



# Oral Squamous Cell Carcinoma in a Free-Ranging Roe Deer (*Capreolus capreolus*)

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## Abstract

**Objective:** We describe a case of squamous cell carcinoma (SCC) in a free-ranging roe deer (*Capreolus capreolus*).

**Methods and Results:** Subject of this paper is an adult male of roe deer (*Capreolus capreolus*), found in Aosta Valley Region (North-west Italy), with an evident swelling at the right mandibular region. By applying routine histological (Haematoxylin and Eosin staining) and immunohistochemical (antibodies detection by diaminobenzidine DAB and counterstaining by Mayer's Haematoxylin) techniques, the tumor appeared to be formed by trabeculae and islands of squamous epithelial cells, growing from the oral mucosa, involving soft and bone tissues and surrounded by a desmoplastic reaction; it was also observed a pseudo-glandular pattern due to the formation of cysts filled with acantholytic keratinocytes; strong anisokaryosis and anisocytosis, with a quite low mitotic activity (about 1 division per high-power – 400x - field), characterized the neoplastic cells. Epithelial trabeculae were positive for cytokeratin and negative for vimentin, last one well expressed by the fibroblasts in the desmoplastic stroma. By routine bacteriological examination (Blood Agar and MacConkey agar incubated over 48 h at 37 °C in aerobic and anaerobic atmosphere) overlapping infection of neoplastic mass by *Trueperella pyogenes* was found. Based on microscopical features the tumor was classified as acantholytic SCC.

**Discussion and conclusions:** SCC is a malignant tumor of epidermal origin well known in domestic animals, especially in cats where it's the most prevalent oral neoplasm, and wild fauna, such as fish, birds, reptiles and various classes of mammals. To our knowledge, this is the first report of a similar neoplasia in roe deer; adding SCC to the list of tumors that can affect the alpine wild ruminants contributes to the study and knowledge of neoplastic processes in all wildlife.

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**Keywords:** Squamous cell carcinoma; Roe deer; *Capreolus capreolus*



## Introduction

Squamous cell carcinoma (SCC) is a malignant tumor of epidermal origin well known in domestic animals, especially in cats where it's the most prevalent oral neoplasm, generally found at mandibular, maxillary or sublingual regions. As it happens for other types of neoplasia, it's reported sporadically in different wild animals, free-ranging or captive, as fish [1,2], birds [3], reptiles [4,5] and various classes of mammals: cetaceans such as *Tursiops truncatus* [6], marsupials such as *Isoodon macrourus* [7], rodents such as *Agouti paca* [8], rhinocerotids, such as *Rhinoceros unicornis* [9], felids, such as *Leopardus pardalis* [10], *Panthera tigris* [11], *Panthera tigris altaica* [12], *Panthera leo* [13], *Panthera pardus pardalis* [14] different species of *Lynx* [15-17] and ruminants; among these, it has been observed in different species of cervidae, such as *Cervus elaphus* [18], *Elaphurus davidianus* [19], *Odocoileus virginianus* [20], *Cervus nippon pseudaxis* [21] and *Rangifer tarandus tarandus* [22]. Aim of this work is to describe the gross and microscopic findings observed in the first case, to our best knowledge, of an oral squamous cell carcinoma in a free-ranging roe deer (*Capreolus capreolus*).

## Case details

Subject of this report is an adult male of roe deer (it was not possible to define exactly the age due to the abnormal consumption of the teeth) found by the rangers in Aosta Valley Region (North-west Italy), at 1100 m of altitude; due to cachexia and an evident swelling at the right mandibular region without skin ulcers or fistulas (Fig. 1A), the animal was recovered in a wildlife recovery center and subsequently euthanized for the poor conditions and prognosis. At the necropsy, a globular mass of about 15 cm in diameter, involving the intermediate portion of the right mandibular body (Fig. 1B), was detected; the neoformation contained various pus collections, associated with vegetables fragments, draining in the oral cavity at the level of the first molar and causing luxation and loosening of the other teeth of the same dental arch (Fig. 1C). No other pathological findings were observed, apart from a conspicuous infestation by intestinal Trichostrongilidae (the eggs were observed by parasitological analysis with flotation in 100% zinc sulphate solution).

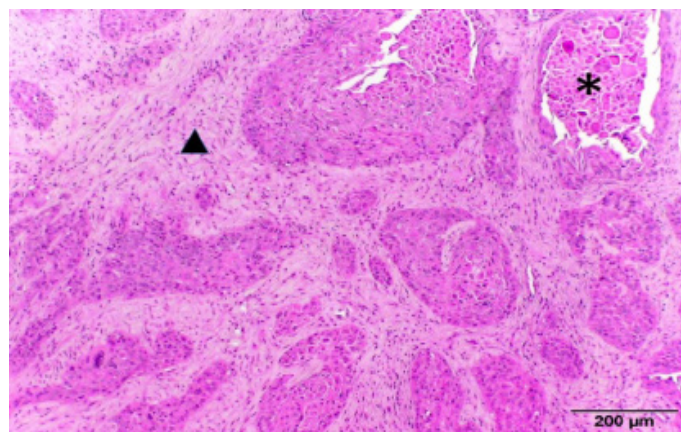


**Figure 1:** Male roe deer (*Capreolus capreolus*) showing cachexia and an extensive enlargement at the right mandibular region; **B** - Large mass involving the intermediate portion of the right mandibular body; **C**-Note the loosening of teeth, vegetable fragments and ulcers at the surface of the neoformation (partially removed from mandibular body).

Bacteriological examination of the pus collected inside the mandibular mass was performed by Blood Agar and MacConkey agar over 48 h at 37 °C in aerobic and anaerobic atmosphere; colonies of interest were characterized by morphology, catalase and oxidase test, Gram staining and typing by API Biomerieux biochemical galleries. Tissue samples from the lesion were fixed in 10% neutral buffered formalin and, after decalcification of the ossified areas by Osteosoft (Merck, Darmstadt, Germany), paraffin-embedded and cut into 3 µm thickness sections for histological and immunohistochemical examinations. Some sections were stained with Haematoxylin and Eosin, additional sections were subjected to immunohistochemistry using primary antibodies specific for cytokeratin AE1/AE3 (1:200 dilution; Dako, Santa Clara, California, USA) and for vimentin V9 (1:50 dilution; Dako, Santa Clara, California, USA); labelling with each antibody was detected by 3,3'-diaminobenzidine (DAB), counterstaining slides with Mayer's Haematoxylin.

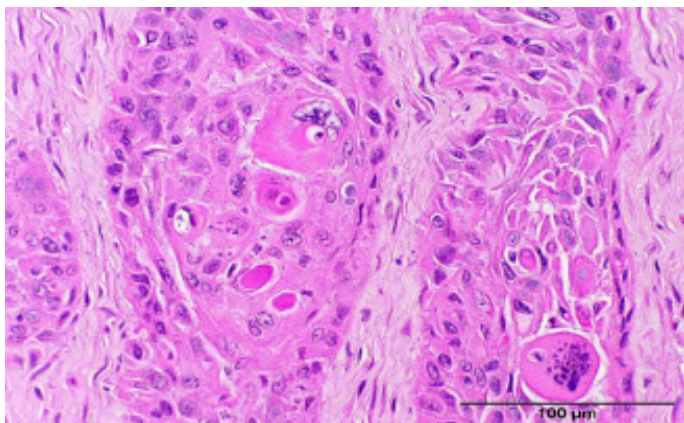
Bacteriological examination highlighted coryneform bacilli, partially hemolytic, Gram positive, catalase and oxidase negative, later identified as *Trueperella pyogenes*.

Histologically, a non-capsulated and ulcerated soft tissue neoplasia has been observed. Neoplastic cells were arranged in nests and anastomosing trabeculae, growing from oral mucosa and associated with abundant stromal fibrous connective tissue (desmoplasia), with initial extending into the mandibular bone tissue. There was a widespread tendency to keratinization, without corneal pearls, but with single keratinized cells or wider areas occupied by numerous squamous keratinocytes; dissociation and degeneration of squamous neoplastic cells resulted in cysts, lined with a single layer of keratinizing epithelial cells and filled with large number of acantholytic keratinocytes, often invaded by neutrophils, with an extensive pseudo-glandular pattern (Figure 2).



**Figure 2:** Islands and cords of neoplastic epithelial cells, with diffuse desmoplastic reaction (triangle) and pseudo-glandular features with acantholytic keratinocytes (asterisk). HE.

The cellular elements were characterized by abundant eosinophilic cytoplasm, oval nuclei with small clumped chromatin, high anisocytosis and anisokaryosis, with the presence of "giant" cells with huge nuclei, mitotic index of about 1 division per high-power (400x) field (Fig. 3); it were also observed large lytic areas with bacterial aggregates, superficial ulcers covered by serocellular crusts with abundant neutrophils and numerous protozoan cysts (*Sarcocystis* spp.) in the annexed muscle layers.

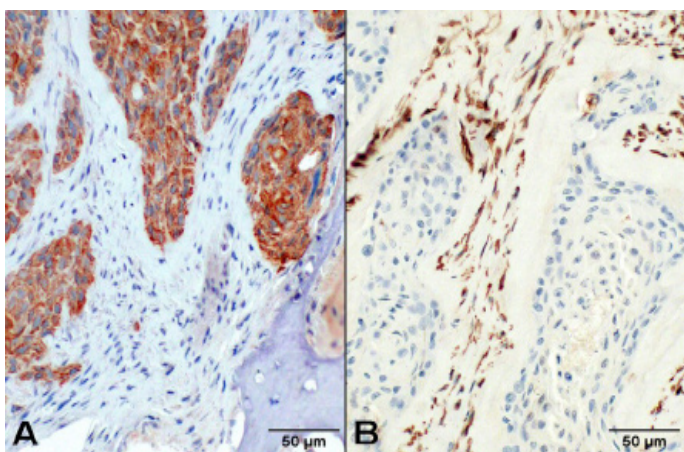


**Figure 3:** Marked cytoplasmic eosinophilia, anisocytosis and anisokaryosis of neoplastic squamous cells. HE.

The cytoplasm of neoplastic cells was strongly positive for cytokeratin (Fig. 4A) and negative for vimentin (Fig. 4B), last one well expressed by the cells of desmoplastic connective stroma.

No metastasis was found in vascular and lymphatics lumen, in regional lymph nodes or in thoracic and abdominal organs.

Based on histological features, and particularly on the pseudo-glandular pattern expressing cysts filled with acantholytic keratinocytes, we classified the neoplasia as acantholytic SCC.



**Figure 4:** Neoplastic cells are strongly positive for cytokeratin (A) and negative for vimentin (B), last one widely expressed by desmoplastic stroma. IHC.

### Discussion and conclusions

In roe deer (*Capreolus capreolus*) different types of neoplasia are signaled; in a surveillance performed in Switzerland [23], the authors refers 32 cases of tumors, observed in lymph nodes (lymphosarcoma, histiocytic sarcoma), head (spindle cell sarcoma, melanoma, ossifying fibroma, osteosarcoma, fibrosarcoma, round cell tumor, carcinoma of salivary gland), ovary (carcinoma/teratoma, granulosa cell tumor), skin (fibroma, fibrosarcoma, fibropapilloma), lung (carcinoma) and liver (cholangiocarcinoma, hepatocellular carcinoma), kidney (renal adenocarcinoma, adenoma) other than some neoplasia defined as undetermined. A similar study in Sweden [24] showed 19 cases of tumors, overlapping to those observed in Switzerland a part two cases of hemangiosarcoma and one of rhabdomyosarcoma. Other single reports refer the same type of some neoplasms observed in Switzerland and Sweden, in other words adenoma [25], teratoma [26], lymphosarcoma [27], viral fibropapillomas

[28] and many cases of hepatocellular tumor, observed in Britain, probably associated with high levels of spruce needles, buds and twig tips (rich in tannins and terpenes) in the diet [29].

Different hypothesis about the causes of the SCC in roe deer can be advanced considering the various risk factors reported for this type of tumor in people and animals, including sex (male are more affected than females), age (cat and dogs of average 10-11 years old are most affected), chronic inflammations, ultraviolet light, papillomavirus infection, bracken fern ingestion, carcinogens in tobacco, coal tar, soot, arsenic and smegma [30, 31]; further anamnestic information, unfortunately often unavailable in the case of free-living animals, would be necessary to understand the exact pathogenesis of the neoplasia we have described.

### Conclusion

In conclusion, to our best knowledge based on the references found, this is the first report of SCC in roe deer; adding this type of cancer to the list of tumors that can affect the alpine wild ruminants contributes to the study and knowledge of neoplastic processes in all wildlife.

### References

1. Mawdesley-Thomas LE, Bucke D. Squamous cell carcinoma in a gudgeon (*Gobio gobio*, L.). *Pathol Vet.* 1967; 4: 484-489.
2. Wildgoose WH. Papilloma and squamous cell carcinoma in koi carp (*Cyprinus carpio*). *Vet. Rec.* 1992; 130: 153-157.
3. Ramis A, Gibert X, Majó N, Grifols J. Metastatic oral squamous cell carcinoma in a Montagu's harrier (*Circus pigargus*). *J. Vet. Diagn. Invest.* 1999; 11: 191-194.
4. Anderson ET, Kennedy-Stoskopf S, Sandy JR, Dorn B, Boyette T, et al. Squamous Cell Carcinoma with Vascular Invasion in a Diamondback Rattlesnake (*Crotalus adamanteus*). *J. Zoo Wildlife Med.* 2010; 41: 745-749.
5. Hill AG, Denis MM, Pyne M (2016) Squamous cell carcinoma with hepatic metastasis in a saltwater crocodile (*Crocodylus porosus*). *Aust Vet J.* 2016; 94: 83-86.
6. Ewing RY, Mignucci-Giannoni AA. A Poorly Differentiated Pulmonary Squamous Cell Carcinoma in a Free-Ranging Atlantic Bottlenose Dolphin (*Tursiops truncatus*). *J. Vet. Diagn. Invest.* 2003; 15: 162-165.
7. Beck AP, Shima AL, Bennett MD, Johnson LK. Metastatic Squamous Cell Carcinoma in a Northern Brown Bandicoot (*Isodon macrourus*). *Vet Sci.* 2017; 14: 4.
8. Luppi MM, Malta MC, Costa ME, Motta RO, Santos RL. Multicentric squamous cell carcinoma in a paca (*Agouti paca*) resembling Bowen's disease. *J. Zoo Wildlife Med.* 2008; 39: 244-247.
9. Naik SN, Ishwad CS, Karawale MS, Wani MV. Squamous cell carcinoma in an Indian rhinoceros. *Vet Rec.* 1986; 118: 590-591.
10. Leme MCM, Martins AMCRF, Bodini MES, Carvalho PR, Portugal MASC. Carcinoma de células escamosas em uma jaguatirica (*Leopardus pardalis*). *Arq Inst Biol.* 2003; 70: 217-219.
11. Kloft HM, Ramsay EC, Sula MM. Neoplasia in Captive *Panthera* Species. *J Comp Pathol.* 2019; 166: 35-44
12. de Oliveira AR, de Carvalho TF, Arenales A, Tinoco HP, Coelho CM, et al. Mandibular squamous cell carcinoma in a captive Siberian tiger (*Panthera tigris altaica*). *Braz. J. Vet. Path.* 2018; 11: 97-101.
13. Mwase M, Mumba C, Square D, Kawarai S, Madarambe H. Cu-

- taneous squamous cell carcinoma presenting as a wound with discharging sinus tracts in a wild African lion (*Panthera leo*). J Comp Pathol. 2013; 149: 520-523.
14. Napier JE, Lund MS, Armstrong DL, McAloose. A retrospective study of morbidity and mortality in the North American Amur leopard (*Panthera pardus orientalis*) population in zoologic institution from 1992 to 2014. J. Zoo Wildlife Med. 2018; 49: 70-78.
  15. Altamura G, Eleni C, Meoli R, Cardeti G, Friedrich KG, et al. Tongue squamous cell carcinoma in a European lynx (*Lynx lynx*): papillomavirus infection and histologic analysis. Vet Sci. 2011; 5: 1-6.
  16. Gunson DE, Klein LV, Reid CF. Gingival squamous cell carcinoma in a Canadian lynx. J Am Vet Med Assoc. 1978; 173: 1228-1230.
  17. Sladakovic I, Burnum A, Blas-Machado U, Kelly LS, Garner BC, et al. Mandibular Squamous Cell Carcinoma in a Bobcat (*Lynx rufus*). J. Zoo Wildlife Med. 2016; 47: 370-373.
  18. Ulrich R, Teifke JP, Voigt U, Seehusen F. Oral squamous cell carcinoma in a red deer (*Cervus elaphus*). J. Wildlife Dis. 2014; 50: 113-116.
  19. Agrimi U, Morelli L, Di Guardo G. Squamous cell carcinoma of the skin in a Père David's deer (*Elaphurus davidianus*). J. Wildlife Dis. 1993; 4: 616-617.
  20. Stroud RK, Amundson TE. Squamous cell carcinoma in a free-ranging white-tailed deer (*Odocoileus virginianus*). J. Wildlife Dis. 1983; 19: 162-164.
  21. Ensley PK, Janssen DL, Anderson MP. Squamous cell carcinoma in an Indochina sika deer. J Am Vet Med Assoc. 1980; 177: 932.
  22. Gonzalez-Alonso-Alegre EM, Rodriguez-Alvaro A, Martinez-Nevado E, Martinez-de-Merlo EM, Sanchez-Maldonado B. Conjunctival squamous cell carcinoma in a reindeer (*Rangifer tarandus tarandus*). Vet Ophthalmol. 2013; Suppl 1: 113-116.
  23. Pewsner M, Origgi FC, Frey J, Ryser-Degiorgis MP. Assessing Fifty Years of General Health Surveillance of Roe Deer in Switzerland: A Retrospective Analysis of Necropsy Reports. PLoS One. 2017; 19; 12: e0170338.
  24. Aguirre AA, Bröjer C, Mörner T. Descriptive epidemiology of roe deer mortality in Sweden. J. Wildlife Dis. 1999; 35: 753-762.
  25. Craig WA. Adenoma in a British roe deer (*Capreolus capreolus*). Vet. Rec. 1979; 104: 214-215.
  26. Barlow AM, Couper D. Cutaneous teratoma in a wild roe deer (*Capreolus capreolus*) in the UK. Vet. Rec. 2006; 159: 211-212.
  27. Woodford M. Lymphosarcoma in a wild roe deer. Vet Rec. 1966; 79: 74.
  28. Rajský D, Rajský M, Garaj P, Kropil R, Ivan M, et al. Emergence and expansion of roe deer (*Capreolus capreolus*) fibropapillomatosis in Slovakia. Eur J Wildlife Res. 2016; 62: 43-49.
  29. de Jong CB, van Wieren SE, Gill RM, Munro R. Relationship between diet and liver carcinomas in roe deer in Kielder. Forest and Galloway Forest. Vet Rec. 2004; 155: 197-200.
  30. Brown CC, Baker DC, Barker IK. Alimentary system. In: Pathology of domestic animals Jubb, Kennedy, and Palmer's, 5th Edit., Maxie MG, Elsevier, Edinburgh, Great Britain. 2007; 3-296.
  31. Goldschmidt MH, Shofer FS. Skin Tumors of the dog and cat. 1th Edit., Butterworth-Heinemann, Oxford. 1992: 38-40.