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Disease in wildlife or exotic species

Epithelial membrane antigen-reactive feline chordoid meningioma in a European wildcat (*Felis silvestris*)

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ABSTRACT

Meningioma is the most frequent intracranial neoplasm in cats. Here we describe the first case of chordoid meningioma (CM), a rare grade II meningioma subtype, in a 5.5-year-old European wildcat (*Felis silvestris*) from a Swiss zoo. The wildcat was found dead after a clinical history of neurological signs and clinical suspicion of a carcinoma in the right external ear canal with concurrent chronic otitis. Post-mortem examination revealed a large intracranial, extra-axial and intradural neoplasm that invaded into the right ear canal and had histological features compatible with CM, which has been only reported in humans and dogs. Neoplastic cells expressed vimentin but were negative for glial fibrillary acidic protein, S100 and pancytokeratin. Immunohistochemistry revealed epithelial membrane antigen (EMA) expression in neoplastic cells. To the best of our knowledge, we provide the first evidence of EMA expression in feline meningioma.

Meningioma is the most common primary intracranial brain tumour in pet animals, accounting for approximately 60% and 50% of primary brain tumours in cats [1] and dogs [2,3], respectively. The tumour originates from meningothelial (arachnoid cap) cells, which line the arachnoid villi [4]. Macroscopically, feline meningiomas are usually described as extra-axial, well-defined, hard to solid, compressively growing, yellow to grey tumours that occur solitarily or as multiple masses, with broad attachment to the meninges [5]. Due to the comparatively outdated Histological Classification of Tumors of the Nervous System of Domestic Animals by Koestner et al [6], provided by the World Health Organization (WHO), veterinary pathologists usually rely on the human WHO Classification of Tumors of the Central Nervous System by Louis et al [7] for classification and grading of meningioma. The human WHO classification divides meningioma into three grades and 15 histological subtypes on the basis of mitotic count (MC), features of malignancy and/or their predominant histological appearance: (a) benign (grade I) meningiomas including meningothelial, fibrous (fibroblastic), microcystic, transitional, psammomatous, angiomatous (includes haemangioblastic and angioblastic), secretory, metaplastic and lymphoplasmacyte-rich histotypes; (b) atypical (grade II) meningiomas including clear cell and chordoid subtypes; and (c) anaplastic (grade III) meningiomas including rhabdoid and papillary subtypes [7,8]. The presence of brain invasion or a MC $\geq$4 per 10 high-power fields (HPFs) is sufficient for diagnosis of atypical meningiomas. In the absence of one of these two stand-alone criteria, three out of five histological criteria (sheeting, high cellularity, spontaneous necrosis, prominent nucleoli and small cells) are required for diagnosis [9]. Additionally, meningiomas with predominant clear cell or chordoid morphology are considered as atypical, due to their high recurrence rate, especially after subtotal resection [7]. A MC $\geq$20 per 10 HPFs or frank carcinomatous or sarcomatous histological features determine grade III meningiomas [9].

Histologically, feline meningioma is considered a relatively uniform tumour, which most commonly occurs as grade I. Fibrous, transitional and psammomatous meningiomas are the most common histological subtypes in the cat, while others are extremely rare [10–14]. Additionally, cholesterol clefts and a prominent fibrous stroma are frequent [5]. There is a lack of specific molecular markers for meningiomas in animals. Expression of vimentin, E-cadherin and CD34 are supportive of a diagnosis of canine meningioma [15]. Additionally, cytokeratin, glial fibrillary acidic protein (GFAP), β-catenin, glucose transporter I, S100, laminin, claudin-

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I, protein-gene-product 9.5 and progesterone receptor are expressed to variable degrees in canine and feline meningiomas [10–12,15]. Epithelial membrane antigen (EMA) is a commonly used marker for human meningiomas [16–19] and has been recently established as a marker for canine meningioma [20], but not yet in cats.

We now describe the first case of feline chordoid meningioma (CM) in a European wildcat (*Felis silvestris)* and present the first evidence of EMA expression in formalin-fixed, paraffin-embedded (FFPE) sections of feline meningioma, indicating this antigen as a potential immunohistochemical marker for feline meningioma.

A 5.5-year-old intact female European wildcat was found dead in a Swiss zoo and submitted for necropsy to the Institute of Veterinary Pathology, Bern. The cat had a history of emaciation and neurological signs including head tilting to the right, occasional nystagmus and ataxia of the hindlimbs. Clinical signs improved following administration of antibiotics, steroids and non-steroidal anti-inflammatory drugs but relapsed 2 months after initial onset. A second examination revealed a polypoid proliferation in the right external ear canal, which was punctured and aspirated under general anaesthesia. In May–Grünwald–Giemsa-stained cytological preparations, two distinct cell populations were observed: clusters of large polymorphic cells and spindloid cells with elongated processes. Both cell populations had marked anisokaryosis and anisocytosis with some having large nucleoli. These two populations were admixed with numerous macrophages and neutrophils. All cells were embedded in abundant, granular, reddish background material. The cytological preparation was initially interpreted as a poorly differentiated carcinoma with associated suppurative inflammation.

The cat died during the following night despite therapy and was sent for post-mortem examination. Necropsy revealed a gelatinous, beige, non-encapsulated, well-demarcated, extra-axial and intradural nodular mass (2.5 × 1.0 × 1.0 cm) with a smooth surface in the rostral fossa, right of the hypophysis and broadly attached to the dura (Fig. 1). The mass severely compressed and displaced the adjacent right parietal and temporal lobes resulting in a shift of the brain midline to the left. On the external surface of the right tympanic membrane, there was a red beige, mottled, firm, non-encapsulated mass (0.2 × 0.2 × 0.2 cm). A smooth, egg-shaped mass (2 × 1 × 1 cm) bulged from the oropharyngeal mucosa. No obvious connection between these three masses was found macroscopically. Other organs were macroscopically unremarkable.

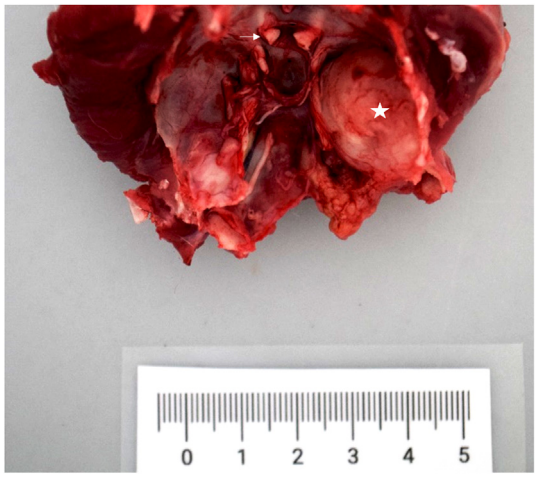

Fig. 1. Chordoid meningioma, brain, European wildcat. Red-beige, round, well-defined, gelatinous mass (asterisk) in right rostral fossa, attached with a broad base to dura mater on right of optic chiasm (arrow).

Samples from the masses, the entire brain and spinal cord were immediately fixed for 24 h in 4% neutral buffered formaldehyde and tissue slices were embedded in paraffin, cut at 4 μm and stained with haematoxylin and eosin (HE) for histological evaluation. Histological analysis of the mass located in the skull revealed a non-encapsulated proliferation of two morphologically very distinct neoplastic cell populations. The first consisted of polygonal cells that were arranged in loose chords and had broad intercellular contacts and a moderate amount of eosinophilic cytoplasm with distinct cell borders. Neoplastic cells had one to multiple, centrally located, large, round nuclei with coarsely stippled chromatin and up to four nucleoli (Fig. 2). The second population was composed of spindloid to stellate cells that were arranged in loose bundles and had eosinophilic cytoplasm with elongated cell processes and less distinct cell borders. Most of these cells had one elongated nucleus with finely stippled chromatin and up to two small nucleoli. Both cell populations were embedded in abundant Alcian blue-positive mucinous matrix (Fig. 3). Anisocytosis and anisokaryosis were prominent and six mitotic figures were detected per 10 HPFs. Multifocally, the tumour was disrupted by confluent areas of necrosis and clustered cholesterol clefts. Abundant neutrophils and fewer lymphocytes, occasionally grouped as lymph follicle-like structures, infiltrated the mass. A few swollen axons and mild gliosis in the adjacent right temporal lobe were compatible with chronic compression. The mass affecting the right tympanic membrane was composed of similar neoplastic cells that grew into the right internal and middle ear towards the external ear canal. The dermis of the canal was infiltrated by abundant lymphocytes.

The pharyngeal mass was diagnosed as granulomatous pharyngitis and concurrent myositis without evidence of invasion by neoplastic cells. No infectious agents were found using Ziehl–Neelsen and periodic–acid Schiff special stains.

Although the histological features were most compatible with a CM, chordoid meningioma, chondrosarcoma, myxoma/myxosarcoma, myxoid ependymoma and peripheral nerve sheath tumour (PNST) were considered as differential diagnoses. For further characterization of the tumour, immunohistochemistry with antibodies against vimentin, GFAP, cytokeratin, S100 and EMA was performed. Applied protocols and expected labelling properties of meningioma and differential diagnoses are presented in Supplementary Table 1. For evaluation of EMA expression, a recently published canine immunohistochemistry protocol [20] was optimized using four grade I feline fibrous meningioma cases as positive controls, which all labelled positive. As negative controls, the primary antibody was replaced by non-specific mouse or rabbit IgG at the corresponding protein concentration. All neoplastic cells had strong cytoplasmic expression of vimentin and

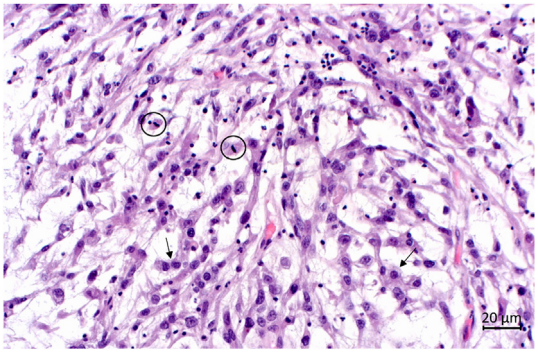

Fig. 2. Chordoid meningioma, brain, European wildcat. Cranial mass. Pleomorphic to epithelioid neoplastic cells with distinct cell borders have broad intercellular connections and are loosely arranged in short chains (arrows) embedded in abundant, pale mucinous matrix. Mitotic figures (circles). HE.

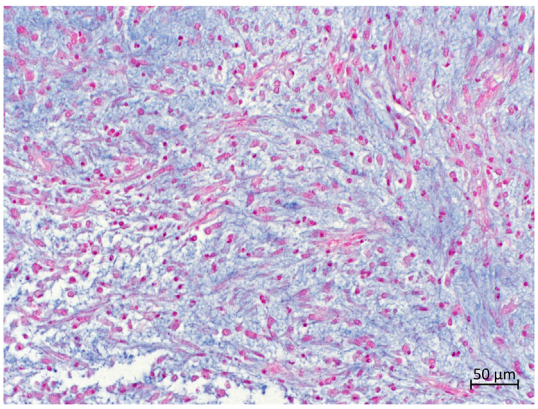

Fig. 3. Chordoid meningioma, brain, European wildcat. Abundant bluish extracellular material. Alcian blue.

approximately 70% of both neoplastic cell populations in both the intracranial and ear masses had weak to moderate membranous labelling for EMA (Fig. 4). Neoplastic cells were immunonegative for GFAP, S100 and pancytokeratin (Supplementary Table 1).

A final diagnosis of a CM with extracranial expansion into the right ear and concurrent otitis externa was made on the basis of macroscopic features, cellular morphology, the presence of an abundant mucinous matrix and the combined expression of vimentin and EMA. The MC >4 and confluent necrosis, together with aggressive, infiltrative growth, further supported a diagnosis of grade II (atypical) meningioma subtype.

In comparison with their histologically diverse canine counterparts [10,13], feline meningiomas are relatively uniform in histological appearance [10–14], and are benign; metastasis has not been described in this type of neoplasm in cats [21]. Despite the relative ease of surgical removal [14], feline meningiomas tend to recur [1,14]. The anatomical location and the broad contact of the present neoplasm with the dura is supportive of a meningioma diagnosis. However, this tumour had a gelatinous consistency rather than the typical firm macroscopic appearance [5]. Histologically, it was characterized by two distinct cell populations and abundant extracellular mucin, and lacked the usual hallmarks of feline meningioma (whorling, prominent fibrous component, psammoma bodies) [5]. In humans and dogs, subtypes with such prominent mucin formation are reported as CM [17–19,22] or myxoid meningioma (MM), a very rare variant of the benign grade I metaplastic subtype [22–25]. Neoplastic cells in CM are described as polyhedral to epithelioid cells arranged in chords, trabeculae or nests [17,18,24–27], which is consistent with our findings in this case (Fig. 3). Inflammatory infiltrates are common

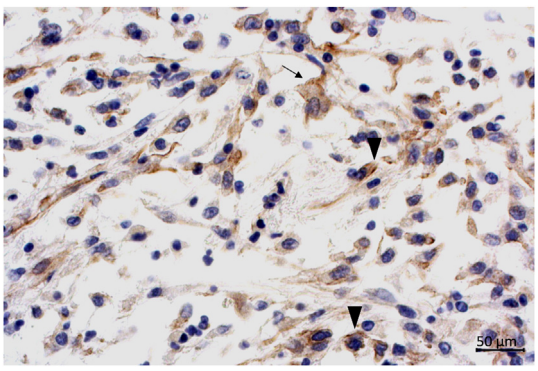

Fig. 4. Chordoid meningioma, brain, European wildcat. Cytoplasmic (arrow) and occasional membranous (arrowheads) immunolabelling of EMA in neoplastic cells. IHC.

in CM [17]. Histologically, differentiation from MM may be challenging [14,17,24,28]. The overall comparatively malignant appearing cellular morphology and aggressive growth, together with the presence of neoplastic cells arranged in cords, allowed a MM to be discounted. Reported canine CM cases have been graded inconsistently as benign, atypical or anaplastic depending on MC, and metastases have not been described [26,27]. According to the human WHO classification, the presented case is graded as atypical, as it meets the criteria of a moderate MC (4–19), prominent necrosis and aggressive growth. Other differential diagnoses for CM range from chordoma, myxoma, myxosarcoma, chondrosarcoma, myxopapillary ependymoma and PNST to metastatic carcinoma [17,19] (Supplementary Table 1). PNST was discounted as basal membranes were not detected on neoplastic cells using Gordon and Sweet's reticulin stain [4]. Fundamental to further differential diagnoses is the analysis of expressed marker proteins using immunohistochemistry (Supplementary Table 1). In this case, the positive reaction to EMA (Fig. 4) and vimentin, together with negative results for cytokeratin, S100 and GFAP, supports the diagnosis of a meningioma. EMA, also called Mucin 1, is a transmembrane glycoprotein expressed in many epithelial tissues at basal level [29]. In non-carcinomatous neoplasms, it is commonly used as a marker for human meningioma [16–19,29] and has only recently been shown to label canine meningiomas in a case series [20]. The labelling pattern of EMA is described as cytoplasmatic [30] or membranous [20]. Both patterns were present in the current case.

The aggressive growth towards the external ear in the present case is unusual for meningioma in cats and extracranial expansion has been reported only in few feline cases [31,32]. Expansion into the external ear has been described in only one dog [33]. There are rare reports of primary meningiomas in humans arising in the internal ear, middle ear, temporal bone or geniculate ganglion, which are proposed to have originated from displaced arachnoid cells and the arachnoid lining of the canal. These tumours might also have an intracranial portion [34]. Therefore, we cannot exclude the ear as the primary site of origin of this meningioma.

In conclusion, we have presented the first evidence of EMA expression in feline meningioma, which is widely used for diagnosis of meningioma and has been recently described in canine meningioma. Investigation of EMA expression in a large series of feline meningioma and other central nervous system tumours is required to demonstrate the specificity of this marker and to confirm its value as a diagnostic marker for feline meningioma.

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Declaration of competing interests

The authors declared no conflicts of interest in relation to the research, authorship or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2023.01.007.

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