of the confouding factor with the highest P-value, the weight, from the analysis. Goodness of fit: Hosmer and Lemeshow test: p=0.39, no outliers were removed from the analysis, area under the curve of the ROC curve: 0.71=moderate predictive value.

	Odds ratio (95 % Confidence Interval)	P-value
No access to outdoor	Reference	-
Access to outdoor less than once a week	0.93 (0.54-1.59)	0.78
Access to outdoor more than once a week	0.66 (0.39-1.14)	0.13
Presence of incisors disease	2.32 (1.26-4.24)	0.006
Age	1.33 (1.22-1.46)	<0.001

ratio: 1.33; 95% Confidence Interval: 1.22-1.46; P-value<0.001). The presence of cheek teeth disease was significantly associated with the presence of incisors disease (Odds ration=2.32; 95% Confidence Interval: 1.26-4.24; P-value=0.006). The goodness of fit of the model was acceptable (Hosmer and Lemeshow test, P=0.39) after removing the confounding factor with the highest P-value: the weight (Before removing the weight: Hosmer and Lemeshow test P=0.04). The area under the curve of the ROC curve was 0.71, indicating a moderate predictive value of the model.

Discussion

The present case-control study highlighted that the frequency of access to an outdoor enclosure is not significantly associated with the presence of dental disease (all P-values>0.05). There was a statistically significant association between the presence of dental disease or cheek teeth malocclusion and age (P-values<0.001). Many authors have suggested that exposure to UVB could decrease the risk of dental disease in companion rabbits [18,26,27]. The results of the current survey contrast with this hypothesis since rabbits given access to outdoor enclosures, and to natural UVB radiation did not have a lower risk of dental disease than rabbits kept indoors. There was no significant association between access to outdoor enclosures and the presence of incisors or cheek teeth diseases either.

First, it is possible that companion rabbits in our study received an appropriate amount of vitamin D in their diet. Most (80%) had access to pellets containing vitamin D. In such case, exposure to UVB radiations would not have any additional impact on vitamin D blood levels and calcium metabolism. This would also mean that hypovitaminosis D may not cause dental disease in our sample. Of note, the prevalence of dental disease observed in the present study was similar to those described in other populations [8,9]. Although the brand and approximative amount of pellets offered to rabbits were recorded, calculating the amount of dietary vitamin D eaten by rabbits was beyond the scope of the present study. Variations in nutritional value of hay [28-30] and the selective feeding habits of rabbits [31] would have resulted in too much imprecision regarding the input of dietary vitamin D.

Conversely, exposure to UVB might be insufficient in all three groups of rabbits studied here. The duration of exposure to synthesize enough vitamin D from a given light irradiance is known in humans, but not in rabbits [32,33]. Crepuscular or nocturnal species, such as rabbits, are expected to be very efficient at vitamin D photoconversion, i.e., a short exposure time provides a sufficient amount of vitamin D [34,35]. Even if exposure to UVB is sufficient during summer, it might still be insufficient in winter because of inclement weather and shorter daytime. A seasonal decrease in vitamin D might be sufficient to cause dental disease even in the rabbits offered the most frequent access to sunlight.

Latitude might also have an impact on vitamin D synthesis, as seen in tortoises [36,37]. The same phenomenon could take place in the rabbits of our study; however, France is in the natural geographic range of rabbits (*O. cuniculi*). Further prospective studies documenting vitamin D blood levels in companion rabbits in relation to their diet, season, and exposure to UVB would be warranted to document these hypotheses.

In most mammal species, vitamin D is responsible for calcium homoeostasis, in part through the regulation of the absorption of calcium in the intestine [38]. In contrast, rabbits absorb calcium in their intestine independently from vitamin D concentration, passively [38-44]. Rabbits maintain normocalcemia through urinary excretion of the calcium in excess [44]. The active digestive absorption of calcium, which requires vitamin D, is only used when dietary calcium is low [42,44]. The hypovitaminosis D hypothesis relies on the fact that vitamin D is also involved in normal bone mineralisation [38,45]. Rabbits fed a vitamin D-deficient diet show an inadequate skeletal mineralization and signs of osteomalacia. These lesions are probably caused by hypophosphatemia rather than hypocalcemia [42]. The resulting osteopenia is suspected to weaken bones, especially those surrounding the teeth, resulting in an elongation of reserve crowns and other abnormalities [5,46,47]. However, the impact of hypovitaminosis D on dental health remains to be formally established in rabbits. A dependence of dental health on normocalcemia rather than vitamin D levels might also explain our results.

Age has not been formally identified as a risk factor for dental disease by previous studies while it was a significant factor in the present study with older rabbits having a significantly higher risk of dental disease. In the absence of standardized clinical examination and medical imaging, some rabbits with subclinical dental disease might have been misclassified as "healthy". This might bias the impact of age if such subclinical animals are not evenly distributed in the population. Controlling for age was essential to avoid bias in our study. As a consequence, studies that do not mention rabbits' ages should be interpreted cautiously. Comparison of wild and domestic rabbits is especially dubious in that regard [21]. Age should be included as a confounding factor in further studies. Age, along with hypovitaminosis D, excess of glucocorticoid, excess of dietary phosphorus, neutering and lack of activity can contribute to the development of osteopenia [48-55]. Other age-related factors, such as a chronic lack of abrasive food might also play a role in the pathogenesis of dental disease in rabbits [11].

Breed predispositions could have an influence on the risk of dental disease [5]. Dwarf and small rabbit breeds were more often affected by teeth and jaw lesions than intermediate and large breeds [27]. Conversely, a previous study found no statistical link between the breed and acquired dental disease prevalence [5]. However, in a recent communication of the previous author, Dwarf Lops and Netherland Dwarf were more affected by dental disease [56]. In the present study, information about breed was not deemed reliable. We decided to take into account the weight of rabbits instead [5]. The absence of statistical link between dental disease or incisors disease and weight was consistent with the first results of Harcourt-Brown [5], but not with those of Korn and others [27]. This suggests that the breed might not influence the prevalence of dental disease in our population. Alternatively, the weight may not represent a good proxy for the breed.

The lack of abrasive food is reported to cause dental disease [11]. Dental growth has recently been shown to increase with increasing food abrasiveness in rabbits [7]. This factor had been taken into account in the survey. However, only a minority of rabbits did not accept to eat hay at all. Most such rabbits were old (median age: 6 years of age). The small number of cases and the strong correlation with age precluded further statistical tests. The data we collected was not precise enough to evaluate whether the quantity and abrasiveness of the different food items eaten by each rabbit was appropriate.

The present study was based on an online survey designed for rabbit owners. The survey allowed us to quickly gather data on a large number of animals, but it has some limitations. The answerers were not able to provide us with medically detailed data. A dental examination by a trained vet was not a prerequisite to answer the survey. Consequently, some rabbits considered healthy might have subclinical dental disease. Moreover, access to outdoor once a week for various durations was recorded the same way. These elements could cause the results to be non-significant. In addition, some rabbits were treated by different veterinarians, who may not have the same level of experience with rabbit medicine. As a consequence, we did not discriminate the progressive stages of acquired dental disease from other causes of dental problem, such as genetic factors, trauma, teeth neoplasia, jaw tumor, soft tissue tumor, and foreign bodies [5,27].

Furthermore, the answerers were members of a French association, Marguerite et cie whose purpose is to improve the standard of care of rabbits. As a consequence, the population studied here is unlikely to be representative of the general population of French rabbits.

Conclusion

This study nonetheless allows to conclude that exposure to natural UVB, as it is currently performed by informed owners in France, is inefficient in preventing dental disease in companion rabbits. Further studies are required to determine whether UVB can really prevent dental disease, and, if so, which exposure protocols would be adequate.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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