Intracranial squamous cell carcinoma in an Ovis aries

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Summary
Squamous cell carcinoma (SCC) is a malignant mucoepithelial tumor that affects pets and farm animals. Common sites are dorsal areas and/or areas of poor skin pigmentation exposed to mutagenic ultraviolet (UV) radiation. Novel ovine papillomavirus (OaPV3) was recently described in SCC lesions in Sardinia breed ovines. In 2017, a 7-year-old half-breed aries was presented with symptoms compatible with a vestibular syndrome. The animal was euthanized 1 month after the onset of clinical signs due to a lack of response to treatment and poor prognosis. A complete postmortem examination was performed. Necropsy revealed only a loss of incisors, associated with alveolar necrotic osteomyelitis, and left unilateral purulent nasal discharge. No other thoracic or abdominal lesions were observed. Opening of the skull revealed a cauliflower-like space-occupying mass. Histological examination showed trabecules and islands of squamous, neoplastic epithelial cells with the formation of concentric keratin layers. This raised the suspicion of SCC, which was confirmed with cytokeratin-positive immunostaining. Simplex PCR on the frozen tissue mass was negative for OaPV1, OaPV2, and OaPV3. This case report suggests that SCC, although rare, should be included in the differential diagnosis of cases of vestibular disorder.

Squamous cell carcinoma (SCC) is a common malignant tumor of the skin in both humans (zur Hausen 2009) and animals (Ahmed and Hassanein 2012). It derives from the more superficial epidermal layers (keratinocytes) and mucocutaneous borders; it is noted for its invasive behavior and metastatic abilities. Classification of SCC is based on the histological pattern (acantholytic, spindle cell, verrucous, pseudovascular, adenosquamous) and on the tumor, node, metastasis (TNM) staging system (Weedon *et al.* 2006). Squamous cell carcinoma affects both pets and farm animals, with a reported peak age per species and breed susceptibility (Ladds and Entwistle 1977), though its detection in meat animals is frequently inaccurate because of their short life expectancy (Valentine 2004, Goldschmidt and Hendrick 2002, Ahmed and Hassanein 2012). Its prevalence is variable but is widespread in sheep, with most cases reported from arid and sunny regions such as Western Australia (Daniels and Johnson 1987). Several studies have highlighted a greater susceptibility in breed-selected species characterized by a lack of skin pigmentation e.g., Merinos (Daniels and Johnson 1987) or milk-producing sheep such as Sarda breed (Vitiello *et al.* 2017). The most "common localization" in sheep and goat is dorsally exposed areas and/or areas with poor skin pigmentation and hair, including the head, base of the horn, eyelids, conjunctiva, hind limbs, shoulder, back, abdomen, flank region, vulva, and inner part of the tail (Banadiam *et al.* 2010, Ahmed and Hassanein 2012, Tmumen *et al.* 2016). Other sites (e.g., nasal or intracranial) are considered rare (Zeman and Cho 1986, Banadiam *et al.* 2010, Pace *et al.* 2010).The etiology seems to be multifactorial, although ultraviolet (UV) radiation, especially UVB ray exposure, appears to be the main cause of tumor development due to activation of proto-oncogenes or inactivation of tumor suppression factors expressed in UV irradiated body areas (Kubo *et al.* 2002). Tissues exposed to greater solar radiation produce, as result of tissue damage, a greater amount of reactive oxygen species (ROS) that induce cell damage and possible DNA mutations,

resulting in the development of pre-cancerous lesions (solar keratosis).

In Italy, recent studies of cases of ovine SCC have signaled the involvement of a novel genus of papillomavirus OaPV3 in these lesions (Alberti *et al.* 2010). Papillomavirus can infect the basal layer or skin lesions and cause cutaneous fibropapillomas (OaPV 1 and 2) or rarely progressive skin cancer lesions in humans and animals (Tilbrook *et al.* 1992, Bocaneti *et al.* 2016), suggesting a synergic action with other pro-cancer factors (Vitiello *et al.* 2017). The present report describes a rare and "uncommon localization" of intracranial SCC in a domestic sheep (*Ovis aries*) in Italy.

In 2017, a 7-year-old half-breed aries, bred in Gassino in Turin province, was presented with symptoms suggestive of vestibular syndrome. Detailed clinical findings were head pressing and tilt, circling and loss of sense of balance, pain and difficulty in chewing and walking, and fever. The animal was euthanized one month after the onset of clinical signs due to lack of treatment response and poor prognosis. The carcass was promptly delivered to the laboratory and submitted to complete necropsy. Brain tissue was partly frozen for bacteriological and molecular investigations and partly fixed in 10% buffered formalin solution. Fixed tissue was routinely processed for neuropathological examination; 4 ± 2 µ sections were cut and stained with hematoxylin and eosin (HE) for light microscopy evaluation. Immunohistochemistry investigation for vimentin (clone V9, Dako, Glostrup, DK, dilution 1:150) and cytokeratin (clone AE1/AE3, Dako, Glostrup, DK, dilution 1:50) were performed on selected sections. Tissue samples from the frozen mass were investigated for ovine papillomavirus type 1, 2, and 3 by simplex PCR methods targeted to the L1 gene, as described previously (Vitiello *et al.* 2017). Briefly, DNA was extracted using a commercial kit (QIAmp DNA mini kit, Qiagen, Milan, IT), according to the manufacturer's instructions, and then amplified with sets of primers specific for OaPV type 1, type 2, and type 3. PCR master mix compositions and thermal profiles were applied as reported in Table I. After separation by electrophoresis in agarose gel (2% w/v containing Gel Red nucleic acid stain, Biotium) in TBE buffer 1x at 5 mV/cm for 90 min, the amplification products were visualized by exposure to UV in a Gel Doc apparatus (BioRad, Segrate, Milan, IT).

Postmortem examination revealed rupture and loss of incisors, associated with alveolar necrotic osteomyelitis, and left unilateral purulent nasal discharge; bacteriological and virological examinations isolated no specific pathogens. No thoracic or abdominal lesions were observed. Opening the skull revealed a cauliflower-like space-occupying mass, white-grayish in color, firm to hard, walnut in size, with a broad basis. The mass rested under the left temporal bone, laterally in contact with the petrous portion that showed evident hyperostosis. The pedunculated part of the mass extended into the left cerebral hemisphere, creating a voluminous deep fovea and compression of the underlying cerebral tissue (Figure 1). There was no gross evidence of enlargement of the trigeminal ganglion and no gross evidence of masses in the retrobulbar space, corneal process, frontal sinus, regional lymph nodes or in other organs. Microscopical examination revealed trabeculae and islands of squamous neoplastic epithelial cells with the formation of concentric keratin layers (keratin pearls). The cells showed moderate pleomorphism with abundant eosinophilic cytoplasm, large and hyperchromatic nuclei with prominent nucleoli, usually single (Figure 2). Rare mitotic figures and

Table I. *Set up of PCR detection for OaPV1, 2, and 3 investigations.* Primer pairs, amplicon length, master mix composition, and thermal profile.

Target	Primer pairs	Amplicon length (bp)	Master Mix composition (final volume of 50 µL)	Thermal profile
L1 gene OPV-1	F: CGCCCGTCTCCCTACGGTGC R: CTGCAACGCCTCCGGACCCC	177	PCR Buffer 1X; MgCl2 1.5 mM; Primers concentration: 0.5 µM each; dNTPs: 0.2 mM each; DNA hot-start *Taq* Polymerase 1.25 U/reaction (Platinum *Taq*, Invitrogen); DNA template: 50÷300 ng in 5 µL.	Initial denaturation: 95°C x 5 min 40 cycles: 95 °C x 30 s; 56 °C x 30 s; 72 °C x 30 s Final elongation step: 72 °C x 5 min
L1 gene OPV-2	F: CGCACCACAGCCCAAGGCAC R: TCCAGCGTCCACACGGTCTGA	147		
L1 gene OPV-3	F: AACTGGACTTGTCTTCCATG R: AAAGACTCGGTATTGGGAGG	127		Initial denaturation: 95°C x 5 min 40 cycles: 95 °C x 30 s; 57 °C x 30 s; 72 °C x 30 s Final elongation step: 72 °C x 5 min

bp = base pair; F= forward; R = reverse.

multifocal peritumoral lymphoplasmacytic infiltrates were also detected (Figure 3). Immunohistochemical staining for vimentin was negative (Figure 4); cytokeratin staining showed specific positivity in the neoplastic cells (Figure 5). Histological findings classified the neoplasm as a well differentiated SCC. The simplex PCR analysis for OaPV1, OaPV2 and OaPV3 detected no gene fragments referable to these OaPVs types.

SCC is a common tumor of animals and intracranial localization is rarely described in the literature (Zeman and Cho 1986, Pace *et al.* 1997, Raheja *et al.* 2016). "The Uncommon localizations" of SCC have been demonstrated in cows, in which metastasis spreads from a specific primary site of the skin or muco-cutaneous zone, through a perineural pathway (Mendenhall *et al.* 2007) or from the foramen orbitorotundum, with or without alteration of nearby bone structures (Hinkley 1951, Pace *et al.* 1997, Zeman and Cho 1986). Epidermoid cysts, derived from Rathke's pouch in humans, due to incarceration of vestigial remains of the oropharyngeal ectoderm, are another possible origin for the development of intracranial SCC, involving the sellar region in particular (Pace *et al.* 1997).

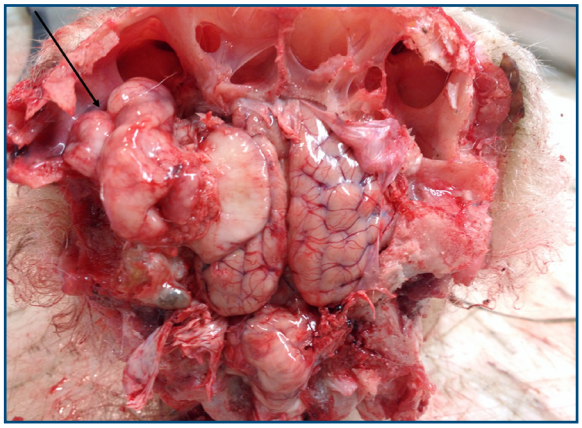

Figure 1. *Gross appearance.* The cauliflower-like mass appears pedunculated and with a broad basis, laterally in contact with the petrous left temporal bone.

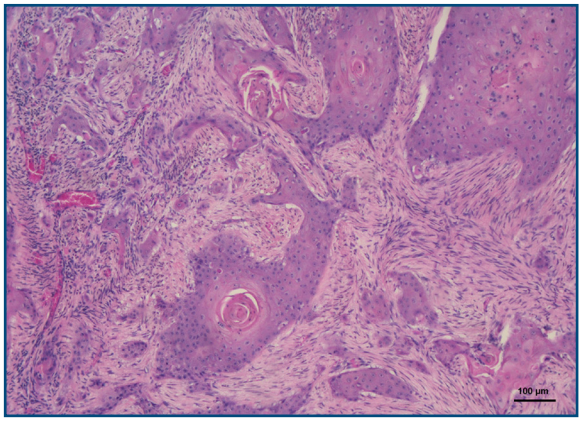

Figure 2. *Microscopical examination.* Trabeculae and islands of squamous, neoplastic epithelial cells associated in concentric keratin layers "keratin pearls". H&E. (original objective10X).

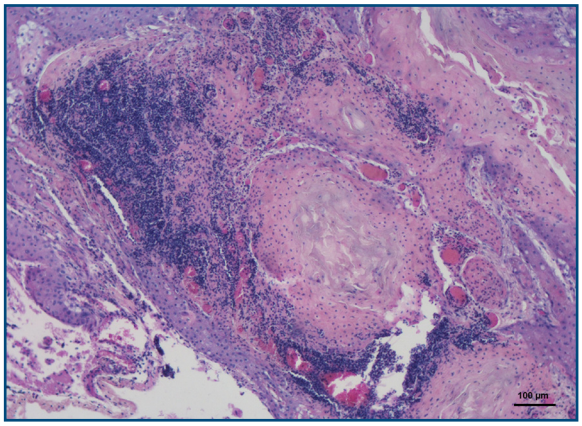

Figure 3. *Microscopical examination.* Peritumoral multifocal lymphoplasmacytic infiltrates. H&E. (original objective10X).

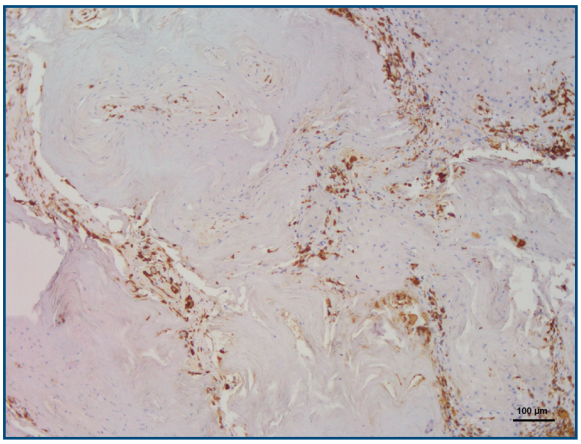

Figure 4. *Immunohistochemical stain negative for vimentin clone V9, IHC (original objective10X).*

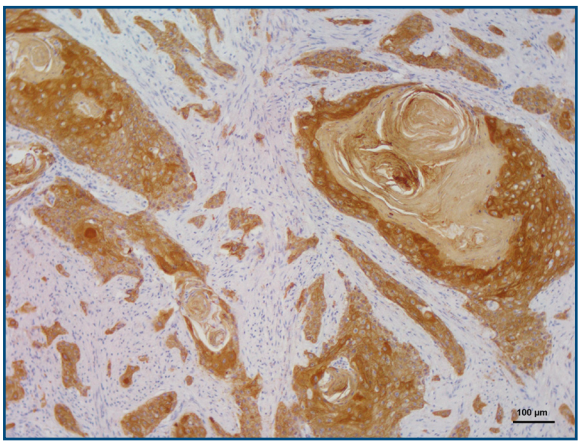

Figure 5. *Immunohistochemical stain positive for cytokeratins AE1/AE3, IHC (original objective10X).*

The possibility of an intracranial primary origin of SCC in the present case cannot be ruled out, considering the absence of macroscopically detectable neoplasms in the oculonasal region, the thoracic and the abdominal cavity, and the large size of the intracranial mass. The clinical signs and the evidence of hyperostosis of the petrosal bone, contiguous laterally to the mass, point to a possible primary source from the medium acoustic meatus. Alternatively, the neoplasm may have originated from the bone labyrinth and induced a vestibular syndrome. No oculofacial abnormality referable to Horner's syndrome could be determined in this animal, unlike previous cases of intracranial SCC (Pace *et al.* 1997).

In contrast to previous studies (Alberti *et al.* 2010, Vitiello *et al.* 2017), our virologic investigation on the frozen tissue did not confirm the presence of papillomavirus nucleic acid. Histological pattern and immunohistochemical staining unequivocally confirmed an epithelial origin of the tumor, thus allowing us to discern between other neoplasms such as meningothelial meningioma or psammomatous meningioma (Pace *et al.* 1997). This case report strongly suggests that SCC, although rare, should be included in the differential diagnosis of cases in which there is a vestibular disorder.

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