Dental Pathology of the Iberian Lynx (Lynx pardinus), Part II: Periodontal Disease, Tooth Resorption, and Oral Neoplasia

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Abstract
The Iberian lynx is an endangered felid that has been subject to an intensive, ongoing conservation program to save it from extinction. Identification of dental pathologies could play an important role in the survival of this endangered species. The prevalence of periodontal bone loss, tooth resorption, and oral neoplasia was evaluated in this species. Skulls of 88 adult specimens of the Iberian lynx (Lynx pardinus) from the Doñana Biological Station (EBD-CSIC), Seville, Spain, were examined macroscopically and full-mouth dental radiographs of all specimens were performed. Different stages of periodontal disease were identified in 81.3% of teeth. Approximately two-thirds (64.8%) of the specimens examined had at least 1 tooth affected by stage 3 or 4 periodontal disease. Maxillary and mandibular incisor teeth accounted for a majority (59.4%) of teeth affected by stage 3 or 4 periodontal disease. Tooth resorption affected 12 teeth (0.63% of evaluated teeth) in 6 skull specimens (6.8% of skulls evaluated). Five of the teeth with resorptive lesions were detected on 1 skull specimen. Six teeth (50%) of those affected by tooth resorption were incisor teeth. Only 1 (1.1%) adult specimen showed signs of oral neoplasia. Our study concluded that there is a high prevalence of periodontal disease that could lead to impaired hunting ability, as well as may represent a threat to overall health and subsequent survival of this endangered species. The extremely low prevalence of tooth resorption in L pardinus could open new pathways in the discovery on the pathogenesis of tooth resorption in felids.

Keywords
Iberian lynx, veterinary dentistry, periodontitis, periodontal disease, tooth resorption, neoplasia, oral tumor

Introduction
There is little information documenting the dental pathology of wild felids. Dental lesions are common in the family Felidae and can be a significant source of morbidity and mortality in the wild.1,2 Recent studies in the lynx3,4 have provided detailed information regarding their dental pathology, hypothesizing that dental lesions in wild felines may be similar to those found in domestic and feral cats.3

Periodontal disease is the most common inflammatory disease of the oral cavity of cats, including plaque-induced inflammatory conditions of the periodontium. Periodontal disease is possibly the most common chronic disease in cats.5,6 References suggest that periodontitis affects the majority (85%-95%) of cats older than 2 years of age, especially those that do not receive oral care.5,6 Periodontitis is diagnosed and staged by clinical and radiological examination.6

Idiopathic tooth resorption is also very common in domestic cats.7 Tooth resorption results in the loss of external tooth structure of permanent teeth due to osteoclastic activity stimulated by an unknown cause.8,11 Frequently it presents clinically as a hard tissue defect at the cementoenamel junction of the tooth covered by hyperplastic gingiva or granulation tissue.8,9 Prevalence rates ranging from 28.5% to 67.0% have been reported in the domestic cat and the incidence increases with increasing age.8,9,12,13,14 Tooth resorption has been shown to occur in feral domestic and wild cats.1,4,8,15 Tooth resorption should be diagnosed by a combination of visual inspection and examination with a dental explorer and intraoral dental radiography.8 Typically representing various forms of external resorption, these lesions are often categorized as either replacement resorption or peripheral inflammatory root resorption.8

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Table 1. Stage of Periodontal Disease, Tooth Resorption, and Oral Neoplasia and Inclusion Criteria.

<table>
<thead>
<tr>
<th>Observation</th>
<th>Inclusion Criteria</th>
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<tr>
<td>Periodontal disease stage 2</td>
<td>Early periodontal disease; less than 25% of attachment loss or, at most, there is a stage 1 furcation involvement in multirooted teeth. The loss of periodontal attachment is less than 25% measured by radiographic determination of the distance of the alveolar margin from the cementoenamel junction relative to the length of the root.</td>
</tr>
<tr>
<td>Periodontal disease stage 3</td>
<td>Moderate periodontal disease; 25%-50% of attachment loss as measured by radiographic determination of the distance of the alveolar margin from the cementoenamel junction relative to the length of the root, or there is a stage 2 furcation involvement in multirooted teeth.</td>
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<tr>
<td>Periodontal disease stage 4</td>
<td>Advanced periodontal disease; more than 50% of attachment loss as measured by radiographic determination of the distance of the alveolar margin from the cementoenamel junction relative to the length of the root, or there is a stage 3 furcation involvement in multirooted teeth.</td>
</tr>
<tr>
<td>Tooth resorption stage 2</td>
<td>Moderate dental hard tissue loss (cementum or cementum and enamel with loss of dentin that does not extend to the pulp cavity).</td>
</tr>
<tr>
<td>Tooth resorption stage 3</td>
<td>Deep dental hard tissue loss (cementum or cementum and enamel with loss of dentin that extends to the pulp cavity); most of the tooth retains its integrity.</td>
</tr>
<tr>
<td>Tooth resorption stage 4</td>
<td>Extensive dental hard tissue loss (cementum or cementum and enamel with loss of dentin that extends to the pulp cavity); most of the tooth has lost its integrity.</td>
</tr>
<tr>
<td>Tooth resorption stage 5</td>
<td>Remnants of dental hard tissue are visible only as irregular radiopacities.</td>
</tr>
<tr>
<td>Oral neoplasia</td>
<td>Evidence of aggressive and extensive bone lysis combined or not with new bone formation; extensive dental hard tissue loss.</td>
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Neoplasms arising in the oral cavity of cats are relatively common. Reports suggest that the most common feline oral neoplasm is squamous cell carcinoma. Marked bone lysis combined with new bone formation are frequent findings detected radiographically in patients with oral squamous cell carcinoma.

The aim of this study was to provide detailed information regarding the prevalence of periodontal disease, tooth resorption, and oral neoplasia of the endangered Iberian lynx (Lynx pardinus) and compare them with results of similar studies assessing Lynx rufus californicus and studies of tooth resorption in the Swedish Eurasian lynx (Lynx lynx). Results of our study can provide new insight for conservation programs of wild and captive Iberian lynx.

Material and Methods

Macroscopic examination of 88 skull specimens from the Doñana Biological Station (EBD-CSIC), Seville, Spain was undertaken. Collections of skulls were obtained from carcass recovery, and donations from the public and other institutions, from 1954 to 2013.

Each skull had been previously labeled with a unique catalog number (EBD), the collection date, collection location and age, sex, and cause of death of the animal, when known. Each skull specimen was categorized as “young adult” (up to 18 months of age), “adult” (range from 18 months to 4 years of age), and “senior adult” (more than 4 years of age). Age status of the skulls was determined based on known age of death and the stage of development of the teeth evaluated through dental radiography. “Juveniles” (with presence of deciduous or mixed dentition) were previously excluded from the study.

The teeth and surrounding bony tissues were inspected with a probe/explorer, according to predefined criteria (Table 1), similar to those established by the American Veterinary Dental College. In order to confirm and classify certain lesions, full-mouth dental radiographs were also obtained.

Periodontal disease was classified based on the degree of severity of attachment loss according to predefined criteria established by the American Veterinary Dental College, modified and adapted to the present study. “Stage 1 periodontitis,” referring to gingivitis, was excluded as this could not be evaluated in the studied skull specimens.

Tooth resorption was classified based on the severity of the resorption according to predefined criteria established by the American Veterinary Dental College. The presence of findings compatible with oral neoplasm was also recorded.

The second part of the study included obtaining full-mouth intraoral dental radiographs of the skulls to confirm and classify detected lesions. The diagnostic value of full-mouth radiography in this species was also assessed. Radiographs were obtained using a portable handheld dental X-ray unit, size 2 intraoral direct digital sensor, and a veterinary diagnostic imaging software. Radiographic views were obtained following the small animal radiographic set technique description of the American Veterinary Dental College, resulting in 10 maxillary and mandibular radiographic views. Additional modified views were obtained for adequate and accurate assessment of areas of special interest. Once the radiographs were obtained, each radiograph was evaluated independently and then compared with the findings of the gross examination. Findings of the macroscopic and radiographic examinations were documented.

The prevalence of dental lesions was compared between skulls from animals of different ages and sex. SAS software, version 9.4 was used for logistic regression analyses. Significance was calculated using the Pearson $\chi^2$ test and the Wilcoxon (rank sums) test. $P < .05$ was considered significant.
Results

Of the 88 skull specimens evaluated, 48.9% were from male Iberian lynx, 33.0% were from female Iberian lynx, and 18.1% were from animals with an unknown gender.

Senior adult Iberian lynx skull specimens comprised 55.7% of the skulls examined, adult Iberian lynx skull specimens comprised 20.4% of the skulls examined, while young adult Iberian lynx skull specimens comprised 23.9% of the skulls examined.

Periodontal Disease

The total number of teeth available for periodontal examination was 1892 (76.8%), out of a potential total of 2464 teeth. Periodontal bone loss was assessed in 81.3% of teeth examined. The majority (64.8%) of the specimens examined had at least 1 tooth affected by stage 3 or 4 periodontal disease. Stage 2 periodontal disease (Figure 1) accounted for 63.1% of evaluated teeth, stage 3 periodontal disease (Figure 2) accounted for 17.4% of evaluated teeth, and stage 4 periodontal disease (Figure 3) accounted for 0.85% of evaluated teeth.

The maxillary and mandibular incisor teeth accounted for most teeth (59.4%) affected by stage 3 or 4 periodontal disease. In this same category, the maxillary premolar and molar teeth accounted for 18.6%, the mandibular premolar and molar teeth accounted for 10.4%, the maxillary canine teeth accounted for 6.1%, and the mandibular canine teeth accounted for 5.5% of teeth.

Senior adult Iberian lynx specimens were more likely to have periodontal disease than the group of young adult and adult specimens (P < .0001). There were no significant differences in prevalence of periodontal disease when comparing males and females.

Tooth Resorption

The total number of teeth available for idiopathic tooth resorption examination was 1915 (77.7%), out of a potential total of 2464 teeth. Tooth resorption affected 12 teeth (0.63% of evaluated teeth) in 6 skull specimens (6.8% of skulls evaluated); 6 of these were the maxillary incisor teeth (Figure 4), 3 of these were the maxillary third premolar teeth (Figure 5), 2 of these were the mandibular third premolar teeth (Figure 6), and 1 of these was the maxillary fourth premolar tooth (Figure 7). Five of the teeth with resorative lesions were detected on a single skull. Stage 2 tooth resorption was detected in 2 mandibular
third premolar teeth and only 1 maxillary incisor tooth. Stage 3 tooth resorption was detected in the maxillary fourth premolar tooth. Stage 4 tooth resorption was detected in 1 maxillary incisor tooth, 1 mandibular incisor tooth, and 2 mandibular third premolar teeth. Tooth resorption stage 5 was detected in 2 maxillary incisor teeth, 1 maxillary third premolar tooth, and 1 mandibular third premolar tooth. Tooth resorption was detected in 5 senior adults (3 male and 2 female) and 1 adult female Iberian lynx specimen. There were no significant differences in prevalence between the age groups and genders.
 Oral Neoplasia

Only 1 adult male specimen showed signs of oral neoplasia on the rostral third of both mandibles (Figure 8), from the left mandibular third premolar tooth to the right mandibular third premolar tooth. Extensive bone lysis combined with mild periosteal new bone formation was detected in this area.
The Don˜ana Biological Station (EBD-CSIC) has the most unique collection of Iberian Lynx (L pardinus) skull specimens in the world. The collection dates of the skulls ranged from 1954 to 2015. The skulls were obtained from carcass recovery, individual and public collections, and other wildlife care and government institutions. Ample information was available from most of the skull specimens examined.

It is important to take into consideration that the number of skull specimens included in this study (n = 88) represents 21.7% of the adult population of the Iberian Lynx in the Iberian Peninsula in 2015. These data imply that even small percentages in the obtained results may be representative. Every single effort to prevent or treat dental pathologies in this species would have decisive effects in the ongoing conservation efforts in this endangered species.

Periodontal bone loss was commonly detected in the present study, affecting 81.3% of teeth evaluated as in previous studies in domestic cats. The majority (64.8%) of the skull specimens had at least 1 tooth affected by stage 3 or 4 periodontal disease. In the study of dental pathology in the California bobcat (L ruﬁcus californicus), periodontal disease affected 7.5% of teeth and 56.0% of specimens. The Iberian lynx had a higher prevalence of periodontal disease and percentage of teeth affected when comparing this population of the California bobcat. One of the possible reasons for this discrepancy can be the different inclusion parameters in both studies, with our study being based on the predefined criteria of periodontal disease established by the American Veterinary Dental College.

The teeth most commonly affected with stage 3 and 4 periodontal disease were the maxillary and mandibular incisor teeth. In the study of dental pathology in the California bobcat, the teeth most commonly affected with periodontal disease were the maxillary fourth premolar tooth and the mandibular first molar tooth in all cases of periodontal disease. In both studies, adult bobcat and senior adult and adult Iberian lynx were more likely to have periodontal disease than young adult bobcats and Iberian lynx, respectively.

Consequences of periodontitis may potentially adversely affect hunting and consuming capabilities, so it has to be taken into consideration in international conservation programs.

Etiology and pathogenesis of different types of tooth resorption are still not clear. External root resorption is classified as surface, replacement, or inflammatory. Surface resorption is self-limiting, reversible, and caused by minor trauma. Replacement resorption results in replacement of the dental hard tissue by bone. Inflammatory resorption is a consequence of inflammation in adjacent tissues, being either peripheral inflammatory root resorption (by osteoclast-activating factors provided by an inflammatory lesion in the adjacent periodontal tissues) or external or internal inflammatory root resorption (stimulated by inflamed or necrotic pulp due to dental trauma). In our study, external inflammatory root resorption (presumably caused by dental trauma as complicated dental fractures and attrition/abrasion with severe exposure of dentin and pulp exposure) was detected (Figures 9 and 10), but these cases were not considered to be true cases of idiopathic root resorption since etiology was apparent.

Tooth resorption detected frequently in cats, and those described in this study of Iberian lynx are included either in replacement resorption or peripheral inflammatory root resorption categories. Tooth resorption affected 12 teeth (0.63% of evaluated teeth) in 6 skull specimens (6.8% of skulls specimens evaluated) of the Iberian lynx. A higher prevalence rate was found in the study of dental pathology in the California bobcat (L. ruﬁcus californicus), where tooth resorption was found in 73 teeth (1.0% of teeth examined), and 9.4% of specimens. In a study of root resorption in the Swedish Eurasian Lynx (L lynx), radiographic signs of root resorption were not observed, except 1 case of external inflammatory root

Figure 9. (A) Gross and (B) radiographic appearance of the right maxillary third incisor tooth with severe attrition/abrasion of the right maxillary first and second incisor teeth. The left maxillary first incisor tooth exhibits severe attrition/abrasion or root fracture with external root resorption, in a senior adult unknown sex Iberian lynx skull (arrows). Note the left maxillary canine tooth with suspected artifactual damage (asterisk).
resorption associated with a periapical lucency on a fractured left maxillary canine tooth. Higher prevalence was found in studies of dental pathology in other wild felids such as the California Mountain Lion (Puma concolor couguar), where tooth resorption was identified in 1.2% of teeth studied and 11.0% of specimens, or in the study of feral cats, where tooth resorption affected 1.2% of teeth and 14.3% of the population studied. In domestic cats, it has been estimated that tooth resorption affects anywhere between 20% and 67% of domestic cats. In one study of the incidence of tooth resorption in a clinically healthy population of 228 domestic cats using a combination of clinical examination and radiography, the prevalence of tooth resorption was 29%. The extremely low prevalence of tooth resorption in the Iberian lynx stands out when compared to other studies that include oral examination and intraoral radiographs in wild felids and domestic cats. The only exception is the study of the Swedish Eurasian Lynx (L lynx) where idiopathic tooth resorptions were not found. The number of specimens of the Swedish Eurasian Lynx study is lower (n = 46) and specimens were obtained in a narrow window of time (1996-1998). One study of the normal morphology and mineralization of feline teeth demonstrated that scanning electron microscopy is ideally suited for high-resolution analysis of the entire surface of feline teeth. Studies to confirm the findings of idiopathic root resorption in the Iberian lynx and other wild felids by analysis using backscattered scanning electron microscopy may be worthwhile, similar to those performed in domestic cats.

The Iberian lynx may provide clues regarding the etiology of root resorption. The fairly controlled living conditions of the Iberian lynx (strict feeding specialists, specific and limited environment and location) makes it an excellent candidate for these studies. Increased blood levels of vitamin D in this species has been implicated as a potential cause in studies in domestic cats and humans. The Iberian lynx is a strict feeding specialist, with the European rabbit (Oryctolagus cuniculus) being the basis of its diet. Since cats are unable to synthesize vitamin D in their skin, diet is an important role if vitamin D plays a role in the etiology of external tooth resorption. Further studies should be developed to determine the influence of these factors on tooth resorption.

Only 1 adult male specimen showed signs of oral neoplasia on the rostral third of both mandibles, including extensive bone lysis and mild perosteal new bone formation. To the authors’ knowledge, this is the first time that oral neoplasia is referenced in the Iberian lynx, with a low prevalence of 1.1% of skull specimens evaluated. Individual case reports of oral squamous cell carcinoma in Lynx rufus and Lynx canadensis have also been described in the literature.

In conclusion, the Iberian lynx exhibits a high prevalence of periodontal bone loss and a very low prevalence of tooth resorption and oral neoplasia. International conservation programs may take into consideration these features and develop actions to specifically study these dental pathologies of the Iberian lynx, specifically to confirm the low prevalence of tooth resorption and whether this species can be helpful to determine the etiology of tooth resorption as seen in domestic and wild felids.

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Materials
b. Nomad Handheld X-Ray System; Aribex, Inc, Orem, Utah, USA.
c. Sopix SD sensor size 2; Satelec—Acteon, Acteon Medico-Dental Iberica, Sentmenat, Barcelona, Spain.
d. Sopro Imaging VET 2.10; Satelec—Acteon, Acteon Medico-Dental Iberica, Sentmenat, Barcelona, Spain.

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