DISEASE IN WILDLIFE OR EXOTIC SPECIES

Short Title: Mammary Neoplasia in a Ring-tailed Lemur

Benign Bilateral Adenomyoepithelioma of the Mammary Gland in a Ring-tailed Lemur (*Lemur catta*)

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Summary

Naturally occurring mammary tumours are uncommon in prosimians. A 20-year-old female ring-tailed lemur (*Lemur catta*) developed bilateral enlargement of the mammary glands. Surgical removal revealed that both masses were comprised of multiple nodules and cystic areas that entirely replaced the normal glands. Histologically, a benign neoplastic biphasic cellular proliferation, composed of luminal–epithelial and basal–myoepithelial components, was identified. Immunohistochemical analysis for expression of cytokeratin (CK) AE1/AE3, CK7, CK5+8, CK14, vimentin, p63 and 14-3-3σ highlighted the biphasic nature of the neoplasm. A low mitotic count, low Ki67 labelling index, expression of oestrogen receptor-α, lack of expression of human epidermal growth factor receptor and a 3-year disease-free period without recurrence supported the benign nature of the tumour. Macroscopically, histologically and immunohistochemically this neoplasm resembled benign adenomyoepithelioma of the breast in women. This is the first complete report of a naturally occurring mammary tumour in a ring-tailed lemur.

Keywords: adenomyoepithelioma; immunohistochemistry; *Lemur catta*; mammary tumour

Most species included in the suborder Prosimii are listed as critically endangered, endangered or vulnerable by the International Union for conservation of Nature and Natural Resources Red List of Threatened Species (http://www.iucnredlist.org). Ring-tailed lemurs (*Lemur catta*) are listed as an endangered species whose wild population is decreasing mainly due to habitat loss, drought and hunting. There are approximately 2,500 captive ring-tailed lemurs (http:// www.iucnredlist.org, accessed 15 March 2017). Naturally-occurring neoplasms in ring-tailed lemurs are described rarely and only as individual cases of a variety of tumours,

including osteochondroma, hepatocarcinoma, cholangiocarcinoma, mixed epithelial and stromal tumour of the kidney, T-cell-rich B-cell lymphoma, osteosarcoma, malignant fibrous histiocytoma and mammary carcinoma (Chang *et al.*, 1979; Wadsworth *et al.*, 1980; Pye *et al.*, 2000; Muller *et al.*, 2007; Remick *et al.*, 2009; Nemeth *et al.*, 2013; Hope *et al.*, 2015). To date, only two documented cases of mammary tumours have been published (Wadsworth *et al.*, 1980), but they lack a detailed histopathological description and follow-up and immunophenotyping of spontaneously arising mammary tumours in prosimians has not been reported previously.

Adenomyoepithelioma (AME) of the breast in women is a rare tumour described for the first time by Hamperl (1970). The tumour is composed of a biphasic benign neoplastic proliferation of luminal and myoepithelial cells. AME may display a heterogeneous pattern due to variable proportions of epithelial and myoepithelial cells with different architectural arrangements (Schmitt *et al.*, 2012). Three variants or patterns of AME are recognized, tubular, spindle cell and lobular (Schmitt *et al.*, 2012; Yoon and Chitale, 2013). Grossly, AME has been described as forming a round to lobulated, well-circumscribed and firm mass (Yoon and Chitale, 2013). Due to its benign behaviour, complete surgical excision alone is usually an adequate treatment (Nadelman *et al.*, 2006). However, malignant transformation of a specific component, either epithelial cells or myoepithelial cells, or both, has been documented (Yang *et al.*, 2014; Korolczuk *et al.*, 2016).

The present report provides a morphological and immunohistochemical description of a case of bilateral mammary AME in a ring-tailed lemur.

A 20-year old female ring-tailed lemur, kept in a local zoo, was presented to the Veterinary Teaching Hospital at the Veterinary College, University of Las Palmas de Gran Canaria (ULPGC), Spain, with a history of bilateral enlargement of the mammary glands.

The glands had been observed to be enlarged for at least 2 months prior to clinical consultation. At presentation, the animal was markedly overweight (3.1 kg; average adult mass 2.2 kg), but a general clinical examination, including radiographical evaluation of the thorax and abdomen, did not reveal any evidence of systemic disease. Haematological and serum biochemical analyses were unremarkable. Bilateral mastectomy was performed and the specimens were immediately submitted for pathological analysis to the Veterinary Anatomopathologic Diagnostic Service of the ULPGC. Gross examination of the left gland revealed a 40 g, lobulated, 11.0 × 5.0 × 10.0 cm mass, with multifocal ulceration of the overlying skin. Well-demarcated, variably-sized, up to 4.0 cm diameter, heterogeneous white to pale yellow nodules were present on the cut surface, replacing the mammary gland parenchyma (Fig. 1). Multifocally there were large (up to 2.0 cm diameter) cystic spaces containing pale yellow, translucent fluid. Within the deep subcutaneous fat, a lymph node was compressed by the tumour. The contralateral mammary gland was conical in shape, weighed 6.0 g and was expanded by a 5.0 × 2.7 × 5.0 cm mass, with no grossly visible signs of skin inflammation. On the cut surface, this second tumour filled the lumen of the mammary ducts with multifocal cystic areas (up to 0.5 cm diameter). Lesions represented 1.29% and 0.19% of the total body weight respectively.

Tissue samples were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE). Tumour cell immunophenotypes and steroid hormone receptors were identified using immunohistochemistry (IHC). Primary antibodies were specific for pan-cytokeratin (Dako, Agilent Technologies, Stockport, UK) and cytokeratin (CK) 5+8 (Euro-Diagnostica, Huntingdon, UK), CK7 (Dako), CK20 (Dako), CK14 (Leica Biosystems, Newcastle upon Tyne, UK), 14-3-3σ (Santa Cruz Biotechnology, Heidelberg, Germany), p63 (Leica Biosystems), vimentin (Dako), Ki67 (Dako), type α oestrogen (Dako) and progesterone

(Neomarkers Inc., Fremont, California, USA) receptors (ORα and PR) and C-erb-B2 (Dako). Antigen–antibody reactions were 'visualized' with the En Vision™ system (Dako) or the Bond Polymer Refine Detection kit (Leica Biosystems). Negative controls were prepared by replacing the primary antibody with normal rabbit serum diluted 1 in 100 in phosphate buffered saline. Qualitative analysis was performed for cytokeratins, vimentin, p63 and 14-3-3σ as described previously (Suarez-Bonnet *et al.*, 2010, 2011). Labelling for ORα and PR was evaluated and the sample was considered positive if at least 1% of tumour nuclei were positive. Human epidermal growth factor receptor (HER)-2 expression was defined as epithelial cell membrane labelling and scored per the guidelines of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) (Wolff *et al.*, 2007). The proliferation index was calculated by counting the nuclei positive for Ki67 in a total of 1,000 neoplastic cells.

Microscopically, both masses shared identical histopathological characteristics of well-demarcated, partially encapsulated, expansile, moderately cellular and multilobulated neoplasms. The neoplasms were composed of two cell populations arranged in tubules and papillary projections (Fig. 2). The first population were cuboidal to columnar luminal epithelial cells, with poorly-demarcated borders, moderate amounts of eosinophilic cytoplasm, an oval nucleus, in a central to basal position, and finely stippled chromatin with a single basophilic nucleolus. The second population consisted of polygonal, hypertrophic to spindle-shaped myoepithelial cells, with indistinct borders, moderate amounts of cytoplasm that varied from clear to intensely eosinophilic suggesting a 'myogenic' appearance. Nuclei were rounded with finely stippled chromatin and, rarely, a single basophilic nucleolus. There was minimal anisocytosis and anisokaryosis and mitotic figures were rare (2 in 20 high power fields; ×400). The epidermis overlying the large mass was focally ulcerated with necrosis and moderate to severe infiltration of neutrophils, restricted to the most superficial areas of the

tumour. The local lymph node seen grossly to be compressed by the tumour exhibited marked lymphoid hyperplasia, but the normal architecture was maintained.

Both cell populations expressed pan-CK with a more intense reaction in the myoepithelial cells. CK7 (Fig. 3) and CK5+8 were expressed only by the epithelial and myoepithelial cell components, respectively. CK20 was negative in both cell compartments. CK14, vimentin, 14-3-3σ (Fig. 4) and p63 proteins highlighted the myoepithelial cells with a cytoplasmic or nuclear labelling pattern. Ki67 was detected in the nuclei of 1% of the cells in both luminal and myoepithelial compartments. ORα was present in >90% of luminal epithelial cells. Incomplete membrane labelling of weak intensity (+1) was observed for HER-2. The pan-CK antibody did not detect epithelial cells within the reactive lymph node. The histological and immunohistochemical findings of the tumour in the present case fulfilled the diagnostic criteria for AME of the breast in women.

To the author's knowledge this is the first report describing in detail the gross, histological and immunohistochemical features of a spontaneously arising mammary tumour in a prosimian. In human breast pathology, AMEs are usually well-circumscribed, expansile masses, ranging from 0.3 to 7 cm and with a cut surface that exhibits a multinodular configuration and may contain areas of cystic change or haemorrhage (Nadelman *et al.*, 2006; Yoon and Chitale, 2013; Korolczuk *et al.*, 2016; Xu *et al.*, 2016; Jones and Fletcher, 2017). Additionally, bilateral benign AME has been described sporadically (Bajpai *et al.*, 2013). Similar features were present in this case, in which neoplastic nodules effaced the mammary gland and compressed a regional lymph node. Microscopically, this case closely resembles the tubular pattern of human AME, illustrated in several publications, which is the most common type in women and considered by some authors to be a variant of intraductal papilloma (Simpson *et al.*, 2004; Nadelman *et al.*, 2006; Yoon and Chitale, 2013; Xu *et al.*, 2016). This neoplasm can progress and become malignant (malignant AME or AME with

carcinoma); which includes those arising from the luminal epithelium (CLE), myoepithelium (CME) or both (EMC), but can also develop as malignant *de novo* (Jones and Fletcher, 2017). Although the histological criteria for malignancy in AME includes the presence of cellular atypia and pleomorphism, necrosis, an invasive growth pattern and a high mitotic count (>5 mitotic figures per 10 HPFs), there are several cases where a histologically benign AME has metastasized, notably to the lungs, where the metastases have shown the same benign appearance as the primary tumour (Nadelman *et al.*, 2006; Korolczuk *et al.*, 2016). In the current case, the tumour exhibited rare mitotic figures, mild anisokaryosis and anisocytosis and necrosis was associated exclusively with epidermal ulceration; most likely related to superficial trauma. The immunohistochemical profile described for human AME includes positivity of the luminal epithelium for pan-CK, CK7, CK8 and for CK5, CK14, vimentin, 14-3-3σ and p63 in the myoepithelium. The Ki67 index varies greatly between benign and malignant lesions ranging from 4 to 90% of positive nuclei (Nadelman *et al.*, 2006; Yoon and Chitale, 2013). PR and HER-2 are consistently negative while ORα-positive nuclei demonstrate weak expression (Yoon and Chitale, 2013; Yang *et al.*, 2014). In the present case, a very similar profile, with the only exception of more abundant ORα-positive luminal cells, was observed. Several other differential diagnoses are listed for AME in women, including malignant tumours. In fact, due to the heterogeneity of this entity, it is difficult with needle core biopsy samples to give a definitive diagnosis. Clear cell carcinomas and adenoid cystic carcinomas may be differentiated by immunolabelling the two cell compartments in the former; the latter has infiltrative borders and a characteristic cribriform histomorphology (Yoon and Chitale, 2013; Yang *et al.*, 2014). Pleomorphic adenomas can mimic AMEs, but these lack the hyaline matrix and the chondroid areas of metaplasia of the former (Schmitt *et al.*, 2012; Yoon and Chitale, 2013).

The benign nature of the AME in this lemur is supported by its histopathological characteristics and by its immunoprofile. 14-3-3σ is an oncoprotein which is expressed in myoepithelial cells in both normal mammary tissue and benign mammary tumours, but is overexpressed in cases of carcinoma (Simpson *et al.*, 2004). Here we show that only myoepithelial cells are positive, which is in accordance with previous studies in both human and canine mammary cancer (Simpson *et al.*, 2004; Suarez-Bonnet *et al.*, 2011). Naturally-occurring mammary tumours in the ring-tailed lemur are extremely rare and can exhibit benign or malignant features. In this case, 3 years following the surgery, the animal has shown no evidence of tumour recurrence and clinical examination is unremarkable. However, given that some 'benign' humans AMEs can develop late metastatic disease, long-term follow-up should be considered for mammary tumours in prosimians.

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Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the research and/or publication of this article.

References

Bajpai J, Punatar SB, Gupta A, Badwe R, Gupta S (2013) Bilateral adenomyoepithelioma of breast. *Journal of Cancer Research and Therapy*, **9**, 523-525.

Chang J, Wagner JL, Kornegay RW (1979) Spontaneous cholangiocarcinoma in a ring-tailed lemur (Lemur catta). *Laboratory Animal Science*, **29**, 374-376.

Hamperl H (1970) The myothelia (myoepithelial cells). Normal state; regressive changes; hyperplasia; tumors. *Current Topics in Pathology*, **53**, 161-220.

Hope KL, Boedeker NC, Gordon SS, Walsh TF (2015) Solitary osteochondroma in a ring-tailed lemur (*Lemur catta*). *Comparative Medicine*, **65**, 348-351.

Jones M, Fletcher J (2017) Malignant adenomyoepithelioma of the breast. *Pathology*.

Korolczuk A, Amarowicz M, Bak K, Korobowicz E, Koncewicz T (2016) Adenomyoepithelioma of the breast with late pulmonary metastases – case report and review of the literature. *Journal of Cardiothoracic Surgery*, **11**, 121.

Muller S, Oevermann A, Wenker C, Altermatt HJ, Robert N (2007) A mixed epithelial and stromal tumor of the kidney in a ringtail lemur (*Lemur catta*). *Veterinary Pathology*, **44**, 243-246.

Nadelman CM, Leslie KO, Fishbein MC (2006) 'Benign' metastasizing adenomyoepithelioma of the breast: a report of 2 cases. *Archives of Pathology and Laboratory Medicine*, **130**, 1349-1353.

Nemeth NM, Blas-Machado U, Cazzini P, Oguni J, Camus MS *et al.* (2013) Well-differentiated hepatocellular carcinoma in a ring-tailed lemur (*Lemur catta*). *Journal of Comparative Pathology*, **148**, 283-287.

Pye GW, Bennett RA, Terrell SP, Ginn PE, McSherry LJ *et al.* (2000). T-cell-rich B-cell lymphoma in a ring-tailed lemur (*Lemur catta*). *Journal of Zoo and Wildlife Medicine*, **31**, 388-393.

Remick AK, Van Wettere AJ, Williams CV (2009) Neoplasia in prosimians: case series from a captive prosimian population and literature review. *Veterinary Pathology*, **46**, 746-772.

Schmitt F, Tan PH, Dabbs D (2012) Myoepithelial and epithelial–myoepithelial lesions. In: *WHO Classification of Tumours of the Breast*, SR Lakhani, IO Ellis, SJ Schnitt, PH Tan, MJ van de Vijver, Eds., IARC Press, Lyon, pp. 120-123.

Simpson PT, Gale T, Reis-Filho JS, Jones C, Parry S *et al.* (2004) Distribution and significance of 14-3-3sigma, a novel myoepithelial marker, in normal, benign, and malignant breast tissue. *Journal of Pathology*, **202**, 274-285.

Suarez-Bonnet A, Herraez P, Martin de las Mulas J, Rodriguez F, Deniz JM *et al.* (2011) Expression of 14-3-3 sigma protein in normal and neoplastic canine mammary gland. *Veterinary Journal*, **190**, 345-351.

Suarez-Bonnet A, Martin de Las Mulas J, Millan MY, Herraez P, Rodriguez F *et al.* (2010) Morphological and immunohistochemical characterization of spontaneous mammary gland tumors in the guinea pig (*Cavia porcellus*). *Veterinary Pathology*, **47**, 298-305.

Wadsworth PF, Gopinath C, Jones DM (1980) Mammary neoplasia in ring-tailed lemurs (*Lemur catta*). *Veterinary Pathology*, **17**, 386-388.

Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC *et al.* (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Journal of Clinical Oncology*, **25**, 118-145.

Xu J, Tang X, Iida Y, Fuchinoue F, Kusumi T *et al.* (2016) Adenomyoepithelioma with carcinoma of the breast: a report of two cases and a review of the literature. *Pathology Research and Practice*, **212**, 130-134.

Yang Y, Wang Y, He J, Pan G, Tuo X *et al.* (2014) Malignant adenomyoepithelioma combined with adenoid cystic carcinoma of the breast: a case report and literature review. *Diagnostic Pathology*, **9**, 148.

Yoon JY, Chitale D (2013) Adenomyoepithelioma of the breast: a brief diagnostic review. *Archives of Pathology and Laboratory Medicine*, **137**, 725-729.

Figure Legends

Fig. 1. Gross features of a mammary adenomyoepithelioma in a ring-tailed lemur. The cut surface exhibits a multinodular, expansile appearance with cyst formation. Note a regional lymph node is compressed by the tumour (arrow).

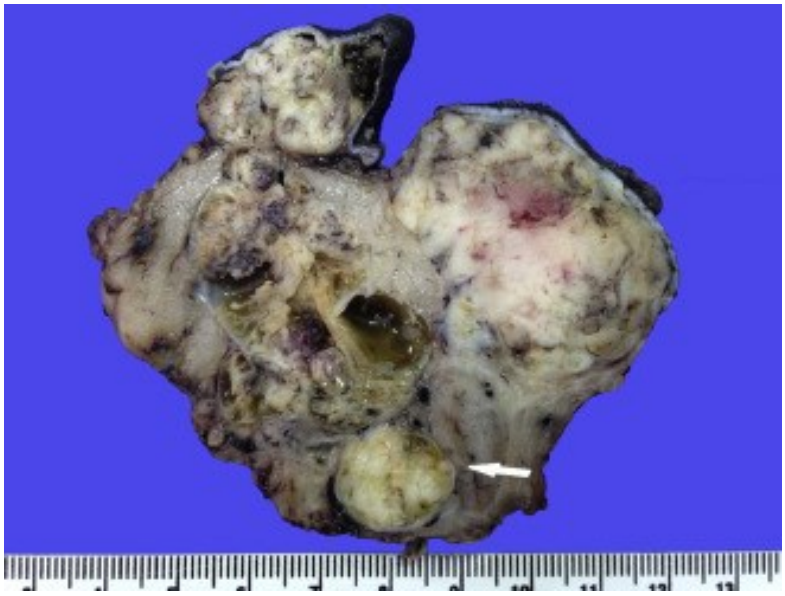

Fig. 2. Neoplastic cells are arranged in papillae (arrows) and tubules (thin arrows). HE. Bar, 200 μm.

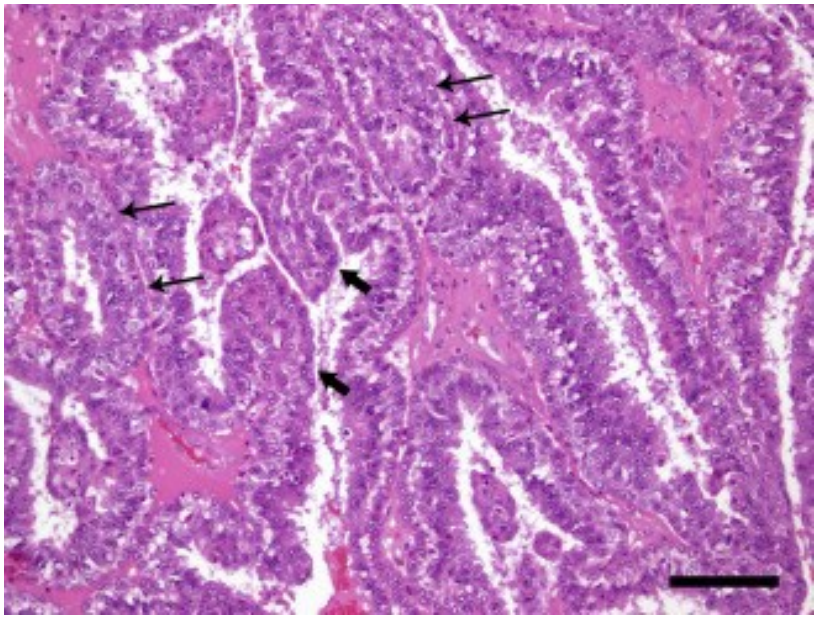

Fig. 3. Anti-CK7 antibody exclusively labels luminal epithelial cells. IHC. Bar, 200 μm.

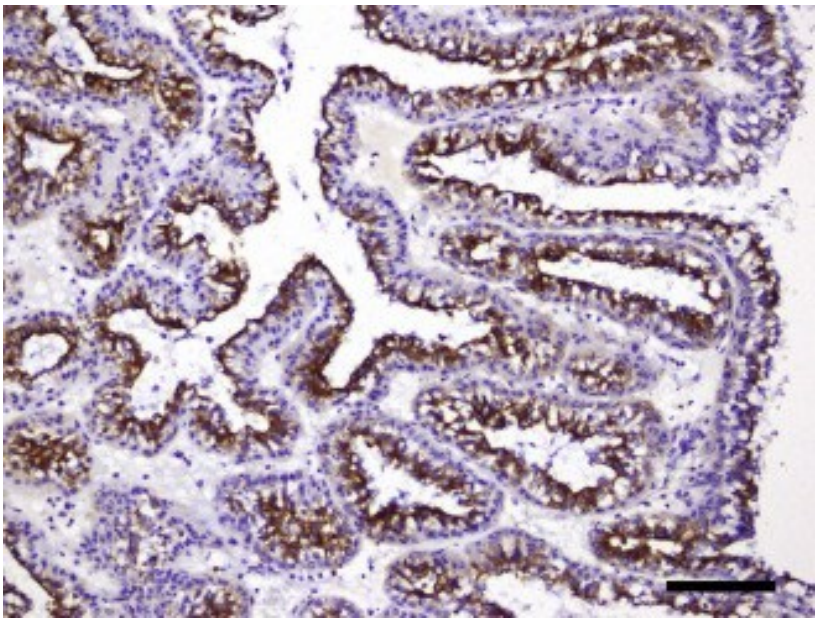

Fig. 4. Anti-14-3-3σ antibody exclusively labels the myoepithelial cells. IHC. Bar, 200 μm.