J. Comp. Path. 2002, Vol. 126, 226–230
doi:10.1053/jcpa.2001.0530, available online at http://www.idealibrary.com on IDEAL®

SHORT PAPER

Spontaneous Squamous Cell Carcinoma of the Tongue and Multiple Bronchioloalveolar Carcinomas in a Virginia Opossum (*Didelphis virginiana*)

D. Y. Kim, M. A. Mitchell*, M. De las Heras†, H. W. Taylor, and D.-Y. Cho

*Department of Pathobiological Sciences and *Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA, and †Departamento de Patología Animal, Facultad de Veterinaria, 50013-Zaragoza, Spain*

Summary

Two primary tumours, squamous cell carcinoma of the tongue and multiple bronchioloalveolar carcinomas, were diagnosed in a Virginia opossum (*Didelphis virginiana*). Two oral masses were located in the right ventrolateral surface of the tongue, near the frenulum, and the lungs contained multiple, widely distributed, nodular masses. Microscopically, the oral masses were composed of invasive cords of pleomorphic, polyhedral cells, typical of squamous cells. The multiple pulmonary masses consisted of non-ciliated, cuboidal, columnar, or occasionally polyhedral cells arranged in an alveolar pattern with multifocal areas of necrosis. This is the first report of spontaneous oropharyngeal squamous cell carcinoma in the Virginia opossum. However, multiple pulmonary adenomas have been reported previously in this species, the lesions being similar to those in sheep pulmonary adenomatosis (jaagsiekte). In the present study, immunohistochemical examination of the pulmonary tumours with a rabbit polyclonal antiserum to jaagsiekte retroviral capsid protein proved negative.

The Virginia opossum (*Didelphis virginiana*), found throughout most of the United States and in parts of Canada and Mexico, is the only native marsupial in North America (Norwak, 1991). Recently, the opossum has become increasingly popular as an animal model for research (La Regina *et al.*, 1988; Liapis *et al.*, 1997; Terman *et al.*, 1999). There is only limited information, however, on the diseases that occur spontaneously in the opossum. Only three cases of neoplasia have been reported in opossums (Sherwood *et al.*, 1969; Barrie and Snyder, 1986; Prater *et al.*, 1999). This communication describes a case of two primary neoplasms in a Virginia opossum: squamous cell carcinoma of the tongue and bronchioloalveolar carcinoma of the lung. Spontaneous oropharyngeal squamous cell carcinoma has not been reported previously in the Virginia opossum. Multiple pulmonary adenomas, however, have been described in this species (Sherwood *et al.*, 1969; Barrie and Snyder, 1986; Prater *et al.*, 1999); because the lesions resembled those of sheep pulmonary adenomatosis (jaagsiekte), a viral aetiology was suggested.

An adult female opossum, having sustained lacerations and injury to the eyes and hind limbs in a road accident, was presented for examination. Routine clinical and laboratory tests revealed haematological results that suggested anaemia and leucopenia (packed cell volume of 36% [normal range 40–50%]; total protein 6·5 mg/dl [5·0–7·5 mg/dl]; white blood cell count $1{\cdot}2 \times 10^3$/

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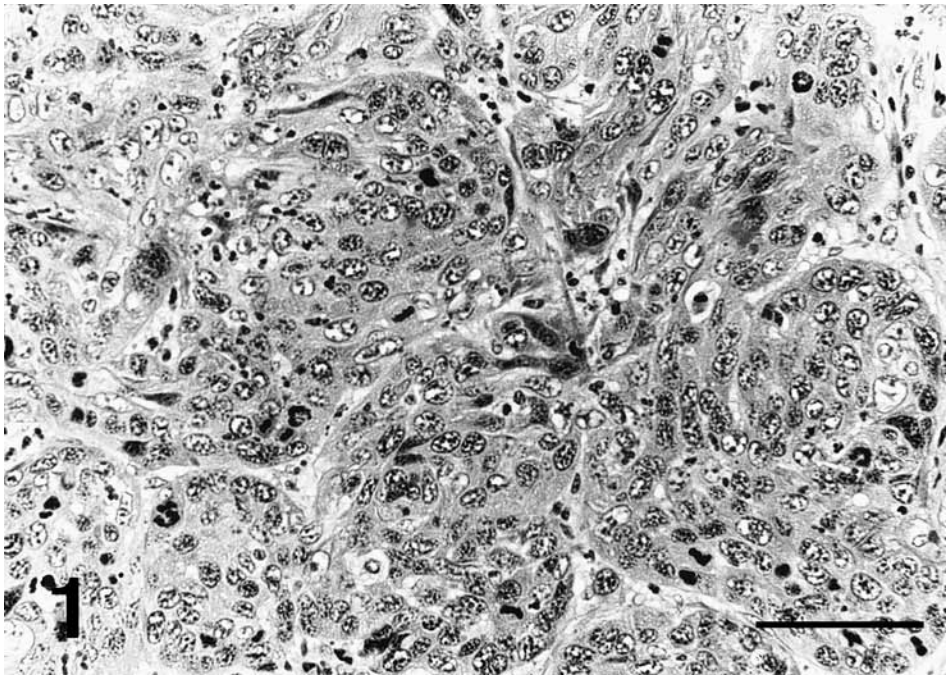

Fig. 1. Islands of malignant squamous cells with numerous mitotic figures and scattered neutrophilic infiltrates. HE. Bar, 20 μm.

ml [6–8 × 10^3/ml]). After supportive care for 16 days the animal was humanely destroyed because of poor prognosis.

At necropsy, the opossum (27·7 kg) was found to be in moderate body condition with minimal postmortem autolysis. There were two, multinodular, white, firm masses (2 × 2 × 3 cm and 2 × 2 × 2 cm) with yellowish white, friable, necrotic centres on the right ventrolateral surface of the tongue near the frenulum. About a quarter of the total substance of the cranioventral lung lobes contained widespread, white nodular masses (diameter 1–5 mm), especially at the periphery. Both submandibular lymph nodes were enlarged (1·5 × 1 × 1 cm) and showed well-demarcated white masses on cut section. Tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned (4 μm) and stained with haematoxylin and eosin (H&E).

Microscopically, the masses from the tongue were composed of irregular cords, trabeculae or islands of polyhedral cells arising from squamous epithelium. These neoplastic cords, which were separated by moderate fibrosis and neutrophilic infiltration, had invaded the submucosal propria and underlying muscle layers aggressively. The tumour cells had vesicular nuclei with wide variation in size and shape, single or double nucleoli, and abundant eosinophilic cytoplasm (Fig. 1). Intercellular bridges and keratin formation were not observed. Mitotic figures occurred frequently (8–10 per × 400 field). Sections of submandibular lymph nodes had metastatic foci composed of tumour cells similar to those seen in the tongue. The multifocal nodular masses in the lungs were non-encapsulated, well demarcated, and composed of non-ciliated, cuboidal, columnar or occasionally polyhedral cells arranged in alveolar pattern with stratified epithelium and multifocal areas of necrosis. The alveoli contained papillary stromal projections lined by the tumour cells (Fig. 2). Occasional areas of fibrosis accompanied by mixed inflammatory cell infiltrates obscured the alveolar pattern (Fig. 3). The cells had centrally located, round to polyhedral, vesiculated nuclei and eosinophilic, hazy or vacuolated cytoplasm. Mitotic figures were rare. Islands of the tumour cells were randomly scattered in the normal alveoli. Some alveoli contained nematodes, which were consistent with *Didelphostrongylus hayesi* (Prestwood *et al.*, 1977), with mild eosinophilic inflammation.

Morphological characteristics of the tumours in the oral cavity were consistent with squamous cell carcinoma. Oropharyngeal squamous cell carcinoma is common in the dog and cat, but few cases have been reported in other animals (Head, 1990). In the dog, it usually affects the tonsil and gingiva and less commonly the tongue (usually the dorsum). In the present case, the squamous cell carcinoma was located on the right ventrolateral

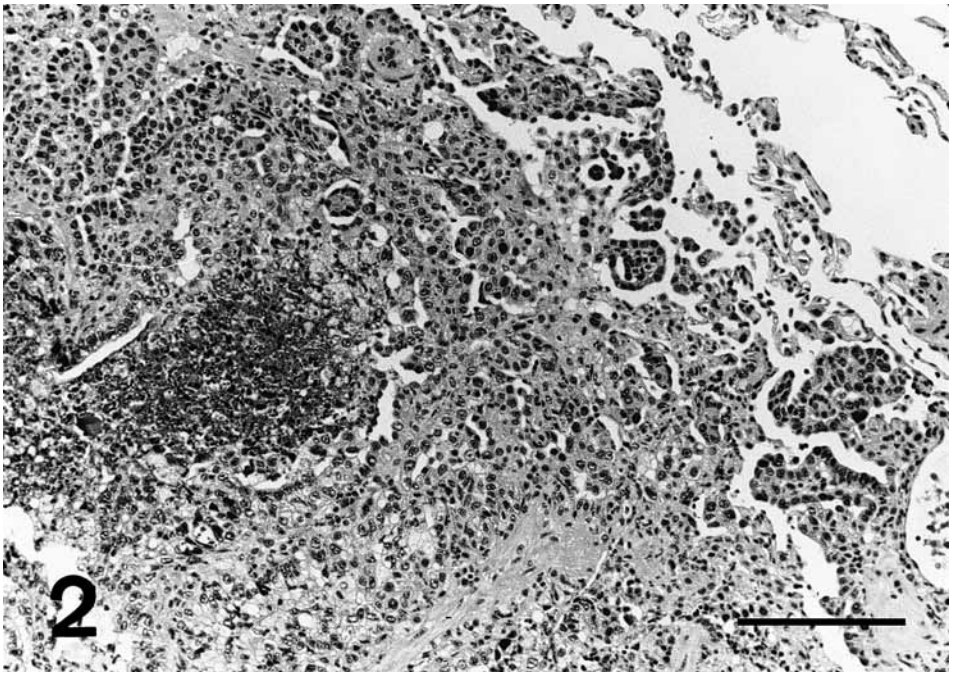

Fig. 2. A well-demarcated but locally infiltrating pulmonary mass composed of cuboidal to polyhedral cells arranged in alveoli with papillary projections and an area of necrosis. HE. Bar, 40 μm.

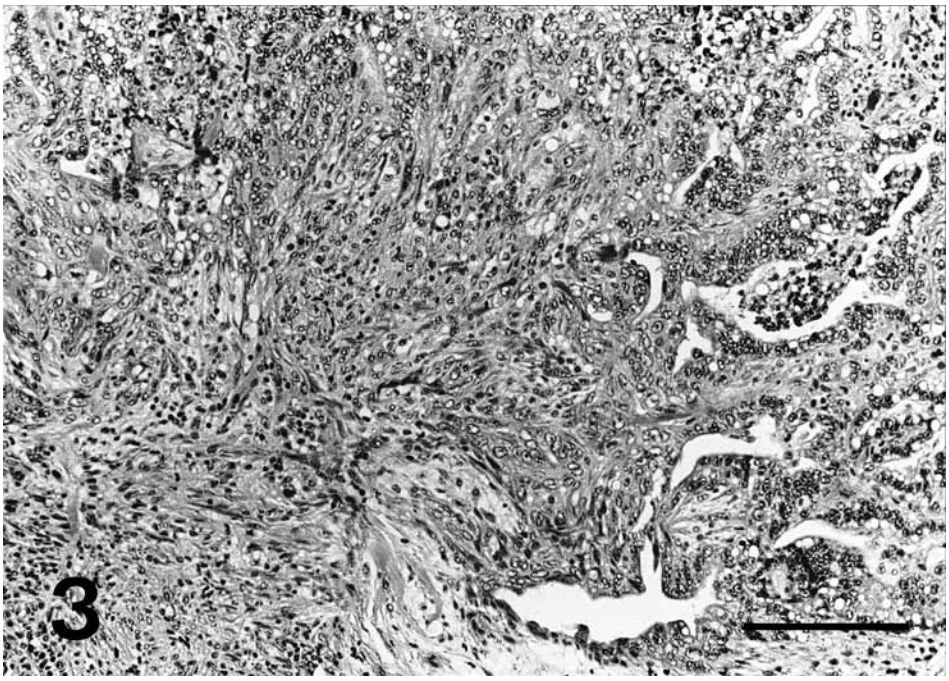

Fig. 3. Multiple layers of neoplastic cells lining the alveoli, and fibrosis accompanied by scattered mixed inflammatory infiltrates within the pulmonary masses. HE. Bar, 40 μm.

surface of the tongue near the frenulum, which is the most common site in the cat (Head, 1990; Barker *et al.*, 1993). The tumour was poorly differentiated as judged by the microscopical appearance of aggressive invasiveness, lack of intercellular bridges, and absence of keratinization. The presence of many neutrophils in the fibrous connective tissue between the neoplastic cords was most likely due to secondary bacterial infection. Metastasis of oropharyngeal squamous cell carcinoma to regional

lymph nodes and even to distant organs is not uncommon. In this case, metastasis only to the regional lymph nodes was found.

Microscopical examination of the multiple pulmonary masses revealed a bronchioloalveolar carcinoma. Such tumours generally originate from alveolar type II cells, secretory bronchiolar (Clara) cells, or both. Malignancy of this tumour was determined on the basis of the infiltrating growth pattern, areas of necrosis and cellular pleomorphism. Sherwood *et al.* (1969) reported multiple pulmonary adenomas in 25% (17 of 68) of wild-captured opossums. They described the multiple adenomas as well-circumscribed papillary tumours made up of non-ciliated, cuboidal-columnar epithelial cells and named the disease "pulmonary adenomatosis", because of gross and histological similarities to the lesions in sheep pulmonary adenomatosis (jaagsiekte), a retrovirus-induced disease characterized by a slowly progressive, low-grade, bronchioloalveolar carcinoma (DeMartini and York, 1997; Garcia-Goti *et al.*, 2000). The complete genomic sequence for the jaagsiekte sheep retrovirus has been determined and the deduced amino-acid sequence suggests that the virus is a unique chimeric virus with type-D capsid sequences and an envelope region, closely related to the type-B viruses (York *et al.*, 1992). Sheep pulmonary adenomatosis occurs in a classical and an atypical form. Histopathologically, the atypical form is essentially the same as the classical form except for the occurrence of fibrosis with severe chronic inflammation in the stroma adjacent to the neoplasm (Garcia-Goti *et al.*, 2000). In the present study, we found multiple bronchioloalveolar carcinomas with occasional areas of fibrosis accompanied by a mixture of inflammatory cells. These findings were similar to those of the atypical form of sheep pulmonary adenomatosis and have not been described previously in the opossum.

Because the pathogenesis of multiple pulmonary carcinomas in the opossum is not understood, an immunohistochemical examination with a rabbit polyclonal antiserum (Palmarini *et al.*, 1995) to jaagsiekte retroviral capsid protein was performed to investigate a possible aetiological relationship with sheep pulmonary adenomatosis. Briefly, selected formalin-fixed tissue sections were immunolabelled with a commercial avidin–biotin peroxidase complex kit (Vectastain ABC kit; Vector Laboratories, Peterborough, U.K.) as recommended by the manufacturers except that, in addition, the sections, after immersion in citrate buffer (pH 6·0), were treated in a microwave oven at 800 watts for 20 min for antigen retrieval. The rabbit polyclonal antiserum diluted 1 in 500 was used as the primary antiserum. Sheep pulmonary adenomatosis tumour tissue sections were used as positive controls. The opossum tissues gave negative results, indicating no aetiological relationship between the disease and jaagsiekte.

The findings reported in this communication gain additional importance from the recent increased use of the opossum as a laboratory animal.

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Received, March 30th, 2001
Accepted, September 10th, 2001