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1	SHORT PAPER
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3	Concurrent Transitional Meningioma and Ceruminous Gland Adenocarcinoma in a Scottish
4	wildcat hybrid (Felis silvestris)
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25 Keywords: Meningioma; Ceruminous gland adenocarcinoma; European wildcat; *Felis*26 *silvestris silvestris*.

27

28 The Scottish wildcat is an isolated sub-population of the European wildcat (Felis silvestris 29 silvestris) and is also considered by some scientists to be a distinct sub-species (Felis silvestris grampia) (Miller, 1912; Beaumont et al., 2001; Kilshaw et al., 2010). The future of 30 31 the European wildcat, and particularly the Scottish wildcat, is uncertain due to habitat loss, hunting and persecution throughout the 18th and 19th centuries (McOrist and Kitchener, 32 1994), and more recently the effects of hybridisation, as a result of inter-breeding with 33 34 domestic cats (Felis silvestris catus), (Daniels et al., 2001; Davis and Gray, 2010; Kilshaw et 35 al., 2010), along with the potential transmission of common infectious diseases from 36 domestic cats (McOrist et al., 1991). Now classified as endangered, they are legally protected 37 under both UK and European legislation (Kilshaw et al., 2010). As such, the majority of 38 published literature is focused on its ecology, genetics and conservation.

39 There have been a limited number of publications regarding pathology and disease of the 40 European wildcat. Most publications are serological studies focusing on the prevalence of 41 common feline viruses (McOrist et al., 1991; Watt et al., 1993; Daniels et al., 1999; 42 Leutenegger et al., 1999; Millán and Rodriguez, 2009; Wasieri et al., 2009), although there 43 are isolated reports dealing with other pathogens such as Chlamydophila sp. (Millán and Rodriguez, 2009), Toxoplasma gondii (McOrist et al., 1991; Herrmann et al., 2013), 44 lungworm (Falsone et al., 2014), hemoplasmas (Willi et al., 2007), and other endoparasites 45 46 (Burt et al., 1980; Krone et al., 2008). Neoplasia has been rarely reported in the European 47 wildcat; from 79 necropsy examinations reported in several articles (Jefferies, 1991; McOrist et al., 1991; Watt et al., 1993; Krone et al., 2008; Wasieri et al., 2009; Hermann et al., 2013; 48

Falsone et al., 2014), only one tumour (a pulmonary lymphoma) was found (Hermann et al.,
2013).

51 This report describes the presentation, gross post-mortem, and histological findings in a 52 Scottish wildcat hybrid with concurrent transitional meningioma and ceruminous gland 53 adenocarcinoma. To the authors' knowledge, there have been no previous reports of 54 meningioma or ceruminous gland adenocarcinoma in the European wildcat (*Felis silvestris* 55 *silvestris*).

56 A 16.5 year old, neutered male, Scottish wildcat hybrid from a zoological collection reportedly suffered from sporadic episodes of incoordination and mild lethargy over a four 57 58 week period. Although this individual was deemed to be a hybrid (Felis silvestris silvestris x 59 *Felis silvestris catus*), rather than a true wildcat, according to the records of the zoological 60 institution, a sample of skeletal muscle was tested using a 35 Single Nucleotide 61 Polymorphism (SNP) marker test developed by the Royal Zoological Society of Scotland 62 from a panel of markers published by Nussberger et al., (2013) in an attempt to confirm this 63 and investigate the degree of hybridisation. Two extracts of DNA were conducted and three 64 replicates of the assay were run but the assay failed to prove or disprove the hybrid status of 65 this individual due to insufficient DNA quality, presumably due to sample degradation. The cat was transferred from a wildlife centre when it was 9 years old, and had since lived with a 66 67 female Scottish wildcat in an outdoor enclosure. Prior medical history was unremarkable. 68 Husbandry and nutrition were considered appropriate for the species. Physical examination 69 under general anaesthesia revealed loose skin, thought to be consistent with poor hydration or 70 recent weight loss, a round bony proliferation on the right stifle joint and a hard mass behind 71 the right ear. Body condition score was considered acceptable (4/9) with a weight of 4.5kg, 72 teeth were in an excellent condition for an old cat and vital parameters (heart and respiratory 73 rate, rectal temperature) were unremarkable. The cat initially appeared to recover well from

74 anaesthesia, but remained recumbent and died 2 hours later. The animal was subsequently

submitted for post-mortem examination at the Royal (Dick) School of Veterinary Studies

76 (The University of Edinburgh, Roslin, Midlothian, UK).

77 Post-mortem examination revealed the entrance to both external ear canals to be obscured by 78 dark grey to blue black, well demarcated, multi-lobular, firm, occasionally cystic, exophytic, 79 polypoid masses ranging from 1 to 5mm in diameter and a cream, well demarcated, 80 60x30x20mm, multi-lobular, firm, subcutaneous mass at the base of the right ear which 81 contained a dark red, central, well circumscribed, depressed area 3mm in diameter on cut 82 section. The right pre-scapular lymph node was moderately enlarged and the brain contained 83 a cream to yellow, well demarcated, multi-lobular, expansile and compressive mass, 84 approximately 15mm in diameter, between the cerebral hemispheres in the region of the falx 85 cerebri (Fig.1). Samples of all gross lesions along with representative samples from all tissues 86 and internal organs were collected, fixed in 10% buffered formalin and routinely processed 87 according to current histological methods. Sections 5µm thick were stained with 88 haematoxylin and eosin. 89 Histopathological examination of the external ear canal masses (Fig.2) revealed a moderately 90 acanthotic, stratified squamous keratinising epithelium elevated by numerous, multi-focal, 91 dilated, cystic, glandular structures lined by a flattened to low cuboidal epithelium. Many 92 cysts contained amorphous, tan to pale brown, granular material (cerumen) and low numbers 93 of large foamy macrophages also contained this granular brown material. Within a mass from 94 the right external ear canal, the superficial dermis was focally expanded by more dense 95 proliferations of polygonal to cuboidal cells forming tubules, acini and fronds. Cells had

96 variably well-defined cell borders, a moderate amount of eosinophilic, finely granular

97 cytoplasm, open-faced, oval to round nuclei with 1-2 prominent nucleoli. Anisocytosis and

98 anisokaryosis were mild with an average of 1 mitotic figure per high power field (x400). Foci

99 of necrosis expanded the centre of these cellular proliferations. Examination of the right ear 100 base mass (Fig.3) was consistent with the pre-existing architecture of a lymph node, 80% of 101 which was effaced by tubules and acini of cells, similar to those described in the mass from 102 the right external ear canal, with evidence of lymphatic invasion. Similar cell proliferation 103 was present in the right pre-scapular lymph node.

104 The histopathological appearance of the auricular lesions was consistent with a diagnosis of 105 bilateral ceruminous gland dilatation and hyperplasia (feline ceruminous cystomatosis) with 106 unilateral (right side) ceruminous gland adenocarcinoma and metastasis to cervical and pre-107 scapular lymph nodes.

108 Sections of cerebral cortex, lateral ventricle and meninges showed the leptomeninges of the 109 cingulate sulcus to be expanded by a large, densely cellular, well demarcated, finely 110 encapsulated, compressive, nodular mass (Fig.4). The mass consisted predominantly of 111 lobules of loosely to densely packed cells forming concentric whorls, occasionally 112 surrounding blood vessels or containing central areas of necrosis. Adjacent cells formed long, 113 interlacing fascicles streaming around the whorls and were supported by a loosely arranged, 114 eosinophilic, fibrillar stroma. Cells were fusiform to polygonal with indistinct cell borders 115 and variable amounts of eosinophilic, wispy, fibrillar cytoplasm. Within the centre of 116 concentric whorls, cells adopted a more epithelioid morphology. Nuclei were round to 117 elongated with loosely packed, finely stippled chromatin and 1 to 2 nucleoli. Anisocytosis 118 and anisokaryosis were moderate with occasional multi-nucleate forms. Mitoses were rare (1 figure per 10 high power fields (x400)). Moderate numbers of neutrophils, occasional 119 120 lymphocytes, plasma cells, pyknotic cells, small clusters of foamy macrophages and 121 occasional acicular clear spaces (cholesterol clefts) were present within the mass. Both the grey matter and white matter tracts of the adjacent cerebral cortex contained mild diffuse 122 123 vacuolation with mild perivascular clearing and spacing (oedema).

124 The histopathological findings of the cerebral mass were consistent with a diagnosis of 125 transitional (mixed) meningioma containing features of both meningothelial (characterised by 126 moderately cellular lobules of polygonal cells) and fibrous tumours (long interlacing fascicles 127 of fusiform cells).

Also identified following post-mortem and histological examination were bilateral stifle joint osteoarthritis, nodular hyperplasia of both thyroid glands, mild hepatic lipidosis, mild, multifocal, chronic cholangiohepatitis and mild, multi-focal, chronic interstitial nephritis with a focal, chronic, renal infarct.

Ceruminous gland adenocarcinomas are the most commonly diagnosed tumour of the external acoustic meatus in cats, accounting for up to 2% of all feline neoplasms (Njaa and Wilcock, 2012) and are more frequently diagnosed than adenomas in domestic cats (Moisan and Watson, 1996; London et al., 1996). They exhibit locally invasive behaviour (London et al., 1996) and metastasis to regional lymph nodes, lungs and viscera can occur in up to 50% of cases (Njaa and Wilcock, 2012). No evidence of pulmonary or visceral metastasis was found in this case.

139 Differentiation between ceruminous adenoma and adenocarcinoma can be challenging unless 140 there is evidence of local invasion or metastatic disease, as in this case (Wilcock et al., 2002). 141 Meningiomas are the most common primary central nervous system (CNS) neoplasm of 142 domestic cats (Koestner & Higgins, 2002; Troxel et al., 2003; Tomek et al., 2006; Motta et 143 al., 2012) typically occurring in cats older than 9 years (Troxel et al., 2003; Tomek et al., 2006) with an increasing incidence with age. Domestic shorthaired cats seem to be 144 145 predisposed but no significant sex predilection has been found (Troxel et al., 2003; Tomek et 146 al., 2006). Transitional meningiomas, as reported here, and fibrous subtypes are most 147 frequently encountered in domestic cats. Meningiomas are typically slow growing, with the 148 exception of the uncommon anaplastic (malignant form), rarely metastatic and approximately 149 50% of cases do not exhibit any clinical signs. To the authors' knowledge this is the first 150 report of meningioma in a European wildcat. There are only isolated additional reports in the 151 literature of central nervous system neoplasia in non-domestic felids, such as meningioma in 152 a Bengal tiger (*Panthera tigris tigris*) (Akin et al., 2013) and intracranial oligodendroglioma 153 in a lion (*Panthera leo*) (Tucker et al., 2008).

The findings reported here pose the question for future studies to determine the incidence of common neoplasms of domestic felines in pure-bred wildcats and their hybrids. This may help to elucidate whether their occurrence may be an associated effect of hybridisation, or purely a reflection of the increasing age of individuals living in zoological collections in comparison to their free living relatives.

159

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164 Lothian, EH55 8PT.

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166 Conflict of Interest Statement: The authors declare no conflict of interest.

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Legend for Figures:



Fig 1. Brain, showing the presence of a nodular mass between the cerebral hemispheres.



Fig 2. Section of tissue from the right external ear canal showing acinar arrangement of neoplastic epithelial cells with central necrosis. Epithelial cells are present within the lumen of the thin-walled vessel at the top of the image. HE.



Fig 3. Section of the right cervical lymph node showing replacement of normal architecture by metastatic neoplastic epithelia forming acinar structures. HE.



Fig 4. Section from the brain mass showing whorls and bundles of neoplastic spindle cells consistent with the transitional form of meningioma. HE.