


REVIEW

The role of COX expression in the prognostication of overall survival of canine and feline cancer: A systematic review

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Abstract

Cyclooxygenase (COX) isoforms-1 and -2 have been extensively investigated in cancer. Although COX-2 is the isoform most studied and has been described in several malignancies associated with histologic criteria of malignancy and worse prognosis, COX-1 has also been linked to some forms of cancer. With the present review our aim was to summarize the current state of knowledge and clarify if and in which type of tumours COX-1 and/or COX-2 expression have real prognostic implications. We searched PubMed database for prognostic studies using predefined inclusion criteria in order to ascertain the prognostic value of COX-1 and COX-2 in malignant neoplasia in dogs and cats. Eighteen studies were analysed. COX-2 was shown to be a negative prognostic factor in canine and feline mammary tumours, canine mast cell tumour, canine melanoma, canine osteosarcoma and canine renal cell carcinoma. COX-1 showed a negative prognostic value in feline oral squamous cell carcinoma (SCC). We found high heterogeneity among studies regarding COX immunohistochemical evaluation methodology even in the same type of neoplasia pointing out the need for its standardization at least by tumour type. The available data support the use of COX-2 as a prognostic factor in canine (mammary carcinoma, mast cell tumour, melanoma, osteosarcoma and renal carcinoma) and feline (mammary carcinoma) cancers. For COX-1, its use is advised in feline oral SCC.

KEYWORDS

cat, COX expression, COX-2, dog, neoplasia, prognosis, tumour

1 | INTRODUCTION

In veterinary medicine, the incidence of cancer is increasing and cancer constitutes nowadays a major cause of morbidity and mortality in small animal practice. Some reasons that could explain this occurrence are related with the advances in diagnostic and treatment techniques, as well as the prophylactic-based clinical practice, which has allowed the improvement of dogs and cats life expectancy (Szweda et al., 2020). Moreover, companion animals share the

human life environment and are under the same cancer predisposing risk factors, developing naturally occurring neoplastic disease, unlike laboratory animals in which cancer has to be induced. All these reasons point dogs and cats with cancer as an important biological model for studying human cancer disease (Davis & Ostrander, 2014; Gardner et al., 2016).

Cancer therapy has evolved enormously in recent years and multimodal treatments are now commonly used. Furthermore, the availability of common treatment modalities in the human field allow

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to better compare prognostic markers and treatment responses between species (Schiffman & Breen, 2015). However, even though all the recent improvements in veterinary cancer therapy, it remains truly important to identify molecular biomarkers that could upgrade the efficacy of oncological diagnostics and provide more accurate prognostic information in animals with tumours.

Cyclooxygenases, a group of enzymes involved in the transformation of arachidonic acid into prostaglandins, have been described as promising biomarkers with increasing relevance in human and veterinary oncology (Carvalho et al., 2018; Guimarães et al., 2014; Hashemi Goradel et al., 2019). Cyclooxygenase (COX)-1 is expressed mainly constitutively, whereas COX-2 expression increases during several pathological processes involving inflammation, pain, fever and several neoplasias (Spugnini et al., 2005). COX-2 overexpression was first described as being involved in human tumour progression, demonstrating an important prognostic value in several human cancers (Méric et al., 2006). Although to a lesser extent than in human medicine, a relevant number of recent studies have also identified COX-2 overexpression in some malignancies in dogs and cats (Doré, 2011) making it a potential prognostic marker and therapeutic target.

To the best of our knowledge no systematic review has been performed to better discriminate these studies on COX expression in the veterinary field with the intent to identify research works that clearly recognises COX isoenzymes as prognostic markers from studies which merely describe COX expression in a particular neoplasia or an association with malignancy criteria but without evaluating the impact on overall survival (OS).

The aim of the present work was to determine whether COX-1 or COX-2 expression have any value as a prognostic marker in canine and feline malignant neoplasms. For this purpose, we reviewed published observational studies analysing COX-1 or COX-2 expression either by immunohistochemistry or molecular quantification and its impact on OS.

2 | METHODS

2.1 | Search strategy and selection criteria

We searched PubMed using the following search terms, either as MeSH terms or free entries: "dog", "cat", "neoplasia", "tumour", "cancer", "cyclooxygenase" and "COX". Studies were eligible if they fulfilled all the following inclusion criteria: (1) were published in English, French, Portuguese or Spanish; (2) were performed in dogs or cats of any age or breed; (3) quantified COX-1 or COX-2 expression by immunohistochemistry or molecular quantification in any malignant neoplasia; (4) consisted of observational studies and (5) evaluated COX association with OS. The study selection procedure was a two-step process: Step 1 (scan read) consisted of title and abstract evaluation, whereas in Step 2 the full study report was evaluated. Both steps were performed by two authors working independently (HG and TRM). Disagreements were solved by consensus. If consensus could not be found, a third author (FLQ) had the final decision on the

inclusion of a particular study. The last search was performed on 20 of October, 2019.

Data collected from studies included type of study and population, type of neoplasia, type of COX evaluated and method of measurement and study results. Data were collected to a standardized data collection sheet. For bias assessment, a methodological classification was used to ascertain the risk of bias based on the criteria described by Laupacis et al. (1994) and the American College of Veterinary Pathologists' Oncology Committee recommended guidelines for evaluation of prognostic studies (Webster et al., 2011). Again, the data collection and risk of bias assessment were performed by two authors (HG and FLQ) working independently and disagreements were solved by consensus. The final report was written according to the recommendations suggested in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (Liberati et al., 2009; Moher et al., 2009).

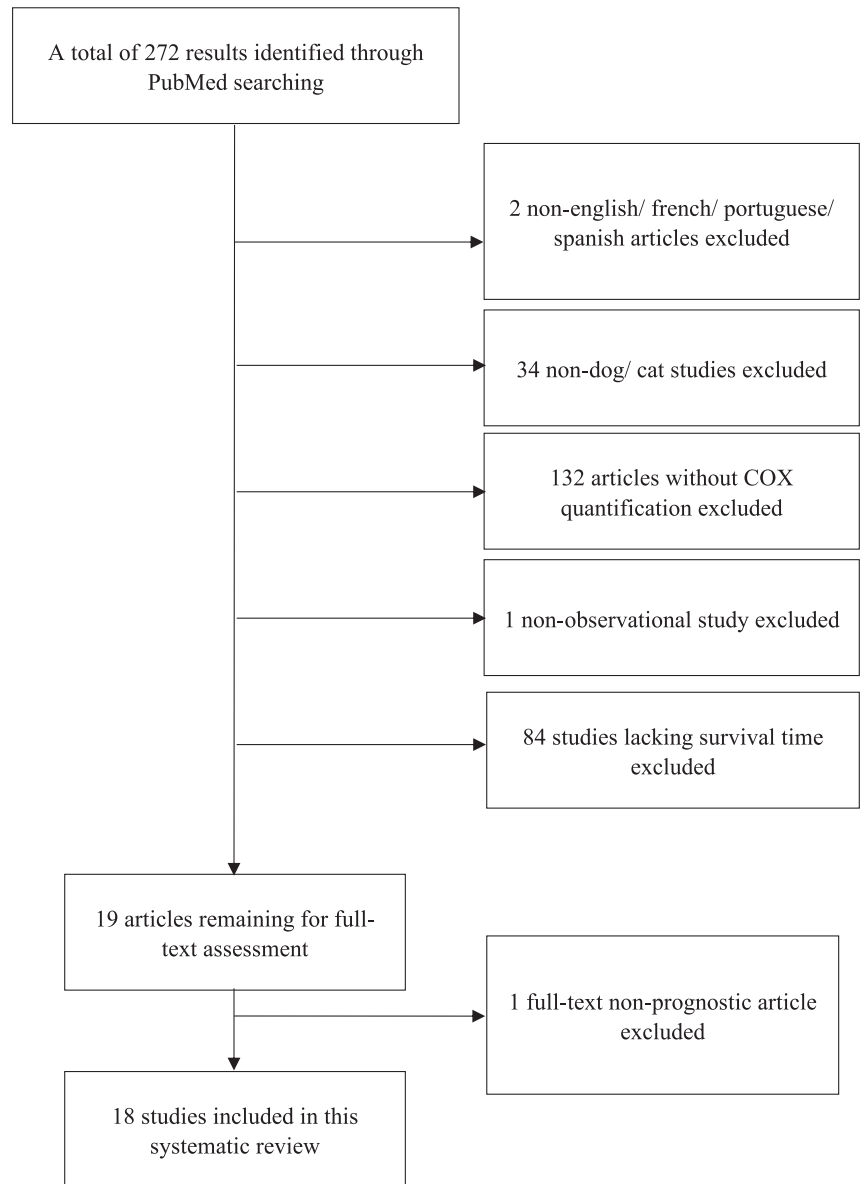
3 | RESULTS

The database search yield 272 results. After reading the title and abstract, 19 were considered relevant. After full-text reading one extra article was excluded for not meeting the inclusion criteria and 18 articles were included for final review (Araújo et al., 2016; Belshaw et al., 2011; Bommer et al., 2012; Carvalho, Pires, et al., 2017; Carvalho, Stoll, et al., 2017; De Campos et al., 2016; Gregório et al., 2017; Hayes et al., 2007; Lavalle et al., 2009, 2012; Martínez et al., 2011; Millanta et al., 2006; Mullins et al., 2004; Nóbrega et al., 2019; Queiroga et al., 2005, 2010; Sorenmo et al., 2004; Vascellari et al., 2013). No extra articles were considered relevant after consulting the references lists of selected studies. A flowchart of the selection process is shown in Figure 1 and a summary of selected studies is described in Table 1.

3.1 | Characteristics of included studies

Of the 18 selected studies, 10 were retrospective (Belshaw et al., 2011; Bommer et al., 2012; Carvalho, Stoll, et al., 2017; De Campos et al., 2016; Gregório et al., 2017; Hayes et al., 2007; Martínez et al., 2011; Mullins et al., 2004; Nóbrega et al., 2019; Sorenmo et al., 2004) and eight were prospective studies (Araújo et al., 2016; Carvalho, Pires, et al., 2017; Lavalle et al., 2009, 2012; Millanta et al., 2006; Queiroga et al., 2005, 2010; Vascellari et al., 2013). Fifteen studies were performed in dogs (Araújo et al., 2016; Belshaw et al., 2011; Carvalho, Pires, et al., 2017; Carvalho, Stoll, et al., 2017; Lavalle et al., 2009; Lavalle et al., 2012; Martínez et al., 2011; Millanta et al., 2006; Mullins et al., 2004; Nóbrega et al., 2019; Queiroga et al., 2005; Queiroga et al., 2010; Sorenmo et al., 2004; Vascellari et al., 2013) and four studies in cats (Bommer et al., 2012; De Campos et al., 2016; Hayes et al., 2007; Millanta et al., 2006), totalling 688 dogs and 145 cats. Eleven different types of tumours were studied: canine mammary tumours (Araújo et al., 2016; Carvalho, Pires, et al., 2017; Lavalle et al., 2009, 2012;

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart of selected studies



Millanta et al., 2006; Queiroga et al., 2005, 2010), feline mammary tumours (De Campos et al., 2016; Millanta et al., 2006), canine mast cell tumours (Gregório et al., 2017; Vascellari et al., 2013), feline transitional cell carcinomas (Bommer et al., 2012), canine nasal carcinomas (Belshaw et al., 2011), canine melanomas (Martínez et al., 2011), feline oral squamous cell carcinomas (Hayes et al., 2007), canine osteosarcomas (Mullins et al., 2004), canine prostatic carcinomas (Sorenmo et al., 2004), canine cutaneous haemangiosarcomas (Nóbrega et al., 2019) and canine renal cell carcinomas (Carvalho, Stoll, et al., 2017). Fourteen studies evaluated COX-2 only (Araújo et al., 2016; Belshaw et al., 2011; Carvalho, Pires, et al., 2017; Carvalho, Stoll, et al., 2017; De Campos et al., 2016; Gregório et al., 2017; Lavallo et al., 2009, 2012; Martínez et al., 2011; Millanta et al., 2006; Mullins et al., 2004; Nóbrega et al., 2019; Queiroga et al., 2005; Vascellari et al., 2013) and four studies evaluated both COX-1 and COX-2 (Bommer et al., 2012; Hayes et al., 2007; Queiroga et al., 2010; Sorenmo et al., 2004). The most common method used to determine COX expression was

immunohistochemistry as the only detection technique (16 studies; Araújo et al., 2016; Belshaw et al., 2011; Bommer et al., 2012; Carvalho, Pires, et al., 2017; Carvalho, Stoll, et al., 2017; De Campos et al., 2016; Gregório et al., 2017; Hayes et al., 2007; Lavallo et al., 2009, 2012; Martínez et al., 2011; Millanta et al., 2006; Mullins et al., 2004; Nóbrega et al., 2019; Queiroga et al., 2010; Sorenmo et al., 2004) whereas one study combined immunohistochemistry with quantitative polymerase chain reaction (qPCR; Vascellari et al., 2013) and another measured COX-2 levels by enzyme immunoassay (Queiroga et al., 2005). COX-2 positivity was ascertained differently in the several studies using immunohistochemistry: one study classified samples as positive or negative (Bommer et al., 2012), another used COX-2 extension only (Martínez et al., 2011), another evaluated intensity, extension and pattern of distribution (Hayes et al., 2007) and another ascertained labelling index and labelling intensity (Nóbrega et al., 2019). However, the majority of studies (13; Araújo et al., 2016; Belshaw et al., 2011; Carvalho, Pires, et al., 2017; Carvalho, Stoll, et al., 2017; De Campos et al., 2016;

TABLE 1 Summary of selected studies

Year/1st Author	Study type/N/Treatment	Tumour	Cox's type and detection technique
2019/Nóbrega, D.F.	Retrospective 60 dogs (30 male + 30 female) Surgery	Canine cutaneous haemangiosarcoma	COX-2 Immunohistochemistry (80% of positive expression)
2017/Carvalho, M.I.	Prospective 109 female dogs Surgery	Canine mammary tumours	COX-2 Immunohistochemistry
2017/Carvalho, S.	Retrospective 30 dogs Surgery ± adjuvant therapy (e.g. cytotoxic drugs or tyrosine kinase inhibitors)	Canine renal cell carcinoma	COX-2 Immunohistochemistry (77% of positive expression)
2017/Gregório, H.	Retrospective 43 dogs Surgery	Canine mast cell tumours	COX-2 Immunohistochemistry (92% of positive expression)
2016/Araújo, M.R.	Prospective 78 dogs Surgery	Canine mammary tumours and paired lymph nodes metastasis	COX-2 Immunohistochemistry
2016/de Campos, C.B.	Retrospective 37 female cats Surgery	Feline mammary tumours and paired lymph nodes metastasis	COX-2 Immunohistochemistry
2013/Vascellari, M.	Prospective 51 dogs (18 males + 30 females + 3 unknown) Surgery ± chemotherapy	Canine cutaneous mast cell tumours	COX-2 Immunohistochemistry (78% of positive expression) Quantitative real-time polymerase chain reaction (qPCR)
2012/Bommer, N.X.	Retrospective 7 cats (4 males + 3 females) Meloxicam ± surgery	Feline transitional cell carcinoma of the urinary bladder	COX-1 and COX-2 Immunohistochemistry (100% and 71% of positive expression respectively)
2012/Lavalle, G.E.	Prospective 29 female dogs Surgery ± carboplatin ± COX inhibitors (piroxicam or firocoxib)	Canine mammary tumours	COX-2 Immunohistochemistry (97% of positive expression)
2011/Belshaw, Z.	Retrospective 42 dogs (17 males + 25 females) Radiotherapy	Canine nasal carcinoma	COX-2 Immunohistochemistry (90% of positive expression)
2011/Martínez, C.M.	Retrospective 41 dogs No treatment information available	Canine melanocytic neoplasms (oral + cutaneous)	COX-2 Immunohistochemistry (88% and 75% of positive expression in oral melanocytic tumours and cutaneous melanocytic tumours respectively)
2010/Queiroga, F.L.	Prospective 27 female dogs Surgery	Canine mammary tumours	COX-1 and COX-2 Immunohistochemistry (100% of positive expression to both)
2009/Lavalle, G.E.	Prospective 46 female dogs Surgery	Canine mammary tumours	COX-2 Immunohistochemistry (100% of positive expression)
2007/Hayes, A.M.	Retrospective 54 cats Surgery ± NSAID, steroids and/or antibiotics	Feline oral squamous cell carcinoma	COX-1 and COX-2 Immunohistochemistry (100% and 67% of positive expression respectively)

Evaluation strategy and cut-off point	Relevance
<p>Labelling index (estimated % of immunolabelled cells) and labelling intensity (weak, moderate or strong) Cut-off point: not defined</p>	COX-2 labelling index ($p = .35$) and labelling intensity ($p = .63$) were not associated with survival
<p>COX-2 score (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–3) scores Cut-off point: >6</p>	High COX-2 expression (>6) was associated with decreased OS ($p < .001$), but was not considered a reliable independent predictor of prognosis
<p>Immunohistochemical score (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–3) scores Cut-off point: >3</p>	<p>High COX-2 score (>3) was associated with decreased OS ($p = .011$) and considered as an independent prognostic factor ($p = .006$)</p> <p>COX-2 staining intensity and % of positive cells were not associated with OS ($p > .05$)</p>
<p>Immunohistochemical score (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–3) scores Cut-off point: >6</p>	<p>High COX-2 intensity was associated with decreased OS ($p = .016$)</p> <p>COX-2 extension and COX-2 score were not associated with OS ($p = .494$ and $p = .196$ respectively)</p>
<p>COX-2 score (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–3) scores Cut-off point: ≥6</p>	COX-2 score was not associated with OS ($p > .05$)
<p>COX-2 score (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–3) scores Cut-off point: ≥6</p>	High COX-2 scores (≥6) were associated with decreased OS with a value trending towards significance ($p = .089$)
<p>COX-2 staining index (0–9) as a result of multiplying the estimated % of positive cells (0–3) and the staining intensity (0–3) scores COX-2 RNA expression levels Cut-off point: not defined</p>	COX-2 expression score and COX-2 RNA expression levels did not show a significant association with mortality ($p = .786$ and $p = .949$ respectively)
<p>Comparison between COX-2-positive versus. -negative expression Cut-off point: not defined</p>	Cats diagnosed with COX-2-positive tumours exhibited shorter survival times than those who had COX-2-negative tumours (mean survival time = 123 days vs. 375 days)
<p>COX-2 immunohistochemical score (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–3) scores Cut-off point: ≥ 6</p>	High COX-2 scores (≥6) were associated with decreased OS ($p = .08$)
<p>Immunohistochemical score (0–12) as a result of multiplying the estimated % of positive cells (1–4) and the staining intensity (0–3) scores Cut-off point: ≥ 2</p>	COX-2 score and survival did not show a significant correlation ($r = -.154$; $p = .331$)
<p>Evaluation of COX-2 extension/ % of positive cells (0–3) Cut-off point: not defined</p>	High COX-2 expression was associated with decreased OS ($p < .001$) and considered as an independent prognostic factor (HR = 2.762; $p = .004$)
<p>Immunohistochemical score (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–3) scores Cut-off point: >6</p>	<p>High COX-2 expression (>6) was associated with decreased DFS ($p = .03$) and OS ($p = .04$), but did not prove to be a significant independent prognostic factor ($p = .067$ and $p = .068$ respectively)</p> <p>COX-1 expression was not associated with DFS or OS</p>
<p>COX-2 score (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–3) scores Cut-off point: ≥ 6</p>	High COX-2 scores (≥6) were associated with decreased OS ($p = .01$)
<p>COX-1 and COX-2 immunohistochemical expression (extension, intensity and distribution pattern) Cut-off point: not defined</p>	<p>COX-1 diffuse distribution pattern was considered a predictive factor for survival (HR = 1; $p = .014$)</p> <p>COX-2 evaluation was not associated with survival</p>

(Continues)

TABLE 1 (Continued)

Year/1st Author	Study type/N/Treatment	Tumour	Cox's type and detection technique
2006/Millanta, F.	Prospective 28 female dogs 47 female cats Surgery	Canine and feline mammary tumours	COX-2 Immunohistochemistry (100% and 96% of positive expression in canine and feline patients respectively)
2005/Queiroga, F.L.	Prospective 25 female dogs Surgery ± antibiotics and steroids	Canine mammary tumours	COX-2 Enzyme immunoassay (EIA)
2004/Mullins, M.N.	Retrospective 44 dogs (24 males + 20 females) Surgery + doxorubicin	Canine appendicular osteosarcoma	COX-2 Immunohistochemistry (77% of positive expression)
2004/Sorenmo, K.U.	Retrospective 35 dogs Treatment with versus. without COX inhibitors (piroxicam or carprofen) and other adjuvant therapies	Canine prostatic carcinoma	COX-1 and COX-2 Immunohistochemistry (94% and 88% of positive expression respectively)

Abbreviations: DFS, disease free survival; OS, overall survival.

Gregório et al., 2017; Lavallo et al., 2009, 2012; Millanta et al., 2006; Mullins et al., 2004; Queiroga et al., 2010; Sorenmo et al., 2004; Vascellari et al., 2013) used scoring systems based on a combination of extension and intensity of staining. Ten studies defined a score grading system ranging 0–12 (Araújo et al., 2016; Belshaw et al., 2011; Carvalho, Pires, et al., 2017; Carvalho, Stoll, et al., 2017; De Campos et al., 2016; Gregório et al., 2017; Lavallo et al., 2009, 2012; Queiroga et al., 2010; Sorenmo et al., 2004) based on the methodologies used to define extension of expression described by other authors (Doré et al., 2003; Queiroga et al., 2007). Nevertheless, one study established a score grading scheme between 0 and 9 (Vascellari et al., 2013), another determined a system that compromised score values between 0 and 16 (Mullins et al., 2004) and, finally, one study used an alternative four-tier score (Millanta et al., 2006) as reported by Ristimäki et al. (2002).

Regarding COX-1, one study classified positive versus negative samples (Bommer et al., 2012), one study evaluated extension, intensity and distribution pattern (Hayes et al., 2007) and two others used a score system ranging 0–12 (Queiroga et al., 2010; Sorenmo et al., 2004) similar to ones used in COX-2.

Results of the studies retrieved for analysis revealed that COX-2 detection was associated with a poorer prognosis in canine (Carvalho, Pires, et al., 2017; Lavallo et al., 2009, 2012; Millanta et al., 2006; Queiroga et al., 2005, 2010) and feline mammary tumours (De Campos et al., 2016; Millanta et al., 2006), canine mast cell tumours (Gregório et al., 2017), canine melanomas (Martínez et al., 2011), canine osteosarcomas (Mullins et al., 2004) and canine renal cell carcinomas (Carvalho, Stoll, et al., 2017). COX-2 was not found of prognostic significance in feline oral SCC (Hayes et al., 2007), canine nasal carcinomas (Belshaw et al., 2011), canine prostatic carcinomas (Sorenmo et al., 2004), canine cutaneous haemangiosarcomas (Nóbrega et al., 2019) and in one study in canine mammary tumours (Araújo et al., 2016) and canine mast cell tumours

(Vascellari et al., 2013). One study with seven cats with bladder TCC described two cats harbouring a negative COX-2 expression tumours which experimented prolonged OS, but no formal statistic test was reported (Bommer et al., 2012).

Our analysis revealed that COX-1 expression showed a positive prognostic value in feline oral SCC (Hayes et al., 2007) but failed to show an impact on prognosis in canine mammary tumours (Queiroga et al., 2010) and canine prostatic carcinomas (Sorenmo et al., 2004).

Several factors contributed to bias in the selected studies, such as lack of representation of the full spectrum of disease, use of a subjective method for COX measurement (immunohistochemistry) performed in a non-blinded way in retrospective studies and lack of adjustment for confounding prognostic variables. Only three studies were classified with a low risk of bias. These studies were related to canine mammary tumours (Carvalho, Pires, et al., 2017), renal carcinomas (Carvalho, Stoll, et al., 2017) and mast cell tumours (Vascellari et al., 2013). Table 2 reports the risk of bias for the different studies.

3.2 | Canine mammary tumours

Seven studies assessed the prognostic value of COX-2 in canine mammary tumours (Araújo et al., 2016; Carvalho, Pires, et al., 2017; Lavallo et al., 2009, 2012; Millanta et al., 2006; Queiroga et al., 2005, 2010) and one evaluated the role of COX-1 (Queiroga et al., 2010). Six studies found COX-2 overexpression to be associated with decreased OS (Carvalho, Pires, et al., 2017; Lavallo et al., 2009, 2012; Millanta et al., 2006; Queiroga et al., 2005, 2010). These studies showed sufficient evidence of the role of COX-2 as a negative prognostic factor in canine mammary cancer, although only two

Evaluation strategy and cut-off point	Relevance
Immunohistochemical score (0–3) based on estimated % of positive cells and staining intensity Cut-off point: ≥ 2	High COX-2 levels were associated with decreased OS ($p = .03$ in dogs and $p = .002$ in cats)
COX-2 concentration (ng/g) Cut-off point: > 60 ng/g	High COX-2 levels (>60) were associated with decreased DFS ($p < .001$) and OS ($p < .001$)
Immunoreactivity score (0–16) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (0–4) scores Cut-off point: not defined	Strong COX-2 expression was associated with decreased OS ($p = .0107$), when the evaluation was carried out based on the following categories: negative (0), poor (1–3), moderate (4–7) and strong (8–16)
Staining index (0–12) as a result of multiplying the estimated % of positive cells (0–4) and the staining intensity (1–3) scores Cut-off point: not defined	COX staining intensity was not associated with survival time ($p > .05$)

studies performed adjustments for variables of confusion and did not find COX-2 as an independent prognostic factor (Carvalho, Pires, et al., 2017; Queiroga et al., 2010). One study did not identify COX-2 as a prognostic factor for this tumour type, but the small number of cases classified with high COX-2 score ($n = 6$) may explain this finding (Araújo et al., 2016). As for COX-1, Queiroga et al. (2010) found it to be of no prognostic value.

3.2.1 | Feline mammary tumours

Two studies appraised the prognostic value of COX-2 in feline mammary tumours (De Campos et al., 2016; Millanta et al., 2006). According to Millanta et al. (2006), high COX-2 levels were significantly associated with decreased OS. The other study (De Campos et al., 2016) seemed to reinforce this premise, but only led to a value

TABLE 2 Risk of bias

Year/1st Author	Representative and well-defined sample of patients at a similar point in the course of the disease?	Follow-up sufficiently long and complete?	Were objective and unbiased criteria used in a blinded way?	Was there adjustment for important prognostic factors?
2019/Nóbrega, D.F.	+	-	-	-
2017/Carvalho, M.J.	+	+	+	+
2017,/Carvalho, S.	+	+	+	+
2017/Gregório, H.	+	+	-	+
2016,/Araújo, M.R.	+	+	-	-
2016/de Campos, C.B.	-	-	-	-
2013/Vascellari, M.	+	+	+	+
2012/Bommer, N.X.	-	+	-	-
2012/Lavalle, G.E.	-	-	+	-
2011/Belshaw, Z.	+	+	+	-
2011/Martínez, C.M.	-	+	+	+
2010/Queiroga, F.L.	+	+	+	-
2009/Lavalle, G.E.	-	+	-	-
2007/Hayes, A.M.	+	+	-	+
2006/Millanta, F.	+	+	+	-
2005/Queiroga, F.L.	+	+	+	-
2004/Mullins, M.N.	+	+	+	-
2004/Sorenmo, K.U.	-	+	-	-

trending towards significance for the same association, albeit with considerably higher risk of bias.

3.2.2 | Canine mast cell tumours

Vascellari et al. (2013) evaluated 51 dogs with cutaneous mast cell tumours of various grades for COX-2 expression by immunohistochemistry and qPCR. The study was prospective with a representative population of all grades of MCT and with an adequate follow-up time (minimum of 12 months). The use of an objective method for quantification of COX-2 (qPCR) contributed to the low risk of bias in this study. They found COX-2 to be of no prognostic value. Contrariwise, Gregório et al. (2017), in a more recent study, described COX-2 intensity, but not extension or score, as a negative prognostic factor for canine cutaneous mast cell tumours. These results were obtained in univariate analysis and the risk of bias was considered higher in this study.

3.2.3 | Feline transitional cell carcinoma

Bommer et al. (2012) evaluated a small number of cats with urinary bladder TCC for COX-1 and COX-2 expression. The small population prevented any statistical significance regarding the prognostic value of COX-1 and COX-2, but the authors reported a median survival time of 123 days for COX-2-positive cats ($n = 5$) and 375 days for COX-2-negative cats ($n = 2$). All seven cats were positive for COX-1 preventing any conclusion regarding its prognostic value. The retrospective nature of the study combined with the small number of patients treated in different ways poses this study with a high risk of bias.

3.2.4 | Canine nasal carcinoma

A single study looked into the role of COX-2 in canine nasal cell carcinoma (Belshaw et al., 2011). This study found that the majority of nasal carcinomas (adenocarcinoma, papillary carcinoma, TCC and anaplastic carcinoma) expressed COX-2 but no association was found between COX-2 score and OS.

3.2.5 | Canine melanoma

Martínez et al. (2011) described that melanomas with higher COX-2 scores had a significant lower OS and COX-2 was found to be of independent prognostic value.

3.2.6 | Feline oral SCC

Hayes et al. (2007) found COX-2 to be of no prognostic value, while COX-1 pattern of distribution was found to be an independent negative prognostic factor.

3.2.7 | Canine appendicular osteosarcoma

When analysing COX-2 expression, Mullins et al. (2004) found that COX-2 overexpression was associated with decreased OS in dogs with appendicular osteosarcoma, despite the retrospective nature of the study and the fact that multivariate analysis was not performed.

3.2.8 | Canine prostatic carcinoma

One study evaluated the role of COX-1 and COX-2 in dogs with prostatic carcinoma (Sorenmo et al., 2004). No association was found between COX-1 and COX-2 intensity and OS, excluding them from being relevant prognostic factors in this disease.

3.2.9 | Canine cutaneous haemangiosarcoma

Nóbrega et al. (2019) showed a frequent COX-2 expression in these neoplasms, contrary to what was previously described by Heller et al. (2005) who did not identify positive staining for COX-2 in any of the 19 canine haemangiosarcomas studied.

The authors did not find a correlation between the COX-2 immunolabelling and survival, which prevented COX-2 expression from having prognostic value.

3.2.10 | Canine renal cell carcinoma

Carvalho, Stoll, et al. (2017) also described an association between COX-2 expression and survival. In this study, although COX-2 staining and the percentage of positive cells were not associated with OS, high COX-2 score was significantly associated with OS and was even considered as an independent prognostic factor at multivariate analysis.

4 | DISCUSSION

The scientific knowledge supporting that inflammation and cancer share many common pathways (Demaria et al., 2010; Raposo et al., 2015), associated with the fact that chronic inflammatory states can lead to cancer development (Pesic & Greten, 2016), plus the findings in human medicine literature suggesting that the chronic use of NSAID is associated with lower incidence of cancer (Cooper et al., 2010) has led to an increased interest in investigating COX as a prognostic marker with potential therapeutic value in the veterinary oncology setting. This systematic review showed that both isoforms of COX (COX-1 and COX-2) have been evaluated as a prognostic biomarker in several canine and feline malignancies in a total of 18 studies, attesting the relevance of the subject in veterinary oncology.

Biomarkers are biological molecules that signal a process, condition or disease and have multiple purposes such as risk assessment, staging, therapeutic response assessment and prognosis (Henry & Hayes, 2012). Ideally, a biomarker should help the clinician to classify a patient into a specific group with individualized treatment and prognostic specificities. Several biomarkers are already used in veterinary oncology mainly to ascertain prognosis. These include markers of several hallmarks to carcinogenesis such as Ki-67 (proliferation; Araújo et al., 2016; Carvalho et al., 2016; Carvalho, Pires, et al., 2017; Gregório et al., 2017; Martínez et al., 2011; Millanta et al., 2006; Vascellari et al., 2013), BRAF (promotion; Mochizuki et al., 2015) and VEGF (angiogenesis; Carvalho, Pires, et al., 2017; De Campos et al., 2016; Millanta et al., 2006; Nóbrega et al., 2019).

Cyclooxygenase-2 expression has been mainly used to determine prognosis of particular malignancies as it is involved in several hallmarks of carcinogenesis which in theory makes it an indicator of worse prognosis. Moreover, heightened interest arises from the fact that it constitutes a target for currently available non-steroidal anti-inflammatory drugs, which can prove the clinical benefit of this type of therapy and the importance of COX-2 as a potential biomarker of therapeutic response (Szweda et al., 2020).

One of the aspects that we identified as a possible origin of bias was the method of identification and quantification of COX. Quantification of COX in tissue samples can be performed by immunohistochemistry—a semi-quantitative method—or molecular quantification which constitutes a truly quantitative and therefore objective method. The former has the advantage of being performed in routine paraffin-embedded samples allowing to be carried out in regular laboratories with stored samples. This may be the reason why the vast majority of the studies used this methodology. However, quantifying a protein through colour intensity and extension is inherently subjective as it depends on the observer, the antibody used and the tissue processing technique, which translates into a final result that is categorical as opposed to a continuous result obtained from a truly quantitative method that in turn favours statistical analysis. In addition, the delineation of the respective range for these categorical results is not standardized which makes the comparison between studies hard to perform. In order to avoid these pitfalls, guidelines for immunohistochemistry have been proposed (Ramos-Vara et al., 2008) and digital-assisted immunohistochemistry (Rizzardi et al., 2016) and other methods such as ELISA (Queiroga et al., 2005) and qPCR (Vascellari et al., 2013) have been used. In our results, all but two studies used semi-quantitative immunohistochemistry. Vascellari et al. (2013) used qPCR to quantify COX-2 on mast cell tumours samples and Queiroga et al. (2005) used ELISA to detect COX-2 on mammary tumour samples. These methodologies clearly contributed to a decreased risk of bias on both studies.

In canine mammary tumours, COX-2 has been found to be associated with histologic criteria of malignancy and aggressiveness (Doré et al., 2003; Queiroga et al., 2010, 2011). COX-2 inhibitors are already incorporated in metronomic chemotherapy protocols in women

(Perroud et al., 2013) and therapeutic protocols in female dogs (Lavalle et al., 2012; M. Souza et al., 2009). Selected studies (Carvalho, Pires, et al., 2017; Lavalle et al., 2009, 2012; Millanta et al., 2006; Queiroga et al., 2005, 2010) showed evidence enough to use COX-2 as a negative prognostic marker in canine mammary malignant tumours, although some caution should be taken as canine mammary cancer constitutes a large spectrum of disease with different histologic types and none of the studies addressed specific histological types individually.

As for feline mammary tumours they are often associated with malignant behaviour (Zappulli et al., 2005) and COX-2 is frequently expressed in these neoplasms (Sayasith et al., 2009). COX-2 inhibitors are being used in queens, but current results have failed to demonstrate prognostic benefit in this species (Borrego et al., 2009). The two studies that addressed the role of COX-2 in feline mammary disease (De Campos et al., 2016; Millanta et al., 2006) evidenced the prognostic value of this biomarker in feline populations.

Although COX-2 has been identified as a potential negative prognostic factor in feline mammary cancer, further studies are needed, particularly based on a multivariate analysis, to draw conclusions with stronger evidence.

Canine mast cell tumours are the most common skin neoplasia in dogs and they can have a wide range spectrum of clinical behaviour specially in tumours of intermediate grade, so new prognostic markers could be of extreme value (Blackwood et al., 2012). Two studies had conflicting conclusions on the role of COX-2 in OS. These opposite results could be explained through the unequal representation of neoplasms by tumour grade, according to Patnaik grading system, in the two studies. The most recent study (Gregório et al., 2017) had a more uniform distribution (30.0% grade I, 34.0% grade II and 36.0% grade III) in comparison with the first one described (Vascellari et al., 2013; 52.8% grade I, 41.5% grade II and 5.7% grade III). The lower percentage of grade III mast cell tumours, obtained by Vascellari et al. (2013), may have influenced COX-2 expression and consequently its association with survival. Despite that fact, COX-2 score and COX-2 extension did not show a significant association with the prognosis in both studies, so to date there is only evidence of the prognostic value of COX-2 intensity in these neoplasms.

These results suggest the potential role of COX-2 in the prognosis of mast cell tumours, proposing COX-2 intensity evaluation as the most accurate approach to perform, instead of COX-2 score quantification as has been frequently done for other tumours. More studies are needed to precisely clarify the role of COX-2 in MCTs either by analysing specific Patnaik grades (Patnaik et al., 1984) or by using the new proposed grading scheme by Kiupel (Kiupel et al., 2011).

Feline TCC is a relatively uncommon disease in cats (Cannon & Allstadt, 2015). COX inhibitors are routinely used with efficacy in canine disease variant (Knapp et al., 1994; McMillan et al., 2011) causing special interest in evaluating the role of COX in feline disease. However, the small number on animals enrolled in the single study selected (Bommer et al., 2012) prevented any conclusions on its prognostic role in feline TCC.

Nasal carcinoma has a poor prognosis in dogs. Several prognostic factors have been identified such as epistaxis (Rassnick et al., 2006),

age (>10 years; LaDue et al., 1999), duration of clinical signs (LaDue et al., 1999) and clinical stage (LaDue et al., 1999). The single study that addressed COX-2 expression in canine nasal carcinoma failed to prove a role for COX-2 as a prognostic factor in this disease (Belshaw et al., 2011).

Canine melanoma is a relatively common canine neoplasia. While melanoma located in the skin generally has a good prognosis, oral and nail bed melanomas are considered to have a poor prognosis (Smedley et al., 2011; Spangler & Kass, 2006). Additional to anatomic location, several other pathological prognostic markers have been identified, with mitotic index and Ki67 being the most popularly used (Smedley et al., 2011). COX-2 has been identified in canine melanomas and associated with clinicopathological characteristics of malignancy (Gregório et al., 2016; Martínez et al., 2011; Pires et al., 2010). Although findings from one single study (Martínez et al., 2011) showed sufficient evidence of the prognostic impact of COX-2 in canine melanomas, it should be noted that no differentiation between oral and cutaneous melanocytic lesions was made in this study. Given the different prognosis described for each anatomical location, it would have been interesting if the prognostic assessment of COX-2 was done separately for each group. This could potentially prove helpful identifying the small subset of oral melanomas with better prognosis and likewise identify the less common malignant cutaneous melanomas.

Feline oral SCC is a serious disease with poor prognosis (Bilgic et al., 2015). One study (Hayes et al., 2007) identified a possible role of COX-1 in the carcinogenesis of feline oral SCC and showed to have a moderate risk of bias due to the subjectivity associated with the classification of the distribution pattern used to evaluate COX-1.

Osteosarcomas are tumours with aggressive biological behaviour in dogs and humans and are generally associated with a poor prognosis (Simpson et al., 2017). One study (Mullins et al., 2004) looked at the role of COX-2 as prognostic factor and found it to be a possible negative prognostic marker in canine osteosarcoma. The high risk of bias precludes its use as a prognostic factor without further studies being developed.

Prostatic carcinoma in dogs is an aggressive disease where COX-2 is consistently expressed and where COX inhibitors are routinely used in treatment (L'Eplattenier et al., 2007). The results from one selected study (Sorenmo et al., 2004) did not find an association between COX-1 and COX-2 expression and OS. This study had a high risk of bias due to retrospective nature of the study and heterogeneity of the studied population with regard to clinical staging and treatments performed.

Cutaneous haemangiosarcoma is a neoplasia that has been associated with exposure to ultraviolet radiation in dogs. Although prognosis is good for localized superficial lesions, a much worse prognosis is described for deep and invading lesions (Smith, 2003). One study (Nóbrega et al., 2019) addressed the role of COX-2 as prognostic marker. This study had a high risk of bias due to its retrospective nature and due to the subjective evaluation of COX-2 expression, mainly with regard to its labelling intensity. Further studies of a prospective nature are needed to properly assess the prognostic

value of this biomarker in canine cutaneous haemangiosarcoma, but currently there is not enough evidence to support COX-2 as a biomarker in canine cutaneous haemangiosarcoma.

Canine renal cell carcinoma has been considered the most frequent primary renal tumour in dogs (Bryan et al., 2006). The prognostic value of histological features, as the mitotic index, has already been reported (Edmondson et al., 2015). One study (Carvalho, Stoll, et al., 2017) with low risk of bias found COX-2 score as an independent negative prognostic factor. Although ideally further studies should corroborate these results, there is enough evidence to support COX-2 score as a biomarker of prognosis in this disease.

Cyclooxygenase-2 overexpression was detected in all neoplasms in the selected studies. In fact, COX-2 and its end product PGE₂ have long been associated with several hallmarks of cancer such as promotion, invasion, apoptosis inhibition and metastasis (Wang & Dubois, 2010), and consistently described as associated with other characteristics and markers of malignancy in various canine and feline cancers (Doré, 2011), suggesting it could be used as an important prognostic marker and potential therapeutic target. Very few studies looked into the prognostic value of COX-1 probably due to its ubiquitous expression in several neoplasms both benign and malignant, but one study reported the importance to analyse the staining pattern (Hayes et al., 2007) and it would be interesting to analyse this in other neoplasms. Unfortunately, most studies used an immunohistochemistry score to report COX levels, which is inherently subjective apart from the fact that score methodology calculation and cut-off points differed between studies making it hard for generalizations or, ideally, a meta-analysis performance. This constituted one of the main limitations of studies in our review alongside with small number of patients in each study and the lack of adjustments for confounding variables to ascertain the real and independent prognostic value of COX. This could be attributed to the general difficulty in enrolling large number of cases in veterinary studies, an essential requisite to adjust for confounding variables.

Finally, we described a difference in the prognostic value of COX-2 expression according to the tumour histotype. A possible explanation may be related to the role of COX-2/PGE₂ pathway in the carcinogenesis process (Szveda et al., 2020). One can hypothesize that when it is involved in steps associated with increase tumour aggressiveness such as invasion and metastasis in opposition to tumour initiation, for example, its expression would be associated more clearly with prognosis. In addition, in other tumours other metabolic pathways could have a more important role in carcinogenesis bypassing the relevance of COX-2 in those same tumours. Even so, further studies on the oncogenesis and pathogenesis are needed to fully clarify this issue.

5 | CONCLUSION

In this review, we analysed the role of COX-1 and -2 as prognostic factors in canine and feline malignant neoplasms. We concluded that COX-2 showed to be of negative prognostic value in canine and

feline mammary tumours and in canine mast cell tumour, melanoma, osteosarcoma and renal cell carcinoma. Additionally, COX-1 distribution pattern also showed to be a negative prognostic factor in feline oral SCC. We also found that the main obstacles to more widespread use of COX as prognostic marker was the subjectivity associated with COX quantification which unquestionably contributes to the increased risk of bias in many studies and inherently hinders reproducibility among pathologists.

The findings of the present study prove the rationality of the use of COX enzymes (mostly COX-2) to predict the survival of animals especially in tumours with a wide spectrum of disease such as mammary cancer, where they can prove to be useful to better discriminate more aggressive forms of disease. Further studies still are needed to ascertain the prognostic value of COX in other canine and feline malignancies, ideally with standardized measurement methods.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTION

Hugo Gregório: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Writing-original draft. **Tomás R Magalhães:** Formal analysis; Investigation; Methodology; Writing-review & editing. **Isabel Pires:** Investigation; Software; Supervision. **Justina Prada:** Investigation; Validation. **Maria Isabel Carvalho:** Investigation; Validation. **Felisbina L Queiroga:** Conceptualization; Formal analysis; Funding acquisition; Investigation; Project administration; Resources; Supervision; Writing-review & editing.

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REFERENCES

- Araújo, M. R., Campos, L. C., Damasceno, K. A., Gamba, C. O., Ferreira, E., & Cassali, G. D. (2016). HER-2, EGFR, Cox-2 and Ki67 expression in lymph node metastasis of canine mammary carcinomas: Association with clinical-pathological parameters and overall survival. *Research in Veterinary Science*, 106, 121–130. <https://doi.org/10.1016/j.rvsc.2016.03.020>
- Belshaw, Z., Constantio-Casas, F., Brearley, M. J., Dunning, M. D., Holmes, M. A., & Dobson, J. M. (2011). COX-2 expression and outcome in canine nasal carcinomas treated with hypofractionated radiotherapy. *Veterinary and Comparative Oncology*, 9(2), 141–148. <https://doi.org/10.1111/j.1476-5829.2010.00243.x>
- Bilgic, O., Duda, L., Sánchez, M. D., & Lewis, J. R. (2015). Feline oral squamous cell carcinoma: Clinical manifestations and literature review. *Journal of Veterinary Dentistry*, 32(1), 30–40. <https://doi.org/10.1177/089875641503200104>
- Blackwood, L., Murphy, S., Buracco, P., De Vos, J. P., De Fornel-Thibaud, P., Hirschberger, J., Kessler, M., Pastor, J., Ponce, F., Savary-Bataille, K., & Argyle, D. J. (2012). European consensus document on mast cell tumours in dogs and cats. *Veterinary and Comparative Oncology*, 10(3), e1–e29. <https://doi.org/10.1111/j.1476-5829.2012.00341.x>
- Bommer, N. X., Hayes, A. M., Scase, T. J., & Gunn-Moore, D. A. (2012). Clinical features, survival times and COX-1 and COX-2 expression in cats with transitional cell carcinoma of the urinary bladder treated with meloxicam. *Journal of Feline Medicine and Surgery*, 14(8), 527–533. <https://doi.org/10.1177/1098612X12442041>
- Borrego, J. F., Cartagena, J. C., & Engel, J. (2009). Treatment of feline mammary tumours using chemotherapy, surgery and a COX-2 inhibitor drug (meloxicam): A retrospective study of 23 cases (2002–2007). *Veterinary and Comparative Oncology*, 7(4), 213–221. <https://doi.org/10.1111/j.1476-5829.2009.00194.x>
- Bryan, J. N., Henry, C. J., Turnquist, S. E., Tyler, J. W., Liptak, J. M., Rizzo, S. A., Sfiligoi, G., Steinberg, S. J., Smith, A. N., & Jackson, T. (2006). Primary renal neoplasia of dogs. *Journal of Veterinary Internal Medicine*, 20(5), 1155–1160. <https://doi.org/10.1111/j.1939-1676.2006.tb00715.x>
- Cannon, C. M., & Allstadt, S. D. (2015). Lower urinary tract cancer. *Veterinary Clinics of North America: Small Animal Practice*, 45(4), 807–824. <https://doi.org/10.1016/j.cvsm.2015.02.008>
- Carvalho, M. I., Bianchini, R., Fazekas-Singer, J., Herrmann, I., Flickinger, I., Thalhammer, J. G., Pires, I., Jensen-Jarolim, E., & Queiroga, F. L. (2018). Bidirectional regulation of COX-2 expression between cancer cells and macrophages. *Anticancer Research*, 38(5), 2811–2817. <https://doi.org/10.21873/anticancer.12525>
- Carvalho, M. I., Pires, I., Prada, J., Lobo, L., & Queiroga, F. L. (2016). Ki-67 and PCNA expression in canine mammary tumors and adjacent nonneoplastic mammary glands: Prognostic impact by a multivariate survival analysis. *Veterinary Pathology*, 53(6), 1138–1146. <https://doi.org/10.1177/0300985816646429>
- Carvalho, M. I., Pires, I., Prada, J., Raposo, T. P., Gregório, H., Lobo, L., & Queiroga, F. L. (2017). High COX-2 expression is associated with increased angiogenesis, proliferation and tumoural inflammatory infiltrate in canine malignant mammary tumours: A multivariate survival study. *Veterinary and Comparative Oncology*, 15(2), 619–631. <https://doi.org/10.1111/vco.12206>
- Carvalho, S., Stoll, A. L., Priestnall, S. L., Suarez-Bonnet, A., Rassnick, K., Lynch, S., Schoepper, I., Romanelli, G., Buracco, P., Atherton, M., de Merlo, E. M., & Lara-Garcia, A. (2017). Retrospective evaluation of COX-2 expression, histological and clinical factors as prognostic indicators in dogs with renal cell carcinomas undergoing nephrectomy. *Veterinary and Comparative Oncology*, 15(4), 1280–1294. <https://doi.org/10.1111/vco.12264>
- Cooper, K., Squires, H., Carroll, C., Papaioannou, D., Booth, A., Logan, R. F., Maguire, C., Hind, D., & Tappenden, P. (2010). Chemoprevention of colorectal cancer: Systematic review and economic evaluation. *Health Technology Assessment*, 14(32), 1–206. <https://doi.org/10.3310/hta14320>
- Davis, B. W., & Ostrander, E. A. (2014). Domestic dogs and cancer research: A breed-based genomics approach. *ILAR Journal*, 55(1), 59–68. <https://doi.org/10.1093/ilar/ilu017>
- De Campos, C. B., Damasceno, K. A., Gamba, C. O., Ribeiro, A. M., Machado, C. J., Lavalle, G. E., & Cassali, G. D. (2016). Evaluation of prognostic factors and survival rates in malignant feline mammary gland neoplasms. *Journal of Feline Medicine and Surgery*, 18(12), 1003–1012. <https://doi.org/10.1177/1098612X15610367>
- de M. Souza, C. H., Toledo-Piza, E., Amorin, R., Barboza, A., & Tobias, K. M. (2009). Inflammatory mammary carcinoma in 12 dogs: Clinical features, cyclooxygenase-2 expression, and response to piroxicam treatment. *The Canadian Veterinary Journal*, 50(5), 506–510.

- Demaria, S., Pikarsky, E., Karin, M., Coussens, L. M., Chen, Y.-C., El-Omar, E. M., Trinchieri, G., Dubinett, S. M., Mao, J. T., Szabo, E., Krieg, A., Weiner, G. J., Fox, B. A., Coukos, G., Wang, E., Abraham, R. T., Carbone, M., & Lotze, M. T. (2010). Cancer and inflammation: Promise for biologic therapy. *Journal of Immunotherapy*, 33(4), 335–351. <https://doi.org/10.1097/CJI.0b013e3181d32e74>
- Doré, M. (2011). Cyclooxygenase-2 expression in animal cancers. *Veterinary Pathology*, 48(1), 254–265. <https://doi.org/10.1177/0300985810379434>
- Doré, M., Lanthier, I., & Sirois, J. (2003). Cyclooxygenase-2 expression in canine mammary tumors. *Veterinary Pathology*, 40(2), 207–212. <https://doi.org/10.1354/vp.40-2-207>
- Edmondson, E. F., Hess, A. M., & Powers, B. E. (2015). Prognostic significance of histologic features in canine renal cell carcinomas: 70 nephrectomies. *Veterinary Pathology*, 52(2), 260–268. <https://doi.org/10.1177/0300985814533803>
- Gardner, H. L., Fenger, J. M., & London, C. A. (2016). Dogs as a model for cancer. *Annual Review of Animal Biosciences*, 4, 199–222. <https://doi.org/10.1146/annurev-animal-022114-110911>
- Gregório, H., Raposo, T., Queiroga, F. L., Pires, I., Pena, L., & Prada, J. (2017). High COX-2 expression in canine mast cell tumours is associated with proliferation, angiogenesis and decreased overall survival. *Veterinary and Comparative Oncology*, 15(4), 1382–1392. <https://doi.org/10.1111/vco.12280>
- Gregório, H., Raposo, T. P., Queiroga, F. L., Prada, J., & Pires, I. (2016). Investigating associations of cyclooxygenase-2 expression with angiogenesis, proliferation, macrophage and T-lymphocyte infiltration in canine melanocytic tumours. *Melanoma Research*, 26(4), 338–347. <https://doi.org/10.1097/CMR.0000000000000262>
- Guimarães, M. J., Carvalho, M. I., Pires, I., Prada, J., Gil, A. G., Lopes, C., & Queiroga, F. L. (2014). Concurrent expression of cyclo-oxygenase-2 and epidermal growth factor receptor in canine malignant mammary tumours. *Journal of Comparative Pathology*, 150(1), 27–34. <https://doi.org/10.1016/j.jcpa.2013.07.005>
- Hashemi Goradel, N., Najafi, M., Salehi, E., Farhood, B., & Mortezaee, K. (2019). Cyclooxygenase-2 in cancer: A review. *Journal of Cellular Physiology*, 234(5), 5683–5699. <https://doi.org/10.1002/jcp.27411>
- Hayes, A. M., Adams, V. J., Scase, T. J., & Murphy, S. (2007). Survival of 54 cats with oral squamous cell carcinoma in United Kingdom general practice. *Journal of Small Animal Practice*, 48(7), 394–399. <https://doi.org/10.1111/j.1748-5827.2007.00393.x>
- Heller, D. A., Clifford, C. A., Goldschmidt, M. H., Holt, D. E., Manfredi, M. J., & Sorenmo, K. U. (2005). Assessment of cyclooxygenase-2 expression in canine hemangiosarcoma, histiocytic sarcoma, and mast cell tumor. *Veterinary Pathology*, 42(3), 350–353. <https://doi.org/10.1354/vp.42-3-350>
- Henry, N. L., & Hayes, D. F. (2012). Cancer biomarkers. *Molecular Oncology*, 6(2), 140–146. <https://doi.org/10.1016/j.molonc.2012.01.010>
- 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., ... Miller, R. (2011). Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Veterinary Pathology*, 48(1), 147–155. <https://doi.org/10.1177/0300985810386469>
- Knapp, D. W., Richardson, R. C., Chan, T. C., Bottoms, G. D., Widmer, W. R., DeNicola, D. B., Teclaw, R., Bonney, P. L., & Kuczek, T. (1994). Piroxicam therapy in 34 dogs with transitional cell carcinoma of the urinary bladder. *Journal of Veterinary Internal Medicine*, 8(4), 273–278. <https://doi.org/10.1111/j.1939-1676.1994.tb03232.x>
- LaDue, T. A., Dodge, R., Page, R. L., Price, G. S., Hauck, M. L., & Thrall, D. E. (1999). Factors influencing survival after radiotherapy of nasal tumors in 130 dogs. *Veterinary Radiology Ultrasound*, 40(3), 312–317. <https://doi.org/10.1111/j.1740-8261.1999.tb00367.x>
- Laupacis, A., Wells, G., Richardson, W. S., & Tugwell, P. (1994). Users' guides to the medical literature. V. How to use an article about prognosis. *Journal of the American Medical Association*, 272(3), 234–237. <https://doi.org/10.1001/jama.1994.03520030076032>
- Lavalle, G. E., Bertagnolli, A. C., Tavares, W. L., & Cassali, G. D. (2009). Cox-2 expression in canine mammary carcinomas: Correlation with angiogenesis and overall survival. *Veterinary Pathology*, 46(6), 1275–1280. <https://doi.org/10.1354/vp.08-VP-0226-C-FL>
- Lavalle, G. E., De Campos, C. B., Bertagnolli, A. C., & Cassali, G. D. (2012). Canine malignant mammary gland neoplasms with advanced clinical staging treated with carboplatin and cyclooxygenase inhibitors. *In Vivo*, 26(3), 375–379.
- L'Eplattenier, H. F., Lai, C. L., van den Ham, R., Mol, J., van Sluijs, F., & Teske, E. (2007). Regulation of COX-2 expression in canine prostate carcinoma: Increased COX-2 expression is not related to inflammation. *Journal of Veterinary Internal Medicine*, 21(4), 776–782. <https://doi.org/10.1111/j.1939-1676.2007.tb03021.x>
- Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., Clarke, M., Devereaux, P. J., Kleijnen, J., & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLoS Med*, 6(7), e1000100. <https://doi.org/10.1371/journal.pmed.1000100>
- Martínez, C. M., Penafiel-Verdu, C., Vilafranca, M., Ramírez, G., Méndez-Gallego, M., Buendía, A. J., & Sánchez, J. (2011). Cyclooxygenase-2 expression is related with localization, proliferation, and overall survival in canine melanocytic neoplasms. *Veterinary Pathology*, 48(6), 1204–1211. <https://doi.org/10.1177/0300985810396517>
- McMillan, S. K., Boria, P., Moore, G. E., Widmer, W. R., Bonney, P. L., & Knapp, D. W. (2011). Antitumor effects of deracoxib treatment in 26 dogs with transitional cell carcinoma of the urinary bladder. *Journal of the American Veterinary Medical Association*, 239(8), 1084–1089. <https://doi.org/10.2460/javma.239.8.1084>
- Méric, J. B., Rottey, S., Olausson, K., Soria, J. C., Khayat, D., Rixe, O., & Spano, J. P. (2006). Cyclooxygenase-2 as a target for anticancer drug development. *Critical Reviews in Oncology/Hematology*, 59(1), 51–64. <https://doi.org/10.1016/j.critrevonc.2006.01.003>
- Millanta, F., Citi, S., Della Santa, D., Porciani, M., & Poli, A. (2006). COX-2 expression in canine and feline invasive mammary carcinomas: Correlation with clinicopathological features and prognostic molecular markers. *Breast Cancer Research and Treatment*, 98(1), 115–120. <https://doi.org/10.1007/s10549-005-9138-z>
- Mochizuki, H., Kennedy, K., Shapiro, S. G., & Breen, M. (2015). BRAF Mutations in canine cancers. *PLoS One*, 10(6), e0129534. <https://doi.org/10.1371/journal.pone.0129534>
- Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med*, 6(7), e1000097. <https://doi.org/10.1371/journal.pmed.1000097>
- Mullins, M. N., Lana, S. E., Dernell, W. S., Ogilvie, G. K., Withrow, S. J., & Ehrhart, E. J. (2004). Cyclooxygenase-2 expression in canine appendicular osteosarcomas. *Journal of Veterinary Internal Medicine*, 18(6), 859–865. <https://doi.org/10.1111/j.1939-1676.2004.tb02633.x>
- Nóbrega, D. F., Sehaber, V. F., Madureira, R., & Bracarense, A. (2019). Canine Cutaneous haemangiosarcoma: Biomarkers and survival. *Journal of Comparative Pathology*, 166, 87–96. <https://doi.org/10.1016/j.jcpa.2018.10.181>
- Patnaik, A. K., Ehler, W. J., & MacEwen, E. G. (1984). Canine cutaneous mast cell tumor: Morphologic grading and survival time in 83 dogs. *Veterinary Pathology*, 21(5), 469–474. <https://doi.org/10.1177/030098588402100503>
- Perroud, H. A., Rico, M. J., Alasino, C. M., Queralt, F., Mainetti, L. E., Pezzotto, S. M., Rozados, V. R., & Scharovsky, O. G. (2013). Safety and therapeutic effect of metronomic chemotherapy with

- cyclophosphamide and celecoxib in advanced breast cancer patients. *Future Oncology*, 9(3), 451–462. <https://doi.org/10.2217/fon.12.196>
- Pesic, M., & Greten, F. R. (2016). Inflammation and cancer: Tissue regeneration gone awry. *Current Opinion in Cell Biology*, 43, 55–61. <https://doi.org/10.1016/j.ccb.2016.07.010>
- Pires, I., Garcia, A., Prada, J., & Queiroga, F. L. (2010). COX-1 and COX-2 expression in canine cutaneous, oral and ocular melanocytic tumours. *Journal of Comparative Pathology*, 143(2–3), 142–149. <https://doi.org/10.1016/j.jcpa.2010.01.016>
- Queiroga, F. L., Alves, A., Pires, I., & Lopes, C. (2007). Expression of Cox-1 and Cox-2 in canine mammary tumours. *Journal of Comparative Pathology*, 136(2–3), 177–185. <https://doi.org/10.1016/j.jcpa.2007.01.010>
- Queiroga, F. L., Perez-Alenza, M. D., Silvan, G., Pena, L., Lopes, C., & Illera, J. C. (2005). Cox-2 levels in canine mammary tumors, including inflammatory mammary carcinoma: Clinicopathological features and prognostic significance. *Anticancer Research*, 25(6b), 4269–4275.
- Queiroga, F. L., Pires, I., Lobo, L., & Lopes, C. S. (2010). The role of Cox-2 expression in the prognosis of dogs with malignant mammary tumours. *Research in Veterinary Science*, 88(3), 441–445. <https://doi.org/10.1016/j.rvsc.2009.10.009>
- Queiroga, F. L., Pires, I., Parente, M., Gregório, H., & Lopes, C. S. (2011). COX-2 over-expression correlates with VEGF and tumour angiogenesis in canine mammary cancer. *The Veterinary Journal*, 189(1), 77–82. <https://doi.org/10.1016/j.tvjl.2010.06.022>
- Ramos-Vara, J. A., Kiupel, M., Baszler, T., Bliven, L., Brodersen, B., Chelack, B., West, K., Czub, S., Del Piero, F., Dial, S., Ehrhart, E. J., Graham, T., Manning, L., Paulsen, D., & Valli, V. E. (2008). Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. *Journal of Veterinary Diagnostic Investigation*, 20(4), 393–413. <https://doi.org/10.1177/104063870802000401>
- Raposo, T. P., Beirão, B. C., Pang, L. Y., Queiroga, F. L., & Argyle, D. J. (2015). Inflammation and cancer: Till death tears them apart. *The Veterinary Journal*, 205(2), 161–174. <https://doi.org/10.1016/j.tvjl.2015.04.015>
- Rassnick, K. M., Goldkamp, C. E., Erb, H. N., Scrivani, P. V., Njaa, B. L., Gieger, T. L., Turek, M. M., McNeil, E. A., Proulx, D. R., Chun, R., Mauldin, G. E., Phillips, B. S., & Kristal, O. (2006). Evaluation of factors associated with survival in dogs with untreated nasal carcinomas: 139 cases (1993–2003). *Journal of the American Veterinary Medical Association*, 229(3), 401–406. <https://doi.org/10.2460/javma.229.3.401>
- Ristimäki, A., Sivula, A., Lundin, J., Lundin, M., Salminen, T., Haglund, C., Joensuu, H., & Isola, J. (2002). Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Research*, 62(3), 632–635.
- Rizzardi, A. E., Zhang, X., Vogel, R. I., Kolb, S., Geybels, M. S., Leung, Y.-K., Henriksen, J. C., Ho, S.-M., Kwak, J., Stanford, J. L., & Schmechel, S. C. (2016). Quantitative comparison and reproducibility of pathologist scoring and digital image analysis of estrogen receptor β immunohistochemistry in prostate cancer. *Diagnostic Pathology*, 11(1). <https://doi.org/10.1186/s13000-016-0511-5>
- Sayasith, K., Sirois, J., & Doré, M. (2009). Molecular characterization of feline COX-2 and expression in feline mammary carcinomas. *Veterinary Pathology*, 46(3), 423–429. <https://doi.org/10.1354/vp.08-VP-0161-D-FL>
- Schiffman, J. D., & Breen, M. (2015). Comparative oncology: What dogs and other species can teach us about humans with cancer. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1673). <https://doi.org/10.1098/rstb.2014.0231>
- Simpson, S., Dunning, M. D., de Brot, S., Grau-Roma, L., Mongan, N. P., & Rutland, C. S. (2017). Comparative review of human and canine osteosarcoma: Morphology, epidemiology, prognosis, treatment and genetics. *Acta Veterinaria Scandinavica*, 59(1). <https://doi.org/10.1186/s13028-017-0341-9>
- Smedley, R. C., Spangler, W. L., Esplin, D. G., Kitchell, B. E., Bergman, P. J., Ho, H.-Y., Bergin, I. L., & Kiupel, M. (2011). Prognostic markers for canine melanocytic neoplasms: A comparative review of the literature and goals for future investigation. *Veterinary Pathology*, 48(1), 54–72. <https://doi.org/10.1177/0300985810390717>
- Smith, A. N. (2003). Hemangiosarcoma in dogs and cats. *Veterinary Clinics of North America: Small Animal Practice*, 33(3), 533–552. [https://doi.org/10.1016/S0195-5616\(03\)00002-0](https://doi.org/10.1016/S0195-5616(03)00002-0)
- Sorenmo, K. U., Goldschmidt, M. H., Shofer, F. S., Goldkamp, C., & Ferracone, J. (2004). Evaluation of cyclooxygenase-1 and cyclooxygenase-2 expression and the effect of cyclooxygenase inhibitors in canine prostatic carcinoma. *Veterinary and Comparative Oncology*, 2(1), 13–23. <https://doi.org/10.1111/j.1476-5810.2004.00035.x>
- Spangler, W. L., & Kass, P. H. (2006). The histologic and epidemiologic bases for prognostic considerations in canine melanocytic neoplasia. *Veterinary Pathology*, 43(2), 136–149. <https://doi.org/10.1354/vp.43-2-136>
- Spugnini, E. P., Porrello, A., Citro, G., & Baldi, A. (2005). COX-2 overexpression in canine tumors: Potential therapeutic targets in oncology. *Histology and Histopathology*, 20(4), 1309–1312. <https://doi.org/10.14670/HH-20.1309>
- Szweda, M., Rychlik, A., Babińska, I., & Pomianowski, A. (2020). Cyclooxygenase-2 as a biomarker with diagnostic, therapeutic, prognostic, and predictive relevance in small animal oncology. *Journal of Veterinary Research*, 64(1), 151–160. <https://doi.org/10.2478/jvetres-2020-0018>
- Vascellari, M., Giantin, M., Capello, K., Carminato, A., Morello, E. M., Vercelli, A., Granato, A., Buracco, P., Dacasto, M., & Mutinelli, F. (2013). Expression of Ki67, BCL-2, and COX-2 in canine cutaneous mast cell tumors: Association with grading and prognosis. *Veterinary Pathology*, 50(1), 110–121. <https://doi.org/10.1177/0300985812447829>
- Wang, D., & Dubois, R. N. (2010). Eicosanoids and cancer. *Nature Reviews Cancer*, 10(3), 181–193. <https://doi.org/10.1038/nrc2809>
- Webster, J. D., Dennis, M. M., Dervisis, N., Heller, J., Bacon, N. J., Bergman, P. J., Bienzle, D., Cassali, G., Castagnaro, M., Cullen, J., Esplin, D. G., Peña, L., Goldschmidt, M. H., Hahn, K. A., Henry, C. J., Hellmén, E., Kamstock, D., Kirpensteijn, J., Kitchell, B. E., ... Kiupel, M. (2011). Recommended guidelines for the conduct and evaluation of prognostic studies in veterinary oncology. *Veterinary Pathology*, 48(1), 7–18. <https://doi.org/10.1177/0300985810377187>
- Zappulli, V., De Zan, G., Cardazzo, B., Bargelloni, L., & Castagnaro, M. (2005). Feline mammary tumours in comparative oncology. *Journal of Dairy Research*, 72(S1), 98–106. <https://doi.org/10.1017/S0022029905001263>

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