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NEOPLASTIC DISEASE

Association between Degree of Anaplasia and Degree of Inflammation with the Expression of COX-2 in Feline Injection Site Sarcomas

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Summary

Feline injection site sarcomas (FISSs) are mesenchymal neoplasms that develop at the sites of delivery of vaccines or other injectable products. Vaccine adjuvants can trigger an intense and persistent inflammatory response that may lead to neoplastic transformation. The proinflammatory role of cyclo-oxygenase (COX)-2 is well known and its overexpression has prognostic value in multiple neoplastic processes. One hundred and seventeen FISSs were evaluated for the degree of inflammation and anaplasia. Immunohistochemistry was used to determine the expression of COX-2 in these sarcomas. There was a significant association between the degree of inflammation and the expression of COX-2 by neoplastic cells. COX-2 expression was lower in tumours with higher degrees of anaplasia. These findings may be useful in predicting the sensitivity of FISSs to treatment with COX-2 inhibitors. The potential therapeutic use of such agents could then be restricted to tumours with lower degrees of anaplasia.

Keywords: cat; cyclo-oxygenase-2; feline injection site sarcoma; immunohistochemistry

Introduction

Feline injection site sarcomas (FISSs) are neoplasms of mesenchymal origin, which develop at sites of injection of vaccines or other products (Hendrick *et al.*, 1992; Hendrick and Brooks, 1994; Goldschmidt and Hendrick, 2002; Shaw *et al.*, 2009). FISS was first described in 1991 in the USA by Hendrick and Goldschmidt (1991) and, subsequently, these tumours were diagnosed in most continents (Hendrick and Brooks, 1994; Burton and Mason, 1997; Nieto *et al.*, 2003; Chang *et al.*, 2006).

There are different types of FISS, the most frequent being fibrosarcomas (Hendrick *et al.*, 1992; Goldschmidt and Hendrick, 2002; Wilcock *et al.*, 2012) (Fig. 1). FISSs are frequently associated with peritumoural inflammatory cells, mainly lymphocytes and macrophages (Hendrick *et al.*, 1992, 1998; Hendrick and Brooks, 1994; Doddy *et al.*, 1996; Madewell *et al.*, 2001; Goldschmidt and Hendrick, 2002) (Fig. 2). The diagnosis of FISS depends on its histological appearance and anatomical location,

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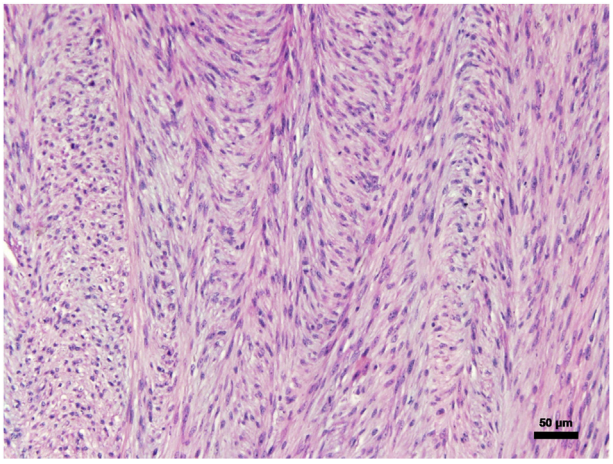

Fig. 1. Feline fibrosarcoma associated with an injection site. Characteristic pattern of interleaved bundles of fibroblasts are shown. HE.

which usually coincides with injection sites (Wilcock *et al.*, 2012).

FISSs are highly invasive and recurrent neoplasms that may metastasize (Hendrick and Brooks, 1994; Hendrick *et al.*, 1994; Hershey *et al.*, 2000, 2005; Goldschmidt and Hendrick, 2002; Deim *et al.*, 2008). The incidence is estimated to be somewhere between 1 in 1,000 and 1 in 10,000 injected cats (Kass *et al.*, 1993; Goldschmidt and Hendrick, 2002; Wilcock *et al.*, 2012). The tumours may become apparent clinically between 3 months and 3 years after injection (Hendrick *et al.*, 1992); however, this interval can be as long as 13–15 years (Wilcock *et al.*, 2012).

There is no sex predisposition for FISS and the average age of presentation is 8.1 years. Sarcomas not associated with inoculation sites occur in cats with a mean age of 9.2 years (Hendrick and Brooks, 1994; Goldschmidt and Hendrick, 2002).

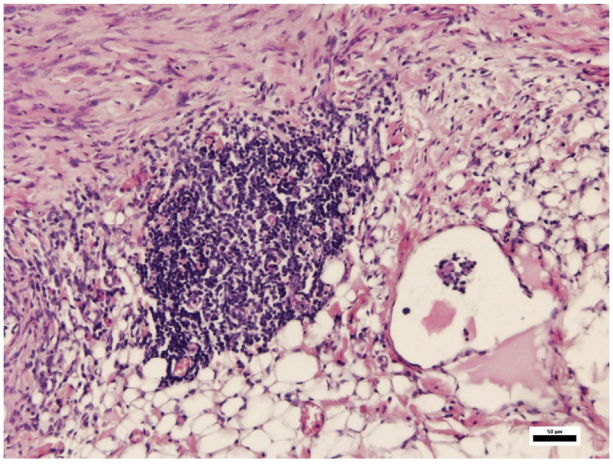

Fig. 2. Peritumoural inflammatory response in a feline sarcoma associated with an inoculation site. HE.

One of the suggested origins of FISS is the malignant transformation of reactive fibroblasts occurring within the background of a chronic inflammatory response at the sites of injection (Wilcock *et al.*, 2012). The main injectable products that have been associated with FISS are vaccines, and in particular, adjuvanted vaccines such as those against rabies and the feline leukaemia virus (FeLV) (Hendrick *et al.*, 1992; Deim *et al.*, 2008). Aluminum-based adjuvants cause foreign body granulomas in man and this reaction is similar to that found at injection sites in cats (Hendrick and Dunagan, 1991; Hendrick *et al.*, 1992). The presence of peritumoural aggregates of macrophages with content corresponding to aluminum supports the diagnosis of a sarcoma associated with vaccination (Hendrick *et al.*, 1992; Goldschmidt and Hendrick, 2002). It is postulated that aluminum adjuvant generates a persistent inflammatory response that induces the synthesis of growth factors and cytokines, which in turn stimulate the proliferation of fibroblasts and myofibroblasts. However, FISS is also related to the injection of vaccines containing other adjuvants and vaccines without adjuvant (Kass *et al.*, 1993; Deim *et al.*, 2008), but is always associated with chronic inflammatory processes. Persistent inflammation at injection sites would lead to development of a reparative response that in some cases would lead to induction of a neoplasm (Hendrick *et al.*, 1992; Kass *et al.*, 1993; Woodward, 2011). This hypothesis is based on the finding of microscopical sarcomatous foci within granulomatous inflammatory responses at injection sites (Hendrick *et al.*, 1992).

Fibroblasts can undergo neoplastic transformation through different mechanisms, including the activation of oncogenes and inactivation of tumour suppressor genes (O'Byrne and Dalgleish, 2001). Chronic inflammation can induce the production of free radicals and metabolites that cause DNA damage and mutations, acting as an initiator of carcinogenesis. The environment provided by chronic inflammation, coupled with a genetic predisposition, alters the susceptibility to carcinogenic injuries (O'Byrne and Dalgleish, 2001; Nieto *et al.*, 2003).

As a result of the inflammatory process, phospholipase A2 induces the release of arachidonic acid, which in turn may be metabolized following one of the two well-known pathways: lipoxygenase or cyclooxygenase (COX). The latter pathway involves COX-1 and COX-2 expression. COX-1 expression is constitutive in most tissues, while COX-2 expression is constitutive in the placenta, the macula densa of the kidney and in the brain, and is inducible in

other locations, by different inflammatory cytokines, such as interleukin (IL)-1, tumour necrosis factor (TNF)-α, interferon (IFN)-γ, and under hypoxic states (Williams *et al.*, 1999; Araki *et al.*, 2003; Greenhough *et al.*, 2009).

COX-2 expression is found in mesenchymal neoplasms, such as Kaposi's sarcoma, rhabdomyosarcoma, osteosarcoma and chondrosarcoma (Xu *et al.*, 2006; Rodríguez *et al.*, 2008; Sharma-Walia *et al.*, 2010, 2012; Carmody Soni *et al.*, 2011). COX-2 expression has also been reported in FISS (Magi *et al.*, 2010; Carneiro *et al.*, 2018). Magi *et al.* (2010) found no association between the expression of COX-2 and the histological grade of the sarcomas; however, neither study evaluated the association between the degree of inflammation and the expression of COX-2. The aim of the present study was to determine the presence of COX-2 in different types of FISS and to relate its expression to the degree of anaplasia (DA) and degree of inflammation (DI).

Materials and Methods

One hundred and seventeen samples diagnosed as FISS between 2011 and 2012 by two experienced pathologists were selected. Four of the 117 lesions were tumour recurrences.

Histopathology

Tissue samples were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (3–5 μm) were stained with haematoxylin and eosin (HE). Samples were examined in order to establish the degree of anaplasia and to evaluate the peritumoural inflammatory response. The DA was determined using a protocol proposed by Couto *et al.* (2002), in which cell differentiation, mitotic index (MI) and degree of necrosis are considered (Table 1). By combining these characteristics, three grades of anaplasia were defined (Table 2).

Table 1
Criteria for scoring the degree of anaplasia

Criterion	*Score*	*Description*
Differentiation	1	Similar cells to those of the tissue of origin
	2	Cells with defined histological phenotype
	3	Poorly-differentiated cells
Mitotic index	1	1–9 mitotic figures/10 fields at ×400
	2	10–19 mitotic figures/10 fields at ×400
	3	20 or more mitotic figures/10 fields at ×400
Necrosis	1	Absence of necrosis
	2	Necrosis <50% of the total area
	3	Necrosis >50% of the total area

After Couto *et al.* (2002).

Table 2
Definition of the degree of anaplasia

Degree of anaplasia	*Score*
I	3–4
II	5–6
III	7–9

After Couto *et al.* (2002).

The DA was scored following these criteria: 1, tumours comprised of cells that were similar to differentiated cells; 2, tumours with cells having defined histological phenotype; and 3, tumours with poorly differentiated cells (Couto *et al.*, 2002).

The MI was established as the average number of mitosis in 10 high-power (×400) fields. Areas with extensive areas of inflammation and necrosis were excluded from the analysis. Scoring of sarcomas for MI was: 1, MI between 1 and 9; 2, MI between 10 and 19; and 3, MI ≥20 (Couto *et al.*, 2002).

Scoring for the necrosis was: 1, no necrosis; 2, <50% necrosis; and 3, >50% necrosis (Couto *et al.*, 2002).

The sum of the scores obtained for each criterion constituted the final score, according to which one of three degrees of anaplasia was assigned to each tumour (Table 2) (Couto *et al.*, 2002).

The DI was established by evaluating the peritumoural lymphoid infiltrate at ×40 magnification, according to the criteria described by Couto *et al.* (2002): 0, absence of peritumoural lymphoid infiltrate; 1, sparse lymphoid infiltrate (requires careful searching); 2, diffuse infiltrate or small aggregates in <50% of the fields; and 3, lymphoid aggregates in 50% of the fields or more. Since one of the samples obtained by incisional biopsy had a non-representative peritumoural area, it was excluded from this evaluation.

Immunohistochemistry

Sections (3 μm) were mounted on glass slides with a positive charge (HistoSystem, Rosario, Argentina), dewaxed, rehydrated and incubated in 3% H_2O_2 in methanol to inhibit endogenous peroxidase for 30 min at room temperature. Slides were then washed with phosphate buffered saline (PBS, pH 7.4). Antigen retrieval was performed with two cycles of microwave heating at 800 W, for 3 min each in citrate buffer, pH 6.0. Non-specific binding sites were blocked by incubating sections with 1% bovine serum albumin (BSA) for 30 min at room temperature in a humid chamber. The primary antibody was

incubated overnight at 4°C in a humid chamber. The COX-2 polyclonal primary antibody (Cayman Chemical, Ann Arbor, Michigan) was used for the detection of COX-2 at a dilution of 1 in 200 in 0.1% BSA, followed by anti-rabbit EnVision System™ (Dako, Carpinteria, California, USA). This antibody was previously successfully used in feline tissue (Newman and Mrkonjich, 2006; Bardag *et al.*, 2012). As a negative control, the antibody was replaced by PBS. As a negative tissue control, normal cat skin obtained from animals referred for necropsy examination was used. In addition, the internal negative controls were the non-neoplastic tissues of each sample. As a positive control, the macula densa of sections of dog kidney was used (Khan *et al.*, 2001). The chromogen was 3, 3′-diaminobenzidine tetrahydrochloride (Dako) and haematoxylin was used for counterstaining.

In order to determine the susceptibility of the COX-2 antigen to prolonged exposure to formalin, immunohistochemistry (IHC) was also tested using normal dog kidney sections fixed in 10% neutral buffered formalin for 24 h, 1 week or 1 month, with or without antigenic retrieval.

COX-2 immunolabelling was evaluated by estimating the percentage of cells with positive cytoplasmic labelling by counting 10 fields at ×400 magnification. Images were captured using an analogue video camera (Sony DXC-390, Tokyo, Japan) mounted on a trinocular microscope (CX31, Olympus, Japan) and connected to a computer. Captured images were processed using a digital image analyzer (ImagePro Plus v6.3, Media Cybernetics, Rockville, Maryland, USA).

Statistical Analysis

COX-2 immunolabelling was expressed as a percentage. In all cases it was compared with the DI and the DA by use of analysis of variance (ANOVA) from a Bayesian perspective. To comply with the theoretical assumptions of the ANOVA, mandatory transformation of the data was done. In the case of the percentage variables, the chosen transformation was arcsine. Prior non-informative distributions (PNI) were used and 20,000 iterations were run, discarding the first 1,000. For analysis, the R software and the free software WinBUGS (Campbell and Thompson, 2012; Risso and Risso, 2017) were used. Significant results were considered at $P < 0.05$.

We performed a Bayesian correlation test (Bayesian Correlation Matrix) to evaluate whether there was an association between DA and DI variables (Risso and Risso, 2017). Significant results were considered at $P < 0.05$.

Results

Tumours graded as DA II were the most numerous in the study (Table 3). Three of the recurrent tumours had the same DA (DA II) as the primary tumour and one had a lower DA (DA I) in comparison with the primary lesion. Most of the tumours had grade 3 inflammation (Table 4). Three of the recurrent tumours had the same DI as the primary tumour (grade 3) and one had a lesser DI (grade 1) than the primary tumour that was grade 2. There was no significant association between DA and DI ($r = -0.110$, $df = 114$, $P = 0.203$).

The intensity of COX-2 labelling in the macula densa of samples of kidney subjected to different fixation times was similar in all cases, both with or without antigen retrieval (Fig. 3).

COX-2 immunolabelling was positive in 56.4% of the cases (Fig. 4) and negative in 43.6%. No variation was observed in the expression of COX-2 within the tumours in relation to the areas of inflammation, nor were differences observed in COX-2 expression in inflammatory infiltrates located at the periphery or the centre of the tumours (areas free of necrosis).

The percentage of COX-2 immunolabelling showed significant differences between tumours with DI 1, 2 and 3 ($P < 0.05$, ANOVA, $n = 116$). The expression of COX-2 was highest in tumours of DI grade 3, followed by those of grade 2 and then grade 1. In contrast, the percentage of COX-2 immunolabelling showed no significant difference between DA groups 1 and 2; however, the lowest expression of COX-2 was observed in DA group 3 ($P < 0.05$, ANOVA, $n = 117$).

Discussion

It is suggested that FISS originates from the malignant transformation of fibroblasts as a result of chronic inflammation occurring at sites of injection, in particular injection with adjuvanted vaccines (Hendrick and Dunagan, 1991; Hendrick *et al.*, 1992; Kass *et al.*, 1993; Goldschmidt and Hendrick, 2002; Deim *et al.*, 2008; Wilcock *et al.*, 2012). The present study reports that COX-2 is expressed within these tumours, a result that is in accordance with the investigations of Magi *et al.* (2010) and Carneiro *et al.*

Table 3
Distribution of tumours according to the degree of anaplasia

Degree of anaplasia	*Percentage of cases*
I	29.91
II	64.1
III	5.98

Table 4
Distribution of tumours according to the degree of inflammation

Degree of inflammation	*Percentage of cases*
I	15.52
II	36.21
III	48.27

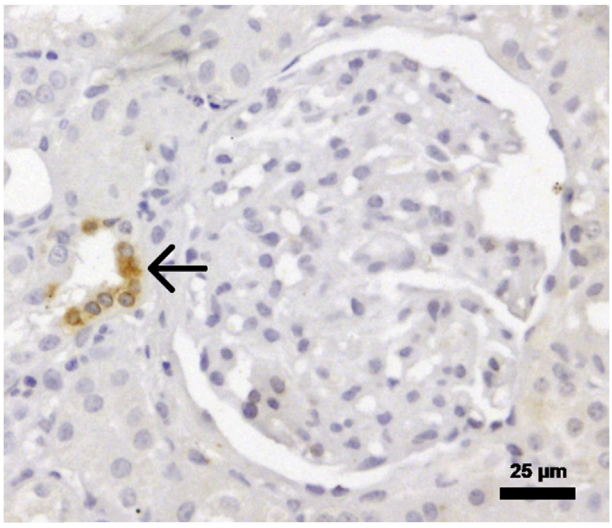

Fig. 3. Expression of COX-2 in the macula densa of a section of canine kidney (arrow). IHC.

(2018), but in contrast to the results of Beam *et al.* (2003). A possible explanation for the discrepancy may be the use of different immunohistochemical protocols in the separate studies. The immunohistochemical technique (i.e. biotin–avidin immunoperoxidase) used in previous investigations was different from that use in the present work; the primary antibody used was also different. Although antigen retrieval was also done in a microwave oven, the previous studies used two high-power cycles of 10 min instead of the 3 min used herein.

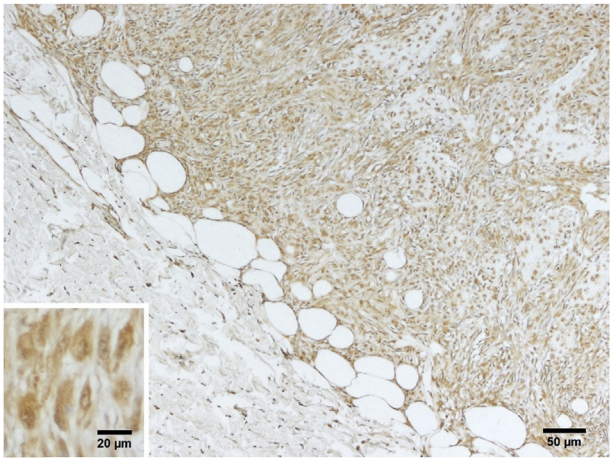

Fig. 4. Expression of COX-2 in a feline sarcoma associated with an injection site. Inset: cytoplasmic expression of COX-2 by tumour cells. IHC.

Beam *et al.* (2003) suggested that fixation time was one of the possible causes of the absence of COX-2 immunolabelling in their study and different antigens, or the same antigen located in different compartments, are known to have different susceptibilities to fixation (Nieto *et al.*, 2003; Ramos-Vara, 2005; Ramos-Vara and Miller, 2014). However, in the present work we demonstrated that COX-2 expression was not susceptible to prolonged fixation time of up to 1 month.

COX-2 is a proinflammatory molecule (Williams *et al.*, 1999; Cha and DuBois, 2007) and in the present study we showed that there is a close relationship between COX-2 expression and inflammation. From our results, we concluded that the expression of COX-2 is directly proportional to the DI, as it was greater in the samples with the highest DI.

Most of the FISSs reported here were associated with abundant inflammation (grade 3) and three of the four samples of tumour recurrence showed the same DI as the primary tumours (grade 3). This observation is in accordance with the findings of Couto *et al.* (2002). Overexpression of COX-2 is reported to be associated with tumour proliferation and invasion, inhibition of apoptosis, suppression of immune surveillance and angiogenesis (Williams *et al.*, 1999; Costa *et al.*, 2002; Rodríguez *et al.*, 2008). This enzyme participates in the synthesis of multiple arachidonic acid derivatives, including prostaglandin E_2, which is related to carcinogenic processes (Williams *et al.*, 1999; O'Byrne and Dalgleish, 2001; Nieto *et al.*, 2003; Cha and DuBois, 2007; Rodríguez *et al.*, 2008).

The chronic inflammation present in human Kaposi's sarcoma is also associated with COX-2 expression, which participates in tumour progression (Sharma-Walia *et al.*, 2010). COX-2 expression has a prognostic value in numerous neoplastic processes, including colorectal cancer and human and canine mammary tumours (Couto *et al.*, 2002; Lavalle *et al.*, 2009; Wang and DuBois, 2010). In the present study, highly undifferentiated tumors (DA III) showed lower COX-2 expression, which may reflect a loss of the capacity for synthesis of such substances in highly anaplastic cells (Cullen *et al.*, 2002).

One of the possible treatments for FISS is therapy with COX-2 inhibitors. This therapy has shown favourable results in human and veterinary oncology (Cha and DuBois, 2007; Wang and DuBois, 2010; Nardi *et al.*, 2011). In veterinary medicine, COX-2

inhibitors have shown antineoplastic effects in canine tumours including bladder transitional cell carcinomas and oral squamous cell carcinomas. In addition, favourable results were observed after their use in the treatment of dogs with rectal polyps and inflammatory mammary carcinomas (Nardi *et al.*, 2011). Magi *et al.* (2010) found no association between the expression of COX-2 and the DA; however, in the present study we found that sarcomas of higher DA showed a lower expression of the enzyme. This could make them less susceptible to therapy with COX-2 inhibitors.

In the future, it will be necessary to evaluate the prognostic value of COX-2 expression and the therapeutic value of the use of its inhibitors in FISS. If the usefulness of these inhibitors is verified, the DA and DI would have a predictive value regarding susceptibility to this type of treatment for FISS. Although the primary treatment of choice in these cases is surgery with a wide excisional margin, protocols that include COX-2 inhibitors could contribute to reduction in tumour size before surgery, to extend the survival period in unresectable tumours and to extend the tumour-free period (between recurrences) when radical surgery is not feasible.

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References

Araki Y, Okamura S, Hussain SP, Nagashima M, He PJW *et al.* (2003) Regulation of cyclooxygenase-2 expression by the Wnt and Ras pathways. *Cancer Research*, **63**, 728–734.

Bardag M, Fondevila D, Ferrer L (2012) Immunohistochemical detection of COX-2 in feline and canine actinic keratoses and cutaneous squamous cell carcinoma. *Journal of Comparative Pathology*, **146**, 11–17.

Beam SL, Rassnick KM, Moore AS, McDonough SP (2003) An immunohistochemical study of cyclooxygenase-2 expression in various feline neoplasms. *Veterinary Pathology*, **40**, 496–500.

Burton G, Mason KV (1997) Do postvaccinal sarcomas occur in Australian cats? *Australian Veterinary Journal*, **75**, 102–108.

Campbell JID, Thompson VA (2012) More Power 6.0 for ANOVA with relational confidence intervals and Bayesian analysis. *Behavior Research Methods*, **44**, 1255–1265.

Carmody Soni EE, Miller BJ, Scarborough MT, Reith J, Gibbs CP (2011) Cyclooxygenase-2 expression is not associated with clinical outcome in synovial sarcoma. *Oncology Reports*, **26**, 1513–1517.

Carneiro CS, Queiroz GF, Pinto AC, Dagli ML, Matera JM (2018) Feline injection site sarcoma: immunohistochemical characteristics. *Journal of Feline Medicine and Surgery*, https://doi.org/10.1177/1098612X18774709 [Epub ahead of print].

Cha YI, DuBois RN (2007) NSAIDs and cancer prevention: targets downstream of COX-2. *Annual Reviews of Medicine*, **58**, 239–252.

Chang HW, Ho SY, Lo HF, Tu YC, Jeng CR *et al.* (2006) Vaccine-associated rhabdomyosarcoma with spinal epidural invasion and pulmonary metastasis in a cat. *Veterinary Pathology*, **43**, 55–58.

Costa C, Soares R, Reis-Filho JS, Leitão D, Amendoeira I *et al.* (2002) Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. *Journal of Clinical Pathology*, **55**, 429–434.

Couto SS, Griffey SM, Duarte PC, Madewell BR (2002) Feline vaccine-associated fibrosarcoma: morphologic distinctions. *Veterinary Pathology*, **39**, 33–41.

Cullen JM, Page R, Misdorp W (2002) An overview of cancer pathogenesis, diagnosis, and management. In: *Tumors in Domestic Animals*, 4th Edit., DJ Meuten, Ed., Wiley Blackwell, Hoboken, pp. 3–44.

Deim Z, Pálmai N, Cserni G (2008) Feline vaccine-associated fibrosarcoma induced by aluminium compound in two cats: short communication. *Acta Veterinaria Hungarica*, **56**, 111–116.

Doddy FD, Glickman LT, Glickman NW, Janovitz EB (1996) Feline fibrosarcomas at vaccination sites and non-vaccination sites. *Journal of Clinical Pathology*, **114**, 165–174.

Goldschmidt MH, Hendrick MJ (2002) Tumors of the skin and soft tissues. In: *Tumors in Domestic Animals*, 4th Edit., DJ Meuten, Ed., Wiley Blackwell, Hoboken, pp. 45–117.

Greenhough A, Smartt HJM, Moore AE, Roberts HR, Williams AC *et al.* (2009) The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumor microenvironment. *Carcinogenesis*, **30**, 377–386.

Hendrick MJ, Goldschmidt MH (1991) Do injection site reactions induce fibrosarcomas in cats? *Journal of the American Veterinary Medical Association*, **199**, 968.

Hendrick MJ, Dunagan CA (1991) Focal necrotizing granulomatous panniculitis associated with subcutaneous injection of rabies vaccine in cats and dogs: 10 cases (1988–1989). *Journal of the American Veterinary Medical Association*, **198**, 304–305.

Hendrick MJ, Goldschmidt MH, Shofer FS, Wang YY, Somlyo AP (1992) Postvaccinal sarcomas in the cat: epidemiology and electron probe microanalytical identification of aluminum. *Cancer Research*, **52**, 5391–5394.

Hendrick MJ, Brooks JJ (1994) Postvaccinal sarcomas in the cat: histology and immunohistochemistry. *Veterinary Pathology*, **31**, 126–129.

Hendrick MJ, Mahaffey EA, Moore FM, Vos JH, Walder EJ (1998) Histological classification of mesenchymal tumors of skin and soft tissues of domestic animals. In: *World Health Organization International*

Classification of Tumors in Domestic Animals, 2nd Edit., World Health Organization, Washington DC, pp. 15–60.

Hendrick MJ, Shofer FS, Goldschmidt MH, Haviland JC, Schelling SH *et al.* (1994) Comparison of fibrosarcomas that developed at vaccination sites and at non-vaccination sites in cats: 239 cases (1991–1992). *Journal of the American Veterinary Medical Association*, **205**, 1425–1429.

Hershey AE, Sorenmo KU, Hendrick MJ, Shofer FS, Vail DM (2000) Prognosis for presumed feline vaccine-associated sarcoma after excision: 61 cases (1986–1996). *Journal of the American Veterinary Medical Association*, **216**, 58–61.

Hershey AE, Dubielzig RR, Padilla ML, Helfand SC (2005) Aberrant p53 expression in feline vaccine-associated sarcomas and correlation with prognosis. *Veterinary Pathology*, **42**, 805–811.

Kass PH, Barnes WG Jr., Spangler WL, Chomel BB, Culbertson MR (1993) Epidemiologic evidence for a causal relation between vaccination and fibrosarcoma tumorigenesis in cats. *Journal of the American Veterinary Medical Association*, **203**, 396–405.

Khan KNM, Stanfield KM, Trajkovic D, Knapp DW (2001) Expression of cyclooxygenase-2 in canine renal cell carcinoma. *Veterinary Pathology*, **38**, 116–119.

Lavalle GE, Bertagnolli AC, Tavares WLF, Cassali GD (2009) Cox-2 expression in canine mammary carcinomas: correlation with angiogenesis and overall survival. *Veterinary Pathology*, **46**, 1274–1280.

Madewell BR, Griffey SM, McEntee MC, Leppert VJ, Munn RJ (2001) Feline vaccine-associated fibrosarcoma: an ultrastructural study of 20 tumors (1996–1999). *Veterinary Pathology*, **38**, 196–202.

Magi GE, Mari S, Renzoni G, Rossi G (2010) Immunohistochemical expression of COX-2 in feline injection site sarcoma. *Veterinary Pathology*, **4**, 340.

Nardi A, Raposo T, Huppes R, Dalek C, Laufer-Amorim R (2011) COX-2 inhibitors for cancer treatment in dogs. *Pakistan Veterinary Journal*, **31**, 275–279.

Newman SJ, Mrkonjich L (2006) Cyclooxygenase-2 expression in feline pancreatic adenocarcinomas. *Journal of Veterinary Diagnostic Investigation*, **18**, 590–593.

Nieto A, Sánchez MA, Martínez E, Rollán E (2003) Immunohistochemical expression of p53, fibroblast growth factor-β, and transforming growth factor-α in feline vaccine-associated sarcomas. *Veterinary Pathology*, **40**, 651–658.

O'Byrne KJ, Dalgleish AG (2001) Chronic immune activation and inflammation as the cause of malignancy. *British Journal of Cancer*, **85**, 473–483.

Ramos-Vara JA (2005) Technical aspects of immunohistochemistry. *Veterinary Pathology*, **42**, 405–426.

Ramos-Vara JA, Miller MA (2014) When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry — the red, brown, and blue technique. *Veterinary Pathology*, **51**, 42–87.

Risso MA, Risso P (2017) Capítulo VIII. WinBUGS, una Simulación Bayesiana. In: *Una Introducción a la Estadística Bayesiana: Uso de Lenguaje R y WinBUGS*, 1st Edit., MA Risso, P Risso, Eds., Vuelta a Casa, La Plata, pp. 19–39.

Rodríguez NI, Hoots WK, Koshkina NV, Morales-Arias JA, Arndt CA *et al.* (2008) COX-2 expression correlates with survival in patients with osteosarcoma lung metastases. *Journal of Pediatric Hematology and Oncology*, **30**, 507–512.

Shaw SC, Kent MS, Gordon IK, Collins CJ, Greasby TA *et al.* (2009) Temporal changes in characteristics of injection-site sarcomas in cats: 392 cases (1990–2006). *Journal of the American Veterinary Medical Association*, **234**, 376–380.

Sharma-Walia N, Patel K, Chandran K, Marginean A, Bottero V *et al.* (2012) COX-2/PGE2: molecular ambassador of Kaposi's sarcoma-associated herpes virus oncoprotein-v-FLIP. *Oncogenesis*, **1**, 1–21.

Sharma-Walia N, Paul AG, Bottero V, Sadagopan S, Veettil MV *et al.* (2010) Kaposi's sarcoma associated herpes virus (KSHV) induced COX-2: a key factor in latency, inflammation, angiogenesis, cell survival and invasion. *PLoS Pathogens*, **6**, 1–30.

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

Wilcock B, Wilcock A, Bottoms K (2012) Feline postvaccinal sarcoma: 20 years later. *Canadian Veterinary Journal*, **53**, 430–434.

Williams C, Mann M, DuBois R (1999) The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene*, **18**, 7908–7916.

Woodward KN (2011) Origins of injection-site sarcomas in cats: the possible role of chronic inflammation. *ISRN Veterinary Science*, **2011**, 1–16.

Xu Z, Choudhary S, Voznesensky O, Mehrotra M, Woodard M *et al.* (2006) Overexpression of Cox-2 in human osteosarcoma cells decreases proliferation and increases apoptosis. *Cancer Research*, **66**, 6657–6664.

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