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Clinicopathological Study of Guinea Pig (*Cavia porcellus*) Mammary Gland Neoplasia

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List of Abbreviations

GP = Guinea pig(s)

IHC = Immunohistochemistry

HPO = Horseradish peroxidase

H&E = Haematoxylin and eosin

Introduction

Guinea pigs (GP) are a relatively common family pet. The UK houses 0.4 million (2017-2018) suggesting that 1% of households own this small mammal (Meredith, 2019). GP are the sixth most common animal seen in clinical practice (see Figure 1) and therefore veterinary practices need to be capable of dealing with challenges particular to GP's; these include restrictions to clinical examinations due to size and anatomy (Nielsen et al., 2019).

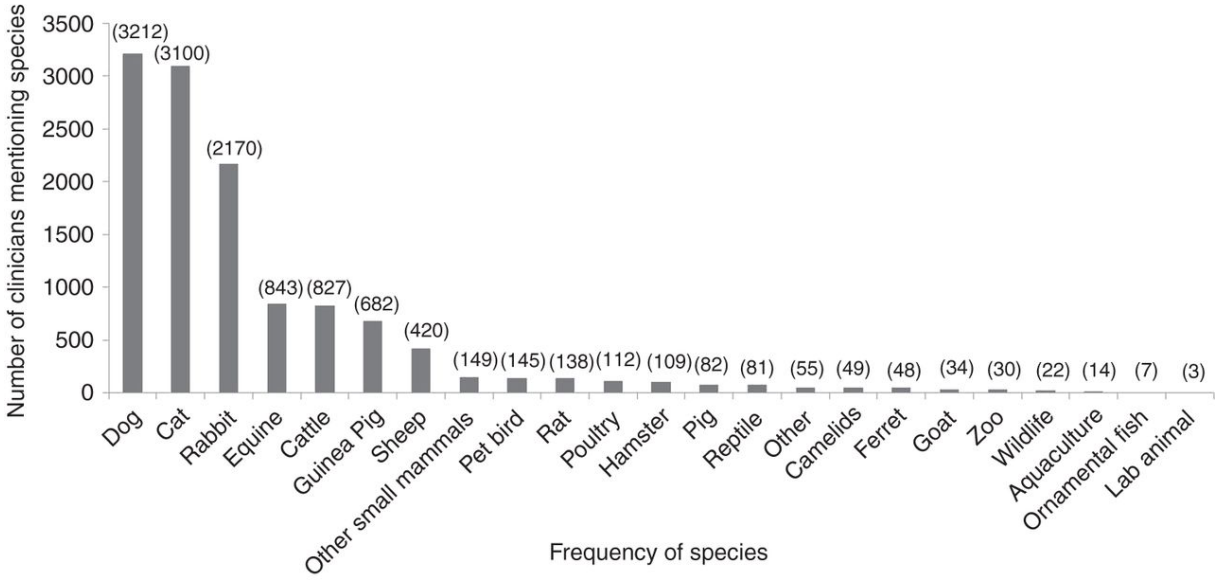


Figure 1. A UK study in 2014 detailing the frequency of different species treated in veterinary practices (Nielsen et al., 2019).

A retrospective Czech study (Minarikova et al., 2015) reviewed disease prevalence in over 1000 GP and only 8.1% were found free of disease. The study spanned a five and a half year period and included primary consultations, referral and routine check up appointments. GP disease often goes unnoticed by the owner which is supported by the relatively high disease prevalence found in this study. The three most common disorders were; dental disease (36.3%), skin disease (33.1%) and ovarian cysts (21.9% of the female population). It is clearly important for the practitioner to be aware of these common problems. GP have important physiological and anatomical differences to other mammals, including closely related members of the Rodentia family. Physiologically, the most important difference is the inability for endogenous vitamin-C synthesis while,

anatomically, dental formulation and development differs from Lagomorphs. Many studies previously detailing GP disease used laboratory counterparts rather than pet animals. Recently, however, research and studies are focusing their attention on the latter (Meredith, 2019). This has reduced the incidence of disorders such as hypovitaminosis-C through better understanding of proper nutrition. Moreover, with increasing knowledge of high fibre dietary requirements and regular dental check ups, the incidence of dental disease is likely to decrease with owners being able to prevent and manage these problems successfully at home.

Neoplasia has previously been reported as uncommon in GP (Barthold, Griffey and Percy, 2016), but with the increased popularity of GP as pets (Veiga-Parga, La Perle and Newman, 2016), the improved quality of care from owners and attendant improved longevity, neoplastic disorders are being seen more frequently (Andrews, 1975). More research geared towards spontaneous neoplastic conditions in GP may be fruitful as there is evidence that it might prove possible to reduce or prevent the clinical manifestations of neoplasia (e.g. through neutering).

There is disparity amongst authors as to which is the most common neoplasm in GP. Greenacre cites bronchogenic papillary adenomas followed by skin and subcutis tumours as the most frequent (Greenacre, 2004). However, according to Andrews et al. mammary adenocarcinomas are secondary to pulmonary adenomas as the most common epithelial tumours. Of cutaneous tumours trichofolliculomas are best known and seen most commonly in practice (Zwart et al., 1981).

Mammary Neoplasms in Guinea Pigs

Studies have been performed in both laboratory and pet animals, with discrepant results.

i. Laboratory Guinea Pigs

A number of studies have detailed neoplasms arising in laboratory GP with varied results. A range of mammary gland tumours has been reported viz, adenocarcinoma, papillary cystadenoma, cystadenoma, adenoma, fibroadenoma, fibrocystadenoma, carcinosarcoma, and liposarcoma) (Hoch-Ligeti et al., 1986).

Rogers and Blumenthal reported 14 GP with spontaneous tumours out of a colony of 4000 laboratory animals; however, none of these tumours were of mammary gland origin. In contrast, in the literature review by the same authors, 12 out of 138 GP had been reported to have mammary gland tumours, suggesting a prevalence of 8.5% (Rogers and Blumenthal, 1960) (Andrews, 1975).

Andrews performed necropsies on 14 GP in which 4 were found to have mammary gland neoplasia (28% prevalence).

Other studies of spontaneous tumours in laboratory GP, with classification details, are summarised in Table A (Appendix).

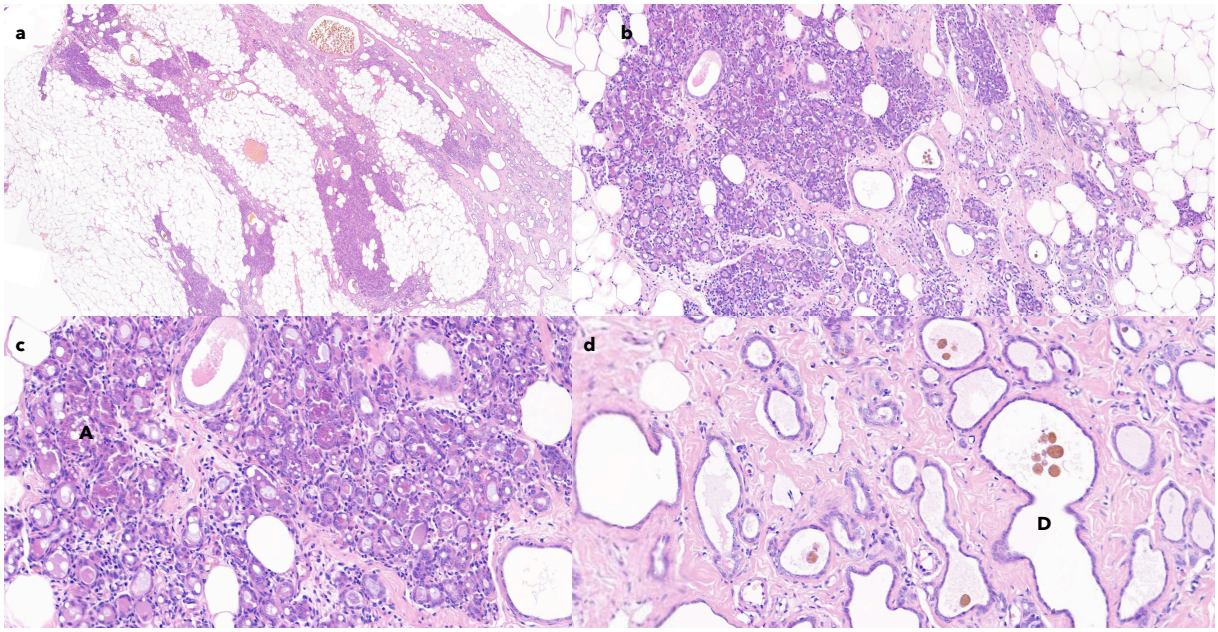
ii. Pet Guinea Pigs

There are few studies of GP tumours in clinical practice. Jelínek et al. evaluated 20 GP neoplasms in which 5 were mammary gland tumours (25%). The disease prevalence was similar to Kitchen et al.'s report of 14 spontaneous tumours in which 4 were found to be of mammary gland origin (29%) (Appendix, Table B).

Histology of the Guinea Pig Mammary Gland

GP have a single pair of mammary glands situated in the left and right inguinal region. In contrast to rabbits, both sexes have visible glands surrounded by a hairless region, although in males the glands are rudimentary. The glands do not have a communicating blood supply and therefore mammectomies are more straightforward. The glands are divided into small lobules, which have numerous small ducts opening into one larger duct connecting to the exterior (Bairbre O'Malley., 2005).

The mammary gland is a modified sweat gland consisting of tubulo-acinar glands and intralobular ducts. When the gland is active, secretory tissue is prominent whereas when inactive only the duct system is apparent. Interlobular ducts lined with bistratified columnar or cuboidal cells drain the lobules into the lactiferous ducts and lactiferous sinuses (teat sinus) at the teat base. The teat sinus leads to the teat canal which is continuous with the skin. The teat sinus has the same epithelium as the interlobular ducts whereas the teat canal is lined by stratified squamous epithelium (Bacha and Bacha, 2017). The ducts are alveoli are under the control of the sex steroids, oestrogen and progesterone (BENSON et al., 1957).



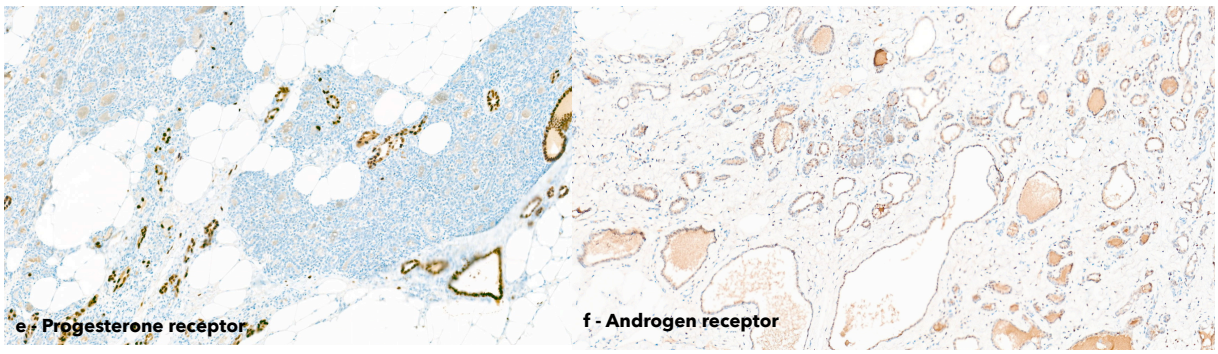


Figure 2. Normal mammary gland tissue stained with haematoxylin and eosin (H&E a-d); ducts (D) and alveoli (A). Immunoexpression of progesterone (e) and androgen receptors (f).

Literature Review

Mammary gland neoplasms are locally invasive, highly vascularised and rarely metastasise. If not completely excised recurrence can occur (Suárez-Bonnet et al., 2009) (Andrews, 1975).

Currently, there is no specific classification system of mammary gland neoplasia in GP. Suárez-Bonnet et al. cites using Misdorps histological classification of mammary gland neoplasia in dogs and cats as a diagnostic reference (see Figure 2). No other study discloses which classification scheme was used, if any. Only two of the included studies used immunohistochemistry (IHC). This morphological lack of standardisation in the diagnosis of mammary gland tumours necessitates a more systematic approach to the categorisation of these neoplasms. In addition, the application of IHC techniques may facilitate classification and understanding.

Histopathological Classification and Nomenclature of Tumours and Dysplasia of the Mammary Gland

1. **Carcinoma**
    1. **Adenocarcinoma**
        A. Tubular
        - Simple type [a]
        - Complex type [b]
        - Simple type
        - Complex type

        B. Papillary cystic
        - Simple type
        - Complex type
    2. **Solid carcinoma**
    - Simple type
    - Complex type
    3. **Spindle cell carcinoma**
    - Simple type

- Complex type

4. **Anaplastic carcinoma**
5. **Squamous cell carcinoma**
6. **Mucinous carcinoma**

2. **Sarcoma**
    1. **Osteosarcoma**
    2. **Fibrosarcoma**
    3. **Osteochondrosarcoma** (fibro-lipo-osteochondrosarcoma) (combined sarcoma)
    4. **Other sarcomas** (liposarcoma)

3. **Carcinosarcoma** (malignant mixed tumour)

4. **Benign or apparently benign tumours**
    1. **Adenoma**
    2. **Papilloma**
    3. **Fibroadenoma**
        A. Pericanalicular
        B. Intracanalicular
        - Non-cellular type
        - Cellular type
        C. Benign mixed tumour
        D. Total fibroadenomatous change
    4. **Benign soft-tissue tumour**

5. **Unclassified tumours**

6. **Benign or apparently benign dysplasias** [c]
    1. **Cyst**
        A. Non-papillary
        B. Papillary
    2. **Adenosis**
    3. **Regular typical epithelial proliferation in ducts or lobules**

4. **Duct ectasia**
5. **Fibrosclerosis**
6. **Gynaecomastia**
7. **Other non-neoplastic proliferative lesions**
    A. Non-inflammatory lobular hyperplasia
    B. Inflammatory lobular hyperplasia

[a] The term "simple" is applied to any type of neoplasm or proliferation composed of cells resembling either secretory epithelial cells or myoepithelial cells
[b] The term "complex" is applied to any type of neoplasm or proliferation composed of cells resembling both secretory epithelial cells and myoepithelial cells
[c] The term "dysplasia" is used as defined in the WHO classification of human breast tumours and not in the sense of certain disorderly proliferations together with a certain degree of cytonuclear atypia

Figure 3. Misdorp's classification of mammary gland neoplasia in dog and cat (Misdorp, ELSE and Hellman, 1999). Highlighted in red are those tumours that have been documented in GP.

Moreover, the distinction between mammary gland tumours and those arising from skin adnexae has not been addressed. The mammary gland is embryonically closely related to the sweat gland, and Allison and Moeller suggested that neoplasms derived from these tissues may readily be confused. Allison and Moeller reported a multilineage adnexal tumour with sebaceous and apocrine differentiation arising in the mammary region. The tumour was difficult to categorise and had features of a complex mammary gland tumour. However as sebaceous glands are not normally found in mammary tissue it was not classified as a mammary gland tumour. Literature reports seldom discuss the normal histology of the mammary gland. This lack of a precise histological understanding of the GP mammary gland further undermines the accurate classification of neoplasms.

Whilst there are reports of spontaneous tumours arising from GP as young as 6 months, most tumours arise in animals over the age of 3 years old (nearly one third over the age of three years will develop a tumour) (Kitchen, Carlton and Bickford, 1975) (Hoch-Ligeti et

al., 1986) (Rogers and Blumenthal, 1960). Previous studies investigated laboratory GP in which the animals were euthanised, often at a young age, and this may account for an observed low incidence of neoplasia (Andrews, 1975).

Interestingly the incidence of mammary gland tumours is higher in males in GP than in other mammals (Andrews, 1975) (Saba et al., 2007). The distribution between sexes is almost equal (Andrews, 1975).

The largest single study of pet GP mammary gland neoplasia to date investigated 10 spontaneous mammary gland tumours and included IHC assessment. 3 out of the 10 tumours were benign (2 simple adenomas and 1 benign mixed tumour) and 7 were malignant (1 simple solid carcinoma and 6 simple tubulopapillary carcinomas). The carcinomas were distinguished from the benign tumours on the basis of possessing at least one of the following features; atypia of the epithelia, an infiltrative growth pattern, high mitotic activity and high cellularity and/ or necrosis. The results of the IHC analysis suggested that sex steroid receptor stimulation may be relevant in the development of the neoplasms (Suárez-Bonnet et al., 2009). Coupled with the unique sex distribution of mammary gland tumours in GP, this may be indicative of an aetiological role in tumour development. While oestrogen and progesterone markers have been analysed, thus far, the expression of the androgen receptor has not been investigated. Biopsies are not usually sent for IHC since it incurs further costs for the owners. Consequently, there is a paucity of data regarding the immunophenotype of spontaneous mammary gland neoplasia in GP.

Tables A-D (Apprendix) list all cases of GP mammary neoplasms in the English veterinary literature; adenocarcinoma is the most frequently reported neoplasm. The mean animal age ranges from 2.3 to 5 years old. While it is understood that sex prevalence is almost equal between female and male GP it seemingly varies greatly between studies: sex prevalence of males affected with mammary neoplasms fluctuates between 16-75% (Suárez-Bonnet et al., 2009) (Andrews, 1975) (Jelínek, 2003).

While there is a generally fair understanding of GP neoplasia, it is evident there is a lack of standardisation pertaining to classification, nomenclature and the disparity between studies using laboratory and pet animals.

Immunohistochemistry

Immunohistochemistry is a laboratory technique that utilises protein antigen expression of target cells for a wide range of purposes, including cell identification. A powerful diagnostic and informative tool there are few publications with immunohistochemical examinations of GP neoplasia (Suárez-Bonnet et al., 2009) (Jelínek, 2003) .

Technical aspects:

The primary paraffin-embedded tissue on the microscope slide is treated carefully with an antibody specific for a known target protein. After binding to the protein, excess antibodies are washed away and a secondary additional antibody, conjugated with the enzyme horseradish peroxidase (HPO), is added to bind to the primary antibody. Finally, the chromogen 3, 3" diaminobenzidine is added, which in the context of antibody binding to its target protein, is transformed by HPO into a brown precipitate that is visualised on light microscopy, highlighting the location of the target protein (Proteinatlas.org, 2019) (see Figure 3).

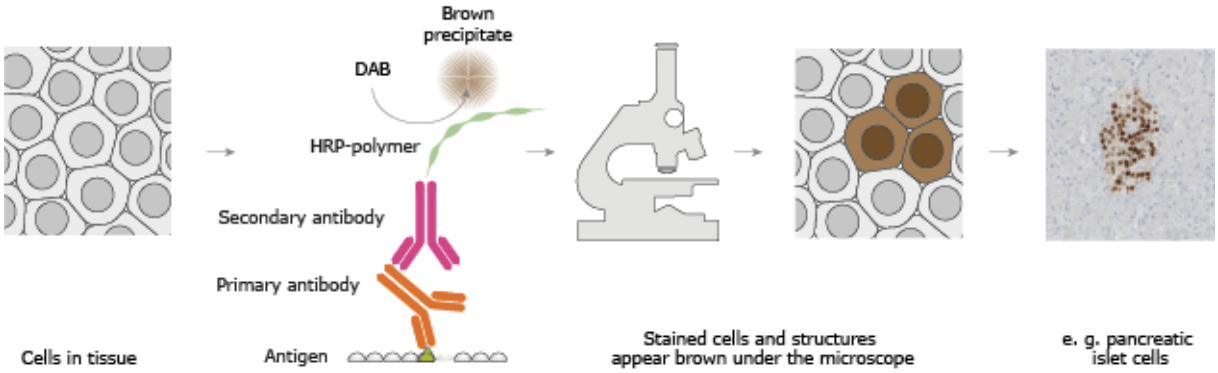


Figure 4. Classical method of IHC (Proteinatlas.org, 2019)

There are a variety of antigen retrieval methods such as enzymatic antigen retrieval, HIER (heat-induced epitope retrieval) and others, which serve to enhance the detection of the target protein.

IHC was developed in the 1930s but it was not until 1942 when the first study surfaced detailing its uses in microbiology and medicine (Sciences et al., 2019) (Durajyan et al., 2012). Since then, the technique has seen widespread adoption in diagnostic surgical pathology. Particularly within the human medical field, the diagnosis and classification of neoplasms is predicated, not only by morphology as seen on routine HE stained slides, but also upon the immunophenotype as characterised by numerous studies reporting panels of antibody applications over many years and reported in the medical literature. Thus, this technique has the potential to increase our understanding of tumours arising in veterinary practice, to derive more stringent diagnostic criteria for these tumours and therefore to contribute to a robust oncology classification in animal pathology. A basis for therapeutic intervention is an additional potential future benefit. Whilst a powerful diagnostic and informative tool there are few publications with immunohistochemical examinations of GP neoplasia (Suárez-Bonnet et al., 2009). These are listed in Tables B_4 and B_5 (Appendix).

Materials and Methods

Aims

To characterise the clinical and pathological features of GP mammary gland neoplasms arising spontaneously in pets.

Case selection

A retrospective search in the pathology archives of Professor János Gál was undertaken for cases labelled as GP tumours. These were resected from GP attending animal clinics of the University of Veterinary Medicine, Budapest and from a private clinic Talpas Állatorvosi Rendelő, Budapest. The biopsies were obtained by surgical excision between the years 2015-2019.

Clinical data

Clinical data recorded included age, sex, neutering status, presenting complaint, operative findings and details, and follow up/ outcome.

Pathology analysis

The gross appearance of the mass and relationship to adjacent tissues were recorded from prosection notes. Each tumour had a single H&E microscope slide prepared following inspection and prosection of the gross tumour. The stained slide was reviewed and the histopathological details recorded. Mitotic index was calculated by counting mitoses in random microscopic fields of tumour and the result expressed per square mm. Immunohistochemistry was performed, according to the protocols below, on each tumour, the panel determined by the morphology of the tumour and the favoured diagnosis. The antibodies used and specific technical methods are listed in Table 1. The resultant stained slides were studied and a semi-quantitive scoring system was used as follows:

No staining - 0
1-25%* 1
26-50%* 2

51-75%* 3

>75%* 4

* Of tumour cell population

Intensity of staining was not assessed as not all immunostains were performed in the same batch.

Table 1: Summary of Sources, Antigen Retrieval Methods, and Dilutions of Antibodies Used

Antibody		Source	Clone	Conditions
EMA	Epithelial membrane antigen - mouse anti-human monoclonal antibody	Leica biosystem	GP 1-4	HIER 20' with ER1 (low pH epitope retrieval solution RTU
Cerb-B2 - Her2	PATHWAY anti-HER-2/ neu (4B5) - rabbit monoclonal primary antibody	Roche: Ventana	4B5	HIER mild with cell conditioning 1 (high pH) RTU
PR	Confirm anti-progesterone receptor - rabbit monoclonal antibody	Roche: Ventana	4B5	HIER standard with cell conditioning 1 (high pH) RTU
ER	Confirm anti-oestrogen receptor - rabbit monoclonal antibody	Roche: Ventana	SP1	HIER standard with cell conditioning 1 (high pH) RTU
AR	Anti-androgen receptor - rabbit monoclonal primary antibody	Cell MARQUE	SP107	HIER with cell conditioning 1 for 32' (high pH), pre-primary peroxidase inhibition RTU
Ep-CAM	Epithelial specific antigen - mouse monoclonal antibody	Cell MARQUE	Ber-Ep4	HIER mild with cell conditioning 1 (high pH) RTU
TTF-1	Anti-thyroid transcription factor 1 - rabbit monoclonal primary antibody	Roche: Ventana	SP141	HIER with cell conditioning 1 for 48' (high pH) RTU

RTU = ready to use antibody

Histological sample preparation

1. The sample was placed in formalin (10% neutral buffered) after excision to prevent putrefaction and tissue disintegration
2. Once ready for histological preparation the sample was removed from the formalin and cut in half using a scalpel blade
3. A section no longer than the breadth of a 5 pence coin was cut from the edge of the two halves. If too thin, the section risks being washed away during the processing while if it were too thick, the tissue sample may be damaged upon being closed in the cassette
4. The sections (representing the centre of the tumour) are then placed in a cassette (appropriately labelled for GP identification)
5. The tissue sample was then processed (find out which machine they use) using the following steps
    1. Dehydration: the sample was immersed in increasing concentrations of alcohol to remove formalin and water
    2. Clearing: xylene was added which removes the alcohol and also allows the paraffin to infiltrate the sample
    3. Embedding: molten paraffin wax surrounded the sample and formed a “paraffin block”. The block provides structural support to the tissue sample and allows thin sectioning
6. The block was chilled using ice cubes for 10 minutes prior to being sectioned to make the tissue not drag while being cut and therefore make the sections more uniform and of a higher quality for analysis
7. A microtome was used to cut 4 micrometer thick sections
8. The tissue ribbons were transferred to a warm water bath to remove some of the paraffin
9. A glass slide was placed underneath the tissue ribbon in the water bath and lifted so that the tissue section holds to the slide
10. The slide was dried for a few hours at 37 degrees which allows any excess paraffin wax to melt
11. The slide was labelled appropriately for GP identification

Staining and IHC

1. The native slides are all stained with H&E
2. After staining a cover slip is mounted and fixed using optical grade glue

All H&E and immunohistochemically stained slides were reviewed by LR and JG.

Results

Table 2: Details of the 7 Cases of Mammary Tumours in Guinea Pigs

Case	Sex/ Neutered	Age (years)	Presentation in the clinic	Follow up	Diagnosis
1	Male/ neutered	2	Mass noted by the owner	NA	Tubulopapillary adenocarcinoma
2	Male	9	October 2017: mass noted by the owner (right mammary gland, general status was good) March 2018: recurrence	NA	Tubulopapillary adenocarcinoma
3	Male	7	Mass noted by the owner	NA	Tubulopapillary adenocarcinoma
4	Male	7	NA	NA	Tubulopapillary adenocarcinoma
5	Male	1	Mass noted by the owner	Not seen since	Carcinosarcoma
6	Male	6	February 2016 mass noted by the owner October 2017: recurrence	NA	Solid grade adenocarcinoma
7	Male	4	Mass noted by the owner	NA	Malignant mixed mammary gland tumour

Table 3: Immunohistochemical Results of Neoplastic Mammary Tumours in Guinea Pig, including Mitotic Index

Case	Size of tumour	Mitotic index	Immunohistochemistry				
			EMA	Cerb-B2	PR	ER	AR
1	Grape sized	7	0	3	4	0	4
2	Not available	2	0	3	4	0	3
3	Not available	1	0	1	4	0	3
4	Plum sized	1	0	3	4	0	3
5	Not available	2	0	1	2	0	2
6	Not available	2	0	0	3	0	0
7	Quail-egg sized	Not available	0	0	4	0	3

Case 1

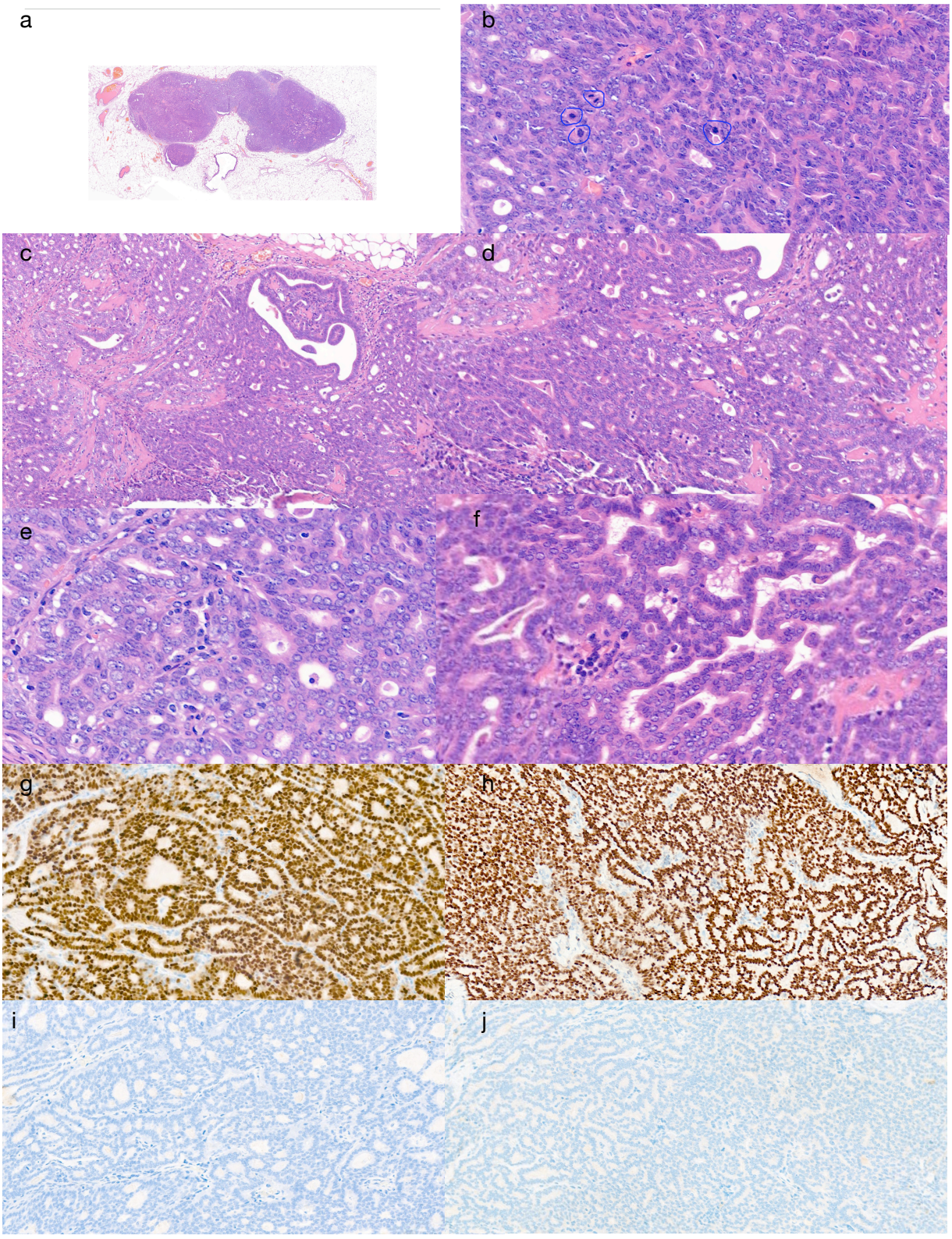


Figure 5. Tubullopapillary adenocarcinoma. Cuboidal-low columnar epithelial cells, with numerous ductal lumina; sometimes cribriform/low papillary. Simple H&E staining (a-f).

Strong progesterone (g) and androgen (h) expression. Negative expression of oestrogen (i) and EMA (j) receptors.

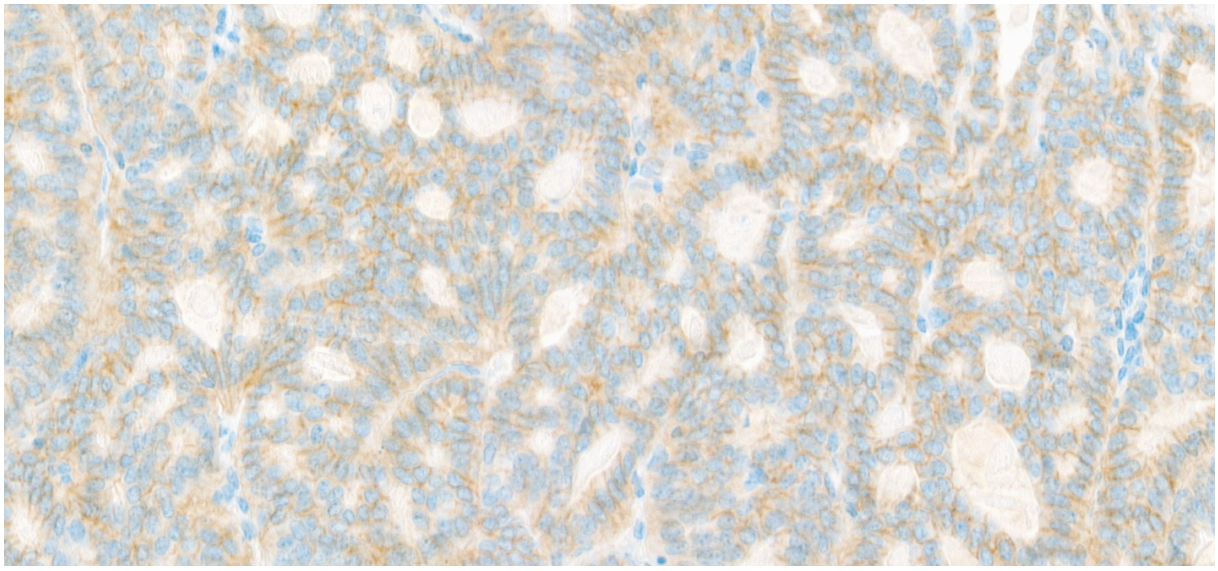
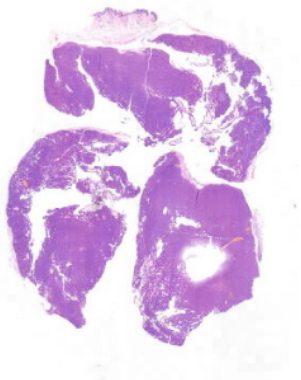


Figure 6. Slight expression of C-erB2 receptor.

Case 1 (Figures 5 and 6) was a multifocal tumour located in subcutaneous fat formed of solid epithelial basaloid cells at the edge of which were dilated ducts. The cells were of a single population, and showed mild pleomorphism and a variable chromatin pattern. The tumour diffusely and strongly expressed progesterone and androgen receptor, but was negative for oestrogen and EMA receptors. C-erB2 was focally and weakly expressed on the cell membrane. Based on the architecture and pathological features of the mass it was diagnosed as a tubulopapillary adenocarcinoma. The mitotic index was 7.

Case 2

a



b

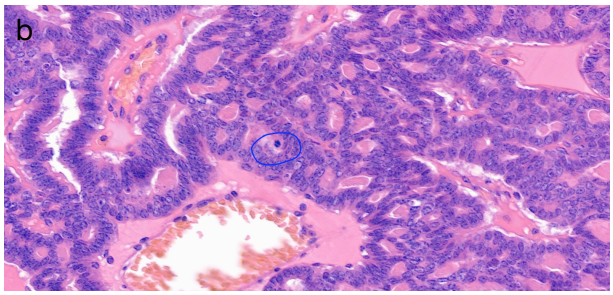


Figure 7. Simple H&E staining, mitoses circled (b). The images are qualitatively identical to the features described in Case 1 but there was conspicuous papillary and tubulopapillary architecture in this case.

Case 2 (Figure 7) was a solid basaloid tumour within the subcutaneous fat. Progesterone receptor was strongly and diffusely expressed as was androgen receptor. EMA and oestrogen receptor were negative. CerbB2 was largely negative but, weak focal membranous expression is suggested. This tumour was diagnosed as a tubulopapillary adenocarcinoma. The mitotic index was 2.

Case 3

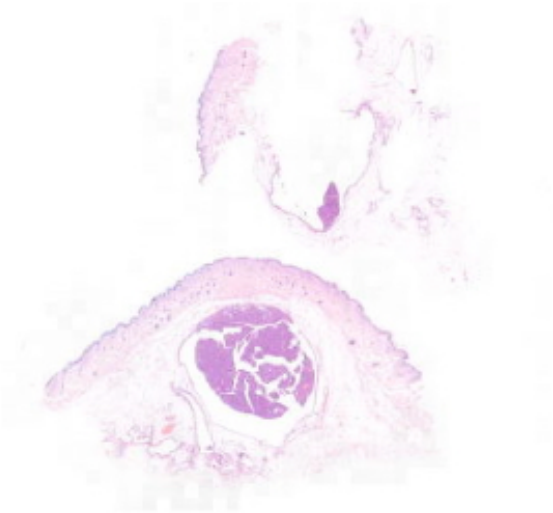


Figure 8. Basaloid epithelial tumour, located within a cystic space in the subcutis which otherwise presented the same morphological features as the previous cases with the addition of multiple papillary proliferations within adjacent dilated ducts.

Progesterone receptor again was strongly expressed with patchy moderate androgen positivity. Oestrogen receptor was negative. CerbB2 was moderately expressed. This tumour was diagnosed as a tubulopapillary adenocarcinoma. The mitotic index was 1.

Case 4

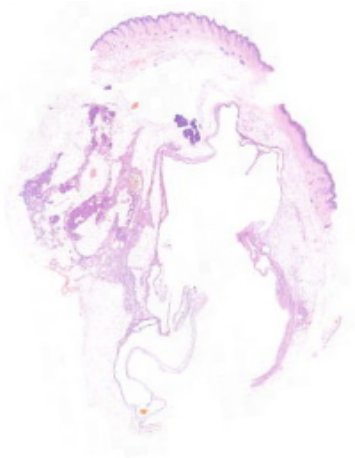


Figure 9. Small basaloid tumour with a tubulopapillary architecture formed of the same mildly pleomorphic cuboidal/columnar cells as previous cases.

Case 4 uniformly and strongly expressed progesterone receptor and moderately androgen receptor. EMA and oestrogen receptor were negative while there was a moderate CerB2 expression. This tumour was diagnosed as a tubulopapillary adenocarcinoma. The mitotic index was 1.

Case 5

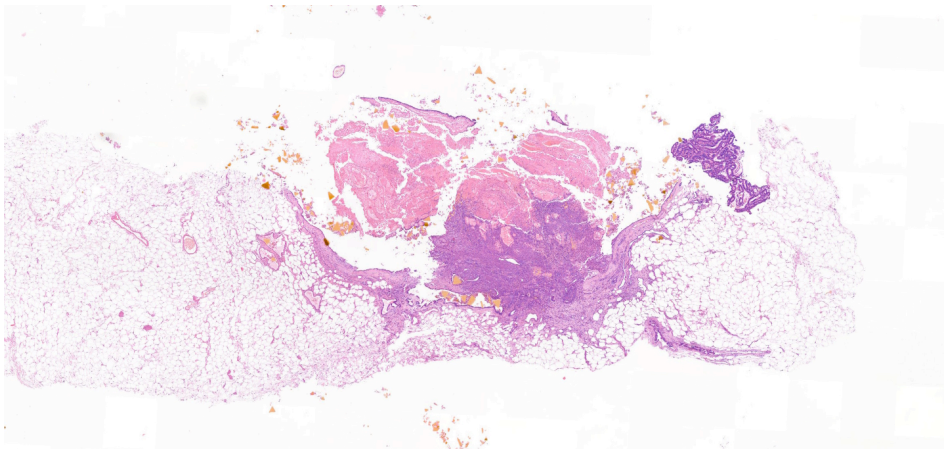


Figure 10. Case 5.

Case 5 differed from the previous four: arising in the subcutaneous fat the tumour had distinct morphological components; the first consisted of tubulopapillary architecture lined by mildly atypical columnar epithelial cells, the second consisted of a dense spindled

cell proliferation. Adjacent and admixed with this proliferation of atypical spindle cells were variably sized ductular structures. The tumour was androgen receptor positive, and, the spindle cell component was focally expresses this receptor. Progesterone receptor was strongly expressed by the epithelial component whilst CerbB2, oestrogen receptor and EMA were negative. This tumour was diagnosed as a carcinosarcoma. The mitotic index was 2.

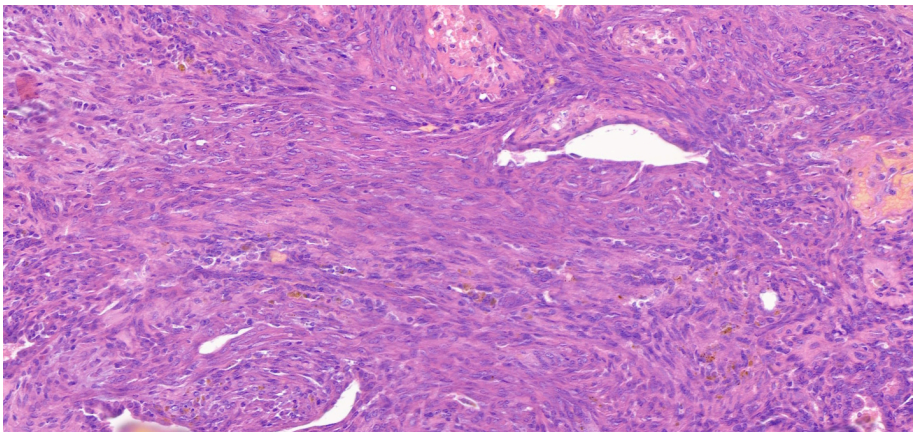


Figure 11. Spindle cell proliferation.

Case 6

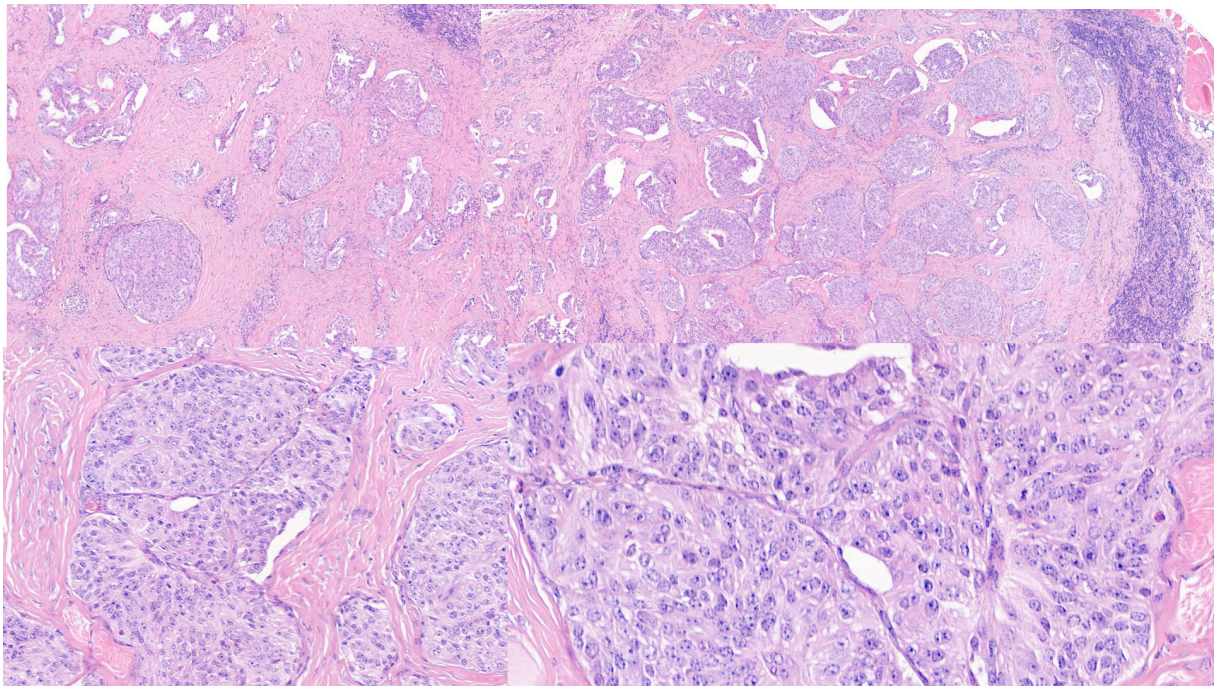


Figure 11. An epithelial tumour within a fibrotic stroma.

The tumour included solid tumour islands but also ducts, and there was marked cytological pleomorphism. There was moderately strong progesterone receptor expression in much of the tumour. Androgen receptor expression was largely negative. EMA, C-erbB2 and oestrogen receptor was negative. This tumour was diagnosed as a solid grade adenocarcinoma. The mitotic index was 2.

Case 7

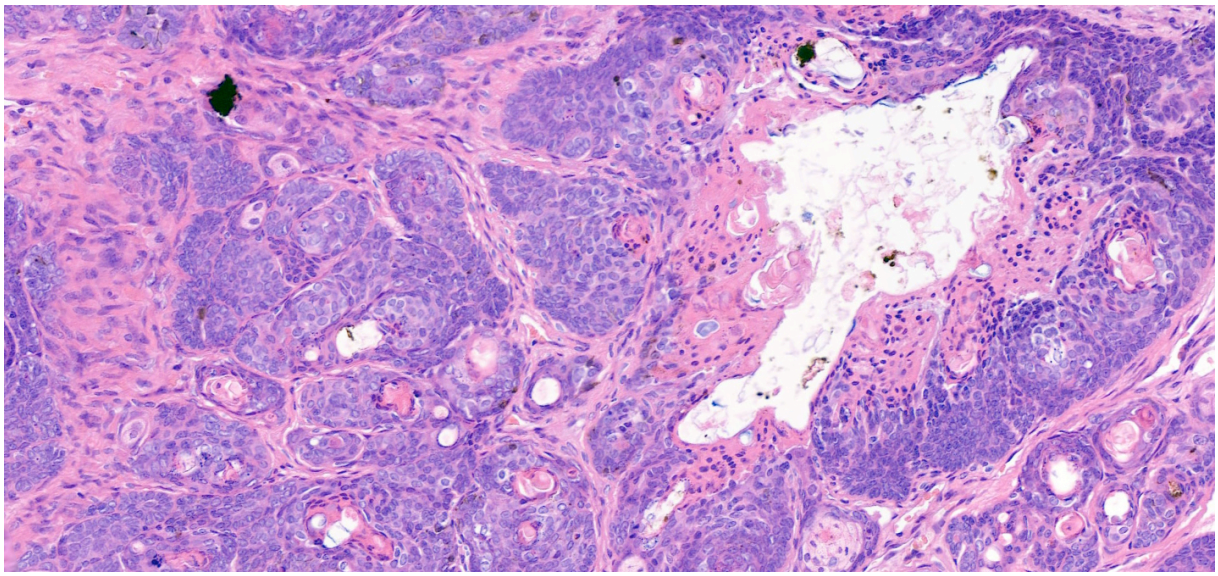


Figure 12. Complex mixed mammary gland tumour.

Case 7 tumour comprised of many cell types. Androgen receptor expression was strongly positive (Figure 13). EMA and oestrogen receptor was negative. The mitotic index was not available for this tumour.

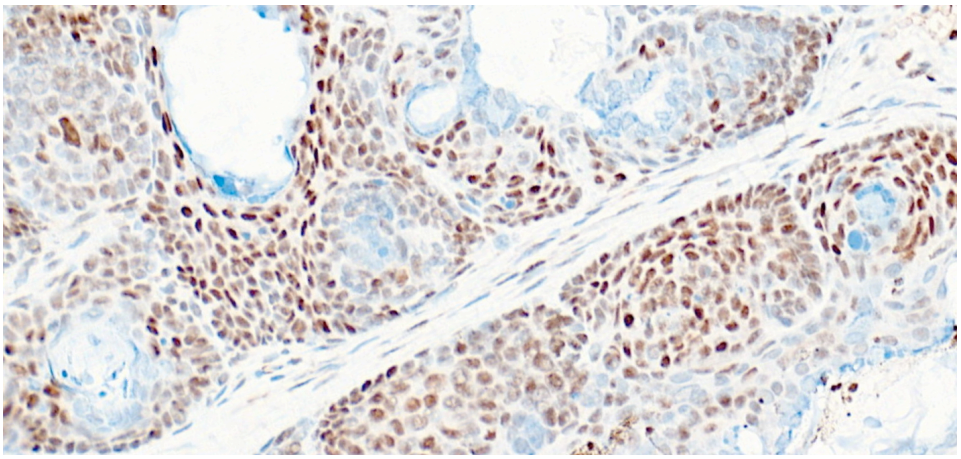


Figure 13. Strong androgen receptor expression in Case 7.

Case 8

Case 8 was rejected as it was found to be a thyroid tumour which is demonstrated by the positive expression of TTF-1 receptor (Figure 14).

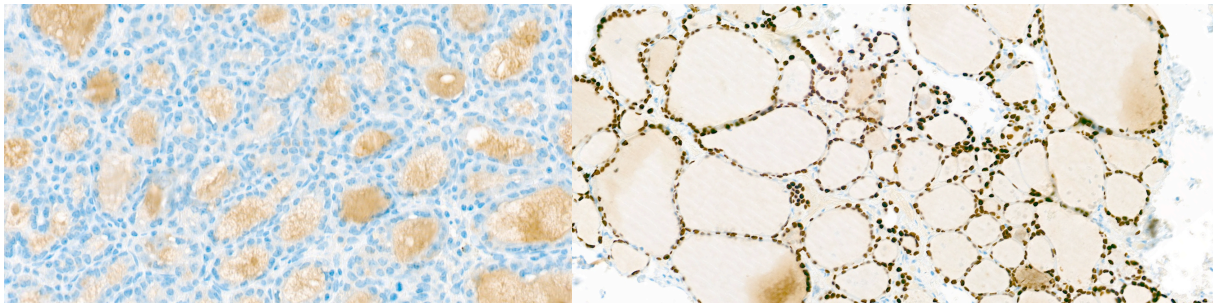


Figure 14. Positive expression of TTF-1 receptor.

Discussion

Seven GP tumours have been described in this study. The mean animal age was 5.14 years old which is consistent with the literature stating that most tumours arise in GP over the age of 3. All tumours were from male GP which is unique to this study; previous studies found an almost equal sex distribution.

Few clinical details were available and follow up information was limited. Thus, few conclusions can be drawn regarding the modes of presentation, effects of neutering and on the prognoses of the tumours.

There was a lack of gross pathological data. Tumour sizes had been recorded using comparisons to objects rather than measurements and the appearance macroscopically was not detailed. No conclusions therefore could be made as to specific neoplasms and their characteristic macroscopic pathological features.

Five of the seven cases were categorised as adenocarcinomas. Four of the adenocarcinomas showed a tubulopapillary conformation with varying degrees of solid foci. The fifth adenocarcinoma was diagnosed as a solid grade adenocarcinoma. This is consistent with the literature which states that malignancies of the GP mammary gland most commonly are a form of adenocarcinoma, typically with a tubulopapillary architecture, albeit often with solid foci.

All seven tumours were classified as malignant. Among the malignancy criteria, those with particular cytological interest were; pleomorphism, hypercellularity (Figure 15), nucleus/cytoplasmic ratio (Figure 16), mitoses/ mitotic index (Figure 17), variable nucleolar size (Figure 15), shape and quantity, vacuolisation, nuclear membrane alterations (Yildirim and Gurel, 2012). Cases 1-4 showed mild cytological pleomorphism, in Case 5 this was more pronounced (Figure 12).

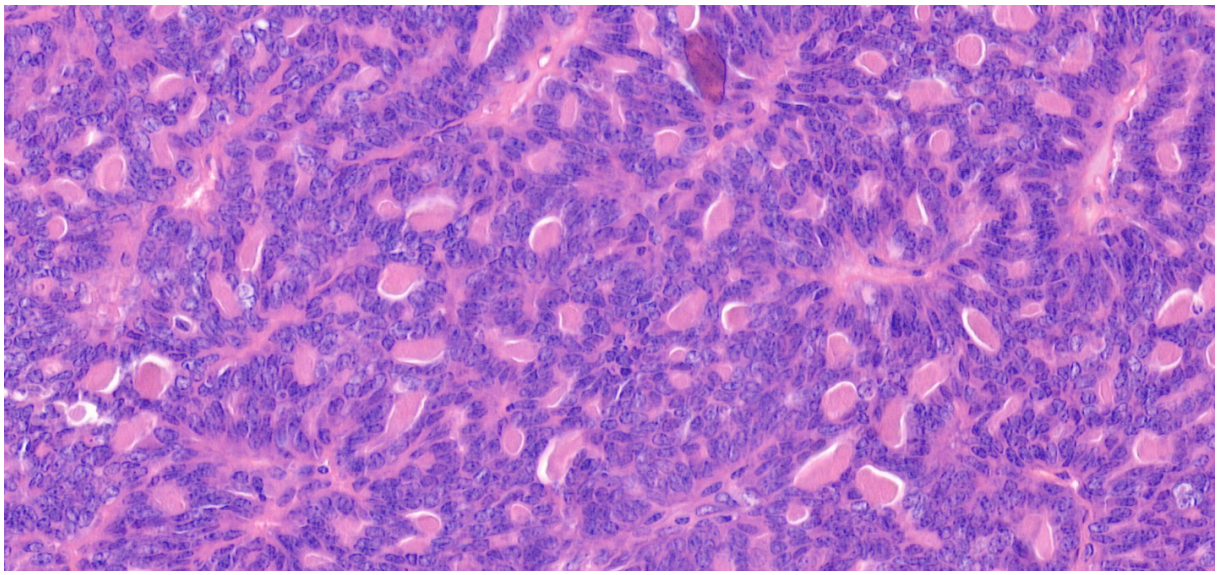


Figure 15. Hypercellularity and variable nuclear sizes evident in Case 2.

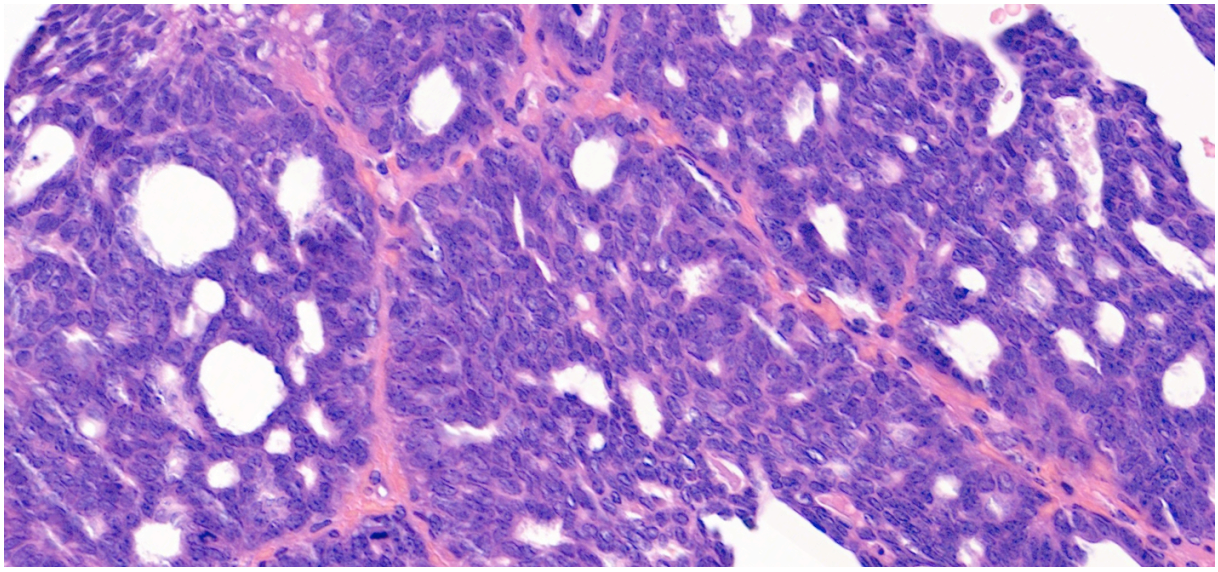


Figure 16. Increased nuclear/ cytoplasmic ratio in Case 4.

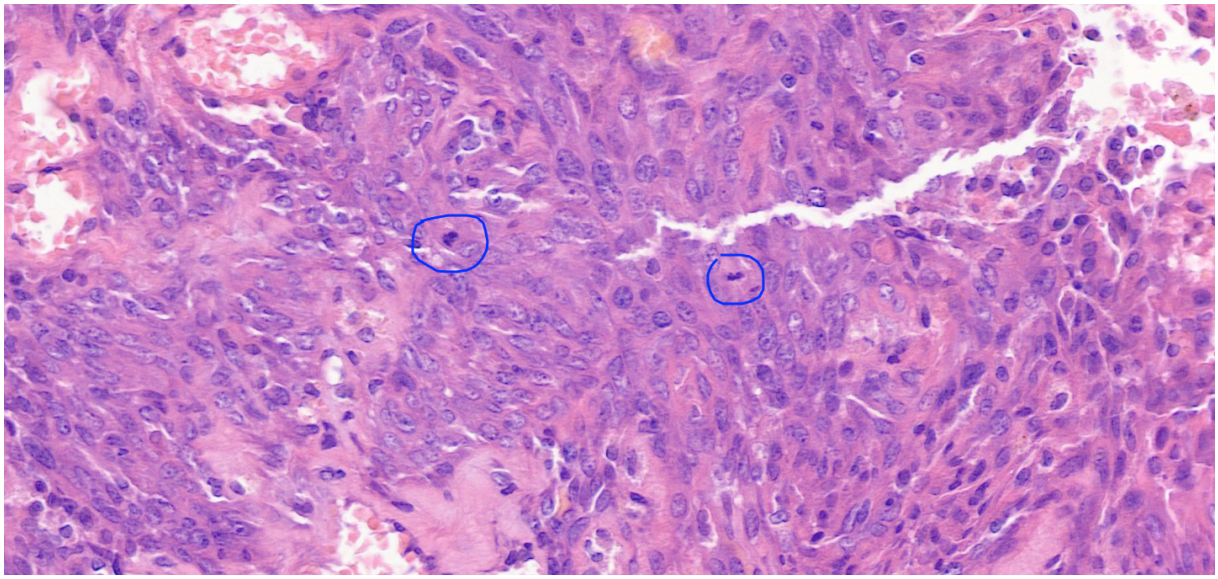


Figure 17. Mitoses shown in blue circles in Case 5.

The stringencies of these criteria of malignancy have not been formally investigated in this study and have seldom been evaluated although we did investigate the number of mitoses per square mm. Mitotic index is defined as the amount of cells undergoing mitosis in a given population of cells (National Cancer Institute, 2019). It is assumed that malignant tumours will be characterised by greater numbers of mitoses when compared to benign tumours although formal comparisons are lacking. Because of this, it is prudent to use mitotic index not as a specific criterion for malignancy but rather as a tool to aid prognostication. Due to the small case number and limited tumour types in this study, it is difficult to say whether mitoses holds any relevance at the moment or that any meaningful comparisons can be drawn. Suárez-Bonnet et al. measured mitoses using high magnification field (40x); however this is not standardised as most microscopic fields vary. Hence in our study mitotic figures were counted per square mm. Nevertheless, the lack of clinical information and follow up precluded accurate evaluation of the significance of mitoses, and indeed tumour morphology, type, or grading as prognostic indices.

Mammary growth and development are dependent on synergy between oestrogen and progesterone (BENSON et al., 1957). All epithelial cells (benign or malignant) within the gland are expected to show both oestrogen and progesterone expression. While all tumour cells showed positive progesterone expression no tumour had oestrogen expression in our study. This differs from the findings of Suárez-Bonnet et al. where all tumour cells showed strong positive oestrogen expression (ER alpha). This can be due to numerous reasons; the tumours may not be the same, the tumours may be morphologically high grade with loss of expression of oestrogen receptor, or reflects the fact that the antibody sources differed between the studies (Dako vs Roche: Ventana). Immunohistochemical reagents have been optimised for human pathology substrates so attention should be paid to sources of antibodies when evaluating animal neoplasms, as not all are likely suitable for use.

Having additional information of neutering status would have been beneficial. Neutering leads to decreased sex steroid production and one may hypothesise that neutering will have a preventive effect on the development of mammary gland neoplasms. This is currently a contentious issue in companion animal medicine. Ovariohysterectomies/

ovariectomies were thought to decrease incidence of canine mammary gland cancer but recent studies have reviewed the literature and undermined this hypothesis (Beauvais, Cardwell and Brodbelt, 2012). Further sound scientific research is needed in this field to draw a valid conclusion between the effects of neutering and development of seemingly sex-steroid dependent mammary gland neoplasms.

Androgen receptor and C-erB2 expressions are demonstrated for the first time and also have aetiological implications. Androgen, a male sex-steroid, was expressed in 6 out of the 7 tumours. As all cases were from males it was hypothesised that all tumours would show androgen expression. Presumably Case 6 had lost the ability to express androgen due to high grade malignancy and poor differentiation of cells (Figure 18). As progesterone and androgen showed marked diffuse expression in the majority of cases these steroid receptors may be an important early driver of the neoplastic process.

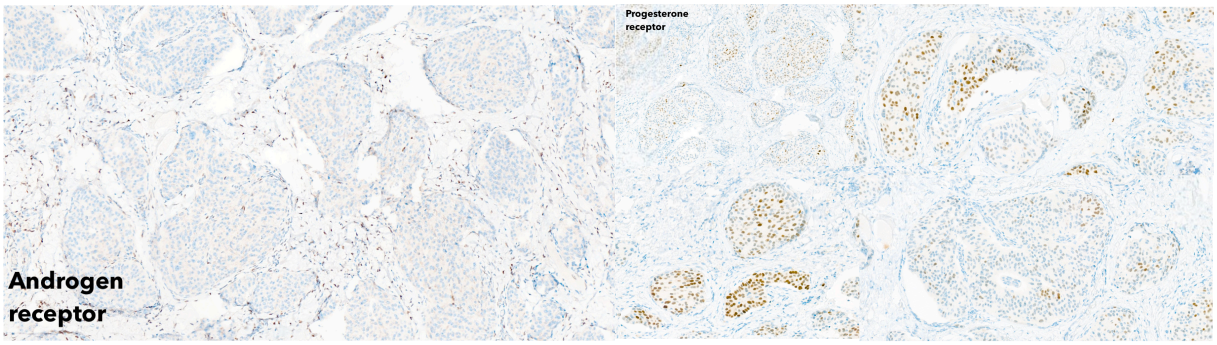


Figure 18. No androgen receptor expression and poor progesterone expression evident in Case 6. Tumour was diagnosed as a solid grade adenocarcinoma.

C-erB2 is a proto-oncogene found in physiologically healthy growing cells (National Cancer Institute, 2019). Its has been extensively studied in human breast, ovarian and pancreatic cancer. It is associated with more malignant neoplasms, treatment resistance and has some prognostic value (Antunes et al., 2004). 5 out of 7 of the cases showed C-erB2 expression albeit most were mild expression (Figure 6). The expression was not diffuse but rather focal which could mean that C-erB2 is more relevant to certain tumour types than others.

Future considerations

Limited clinical details are stated in previous studies (Appendix Tables A-D), many only included age, gender and presentation of the animal (necropsy vs surgical excision). Future research should pay attention to clinical and macroscopic data in order to better understand the clinical significance of tumour types.

While Hoch-Ligeti et al. investigated the effect irradiation had on the development of mammary gland neoplasia few studies have considered the possible aetiological factors in these tumours. The consistent finding of sex steroid receptor expression in our and other studies implicates oestrogen, progesterone and androgen as significant pathogenic agents that is a likely fertile area for future research. The sex-steroids and C-erB2 IHC markers may have practical implications in a diagnostic setting. Not only this, they could help in understanding the pathomechanism behind tumour development. To make any substantive conclusions IHC assessment of greater numbers of tumours is required. Furthermore, molecular analyses of the bases of the observed over-expression would be warranted. For example, the C-erbB2 gene is often amplified or mutated in human breast malignancy and similar mechanisms could be investigated in C-erB2 positive guinea pig mammary tumours.

There is a profound inconsistency with nomenclature when describing neoplasms (Appendix Tables A and B). Most of the literature does not cite which classification system (if any) is used and there is a lack of conformity amongst authors. This makes reviewing and comparing results challenging and makes it difficult to draw meaningful conclusions.

Distinction between mammary gland tumours and those arising from skin adnexae has not been addressed. The Misdorp classification seems to suffice for GP neoplasia but may need future modifications, or a specific GP classification to be developed. For meaningful research and understanding of these neoplasms a classification scheme is required that is universally adopted.

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Appendices

Table A: Tabulated summary of mammary gland neoplasms in laboratory GP.

Author	Case Number	Mammary Gland Neoplasia Prevalence	Results Table Reference
Report of Fourteen Spontaneous Guinea Pig Tumours James B. Rogers	12/138	8.5%	A_1
Mammary gland neoplasia in guinea pig Andrews	4/14	28%	A_2
The biology of the guinea pig Manning	30		A_3
Mammary gland tumours in irradiated guinea pigs Cornelia Hoch-Ligeti	62		NA

Table B: Tabulated summary of mammary gland neoplasms in pet GP.

Author	Case Number	Mammary Gland Neoplasia Prevalence	Results Table Reference
Spontaneous tumours in guinea pigs Jelinke	5/20	25%	B_1
A report of fourteen spontaneous tumours in guinea pigs Kitchen	4/14	28%	B_2
Morphological and immunohistochemical characterisation of spontaneous mammary gland tumours in the guinea pig Suarez	10		B_3-B_5

Table A_1: Rogers and Blumenthal, 1960

Case	Diagnosis	Author
1	Adenocarcinoma (2 cases)	Katase, 1912
2	Adenocarcinoma	Sternberg, 1913
3	Adenocarcinoma	Jones, 1916
4	Adenocarcinoma	Blumensaat and Champy, 1928
5	Adenocarcinoma (2 in males)	Migunow, 1931
6	Adenocarcinoma (1 in male)	Twort and Twort, 1932
7	Lipofibrosarcoma	Murray, 1916
8	Adenoma	Anderson and Lumbrosa, 1933
9	Papillary cystadenoma	Apolant, 1908

Table A_2: Andrew, 1975

Case Age/ sex	Diagnosis	Other histological features
1 Mature/ F	Mammary ductile carcinoma	6 x 3 cm Large areas of necrosis and most of the stroma contained inflammatory cell infiltrates Cystic hemorrhagic mass right inguinal area One week following animal was found dead
2 15 months/ M	Mammary papillary cystadenoma	Areas of necrosis with local cavitation Local invasion not evident Died on experiment
3 18 months/ M	Mammary papillary adenocarcinoma	2.5 x 2 x 1 cm Left mammary gland Mass was locally invasive Virus-like particles found Mass was found 7 months prior to necropsy (experiment)
4 52 months/ M	Mammary papillary cystadenocarcinoma Multiple pulmonary adenomas	5 x 3 x 2.5 cm mass enveloped both mammary glands Numerous tumour nodules varying from 0.2 - 1cm in diameter were scattered throughout the lung lobes Debilitated so was euthanised and necropsied

Table A_3: Wagner and Manning, 1976

Number of cases	Diagnosis	Other comments
10	Adenocarcinoma	16/30 were Hartley strain GP 14/30 were between 10-26 months 5/30 were male 1 adenocarcinomoma and one carcinosarcoma metastasised
4	Papillary cyst adenoma	
1	Cyst adenoma	
3	Adenoma	
8	Fibroadenoma	
2	Fibrocystoadenoma	
1	Carcinosarcoma	
1	Liposarcoma	

Table B_1: Jelínek, 2003

Case Age/ sex	Diagnosis	Other histological features
1 4 years/ M	Adenocarcinoma	2 x 1 x 1 cm Tubular and cystopapillar formations No angioinvasion
2 7 years/ M	Adenocarcinoma	Pancytokeratin antibody (MNF 116, DAKO) cytokeratin K 18 2cm Dome shaped Foci of squamous metaplasia and conspicuous inflammatory cellulation No angioinvasion
3 4 years/ F	Myoepithelial sarcoma	Smooth muscle actin S100 protein Cytokeratins and Vimentin were negative 2 x 1 x 1 cm Mitotic figures were frequent, some atypical
4 2 years/ F	Adenomatous hyperplasia	Cytokeratins, using pancytokeratin antibody MNF 116 (DAKO), revealed positivity only in the epithelium of hair follicles. Epithelium of the tubular formations was negative Smooth muscle actin was positive in majority of the interstitial cells and in some epithelial cells in the adenomatous formations 2 cm No mitotic figures and no cytological abnormalities
5 2 years/ F	Tubular adenoma	Results of immunohistochemistry were the same as in the above mentioned case, only the reaction for actin revealed more myoepithelial cells 1 cm diameter

Table B_2: Kitchen, Carlton and Bickford, 1975

Case Age/ sex	Diagnosis	Other histological features
1 3.5 years/ M	Adenocarcinoma, papillary type	Piling up of cells and loss of polarity Increased nuclear/cytoplasmic ratio Hyperchromatic nuclei
2 3 years/ F	Adenocarcinoma, papillary type	Piling up of cells and loss of polarity Increased nuclear/cytoplasmic ratio Hyperchromatic nuclei Large areas of necroses
3 5 years/ M	Adenoma	No mitotic figures Right inguinal 1 x 1 x 1 cm Ulcerated surface Surrounding tissue contained numerous inflammatory cells (polymorphonuclear leukocytes)
4 7.5 years/ F	Malignant mixed mammary gland tumour with lung metastasis	Frequent/ bizzarre mitotic figures Anaplasia Mass apparent for 7 months Increased nuclear/ cytoplasmic ratio Hyperchromatic nuclei Metastasis to lungs (same features of malignancy)

Table B_3: Suárez-Bonnet et al., 2009

Case	Age	Sex	Diagnosis	Mitotic Index	Other Histological Features	Follow Up
1	2	F	SA	4	Suppurative inflammation	NDA
2	4.5	F	SA	3		18 months free of disease
3	6	F	BMT	0	Suppurative inflammation	24 months free of disease
4	NDA	F	STPC	13		NDA
5	5.5	M	STPC	3	Suppurative inflammation and necroses	16 months free of disease. Natural death
6	4.5	F	STPC	12	Desmoplagia and haemorrhage	NDA
7	4	F	SSC	23	Lymphoplasmocytic inflammation	Death two months after surgery with local recurrence
8	NDA	F	STPC	6	Lymphoplasmocytic inflammation	NDA
9	3.5	M	STPC	11	Suppurative inflammation and necroses	Euthanasia two months after surgery due to recurrence
10	4	F	STPC wLM	9		Death after 5 months months due to recurrence of primary mammary tumour and metastasis to lungs

SA = simple adenoma

BMT = benign mixed tumour

STPC = simple tubulopapillary carcinoma

SCC = simple solid carcinoma

wLM = with lung metastasis

Table B4: Summary of sources, antigen retrieval methods and dilutions of antibodies used (Suárez-Bonnet et al., 2009).

Antibody	Source	Clon	Antigen Retrieval	Dilution
AE1/AE3	Dako	AE1 and AE3	10% pronase	1:100
CK 5+8	Euro-Diagnostics	RCK 102	10% pronase	1:20
CK 8+18	Euro-Diagnostics	NCL-5D3	Citrate buffer	1:20
CK 20	Dako	Ks 20.8	Citrate buffer	1:20
CK 7	Dako	OV-TL 12/30	10% pronase	1:20
CK 14	Novocastra	LL002	Citrate buffer	1:40
Actin	Enzo Life Sciences	HHF35	10% pronase	1:200
Calponin	Dako	CALP	Citrate buffer	1:400
Vimentin	Dako	Vim 3B4	10% pronase	1:100
ER	Dako	ID5	Citrate buffer	1:50
PR	Immunotech	PRA 109	Citrate buffer	1:750

Table B_5: Immunohistochemical results of normal and neoplastic mammary tumours in guinea pig (Suárez-Bonnet et al., 2009).

Tissue	Antibody										
	AE1 / AE3	CK 5+8	CK 8+18	CK 20	CK 7	CK 14	Actin	Calponin	Vimentin	ER_{alpha}	PR
Normal acinar cells	+++	-	+	-	-	-	-	-	-	++	++
Normal ductal cells	+++	-	+++	+++	+++	-	-	-	-	+++	+++
Myoepithelial cells	+++	++	-	-	++	+++	+++	+++	+++	-	-
Neoplastic epithelial cells (benign and malignant)	+++	-	+++	+++	+++	-	-	-	-	+++	+++
Lung metastasis	+++	-	+	+	+++	+++	+++	+++	+++	-	-

CK = cytokeratin

ERalpha = type alpha oestrogen receptor

PR = progesterone receptor

Negative = -

Faint = +

Medium or heterogenous = ++

Strong = +++

Table C: Allison and Moeller, 1993

Number	Age	Sex	Diagnosis	Other Histological Features
1	3	F	Complex adnexal tumour with sebaceous and apocrine differentiation	2.7 x 1.5 x 1.5 cm Right inguinal region Distinctly bilobed It had many features of a complex mammary tumour; however, mammary gland neoplasms do not have a sebaceous component. According to the World Health Organization classification of tumours, complex tumours are composed ofboth secretory and myoepi- thelial components.' This neoplasm contained both a secre- tory component and myoepithelial component, with differ- entiation of the myoepithelial component into adipocytes. Therefore the diagnosis was benign, complex, adnexal tumor with sebaceous and apocrine differentiation.

Table D: Vannevel and Wilcock, 2005

Number	Age	Sex	Diagnosis	Follow Up
1	5 (1.2kg)	F	Bilateral cystic ovaries and mammary fibroadenoma previously Insulinoma	Euthanised due to neurological symptoms relating to the insulinoma
2	5 (1.05kg)	F	Bilateral mammary fibroadenoma previously Insulinoma	Euthanised due to neurological symptoms relating to the insulinoma

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