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AVIAN PAPILLOMA AND SQUAMOUS CELL CARCINOMA: A HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND VIROLOGICAL STUDY

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Abstract

In this retrospective study, we describe the histopathological findings in 7 papillomas and 45 squamous cell carcinomas (SCCs) from psittacine birds, raptors and domestic fowl. The age of affected birds ranged from 3 to 40 years, with median age significantly higher in psittacine birds (P=0.014). The majority of tumours were located on the skin (24/52, 46.2%) or uropygial gland (10/52, 19.2%). Of the SCCs, 30 (66.7%) were well differentiated and 15 (33.3%) were poorly differentiated. SCCs exhibited a significantly higher degree of nuclear pleomorphism (P=0.005) and a greater proportion exhibited surface ulceration (P=0.001) compared to papillomas, however there was no significant difference in mitotic count or inflammation score. The expression of Cox-2 and E-cadherin was investigated by immunohistochemistry. Tumours were assigned a Cox-2 total score (TS) based on the intensity multiplied by the distribution of labelling. The Cox-2 TS was significantly higher in SCC compared to papillomas (P=0.002), but the difference between Cox-2 TS of well and poorly differentiated SCC was not significant.

Cox-2 labelling was predominantly cytoplasmic but some tumours exhibited concurrent membranous and/or perinuclear labelling. SCCs exhibiting membranous labelling had a significantly higher mitotic count (P=0.028). Tumours were determined to be negative for E-cadherin if there was loss of membranous expression in >/= 65% of neoplastic cells. A significantly higher proportion of SCCs were negative for E-cadherin compared to papillomas (P=0.042), but no significant difference was observed between well and poorly differentiated SCCs. Fourteen papillomas and SCC from psittacine birds were also tested by PCR for the presence of *Psittacus erithacus papillomavirus 1* (*PePV1*) and *Psittacid herpesvirus 1* (*PsHV1*), however all samples tested negative. We demonstrate for the first time the expression of Cox-2 and E-cadherin in avian tissues, and suggest that these markers may be useful in differentiating papillomas from SCCs, particularly when sample size is small. We also propose that selective Cox-2 inhibitors may have therapeutic value in the treatment of SCCs in birds.

Introduction

In birds, cutaneous papillomas are common in chaffinches (*Fringilla coelebs*) (Lina *et al.*, 1973) (Osterhaus *et al.*, 1977), and have been described on the beaks and heads of canaries (Dom *et al.*, 1993). Papillomas arising on mucosal surfaces of the oral cavity, pharynx, choanal slit and cloaca are commonly described in psittacine birds such as macaws and cockatoos, a condition commonly referred to as 'internal papillomatosis of parrots' (Gallagher and Sullivan, 1997; Sundberg *et al.*, 1986) (Latimer *et al.*, 1997) (Gartrell *et al.*, 2009).

Squamous cell carcinomas (SCCs) have been reported in the oral cavity, pharynx and oesophagus of domestic fowl (Anderson and Steinberg, 1989; Hatkin *et al.*, 2002; Laura *et al.*, 2016; Vazquez *et al.*, 2003), a Montagu's harrier (Ramis *et al.*, 1999), and an Amazon parrot

(Murtaugh *et al.*, 1986), and from the uropygial gland of penguins (Rettenmund *et al.*, 2015). SCC of the skin is one of the most common skin tumours in broiler chickens and a cause of carcase condemnation at slaughter (Nakamura *et al.*, 2010). These lesions appear to originate from feather follicle epithelium and histologically have a central 'cup'-shaped cavity lined by neoplastic epithelium (Shivaprasad, 2008). Interestingly, in young broilers, dermal SCCs are reported to regress over time, and the term avian keratoacanthoma, due to its resemblance to the human counterpart, has been proposed as a more appropriate name (Hafner *et al.*, 1991; Hafner *et al.*, 1993).

In humans and animals, the expression of molecular markers involved in tumour growth, proliferation, apoptosis, suppression, angiogenesis, invasion and metastasis have been extensively studied for their diagnostic, prognostic and predictive value (Lothaire *et al.*, 2006) (Ramos-Vara, 2016). One molecule extensively investigated for its role in human tumorigeneses is Cox-2 (Greenhough *et al.*, 2009) which has also been studied in a variety of tumours of dogs, cats and horses (Dore, 2011). Cox-2 is usually undetectable in normal skin and epithelial surface but can be induced by mitogens, cytokines, hormones and growth factors. (Dubois *et al.*, 1998). Cox-2 expression has been extensively investigated in human colorectal cancer and the pro-inflammatory and tumour promoting effects of Cox-2 are believed to be mediated through prostaglandin E2 (PGE2) (Wang and Dubois, 2010) with several reports showing that PGE2 promotes tumour growth by stimulating angiogenesis, cell invasion, cell growth and survival (Wang and Dubois, 2006). Cox 1 and Cox-2 overexpression has been detected in cutaneous SCCs in dogs (Bardagi *et al.*, 2012) and oral SCCs in cats (Hayes *et al.*, 2006), and has been associated with progression of SCCs in dogs and cats (Millanta *et al.*, 2016). Another molecule extensively studied in SCCs for its role in epithelial to mesenchymal transition is the intercellular adhesion molecule E-cadherin. Reduced membranous expression

of E-cadherin is frequently reported in human SCCs (Bankfalvi *et al.*, 2002; Jeanes *et al.*, 2008; Re *et al.*, 2018; Zhou *et al.*, 2015), and has been described in dogs (Mestrinho *et al.*, 2015; Nagamine *et al.*, 2017) and horses (Suarez-Bonnet *et al.*, 2018). In birds on the other hand, there is a lack of published information on expression of tumour markers.

Out of the seven papillomaviruses identified in birds to date, three have been associated with cutaneous papillomas and include *Fringilla coelebs papillomavirus 1* (FcPV1) from the common chaffinch (Osterhaus *et al.*, 1977), *Fulmarus glacialis papillomavirus 1* (FgPV1) from a Northern Fulmar (Gaynor *et al.*, 2015) and *Psittacus erithacus papillomavirus 1* (PePV1) from African Grey Parrots (Latimer *et al.*, 1997; O'Banion *et al.*, 1992; Tachezy *et al.*, 2002). The other four avian papillomaviruses have been identified from mucosal swabs, healthy skin and faeces (Truchado *et al.*, 2018). Papillomaviruses are not reported to be associated with mucosal papillomas of psittacine birds; instead there is increasing evidence of the involvement of a herpesvirus, specifically *Psittacid herpesvirus (PsHV)* type 1 (Johne *et al.*, 2002) (Styles *et al.*, 2004) the causative agent of Pacheco's disease (Tomaszewski *et al.*, 2001). It appears that the outcome of infection with *PsHV1* depends on the genotype of the virus and the species of bird exposed (Zsivanovits, 2018). The aetiology of SCCs in birds has not been determined, however in chickens, virus infection e.g. fowlpox virus (Fallavena *et al.*, 2002; Fallavena *et al.*, 1997), chemical irritants (Rigdon and Brashear, 1954), breed, reused litter, and season have all been suggested (Nakamura *et al.*, 2010). No consistent association with a viral agent has been shown to date.

Here we present the histopathological findings in a series of avian papilloma and SCC cases and use these tissues to investigate the expression of Cox-2 and E-cadherin, and the relationship

between the expression of these markers and signalment, location and histopathological findings. We also take the opportunity to screen a selection of the tumours for the presence of papillomavirus and herpesvirus by PCR testing.

Materials and methods

Case selection

The databases of the Anatomical Pathology Diagnostic Service, Campus of the Agrifood, Forestry and Veterinary Sciences, Lleida University, Spain, and the International Zoo Veterinary Group (IZVG) Pathology, Keighley, UK, were searched using the following key words; 'avian', 'papilloma' and 'squamous cell carcinoma', between the time period January 1st, 2004 to December 31st, 2016. A total of 117 cases were identified (34 papillomas, 83 SCCs). Cases with only small amounts of tissue, or where tissue was markedly autolysed or necrotic were excluded. A total of 52 cases of papilloma or SCC were eventually selected.

Histological evaluation

Haematoxylin and eosin (HE) stained tissue sections were examined by two veterinary pathologists (ALJ and SLP). The neoplasms were analysed at the core and at the invasive front, i.e. at the border of the neoplasm with normal dermis or subcutis. Neoplasms were classified histologically into papilloma if the findings were limited to a well demarcated, exophytic proliferation of epidermis or mucosal epithelium, and SCC if showing invasion below the basement membrane (Goldschmidt, 2017). SCCs were categorised as well or poorly differentiated based on the presence or absence, respectively, of stratified layers of epithelial cells undergoing progressive keratinization and the formation of keratin pearls (Goldschmidt,

2017); the degree of nuclear pleomorphism and mitotic count was not taken into account in assessment of differentiation. Examination included determination of mitotic count (MC) by quantification of mitotic figures in 10 high power fields (x400), semi-quantitative scoring of nuclear pleomorphism (none, 0; moderate, 1; marked, 2), semi-quantitative scoring of inflammation within the bulk of the neoplasm, avoiding areas adjacent to ulceration (none, 0; moderate, 1; marked, 2), and recording the presence or absence of surface ulceration.

Immunohistochemistry

Sections of formalin fixed paraffin-embedded tissues were cut at 4µm onto positively charged coated glass slides (SuperFrost Plus, Thermo Fisher Scientific, UK). Immunohistochemical analysis was performed on a BondMax Autostainer (Leica, UK). Tissues were incubated with the following monoclonal antibodies: Cox-2 (clone SP-21, 1:150, Thermo, UK) and E-cadherin (NCH-38, 1:100, Dako, UK). Heat induced antigen retrieval was performed using a pH 6 buffer (Bond ER1; Leica, UK). The Bond Polymer Refine Detection kit (Leica, UK) was used for visualisation. A positive reaction for Cox-2 was indicated by distinct cytoplasmic labelling in the macula densa of normal canine kidney. A positive reaction for E-cadherin was indicated by the presence of distinctive membranous labelling of the epidermis on sections of normal chicken skin. Negative controls were prepared by replacing the primary antibody with Leica Antibody Diluent (Leica, UK) only. Analysis was performed independent of other histological features with a light microscope by two veterinary pathologists (ALJ and SLP) who were blinded to signalment and location. Cell markers were evaluated at the core and at the invasive front of SCC in areas minimally affected by background staining and minimally associated with inflammation, and evaluation of ten high power fields (x400) was undertaken where possible before allocating a score. For Cox-2, a Cox-2 total score (TS) was assigned based on the intensity of staining (none, 0; weak, 1; moderate, 2; strong, 3) multiplied by the percentage of positive tumour cells (0%, 0; <25%, 1; 25-50%, 2; 51-75%, 3; >75%, 4) in the areas

examined. The location of Cox-2 labelling was also recorded for each case; labelling which outlined the cell membrane was categorised as 'membranous'; labelling within the cytoplasm was categorised as 'cytoplasmic; and labelling which outlined the nuclear membrane was categorised as 'perinuclear'. For E-cadherin, labelling intensity was compared to normal epithelium (if available in the section). Labelling which outlined the cell membrane was categorised as 'membranous', and labelling within the cytoplasm was categorised as 'cytoplasmic'. The percentage of neoplastic cells expressing membranous, cytoplasmic, membranous and cytoplasmic, or no E-cadherin labelling was recorded. Tissues were considered negative if >/= 65% of cells in examined areas showed an absence of membranous E-cadherin expression (da Cunha *et al.*, 2016).

Statistical analysis

Results were analysed for any significant relationship between the following: signalment (age, sex, species), location on the body, histopathological features (diagnosis (papilloma or SCC), differentiation of SCCs (well or poor), MC, nuclear pleomorphism score, inflammation score, presence or absence of surface ulceration) and immunohistochemistry (Cox-2 TS, Cox-2 location of labelling, and E-cadherin (positive or negative). The statistical significance of any relationship was tested using IBM SPSS Statistics V25. To test for the significance of a relationship between two categorical variable, a chi-squared test was used; for continuous data, the Student's *t*-test, ANOVA, Mann-Whitney U test or Kruskal-Wallis test was used depending on whether or not the data was normally distributed, and whether two, or more than two groups, were compared. The Pearson's correlation co-efficient was used to define a potential correlation between continuous variables. The significance level for all statistical tests was set at $P < 0.05$.

DNA extraction

Cases for PCR were selected from the psittacine group and were selected based on the quantity and quality of formalin-fixed paraffin embedded tissue available. Four 4µm scrolls of tissue were taken from each block and purification of genomic DNA was carried out using a commercially available kit and following the manufacturer's instructions (QIAamp DNA FFPE tissue kit, Qiagen, UK). To avoid cross contamination, samples were processed individually. DNA was eluted in 50 µL of elution buffer and nuclei acid concentration was determined using a spectrophotometer (DS-11 Spectrophotometer, DeNovix, USA). Extracted DNA was stored at -20^{o}C.

PCR amplification

To confirm the integrity of extracted DNA, a set of primers designed to amplify the 18S rRNA gene in the chicken (*Gallus gallus*) genome was used (Table 1) (Schrenzel *et al.*, 2005).

Target	**Primers**	**Sequence (5' – 3')**	**Reference**
18S rRNA of avian genome	AvHost_m18S_F (forward) AvHost_m18S_R (reverse)	CGCCTGTTTATCAAAAACAT CCGGTCTGAACTCAGATCACGT	(Schrenzel et al., 2005)
PePV DNA	ppv6621fmod (forward) ppv6810r (reverse)	GGGCGGATATGACTTTCTGG GCAGTGCGCACGCCTG	(Styles et al., 2004)
PePV DNA	BconPVF1 (forward) BconPVR1 (reverse)	TYCCWAAGGTSTCTGSAAATCA CCRAAGCCAATATCKSACAT	(Perez-Tris et al., 2011)
PeHV DNA	23Ff5a (forward) 23Fr3 (reverse)	TGCGTGGGGTTAAACTCGGAACTAGAAG GATGTTAGGCTCGTGTAGTCG	(Styles et al., 2004)

Table 1. Details of primer sequences used

The presence of very similar sequence within the African grey parrot (*Psittacus erithacus*) genome was confirmed with a BLAST search. For positive control, an artificial plasmid containing a 729bp synthetic avian papillomavirus gene fragment based on the L1 major capsid protein region (Perez-Tris *et al.*, 2011) (Styles *et al.*, 2004) and another containing a 667bp synthetic psittacine herpesvirus gene fragment which spanned a section of the UL16/UL17 gene (Styles *et al.*, 2004) was assembled using overlapping synthetic oligonucleotides and

PCR, and inserted into pMA-RQ plasmid (GeneArt Plasmid Services, ThermoFisher Scientific, UK).

To amplify papillomavirus DNA, two primers based on L1 capsid protein were used separately: ppv6621fmod and ppv6810r (Styles *et al.*, 2004), and BconPVF1 and BconPVR1 (degenerate primers) (Perez-Tris *et al.*, 2011) (Table 1). Briefly, 50uL of reaction mixture contained 10µL of 1 x buffer, 1.5mM of $MgCl_2$, 0.2mM of dNTPs, 0.025 units of GoTaq® DNA Polymerase (Promega, UK), 0.4µM of forward primer, 0.4µM of reverse primer and 5µL of DNA from biopsies, or $1.5x10^8$ copies of the positive control plasmid per reaction, or PCR-grade water (negative control). The reaction conditions used were as described (Perez-Tris *et al.*, 2011). The reaction involved denaturation at 95ºC for 1 min, 45 PCR cycles at 50ºC for 1 min each, extension at 72ºC for 1 min and a final extension step at 72ºC for 5 min.

To amplify herpesvirus DNA, primers 23Ff5a and 23Fr3 were used (Styles *et al.*, 2004) (Table 1). The reaction mixture was the same as used for the papillomavirus reaction except 2.5mM of $MgCl_2$ was used and (X – To follow) copies of the positive control plasmid per reaction. The reaction conditions were as described (Styles *et al.*, 2004) and involved denaturation at 94ºC for 5 min, 40 PCR cycles at 60ºC for 45 seconds each, extension at 72ºC for 90 seconds, and a final extension step at 72ºC for 5 min.

All PCRs were performed on a TC-412 thermal cycler (Techne, UK). PCR products were visualised on a 1.5% agarose gel containing SafeView Nucleic Acid stain (NBS Biologicals, Cambridgeshire).

Results

<u>Signalment and location</u>

A total of 52 cases were examined. Thirty-two (61.5%) cases were from psittacine and 20 (38.4%) cases were from non-psittacine birds (17 (85%) raptors and 3 (15%) domestic fowl). Age ranged from 3 to 40 years with a median of 10.5 years. The median age was significantly higher in psittacines (18) compared to raptors (10) and domestic fowl (5) (P=0.014). The sex was female in 14 (26.9%), male in 19 (36.5%), and not specified in 19 (36.5%) cases. Tumours were from a range of locations including skin (n=24, 46.2%), uropygial gland (n=10, 19.2%), oropharynx (n=7, 13.5%) and cloaca (n=3, 5.8%) (Appendix 1).

Histopathology

A diagnosis of papilloma was made in 7 (13.5%) (all psittacine) cases (Figure 1A) and SCC in 45 (86.5%) cases (24 (53.3%) psittacines, 18 (40%) raptors and 3 (6.7%) domestic fowl). The median age in papilloma and SCC cases was 7 (min-max; 2-7) and 10.5 (min-max; 1-40) years respectively. The sex distribution was as follows; papillomas (1 (14.3%) male, 1 (14.3%) female, 5 (71.4%) unspecified), SCC (18 (40%) males, 13 (28.9%) females, 14 (31.1%) unspecified). Papillomas were located in the uropygial gland (n=2, 28.6%), cloaca (n=1, 14.3%), oropharynx (n=1, 14.3%), and SCC cases were located in the skin (n=24, 53.3%), uropygial gland (n=8, 17.8%), oropharynx (n=6, 13.3%) and cloaca (n=2, 4.4%) (Table 2).

The median mitotic count of papillomas was 7 (min-max: 1-20)) and 17 for SCCs (min-max: 0-51) (P=0.103). SCCs exhibited a significantly higher degree of nuclear pleomorphism compared to papillomas (P=0.005) but no significant relationship was associated with degree of inflammation (P=0.145). Surface ulceration was present in 3 (42.9%) papillomas and 42 (93.3%) SCCs (P=0.001). Of the SCCs, 30 (66.7%) were well differentiated (Figure 1B) and 15 (33.3%) were poorly differentiated (Figure 2A and 2B). No significant relationship was

found in signalment, location and histopathological features between well and poorly differentiated SCCs.

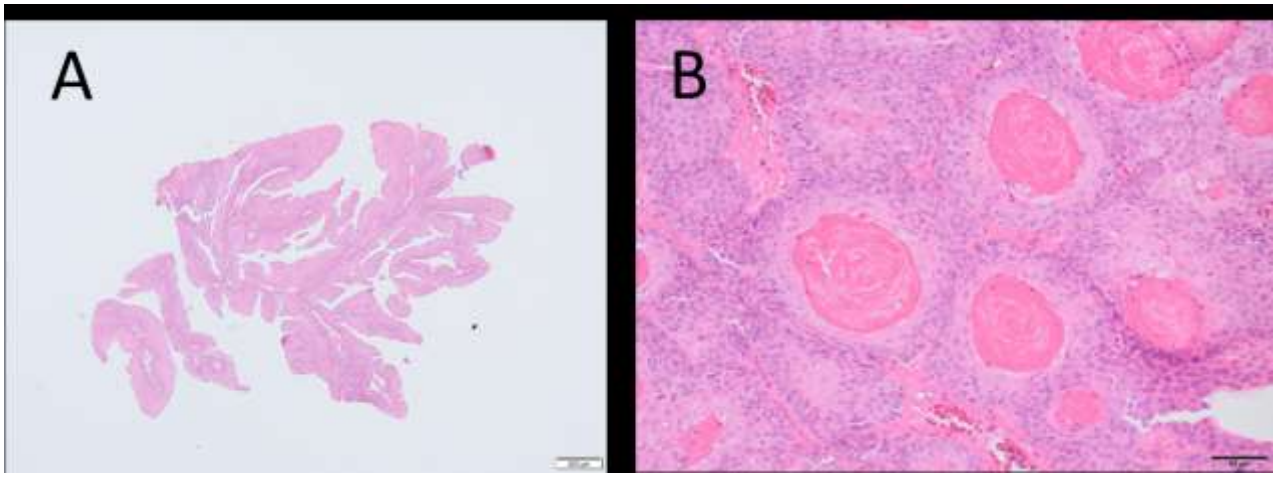

Figure 1. A, Papilloma (Bar = 200µm) B, Formation of keratin pearls in a well-differentiated SCC (Bar = 50µm) (H+E).

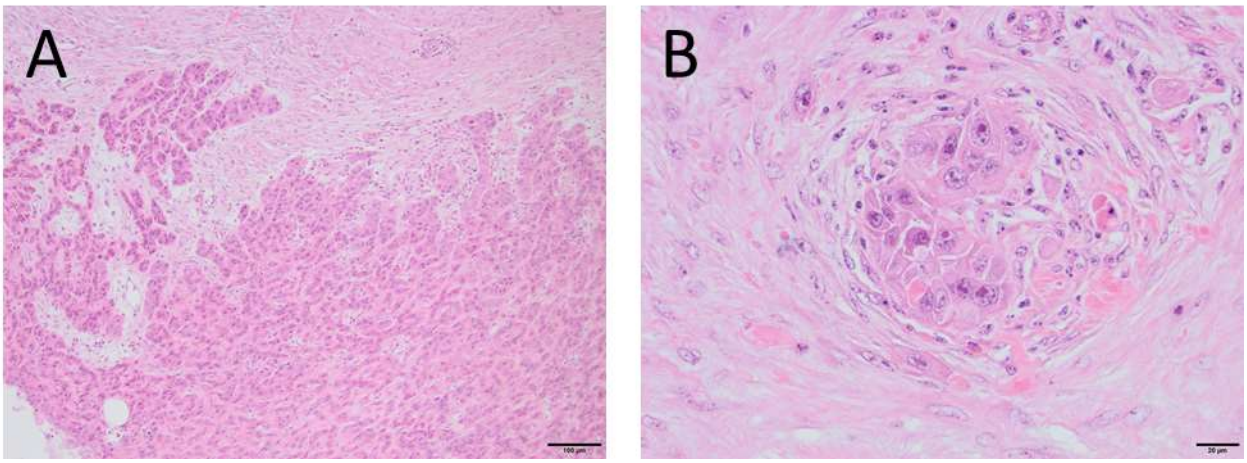

Figure 2. A, Disorganised cords of neoplastic cells in a poorly differentiated squamous cell carcinoma (Bar = 100µm). B, Formation of bizarre nests of neoplastic cells in a poorly differentiated SCC (Bar = 20µm) (H+E).

Immunohistochemistry

Cox-2 labelling in neoplastic cells was predominantly cytoplasmic (Figure 3A). The median Cox-2 TS was significantly higher in SCC (3, min-max: 0-12) compared to papillomas (1, min-max: 0 to 4) (P=0.002). The median Cox-2 TS for well and poorly differentiated SCCs was 3.47 (min-max: 0-12) and 6 (min-max: 1-12) respectively (P=0.070). No significant relationship was detected between Cox-2 TS and signalment, location and histopathological features. Cox-2 membranous labelling was also detected in 12 out of 52 (23%) cases (Figure 3B); 11 (91.7%) of these were SCCs. Of the SCCs expressing membranous Cox-2 labelling, 10/11 (90.9%) were in psittacine birds (P=0.035). The median MC for SCCs exhibiting membranous Cox-2 labelling was significantly higher (24 (min-max; 0-51)) compared to SCCs not expressing membranous labelling (12 (min-max; 0-50)) (P=0.028). No other significant relationship was found between membranous Cox-2 labelling and signalment, location, histopathological and immunohistochemistry results. Cox-2 exhibited perinuclear labelling in 5 out of 52 (9.6%) cases, all of which were SCCs. No significant relationship was found between perinuclear Cox-2 labelling and signalment, location, histopathological features and immunohistochemistry results.

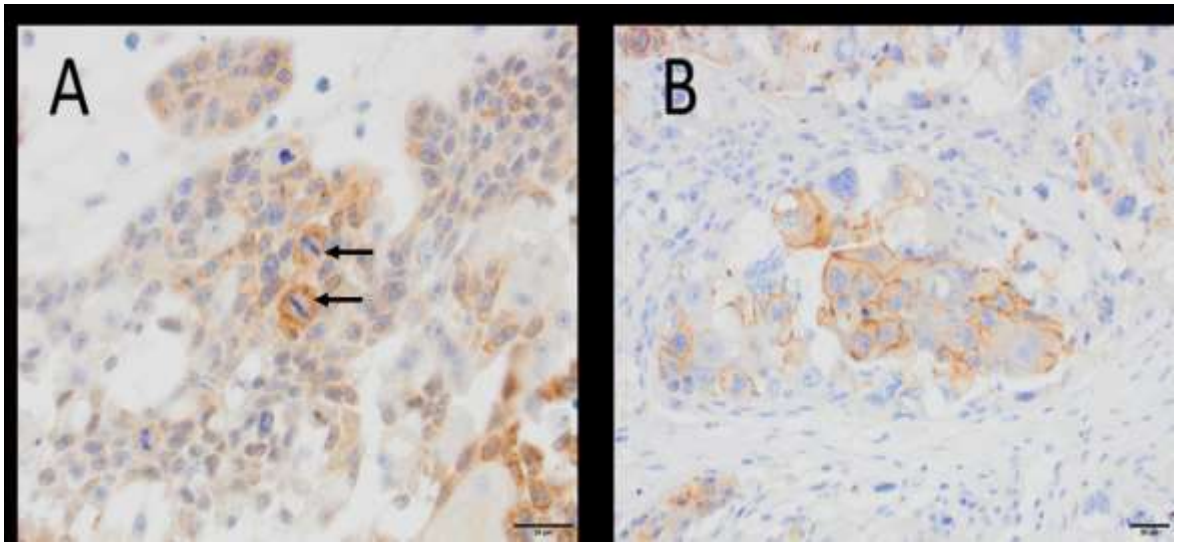

Figure 3. A, Bizarre cords of neoplastic cells exhibiting strong cytoplasmic labelling for Cox-2. Mitotic figures are highlighted with black arrows (Bar = 20µm). B, Nests of poorly-differentiated neoplastic cells exhibiting strong membranous labelling for Cox-2 (Bar = 20µm).

E-cadherin expression was negative in 25 (55.6%) SCCs and one (14.3%) papilloma (P=0.042). E-cadherin expression was negative in 10 (66.7%) poorly, and 15 (50%) well differentiated SCCs (P=0.289) (Figure 4A and 4B). No significant relationship was detected between E-cadherin expression and signalment, location, histopathological features and Cox-2 immunohistochemistry.

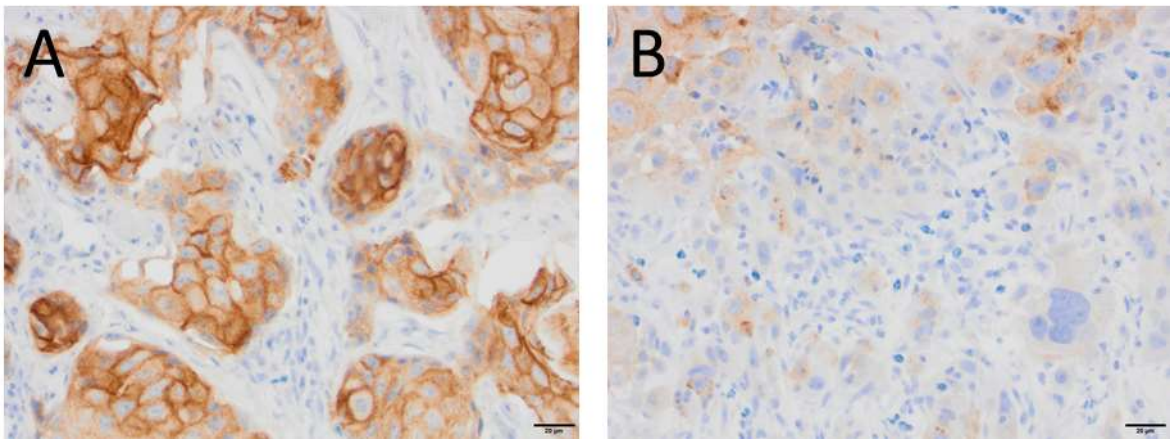

Figure 4. A, Cords and trabeculae of neoplastic cells in a SCC exhibiting membranous labelling for E-cadherin (Bar = 20µm). B, Cords and nests of neoplastic cells in a poorly differentiated SCC exhibiting loss of membranous and variable cytoplasmic labelling for E-cadherin (Bar = 20µm).

PCR

The 18S rRNA gene was amplified from all 14 samples tested, confirming the presence of avian DNA. PePV and PsHV DNA was detected from the positive control; however, all 14 tissues tested negative for PePV and PsHV DNA.

Discussion

In this study, we have successfully demonstrated the expression of Cox-2 and E-cadherin in birds for the first time. To assess the expression, we designed a simple grading scheme with clear categorisation for semi-quantitative scoring of percentage of tumour labelling and intensity of labelling to minimise inter- and intra-observer bias. The expression of Cox-2 was significantly higher in SCCs compared to papillomas as is frequently found in epithelial tumours of humans and other animals (Queiroga *et al.*, 2011; Suarez-Bonnet *et al.*, 2018) but did not differ significantly between well and poorly differentiated SCCs. Cox-2 labelling was predominantly cytoplasmic but in some also exhibited concurrent membranous and perinuclear labelling. The majority of membranous labelling (90.9%) was in psittacine birds, and the mitotic count was found to be significantly higher in those tumours exhibiting membranous Cox-2 labelling. The significance of this is unclear but may reflect a more malignant phenotype. No nuclear labelling was observed. Frequently, Cox-2 expression was identified around areas of inflammation, this being expected as Cox-2 is the inducible isoform of Cox that is rapidly induced by exposure to mitogens, including various cytokines and growth factors, for example interleukin-1α, tumour necrosis factor-α, platelet-derived growth factor, epidermal growth factor, and bacterial lipopolysaccharide (Greenhough *et al.*, 2009; Jones and Budsberg, 2000). These areas were carefully excluded from analysis as described in the materials and methods. In humans, use of non-steroidal anti-inflammatory drugs (NSAIDs) have shown to be associated with a significant reduction in colorectal cancer (Backlund *et al.*,

2005) and has shown promise therapeutically in non-small cell lung cancer (Liao *et al.*, 2005), oesophageal cancer (Liu *et al.*, 2008; Pan *et al.*, 2005) and in the treatment of pre-invasive disease (Steinbach *et al.*, 2000). Selective Cox-2 inhibitors in hairless mice prevented new tumour formation (Fischer *et al.*, 1999). In animals, it is also shown to be beneficial in the treatment of canine prostatic carcinoma (Sorenmo *et al.*, 2004), canine transitional cell carcinoma (Knapp *et al.*, 1994), canine oral squamous cell carcinoma (Schmidt *et al.*, 2001) and in a mucocutaneous SCC in a single horse (Moore *et al.*, 2003). The findings of this study suggest that the use of Cox-2 inhibitors may be a valuable adjunct in the treatment of SCCs in birds, particularly when complete surgical excision might not be possible.

A significantly greater proportion of SCCs were negative for E-cadherin expression, based on the loss of membranous expression >/=65% of neoplastic cells, compared to papillomas. E-cadherin is a cell membrane spanning glycoprotein, which has extracellular regions that bind to the ectodomain of cadherins on neighbouring cells. It is responsible for maintaining cell to cell cohesion and recognition during sorting and tissue reorganisation, cell upon cell locomotion and epithelial polarity (Jeanes *et al.*, 2008). Abnormal E-cadherin expression and the loss of cell-cell contact has been shown in human and animals to be an important step in tumour progression (Berx and Van Roy, 2001; Field, 1992; Nagamine *et al.*, 2017; Zhai *et al.*, 2008). Our findings suggests that loss of adhesion molecule expression is also important in progression of avian SCC. In addition to cell to cell recognition, E-cadherin is linked to a range of fundamental intracellular processes e.g. actin cytoskeleton, cell signalling and trafficking via their cytoplasmic tails that link to a range of proteins e.g. α and β-catenin. The expression of these proteins has been investigated previously in mammalian SCCs (Moles *et al.*, 2016) but has not been investigated in epithelial tumours of birds to date.

As expected, there was a higher degree of nuclear pleomorphism and surface ulceration in SCCs compared to papillomas. Apart from a significantly higher mitotic count in tumours exhibiting membranous Cox-2 expression, mitotic count was not found to be associated with any other feature. Mitotic count in other studies is shown to be associated with higher tumour grades, poorly differentiated tumours and poor prognosis, and forms the basis of many grading criteria's (Dennis *et al.*, 2011; Kiupel *et al.*, 2011; Valli *et al.*, 2011).

Age of affected birds varied widely reflecting the long lifespan of psittacine and raptor birds. Tumours were most commonly located on the skin. This may be because these tumours were visible to owners and identified earlier, and thus perhaps considered better candidates for intervention by clinicians. In psittacine birds, 19% of the tumours were located at or around the uropygial gland, which is not an uncommon location to find papillomas and SCCs (Beaufrere *et al.*, 2007; Hatkin *et al.*, 2002; Rettenmund *et al.*, 2015). The uropygial gland, which is located on the dorsal aspect of the tail is prominent in budgerigars (*Melopsittacus undulates*) and African grey parrots (*Psittacus erithacus)*, and may be exposed to increased levels of ultraviolet light offering a potential reason for the preponderance of tumours at this location. Uropygial gland blockage with secondary infection and self-trauma is also relatively common in parrots, especially malnourished and older birds (Hunt, 2018) and epithelial dysplasia secondary to this may predispose to neoplastic transformation (Rettenmund *et al.*, 2015). It should however be noted that the uropygial gland is absent from some parrots e.g. Amazon parrots (*Amazona* sp.) and other species such as ostriches (*Struthio camelus*) and many pigeons (*Columbidae* sp.) (Sandmeier, 2018).

As clinical history was limited, and follow up information unavailable, we were unable to correlate immunohistochemistry grading with tumour size, presence of metastasis and survival. Carcinomas in mammals commonly metastasise via the lymph node and examination of local lymph node is routinely undertaken for staging in dogs and cats (Biller *et al.*, 2016). "True" lymph nodes equivalent to those in mammals are not present in birds, which have structures known as mural lymph nodes which are located lateral to the lymphatics. These do not interrupt lymph flow as in mammals (Oláh, 2014). Lymphatic invasion would therefore be expected to result in more disseminated metastasis of tumour however metastasis of SCC is rarely reported in birds (Ramis *et al.*, 1999). To aid prediction of the metastatic potential of these tumours, evaluation of proliferative markers such as ki67 or proliferating cell nuclear antigen (PCNA) (Mestrinho *et al.*, 2015), and angiogenic factors such as vascular endothelial growth factor (VEGF) (Millanta *et al.*, 2016), and their relationship to Cox-2 and E-cadherin expression may be informative. With more complete information on tumour progression, size, and survival data, evaluation of a greater range of markers could be undertaken to identify which markers are associated with better or worse prognosis.

In the 14 tumours screened by PCR, no papillomavirus or herpesvirus DNA was detected although positivity of all samples for an avian housekeeping gene control confirmed the presence of adequate DNA. However, the integrity and quantity of DNA extracted from formalin fixed paraffin embedded tissues may be poorer than DNA extracted from fresh samples. Formalin fixation can result in DNA fragmentation and formation of protein–DNA complexes. Autolysis and necrosis can also significantly reduce the integrity of the DNA (Lorenz, 2012). Spectrometry in this case however revealed satisfactory quantities and qualities of extracted DNA, and amplification of 18s rRNA gene was successful in each one. The tissues selected tested negative for *PePV* DNA with both primer sets. Both primer sets were designed

against the L1 major capsid protein of *PePV*, which is reportedly well conserved among human and non-human mammalian papillomavirus species (Bernard *et al.*, 2010). These primers would be expected to amplify papillomaviruses from other species if present. The negative result is supported by the absence of cytological changes typical of papillomavirus infection on histopathology such as cytoplasmic vacuolation, koilocytes and intranuclear inclusion bodies (Latimer *et al.*, 1997). Papillomavirus have been previously described in carcinoma in-situ and malignant SCCs (Eleni *et al.*, 2017; O'Neill *et al.*, 2011), however their role, if any, in the development of malignant tumours is not yet established as they can also be detected in healthy skin in many species (Antonsson and Hansson, 2002). Screening of the remainder for papillomavirus and herpesvirus was not carried out due to the paucity of tissues available.

Conclusion

This study adds significantly to the number of avian SCCs reported in the literature and shows that Cox-2 and E-cadherin is expressed in avian tissues. Increased expression of Cox-2 and reduced membranous expression of E-cadherin is shown to be significantly greater in SCCs compared to papillomas, and these findings could be a useful aid in the diagnosis of these conditions particularly when sample sizes are small. Based on these findings, we propose that use of selective Cox-2 inhibitors may have therapeutic value in SCC. Finally, no association with papillomavirus or herpesvirus was detected.

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References

Anderson, W. I. and Steinberg, H. (1989). Primary glossal squamous-cell carcinoma in a Spanish Cochin hen. *Avian Dis,* **33,** 827-828.

Antonsson, A. and Hansson, B. G. (2002). Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. *J Virol,* **76,** 12537-12542.

Backlund, M. G., Mann, J. R. and Dubois, R. N. (2005). Mechanisms for the prevention of gastrointestinal cancer: the role of prostaglandin E2. *Oncology,* **69 Suppl 1,** 28-32.

Bankfalvi, A., Krassort, M., Buchwalow, I. B., Vegh, A., Felszeghy, E. and Piffko, J. (2002). Gains and losses of adhesion molecules (CD44, E-cadherin, and beta-catenin) during oral carcinogenesis and tumour progression. *J Pathol,* **198,** 343-351.

Bardagi, M., Fondevila, D. and Ferrer, L. (2012). Immunohistochemical detection of COX-2 in feline and canine actinic keratoses and cutaneous squamous cell carcinoma. *J Comp Pathol,* **146,** 11-17.

Beaufrere, H., Brasseur, G. and Heimann, M. (2007). What is your diagnosis? Squamous cell carcinoma of the uropygial gland. *J Avian Med Surg,* **21,** 321-324.

Bernard, H. U., Burk, R. D., Chen, Z., van Doorslaer, K., zur Hausen, H. and de Villiers, E. M. (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology,* **401,** 70-79.

Berx, G. and Van Roy, F. (2001). The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression. *Breast Cancer Res,* **3,** 289-293.

Biller, B., Berg, J., Garrett, L., Ruslander, D., Wearing, R., Abbott, B., Patel, M., Smith, D. and Bryan, C. (2016). 2016 AAHA Oncology Guidelines for Dogs and Cats. *J Am Anim Hosp Assoc,* **52,** 181-204.

da Cunha, I. W., Souza, M. J., da Costa, W. H., Amancio, A. M., Fonseca, F. P., Zequi Sde, C., Lopes, A., Guimaraes, G. C. and Soares, F. (2016). Epithelial-mesenchymal transition (EMT) phenotype at invasion front of squamous cell carcinoma of the penis influences oncological outcomes. *Urol Oncol,* **34,** 433 e419-426.

Dennis, M. M., McSporran, K. D., Bacon, N. J., Schulman, F. Y., Foster, R. A. and Powers, B. E. (2011). Prognostic factors for cutaneous and subcutaneous soft tissue sarcomas in dogs. *Vet Pathol,* **48,** 73-84.

Dom, P., Ducatelle, R., Charlier, G. and de Groot, P. (1993). Papillomavirus-like infections in canaries (Serinus canarius). *Avian Pathol,* **22,** 797-803.

Dore, M. (2011). Cyclooxygenase-2 expression in animal cancers. *Vet Pathol,* **48,** 254-265.

Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van De Putte, L. B. and Lipsky, P. E. (1998). Cyclooxygenase in biology and disease. *FASEB J,* **12,** 1063-1073.

Eleni, C., Corteggio, A., Altamura, G., Meoli, R., Cocumelli, C., Rossi, G., Friedrich, K. G., Di Cerbo, P. and Borzacchiello, G. (2017). Detection of Papillomavirus DNA in Cutaneous Squamous Cell Carcinoma and Multiple Papillomas in Captive Reptiles. *J Comp Pathol,* **157,** 23-26.

Fallavena, L. C., Canal, C. W., Salle, C. T., Moraes, H. L., Rocha, S. L., Pereira, R. A. and da Silva, A. B. (2002). Presence of avipoxvirus DNA in avian dermal squamous cell carcinoma. *Avian Pathol,* **31,** 241-246.

Fallavena, L. C., Rodrigues, N. C., Moraes, H. L., Salle, C. T., da Silva, A. B., Nascimento, V. P. and Rodrigues, O. (1997). Squamous cell carcinoma-like and pox lesions occurring

simultaneously in chorioallantoic membranes of chicken embryos inoculated with materials from squamous cell carcinoma and pox lesions in broiler chickens. *Avian Dis,* **41,** 469-471.

Field, J. K. (1992). Oncogenes and tumour-suppressor genes in squamous cell carcinoma of the head and neck. *Eur J Cancer B Oral Oncol,* **28B,** 67-76.

Fischer, S. M., Lo, H. H., Gordon, G. B., Seibert, K., Kelloff, G., Lubet, R. A. and Conti, C. J. (1999). Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. *Mol Carcinog,* **25,** 231-240.

Gallagher, A. and Sullivan, N. D. (1997). Internal papilloma disease in green-winged macaws (Ara chloroptera). *Aust Vet J,* **75,** 675.

Gartrell, B. D., Morgan, K. J., Howe, L., Munday, J. S. and McFadden, A. M. (2009). Cloacal papillomatosis in the absence of herpesvirus and papillomavirus in a sulphur-crested cockatoo (Cacatua galerita). *N Z Vet J,* **57,** 241-243.

Gaynor, A. M., Fish, S., Duerr, R. S., Cruz, F. N., Jr. and Pesavento, P. A. (2015). Identification of a novel papillomavirus in a Northern Fulmar (Fulmarus glacialis) with viral production in cartilage. *Vet Pathol,* **52,** 553-561.

Goldschmidt, M., and Goldschmidt, KH. (2017). Epithelial and Melanocytic Tumors of the Skin. In: *Tumors in Domestic Animals*, D. Meuten, Ed, Wiley Blackwell, Ames, Iowa, pp. 88-99.

Goldschmidt, M., Munday, JS, Scruggs, JL, Klopfleisch, R, Kiupel, M. (2018). Classification of Epithelial Tumors of the Skin. . In: *Surgical Pathology of Tumors of Domestic Animals*, M. Kiupel, Ed, Davis-Thompson Foundation, Gurnee, Illinois, pp. 25.

Greenhough, A., Smartt, H. J., Moore, A. E., Roberts, H. R., Williams, A. C., Paraskeva, C. and Kaidi, A. (2009). The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis,* **30,** 377-386.

Hafner, S., Harmon, B. G., Rowland, G. N., Stewart, R. G. and Glisson, J. R. (1991). Spontaneous regression of "dermal squamous cell carcinoma" in young chickens. *Avian Dis,* **35,** 321-327.

Hafner, S., Harmon, B. G., Stewart, R. G. and Rowland, G. N. (1993). Avian keratoacanthoma (dermal squamous cell carcinoma) in broiler chicken carcasses. *Vet Pathol,* **30,** 265-270.

Hatkin, J., Styer, E. and Miller, D. (2002). Ingluvial squamous cell carcinoma in a game chicken. *Avian Dis,* **46,** 1070-1075.

Hayes, A., Scase, T., Miller, J., Murphy, S., Sparkes, A. and Adams, V. (2006). COX-1 and COX-2 expression in feline oral squamous cell carcinoma. *J Comp Pathol,* **135,** 93-99.

Hunt, C. (2018). History taking and examination. In: *BSAVA Manual of Avian Practice. A Foundation Manual*, J. a. M. Chitty, D., Ed, British Small Animal Veterinary Association, Quedgeley, Gloucestershire, pp. 136.

Jeanes, A., Gottardi, C. J. and Yap, A. S. (2008). Cadherins and cancer: how does cadherin dysfunction promote tumor progression? *Oncogene,* **27,** 6920-6929.

Johne, R., Konrath, A., Krautwald-Junghanns, M. E., Kaleta, E. F., Gerlach, H. and Muller, H. (2002). Herpesviral, but no papovaviral sequences, are detected in cloacal papillomas of parrots. *Arch Virol,* **147,** 1869-1880.

Jones, C. J. and Budsberg, S. C. (2000). Physiologic characteristics and clinical importance of the cyclooxygenase isoforms in dogs and cats. *J Am Vet Med Assoc,* **217,** 721-729.

Kiupel, M., Webster, J. D., Bailey, K. L., Best, S., DeLay, J., Detrisac, C. J., Fitzgerald, S. D., Gamble, D., Ginn, P. E., Goldschmidt, M. H., Hendrick, M. J., Howerth, E. W., Janovitz, E. B., Langohr, I., Lenz, S. D., Lipscomb, T. P., Miller, M. A., Misdorp, W., Moroff, S., Mullaney, T. P., Neyens, I., O'Toole, D., Ramos-Vara, J., Scase, T. J.,

Schulman, F. Y., Sledge, D., Smedley, R. C., Smith, K., P, W. S., Southorn, E., Stedman, N. L., Steficek, B. A., Stromberg, P. C., Valli, V. E., Weisbrode, S. E., Yager, J., Heller, J. and Miller, R. (2011). Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol,* **48,** 147-155.

Knapp, D. W., Richardson, R. C., Chan, T. C., Bottoms, G. D., Widmer, W. R., DeNicola, D. B., Teclaw, R., Bonney, P. L. and Kuczek, T. (1994). Piroxicam therapy in 34 dogs with transitional cell carcinoma of the urinary bladder. *J Vet Intern Med,* **8,** 273-278.

Latimer, K. S., Niagro, F. D., Rakich, P. M., Campagnoli, R. P., Ritchie, B. W. and McGee, E. D. (1997). Investigation of parrot papillomavirus in cloacal and oral papillomas of psittacine birds. *Vet Clin Pathol,* **26,** 158-163.

Laura, N., Marta, V., Giacomo, B. and Luca, B. (2016). Squamous Cell Carcinoma of the Oropharynx and Esophagus with Pulmonary Metastasis in a Backyard Laying Hen. *Avian Dis,* **60,** 694-697.

Liao, Z., Komaki, R., Milas, L., Yuan, C., Kies, M., Chang, J. Y., Jeter, M., Guerrero, T., Blumenschien, G., Smith, C. M., Fossella, F., Brown, B. and Cox, J. D. (2005). A phase I clinical trial of thoracic radiotherapy and concurrent celecoxib for patients with unfavorable performance status inoperable/unresectable non-small cell lung cancer. *Clin Cancer Res,* **11,** 3342-3348.

Lina, P. H., van Noord, M. J. and de Groot, F. G. (1973). Detection of virus in squamous papillomas of the wild bird species Fringilla coelebs. *J Natl Cancer Inst,* **50,** 567-571.

Liu, J. F., Zhang, S. W., Jamieson, G. G., Zhu, G. J., Wu, T. C., Zhu, T. N., Shan, B. E. and Drew, P. A. (2008). The effects of a COX-2 inhibitor meloxicam on squamous cell carcinoma of the esophagus in vivo. *Int J Cancer,* **122,** 1639-1644.

Lorenz, T. C. (2012). Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *J Vis Exp***,** e3998.

Lothaire, P., de Azambuja, E., Dequanter, D., Lalami, Y., Sotiriou, C., Andry, G., Castro, G., Jr. and Awada, A. (2006). Molecular markers of head and neck squamous cell carcinoma: promising signs in need of prospective evaluation. *Head Neck,* **28,** 256-269.

Mestrinho, L. A., Pissarra, H., Faisca, P. B., Braganca, M., Peleteiro, M. C. and Niza, M. M. (2015). p63 and E-cadherin Expression in Canine Oral Squamous Cell Carcinoma. *Vet Pathol,* **52,** 614-620.

Millanta, F., Andreani, G., Rocchigiani, G., Lorenzi, D. and Poli, A. (2016). Correlation Between Cyclo-oxygenase-2 and Vascular Endothelial Growth Factor Expression in Canine and Feline Squamous Cell Carcinomas. *J Comp Pathol,* **154,** 297-303.

Moles, M. A., Montoya, J. A., Salvago, M. D., Avila, I. R., Campillo, J. J. and Bravo, M. (2016). Implications of Differential Expression of beta-Catenin in Oral Carcinoma. *Anticancer Res,* **36,** 1599-1604.

Moore, A. S., Beam, S. L., Rassnick, K. M. and Provost, R. (2003). Long-term control of mucocutaneous squamous cell carcinoma and metastases in a horse using piroxicam. *Equine Vet J,* **35,** 715-718.

Murtaugh, R. J., Ringler, D. J. and Petrak, M. L. (1986). Squamous cell carcinoma of the esophagus in an Amazon parrot. *J Am Vet Med Assoc,* **188,** 872-873.

Nagamine, E., Hirayama, K., Matsuda, K., Okamoto, M., Ohmachi, T., Uchida, K., Kadosawa, T. and Taniyama, H. (2017). Invasive Front Grading and Epithelial-Mesenchymal Transition in Canine Oral and Cutaneous Squamous Cell Carcinomas. *Vet Pathol,* **54,** 783-791.

Nakamura, K., Ito, M., Fujino, K., Yamamoto, Y., Mase, M., Yamada, M., Kobayashi, H. and Harada, T. (2010). Pathology and microbiology of dermal squamous cell carcinoma in young brown chickens reared on reused litter. *Avian Dis,* **54,** 1120-1124.

O'Banion, M. K., Jacobson, E. R. and Sundberg, J. P. (1992). Molecular cloning and partial characterization of a parrot papillomavirus. *Intervirology,* **33,** 91-96.

O'Neill, S. H., Newkirk, K. M., Anis, E. A., Brahmbhatt, R., Frank, L. A. and Kania, S. A. (2011). Detection of human papillomavirus DNA in feline premalignant and invasive squamous cell carcinoma. *Vet Dermatol,* **22,** 68-74.

Oláh, I., Nagy, N., & Vervelde. (2014). Structure of the avian lymphoid system. . In: *Avian immunology* B. K. a. P. K. Karel A. Schat, Ed, Academic Press, pp. 11-44.

Osterhaus, A. D., Ellens, D. J. and Horzinek, M. C. (1977). Identification and characterization of a papillomavirus from birds (Fringillidae). *Intervirology,* **8,** 351-359.

Pan, C. X., Loehrer, P., Seitz, D., Helft, P., Juliar, B., Ansari, R., Pletcher, W., Vinson, J., Cheng, L. and Sweeney, C. (2005). A phase II trial of irinotecan, 5-fluorouracil and leucovorin combined with celecoxib and glutamine as first-line therapy for advanced colorectal cancer. *Oncology,* **69,** 63-70.

Perez-Tris, J., Williams, R. A., Abel-Fernandez, E., Barreiro, J., Conesa, J. J., Figuerola, J., Martinez-Martinez, M., Ramirez, A. and Benitez, L. (2011). A multiplex PCR for detection of poxvirus and papillomavirus in cutaneous warts from live birds and museum skins. *Avian Dis,* **55,** 545-553.

Queiroga, F. L., Pires, I., Parente, M., Gregorio, H. and Lopes, C. S. (2011). COX-2 over-expression correlates with VEGF and tumour angiogenesis in canine mammary cancer. *Vet J,* **189,** 77-82.

Ramis, A., Gibert, X., Majo, N. and Grifols, J. (1999). Metastatic oral squamous cell carcinoma in a Montagu's harrier (Circus pigargus). *J Vet Diagn Invest,* **11,** 191-194.

Ramos-Vara, J. A. a. B. L. B. (2016). Immunohistochemistry: Fundamentals and Applications in Oncology. In: *Tumours in Domestic Animals*, D. Meuten, Ed, Wiley Blackwell, Ames, Iowa, pp. 44-87.

Re, M., Gioacchini, F. M., Scarpa, A., Cassandro, C., Tulli, M. and Cassandro, E. (2018). The prognostic significance of E-cadherin expression in laryngeal squamous-cell carcinoma: a systematic review. *Acta Otorhinolaryngol Ital,* **38,** 504-510.

Rettenmund, C. L., Newton, A. L. and Calle, P. P. (2015). Uropygial gland squamous cell carcinoma in chinstrap (Pygoscelis antarcticus) and gentoo (Pygoscelis papua) penguins at the Wildlife Conservation Society's Central Park Zoo. *J Zoo Wildl Med,* **46,** 113-119.

Rigdon, R. H. and Brashear, D. (1954). Experimental production of squamous-cell carcinomas in the skin of chickens. *Cancer Res,* **14,** 629-631.

Sandmeier, P. (2018). Anatomy and physiology. In: *BSAVA Manual of Avian Practice. A Foundation Manual*, J. a. M. Chitty, D., Ed, British Small Animal Veterinary Association,, Quedgeley, Gloucestershire, pp. 18-29.

Schmidt, B. R., Glickman, N. W., DeNicola, D. B., de Gortari, A. E. and Knapp, D. W. (2001). Evaluation of piroxicam for the treatment of oral squamous cell carcinoma in dogs. *J Am Vet Med Assoc,* **218,** 1783-1786.

Schrenzel, M. D., Maalouf, G. A., Gaffney, P. M., Tokarz, D., Keener, L. L., McClure, D., Griffey, S., McAloose, D. and Rideout, B. A. (2005). Molecular characterization of isosporoid coccidia (Isospora and Atoxoplasma spp.) in passerine birds. *J Parasitol,* **91,** 635-647.

Shivaprasad, H. (2008). Integumentary System. In: *Avian Histopathology*, O. Fletcher, Ed, American Association of Avian Pathologists, Jacksonville, Florida, pp. 402-403.

Sorenmo, K. U., Goldschmidt, M. H., Shofer, F. S., Goldkamp, C. and Ferracone, J. (2004). Evaluation of cyclooxygenase-1 and cyclooxygenase-2 expression and the effect of cyclooxygenase inhibitors in canine prostatic carcinoma. *Vet Comp Oncol,* **2,** 13-23.

Steinbach, G., Lynch, P. M., Phillips, R. K., Wallace, M. H., Hawk, E., Gordon, G. B., Wakabayashi, N., Saunders, B., Shen, Y., Fujimura, T., Su, L. K., Levin, B., Godio, L., Patterson, S., Rodriguez-Bigas, M. A., Jester, S. L., King, K. L., Schumacher, M., Abbruzzese, J., DuBois, R. N., Hittelman, W. N., Zimmerman, S., Sherman, J. W. and Kelloff, G. (2000). The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med,* **342,** 1946-1952.

Styles, D. K., Tomaszewski, E. K., Jaeger, L. A. and Phalen, D. N. (2004). Psittacid herpesviruses associated with mucosal papillomas in neotropical parrots. *Virology,* **325,** 24-35.

Suarez-Bonnet, A., Willis, C., Pittaway, R., Smith, K., Mair, T. and Priestnall, S. L. (2018). Molecular carcinogenesis in equine penile cancer: A potential animal model for human penile cancer. *Urol Oncol,* **36,** 532 e539-532 e518.

Sundberg, J. P., Junge, R. E., O'Banion, M. K., Basgall, E. J., Harrison, G., Herron, A. J. and Shivaprasad, H. L. (1986). Cloacal papillomas in psittacines. *Am J Vet Res,* **47,** 928-932.

Tachezy, R., Rector, A., Havelkova, M., Wollants, E., Fiten, P., Opdenakker, G., Jenson, B., Sundberg, J. and Van Ranst, M. (2002). Avian papillomaviruses: the parrot Psittacus erithacus papillomavirus (PePV) genome has a unique organization of the early protein region and is phylogenetically related to the chaffinch papillomavirus. *BMC Microbiol,* **2,** 19.

Tomaszewski, E., Wilson, V. G., Wigle, W. L. and Phalen, D. N. (2001). Detection and heterogeneity of herpesviruses causing Pacheco's disease in parrots. *J Clin Microbiol,* **39,** 533-538.

Truchado, D. A., Williams, R. A. J. and Benitez, L. (2018). Natural history of avian papillomaviruses. *Virus Res,* **252,** 58-67.

Valli, V. E., San Myint, M., Barthel, A., Bienzle, D., Caswell, J., Colbatzky, F., Durham, A., Ehrhart, E. J., Johnson, Y., Jones, C., Kiupel, M., Labelle, P., Lester, S., Miller, M., Moore, P., Moroff, S., Roccabianca, P., Ramos-Vara, J., Ross, A., Scase, T., Tvedten, H. and Vernau, W. (2011). Classification of canine malignant lymphomas according to the World Health Organization criteria. *Vet Pathol,* **48,** 198-211.

Vazquez, S., Quiroga, M. I., Aleman, N., Garcia, J. C., Lopez-Pena, M. and Nieto, J. M. (2003). Squamous cell carcinoma of the oropharynx and esophagus in a Japanese bantam rooster. *Avian Dis,* **47,** 215-217.

Wang, D. and Dubois, R. N. (2006). Prostaglandins and cancer. *Gut,* **55,** 115-122.

Wang, D. and Dubois, R. N. (2010). The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene,* **29,** 781-788.

Zhai, B., Yan, H. X., Liu, S. Q., Chen, L., Wu, M. C. and Wang, H. Y. (2008). Reduced expression of E-cadherin/catenin complex in hepatocellular carcinomas. *World J Gastroenterol,* **14,** 5665-5673.

Zhou, J., Tao, D., Xu, Q., Gao, Z. and Tang, D. (2015). Expression of E-cadherin and vimentin in oral squamous cell carcinoma. *Int J Clin Exp Pathol,* **8,** 3150-3154.

Zsivanovits, P. (2018). Infectious Diseases. In: *BSAVA Manual of Avian Practice. A Foundation Manual.*, J. a. M. Chitty, D., Ed, British Small Animal Veterinary Association, Quedgeley, Gloucestershire, pp. 290-291.

Appendix 1: Details of signalment, location and diagnosis

Species	Common name	Scientific name	Age (years)	Sex	Location	Diagnosis
Psittacine						
	African Gray parrot	*Psittacus erithacus*	Unspecified	Unspecified	Cloaca	SCC
	African Gray parrot	*Psittacus erithacus*	Unspecified	Unspecified	Skin - inguinal	SCC
	African Gray parrot	*Psittacus erithacus*	Unspecified	Unspecified	Skin - leg	SCC
	African Gray parrot	*Psittacus erithacus*	21	M	Uropygial gland	SCC
	African Gray parrot	*Psittacus erithacus*	10	M	Skin - head	SCC
	Amazon Parrot	*Amazona sp*	28	M	Skin - body	SCC
	Amazon Parrot	*Amazona sp*	18	M	Unspecified	SCC
	Amazon Parrot	*Amazona sp*	Unspecified	Unspecified	Skin - unspecified	SCC
	Cockatiel	*Nymphicus hollandicus*	11	F	Skin - unspecified	SCC
	Cockatiel	*Nymphicus hollandicus*	Unspecified	M	Uropygial gland	SCC
	Cockatiel	*Nymphicus hollandicus*	Unspecified	Unspecified	Uropygial gland	SCC
	Cockatiel	*Nymphicus hollandicus*	14	F	Uropygial gland	SCC
	Parakeet	Unspecified	3	M	Oropharyngeal	Papilloma
	Parakeet	Unspecified	Unspecified	Unspecified	Unspecified	Papilloma
	Parakeet	Unspecified	Unspecified	Unspecified	Uropygial gland	Papilloma
	Parakeet	Unspecified	Unspecified	Unspecified	Uropygial gland	Papilloma
	Parakeet	Unspecified	10	F	Uropygial gland	SCC
	Parakeet	Unspecified	Adult	F	Uropygial gland	SCC
	Parakeet	Unspecified	Unspecified	Unspecified	Uropygial gland	SCC
	Parrot - unspecified	Unspecified	25	M	Skin - leg	SCC
	Parrot - unspecified	Unspecified	Unspecified	F	Cloaca	Papilloma
	Turquoise fronted amazon	*Amazona aestiva*	35	M	Skin - inguinal	SCC
	Turquoise fronted amazon	*Amazona aestiva*	35	M	Skin - leg	SCC
	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	Papilloma
	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	Papilloma
	Unspecified	Unspecified	Unspecified	Unspecified	Cloaca	SCC
	Unspecified	Unspecified	Unspecified	Unspecified	Oropharyngeal - glottis	SCC
	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	SCC
	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	SCC
	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	SCC
	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	SCC
Raptor						
	Barn owl	*Tyto alba*	Adult	F	Skin - leg	SCC
	Barn owl	*Tyto alba*	15	M	Uropygial gland	SCC
	Bateleur Eagle	*Terathopius ecaudatus*	40	F	Oropharyngeal - oesophagus and pharynx	SCC
	Eagle Owl	*Bubo bubo*	15	M	Skin - unspecified	SCC
	Gyr falcon	*Falco rusticolus*	6	M	Oropharyngeal	SCC
	Gyr X peregrine falcon	*Falco rusticolus X Falco peregrinus*	12	M	Skin - unspecified	SCC
	Harris Hawk	*Parabuteo unicinctus*	13	F	Skin - axillary	SCC
	Harris Hawk	*Parabuteo unicinctus*	18	Unspecified	Skin - body	SCC
	Harris Hawk	*Parabuteo unicinctus*	Adult	M	Skin - inguinal	SCC
	Harris Hawk	*Parabuteo unicinctus*	10	M	Skin - inguinal	SCC
	Harris Hawk	*Parabuteo unicinctus*	10	M	Skin - inguinal	SCC
	Lanner Falcon	*Falco biarmicus*	1	F	Oropharyngeal - tongue	SCC
	Peregrine falcon	*Falco peregrinus*	7	F	Skin - body	SCC
	Peregrine falcon	*Falco peregrinus*	8	M	Skin - inguinal	SCC
	Peregrine falcon	*Falco peregrinus*	5	F	Skin - leg	SCC
	Peregrine falcon	*Falco peregrinus*	Adult	M	Skin - unspecified	SCC
	Turkmenian eagle owl	*Bubo bubo*	10	M	Skin - body	SCC
Domestic fowl						SCC
	Chicken	*Gallus gallus*	4	F	Oropharyngeal - glottis	SCC
	Chicken	*Gallus gallus*	7	F	Oropharyngeal - oral mucosa	SCC
	Chicken	*Gallus gallus*	3	F	Skin - foot	SCC
	Chicken	*Gallus gallus*	6	Unspecified	Skin - foot	SCC