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Fibrosarcomas at Presumed Sites of Injection in Dogs: Characteristics and Comparison with Non-vaccination Site Fibrosarcomas and Feline Post-vaccinal Fibrosarcomas

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Summary

Fifteen fibrosarcomas, surgically excised from presumed sites of injection in dogs, and 10 canine fibrosarcomas excised from sites not used for injection were histologically and immunohistochemically compared with 20 feline post-vaccinal fibrosarcomas. Canine fibrosarcomas from presumed injection sites were of grade I (3), of grade II (4) and grade III (8). Two fibrosarcomas from non-injection sites were of grade I, four of grade II and four of grade III. Feline samples were classified as grade I (2), grade II (4) and grade III (14). All fibrosarcomas from presumed injection sites of both species showed lymphocytic inflammatory infiltration located at the tumour periphery, while two canine fibrosarcomas from non-injection sites showed perivascular inflammatory infiltration within the neoplasm. All samples were immunohistochemically examined for vimentin, smooth muscle actin, muscle specific actin and desmin expression. All tumours were positive for vimentin. Ten canine fibrosarcomas from presumed injection sites and all feline samples contained cells consistent with a myofibroblastic immunophenotype. Aluminium deposits were detected in eight canine fibrosarcomas from presumed injection sites and 11 feline post-vaccinal fibrosarcomas by the aurintricarboxylic acid method. The present study identifies distinct similarities between canine fibrosarcomas from presumed injection sites and feline post-vaccinal fibrosarcomas, suggesting the possibility of the development of post-injection sarcomas not only in cats, but also in dogs.

Introduction

Dogs and cats can sometimes develop subcutaneous inflammatory reactions at sites of injection, and there is some evidence to further suggest that, although other drugs may be involved, those reactions are mainly associated with the use of inactivated virus vaccines containing aluminium-based adjuvants (Hendrick, 1998). In both dogs and cats, the development of necrotizing panniculitis at sites of rabies vaccine administration was first observed by Hendrick and Dunagan (1991). These lesions were characterized by a central area of necrosis rimmed by an inflammatory reaction, often with lymphatic follicles formation. Moreover, in cats a distinctive tumour which developed at sites of rabies and feline leukaemia vaccine administration, was noted by Hendrick and Goldschmidt (1991). Feline post-vaccinal fibrosarcomas (Hendrick et al., 1998) have received a great deal of attention in veterinary literature over the past 10 years. These neoplastic lesions seem to arise in younger cats and seem to be more aggressive, with a higher recurrent rate, than fibrosarcomas arising at other sites (Hendrick, 1998). Histologically, feline post-vaccinal fibrosarcomas are characterized by inflammatory peritumoural infiltration, multinucleated giant cells and myofibroblastic cells (Dubielzig et al., 1993). Grey–brown granular to crystalline foreign material was found within macrophages in the inflammatory foci in 42 of 198 post-vaccinal sarcomas, and in three cases the electron probe X-ray analysis demonstrated that it was composed of aluminium and oxygen (Hendrick et al., 1992). Post-vaccinal fibrosarcomas are believed to arise as a result of proliferation of fibroblasts and myofibroblasts at sites of chronic inflammation induced by the vaccine's adjuvants, its antigens, or both (Macy and Hendrick, 1996).

Fibrosarcoma is the second most prevalent skin tumour in cats, while in dogs it represents a rare tumour (Yager and Wilcock, 1994).

In the present study, 15 cases of canine fibrosarcomas arising at presumed sites of injections and 10 canine fibrosarcomas developing at sites not used for injections (oral cavity, legs) were examined and histologically and immunohistochemically compared with 20 feline post-vaccinal fibrosarcomas.

Materials and Methods

Animals and tissue processing

Paraffin blocks containing fibrosarcomas surgically excised from dogs and cats between 1998 and 2001 were retrieved from the archives of the Histopathology Department of the Istituto Zooprofilattico Sperimentale delle Venezie (northern Italy). Fifteen canine fibrosarcomas, arising at sites commonly used

by veterinarians for subcutaneous injections (back of the neck, inter-scapular region, thorax) comprised the group of 'fibrosarcomas from presumed injection sites'. All dogs had been vaccinated regularly against the most common canine infectious diseases (infectious gastroenteritis, distemper, infectious hepatitis and leptospirosis), and six dogs received also rabies vaccines. Ten canine fibrosarcomas from sites not used for injections and 20 feline post-vaccinal fibrosarcomas, showing typical histopathological characteristics (Hendrick et al., 1998), were examined for comparison. The cats included in the present study had been vaccinated regularly against feline leukaemia virus (FeLV) and other common feline infectious diseases.

For each specimen, 4-μm-thick sections were stained with haematoxylin and eosin and examined microscopically in order to grade the neoplasia and to investigate the presence of an inflammatory reaction. The grading scheme, previously adapted to the dog (Powers et al., 1995) and recently applied to feline post-vaccinal fibrosarcomas (Couto et al., 2002), was based on cellular differentiation, presence and extension of necrosis within the neoplasm and mitotic rate. All fibrosarcomas were scored 1–3 for overall differentiation (1 = tumours closely resembling the mature differentiation; 2 = tumours that had a defined histological phenotype; 3 = poorly differentiated tumours), mitotic rates (1 = 1–9 mitotic figures per ten 400× fields; 2 = 10–19 mitotic figures per ten 400× fields; 3 = 20 or more mitotic figures per ten 400× fields) and necrosis (1 = no necrosis; 2 = < 50% of the total area; 3 = > 50% of the total area). Final scores of three or four were designated grade I; scores of five or six were designated grade II; scores of seven, eight or nine were designated grade III.

A computer program was used for the statistical analysis (STATA). Comparison between canine tumour categories with respect to the grade was performed using the Kruskal–Wallis non-parametric analysis of variance (ANOVA). A level of significance of 0.05 ($P < 0.05$) was used.

Immunohistochemistry

For each sample, 3 μm sections were cut and immunohistochemically stained for vimentin (V9, DAKO, Carpinteria, CA, USA, M0725, 1 : 25), desmin (DE-R-11, DAKO, Carpinteria, CA, USA, M724, 1 : 50), smooth muscle actin (1A4, DAKO, Carpinteria, CA, USA, M851, 1 : 50), and muscle specific actin (MSA) (HHF35, DAKO, Carpinteria, CA, USA, M0635, 1 : 50) (Inter-Species Cross-Reactivity of DAKO antibodies, Code N° 10 145). Each primary antibody was incubated for 30 min at room temperature. Antigen retrieval for desmin and smooth muscle actin was obtained by trypsinization for 30 min at 37°C. The EnVision™ Detection Kit Peroxidase/DAB Rabbit Mouse (DAKO, Carpinteria, CA, USA, K5007) was applied. The sections were counterstained with Mayer's haematoxylin.

Histochemistry

For the detection of aluminium deposits in tissues, the aurintricarboxylic acid method was applied to the sections. Aluminium deposits appeared red under light microscopy (Bonucci, 1981).

Results

Canine fibrosarcomas from presumed injection sites

The average age of dogs with fibrosarcomas at presumed injection sites was 6.2 years (7 months–11 years) (Table 1).

Samples were characterized by a subcutaneous proliferation of neoplastic cells, of a mesenchymal phenotype and a variable degree of pleomorphism and mitotic rate. Neoplasms were sometimes pseudo-encapsulated and showed infiltrative growth. According to the grading scheme introduced, on the basis of cellular differentiation, mitotic rate and extension of necrosis, samples were classified as grade I (3), grade II (4) and grade III (8). All samples exhibited an inflammatory infiltration, mainly composed of lymphocytes, macrophages and plasma cells, localized at the tumour periphery, often in a follicle-like arrangement (Fig. 1).

Immunohistochemically, all fibrosarcomas were strongly positive for vimentin, and negative for desmin. Eight samples showed bundles of cells, mainly located at the tumour periphery, which stained positive for smooth muscle actin and 10 samples contained bundles of cells, which stained

Table 1. Case summaries for 15 dogs with fibrosarcomas from presumed injection sites

Case	Breed	Age (years)	Sex	Location	Vaccine history	Aluminium
1	Collie	5	M	Shoulder	Regularly vaccinated	+
2	Mixed	11	M	Shoulder	Rabies	+
3	Mixed	10	F	Thorax	Regularly vaccinated	–
4	Mixed	10	M	Thorax	Regularly vaccinated	+
5	German Shepherd dog	8	F	Thorax	Rabies	–
6	Mixed	2	M	Back	Regularly vaccinated	–
7	Schnauzer	3	M	Shoulder	Rabies	–
8	Chow-Chow	8	M	Shoulder	Regularly vaccinated	–
9	Golden Retriever	2	M	Shoulder	Regularly vaccinated	+
10	American pit bull	1	F	Shoulder	Regularly vaccinated	–
11	Mixed	6	M	Back	Rabies	+
12	Mixed	10	M	Thorax	Regularly vaccinated	–
13	Siberian Husky	11	M	Shoulder	Rabies	+
14	Drahthaar	7 months	F	Back	Regularly vaccinated	+
15	Irish setter	5	M	Shoulder	Rabies	+

M, male; F, female; regularly vaccinated = vaccinated against the common canine infectious diseases; rabies, vaccinated against the common canine infectious diseases and rabies.

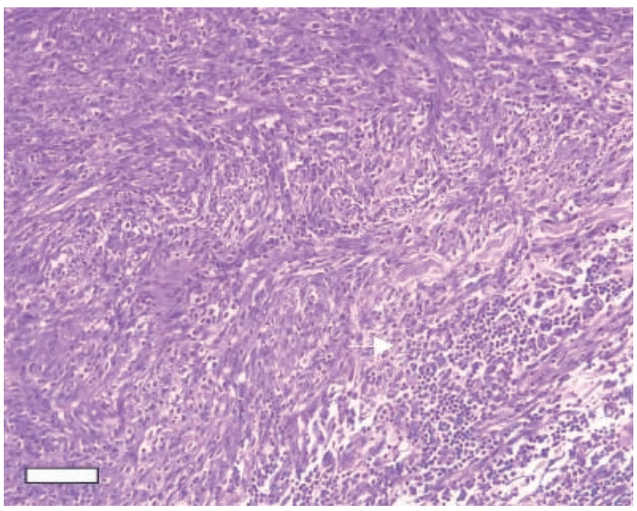

Fig. 1. Canine fibrosarcoma from presumed injection site. The inflammatory reaction (arrow) composed of lymphocytes and rare plasma cells was located at the tumour periphery. HE. Bar = 50 μm.

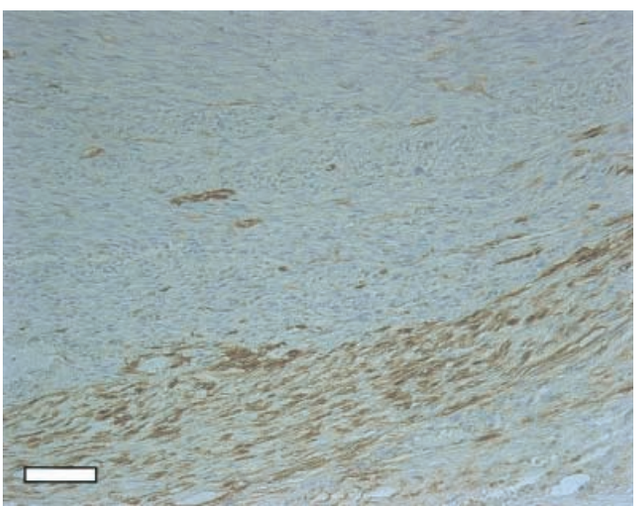

Fig. 2. Canine fibrosarcoma from presumed injection site. Muscle specific actin antigen is expressed by cells located in the tumour periphery. EnVisionTM Detection Kit Peroxidase with HHF35 antibody and haematoxylin counterstain. Bar = 50 μm.

positive for MSA (Fig. 2). These cells showed a fibroblastic phenotype, with abundant cytoplasm and elongated nuclei.

Aluminium deposits were detected in eight fibrosarcomas, both within macrophages and in the fibrous stroma (Table 1; Fig. 3).

Canine fibrosarcomas from sites not used for injection

The average age of dogs with fibrosarcomas from sites not used for injection was 8.4 years (5–11 years) (Table 2).

Two samples were of grade I, four of grade II and four of grade III. Neoplasms were not encapsulated and locally infiltrative. Two fibrosarcomas, from gum and foreleg, showed ulceration of the mucous membrane and cutis, respectively, and perivascular inflammatory infiltration within the neoplastic mass.

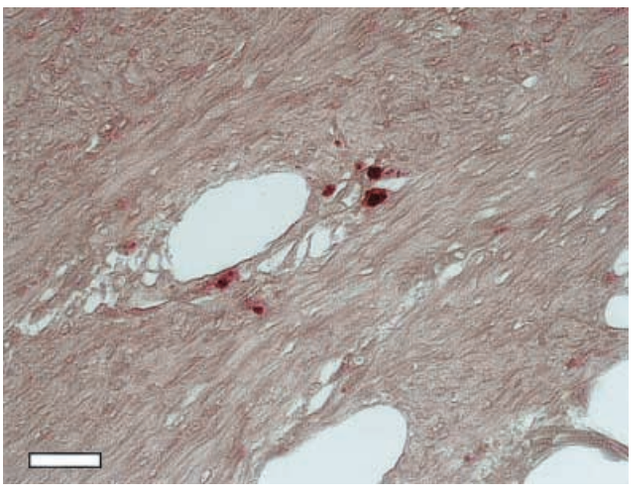

Fig. 3. Canine fibrosarcoma from presumed injection site. Aluminium deposits revealed by the aurintricarboxylic acid method in the fibrous stroma of the excised tumours. Bar = 25 μm.

Table 2. Case summaries for dogs with fibrosarcomas from sites not used for injection

Case	Breed	Age (years)	Sex	Location
1	Mixed	7	F	Gum
2	German shepherd dog	11	F	Foreleg
3	Doberman	10	F	Gum
4	Mixed	11	M	Gum
5	Rottweiler	6	M	Gum
6	Dalmatian	5	F	Hind leg
7	Mixed	7	F	Lip
8	German shepherd dog	6	F	Gum
9	German shepherd dog	11	M	Gum
10	Bloodhound	10	M	Foreleg

M, male; F, female.

When tested by immunohistochemistry, all samples were strongly positive for vimentin and negative for desmin. Single cells positively stained for MSA antigen were detected within two fibrosarcomas. Aluminium deposits were not detected in any sample.

Feline post-vaccinal fibrosarcomas

The average age of cats included in the present survey was 8.4 years (5–13 years) (Table 3). Samples included two fibrosarcomas of grade I, four of grade II and 14 of grade III. All samples showed lymphocytic aggregates at the periphery of the neoplastic proliferation. Multinucleated giant cells were detected in 10 fibrosarcomas.

Immunohistochemically, all samples were strongly positive for vimentin. Bundles of neoplastic cells positive stained for the smooth muscle actin were detected at the periphery of 16 feline fibrosarcomas. Eighteen samples showed cells positive stained for MSA. Only one feline post-vaccinal fibrosarcoma showed few single cells positive for desmin. Aluminium deposits were detected in 11 fibrosarcomas by the aurintricarboxylic acid method.

Table 3. Case summaries for cats with post-vaccinal fibrosarcomas

Case	Breed	Age (years)	Sex	Location
1	DSH	10	M	Shoulder
2	DSH	7	F	Shoulder
3	DSH	ns	M	Shoulder
4	Persian	9	F	Neck
5	DSH	7	F	Shoulder
6	DSH	13	M	Shoulder
7	DSH	9	F	Shoulder
8	Persian	10	F	Shoulder
9	DSH	8	F	Shoulder
10	DSH	6	M	Shoulder
11	DSH	7	F	Shoulder
12	Persian	7	M	Back
13	DSH	9	F	Back
14	DSH	8	M	Shoulder
15	DSH	5	F	Lateral thorax
16	DSH	8	M	Back
17	DSH	7	M	Neck
18	DSH	13	M	Shoulder
19	DSH	10	F	Back
20	DSH	6	M	Lateral thorax

DSH, domestic short haired; ns, non-specified; M, male; F, female.

Discussion

Fibrosarcoma is a rare tumour in dogs, and its most common sites of development are the skin of the trunk and of the proximal limbs as well as the oral cavity (Yager and Wilcock, 1994).

Canine fibrosarcomas arising at presumed sites of subcutaneous injection (shoulder, inter-scapular region, thorax) were examined and morphologically and immunohistochemically compared with canine fibrosarcomas arising at sites not used for injection and feline post-vaccinal fibrosarcomas.

The average age of dogs with fibrosarcomas from presumed injection sites was 6.2 years. The average age of dogs with fibrosarcomas at sites not used for injection was 8.5 years while that of cats was 8.4 years. According to the literature, the average age of cats with fibrosarcomas at sites not used for injection is 12 years (Gross et al., 1992), while post-vaccinal fibrosarcomas are reported to arise in cats with an average age of 8.1 years (Hendrick et al., 1994) and 8.6 years (Doddy et al., 1996), respectively. The average age of dogs with fibrosarcomas, irrespective of the site of development, was reported as 10 years (Gross et al., 1992). The comparison between the average age of the three classes of animals was statistically analysed and no significant difference was detected. Although epidemiological evaluations are not possible due to the limited number of cases included in the present study, the young age of some dogs with presumed post-injection fibrosarcomas supports the hypothesis of an iatrogenic origin.

The three groups of neoplasms were histologically examined for morphological distinctions. The grading scheme applied, was the one used in categorizing canine soft-tissue sarcomas (Powers et al., 1995) and feline post-vaccinal fibrosarcomas (Couto et al., 2002) and allowed the separation of the neoplasms into three classes with increasing malignancy. Histological grading is the most important prognostic factor for human adult soft-tissue sarcomas with regard to the probability of metastasis development and survival rate (Kandel et al., 1999; Mandard et al., 1989). It has been shown that feline post-vaccinal fibrosarcomas exhibit histopathological features consistent with a more aggressive biologic behaviour than fibrosarcomas at sites not used for injection (Doddy et al., 1996). The statistical analysis applied to the tumour grades in this study did not reveal significant differences between the two different groups of canine fibrosarcomas. In both species the fibrosarcomas surgically excised from presumed sites of injection showed an inflammatory response, mainly as lymphatic follicle-like aggregates located at the tumour periphery. In contrast, only two canine fibrosarcomas, excised from the gum and the foreleg, were accompanied by perivascular infiltration of lymphocytes within the neoplasm. In these cases, the inflammatory reaction was probably the consequence of ulceration of the mucous membrane and cutis lining the fibrosarcomas, respectively. The inflammatory response is one of the distinctive features of the feline post-vaccinal fibrosarcomas (Doddy et al., 1996). Data suggest that local inflammation caused by aluminium or other potentially irritant inoculated substances, may predispose tissues to tumour development. Furthermore, feline fibrosarcomas found in vaccine sites are histologically identical to those observed in previously traumatized areas (Smith, 1995). However, the role of lymphocytes in tumourigenesis or host response to neoplasia is still unknown (Couto et al., 2002).

Multinucleated giant cells were detected in 10 feline post-vaccinal fibrosarcomas, whereas they were not detected in any canine sample. The presence of multinucleated giant cells is a common finding in feline fibrosarcomas and is regarded as an indicator of a less differentiated phenotype (Doddy et al., 1996). In human oncology, the presence of multinucleated giant cells is correlated with an aggressive, invasive tumour phenotype and is used as part of a paradigm to estimate prognosis (Couto et al., 2002).

Tumours were tested immunohistochemically for vimentin, actin and desmin expression. All samples were strongly positive for vimentin, thus confirming the mesenchymal origin of the neoplastic cells.

Myofibroblasts are interesting cells identified for the first time in contractile granulation tissue and wounds in the early 1970s (Mentzel and Fletcher, 1997). Ultrastructurally, myofibroblasts are recognized by their features of both fibroblasts and smooth muscle actin. Immunohistochemistry identified four mainly myofibroblastic phenotypes which show, in addition to cytoplasmic β- and γ-actins, immunopositivity for vimentin, vimentin and desmin, vimentin and alpha-smooth muscle actin, or vimentin, alpha-smooth muscle actin, and desmin (Mentzel and Fletcher, 1997). In the present study, immunolabelling of tumours with muscular antigens allowed the identification of bundles of cells with a myofibroblast-like immunophenotype in all the feline and in 10 canine fibrosarcomas from presumed injection sites. These cells were localized at the tumour periphery, often adjacent to lymphatic follicle-like aggregates. It is generally accepted that myofibroblasts represent an important component of numerous benign and malignant mesenchymal neoplasms. In addition to tissue repair process and stromal response to neoplasia, proliferating myofibroblasts are the main cellular component in four pathological settings: reactive lesions, benign tumours, locally aggressive fibromatoses and sarcomas with myofibroblastic differentiation (Mentzel and Fletcher, 1997). Myofibroblasts were previously detected in feline post-vaccinal fibrosarcomas, identified by both immunohistochemistry and electron microscopy (Dubielzig et al., 1993; Madewell et al., 2001).

The function and biological implications of myofibroblasts in tumour growth are far from being clarified. One recent study performed on a rat colorectal tumour model (Lieubeau et al., 1999), suggests that myofibroblasts, due to their contractive properties, are able to form a capsule that enveloped neoplastic nodules, mechanically preventing penetration of T lymphocytes and macrophages into the tumour, while promoting tumour growth and progression. In fact, locomotion and tumour access of immune cells is crucial for the function of the immune system. If this mechanical action should be the same in injection-associated fibrosarcomas, it may account for the presence of abundant lymphocytes along the periphery of the tumours and for their more aggressive biological behaviour than fibrosarcomas at sites not used for injections. In canine fibrosarcomas from non-injection sites, there was no evidence of myofibroblastic differentiation. The single cells positive for MSA, which were observed in two cases, are considered consistent with normal muscular cells entrapped in the neoplastic proliferation.

Aluminium deposits were detected in eight canine fibrosarcomas from presumed injection sites and 11 feline fibrosarcomas by histochemistry. The aurintricarboxylic acid method is a specific method for the identification of aluminium hydroxide deposits in tissues (Bonucci, 1981). Aluminium hydroxide adjuvants are used in many veterinary and human inactivated vaccines. In animals it has been detected at sites of subcutaneous injection for up to 1 year after application (Madewell et al., 2001). Aluminium deposits were previously highlighted in three of 198 feline post-vaccinal fibrosarcomas by electron probe X-ray analysis and ultrastructurally (Hendrick et al., 1992; Madewell et al., 2001), suggesting the role of aluminium-containing adjuvant as irritant in the pathogenesis of these fibrosarcomas. The development of foreign body granulomas caused by aluminium has also been reported in humans (Hendrick et al., 1992; Fawcett and Smith, 1984). All the animals included in the present study received annual vaccinations and underwent surgery soon after the first observation of the neoplastic growth by the owners or veterinarians. Such a special care paid to these pets, assuring a prompt recognition and removal of the nodules, may have guaranteed short intervals between onset of neoplastic growth and histochemical examination, thus resulting in a high percentage of samples containing aluminium deposits. Furthermore, four of eight samples containing aluminium deposits were excised from dogs that had received vaccination against rabies, other than against the most common infectious diseases. The development of necrotizing panniculitis after rabies vaccine administration has already been reported in dogs (Hendrick and Dunagan, 1991). Rabies vaccines have also been associated with the development of fibrosarcomas in cats (Hendrick and Goldschmidt, 1991). Furthermore, it is accepted that substances other than aluminium can be involved in the pathogenesis of these fibrosarcomas. For close to 100 years, investigators have observed that irritation, inflammation and/or wounds are promoters of tumour development (Macy and Hendrick, 1996). Virtually anything that causes a local inflammatory reaction may potentially be responsible for neoplastic initiation (Withrow and MacEwen, 2001). Sarcomas developing at sites of subcutaneous administration of long acting drugs and at sites with deep non-absorbable sutures, as well as ocular post-traumatic sarcomas, are clinical examples that support these findings (Dubielzig, 1984; Dubielzig et al., 1990; Esplin et al., 1999; Buracco et al., 2002).

Although the post-vaccinal fibrosarcoma has been considered as a specific entity in the cat, many similar features were noted in feline and canine samples. In both species, fibrosarcomas arose at the same sites, probably used by practitioners for subcutaneous injections. The lesions were characterized by the proliferation of mesenchymal neoplastic cells, consistent with fibroblasts, with areas of necrosis and peritumoural inflammatory infiltration. Cells with a myofibroblastic phenotype were detected immunohistochemically in fibrosarcomas from presumed injection sites of both species, but not in the canine fibrosarcomas arising at sites not used for injection. Aluminium deposits were noted not only in feline samples, but also in eight canine fibrosarcomas, from presumed injection sites.

In conclusion, the findings of this study support the hypothesis that post-injection fibrosarcomas do not only occur in cats but also in dogs. However, further investigations are needed to elucidate the possible relationship between vaccine administration and fibrosarcoma development at sites of injection in dogs.

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References

Bonucci, E., 1981: Manuale di istochimica. Lombardo Editore, Roma.

Buracco, E., M. Martano, E. Morello, and A. Ratto, 2002: Vaccine associated-like fibrosarcoma in the site of a deep nonabsorbable suture in a cat. Vet. J. **163,** 105–107.

Couto, S. S., S. M. Griffey, P. C. Duarte, and B. R. Madewell, 2002: Feline vaccine-associated fibrosarcoma: morphologic distinctions. Vet. Pathol. **39,** 33–41.

Doddy, F. D., L. T. Glickman, N. W. Glickman, and E. B. Janovits, 1996: Feline fibrosarcomas at vaccination sites and non-vaccination sites. J. Comp. Pathol. **114,** 165–174.

Dubielzig, R. R., K. L. Hawkins, and P. E. Miller, 1993: Myofibroblastic sarcoma originating at the site of rabies vaccination in a cat. J. Vet. Diagn. Invest. **5,** 637–638.

Dubielzig, R. R., 1984: Ocular sarcoma following trauma in three cats. J. Am. Vet. Med. Assoc. **184,** 578–581.

Dubielzig, R. R., J. A. Everitt, J. A. Shadduck, and D. M. Albert, 1990: Clinical and morphologic features of post-traumatic ocular sarcomas in cats. Vet. Pathol. **27,** 62–65.

Esplin, D. G., M. Bigelow, L. D. McGill, and S. R. Wilson, 1999: Fibrosarcoma at the site of a Lufenuron injection in a cat. Vet. Cancer Soc. Newsletter **23,** 8–9.

Fawcett, H. A., and N. P. Smith, 1984: Injection-site granuloma due to aluminium. Arch. Dermatol. **120,** 1318–1322.

Gross, T. L., P. J. Ihrke, and E. J. Walder, 1992: Veterinary Dermatopathology. Mosby Year Book, St. Louis.

Hendrick, M. J., and M. H. Goldschmidt, 1991: Do injection site reactions induce fibrosarcoma in cats? J. Am. Vet. Med. Assoc. **99,** 968.

Hendrick, M. J., and C. A. Dunagan, 1991: Focal necrotizing granulomatous panniculitis associated with subcutaneous injection of rabies vaccine in cats and dogs: 10 cases (1988–1989). J. Am. Vet. Med. Assoc. **198,** 304–305.

Hendrick, M. J., M. H. Goldschmidt, F. S. Shofer, Y. Y. Wang, and A. P. Somlyo, 1992: Postvaccinal sarcomas in the cat: epidemiology and electron probe microanalytical identification of aluminium. Cancer Res. **52,** 5391–5394.

Hendrick, M. J., 1998: Historical review and current knowledge of risk factors involved in feline vaccine-associated sarcomas. J. Am. Vet. Med. Assoc. **213,** 1422–1423.

Hendrick, M. J., F. S. Shofer, M. H. Goldschmidt, J. C. Haviland, S. H. Schelling, S. J. Engler, and J. M. Gliatto, 1994: Comparison of fibrosarcomas that developed at vaccination sites and at nonvaccination sites in cats: 239 cases (1991–1992). J. Am. Vet. Med. Assoc. **205,** 1425–1429.

Hendrick, M. J., E. A. Mahaffey, F. M. Moore, J. H. Vos, and E. J. Walder, 1998: Histological classification of mesenchymal tumors of skin and soft tissues of domestic animals. WHO, Second series, Vol. **II,** AFIP, Washington, D.C.

Kandel, R. A., R. S. Bell, J. S. Wunder, B. O'Sullivan, C. N. Catton, L. M. White, and A. M. Davis, 1999: Comparison between a 2- and 3-grade system in predicting metastatic-free survival in extremity soft tissue sarcoma. J. Surg. Onc. **79,** 77–82.

Lieubeau, B., M. F. Heymann, F. Henry, I. Barbieux, K. Meflah, and M. Gregoire, 1999: Immunomodulatory effects of tumor-associated fibroblasts in colorectal tumor development. Int. J. Cancer **81,** 629–636.

Macy, D. W., and M. J. Hendrick, 1996: The potential role of inflammation in the development of postvaccinal sarcomas in cats. Vet. Clin. North Am. Small Anim. Pract. **26,** 103–109.

Madewell, B. R., S. M. Griffey, M. C. McEntee, V. J. Leppert, and R. J. Munn, 2001: Feline vaccine associated fibrosarcoma: an ultrastructural study of 20 tumors (1996–1999). Vet. Pathol. **38,** 196–202.

Mandard, A. M., J. F. Petiot, J. Marnay, J. C. Mandard, J. Chasle, E. de Ranieri, P. Dupin, P. Herlin, J. de Ranieri, A. Tanguy, N. Boulier, and J. S. Abbattucci, 1989: Prognostic factors in soft tissue sarcomas. A multivariate analysis of 109 cases. Cancer **63,** 1437–1451.

Mentzel, T., and C. D. M. Fletcher, 1997: The emerging role of myofibroblasts in soft tissue neoplasia. Am. J. Clin. Pathol. **107,** 2–4.

Powers, B. E., P. J. Hoppes, and E. J. Ehrhart, 1995: Tumor diagnosis, grading and staging. Semin. Vet. Med. Surg. (Small Anim.) **10,** 158–157.

Smith, C. A., 1995: Are we vaccinating too much. J. Am. Vet. Med. Assoc. **207,** 421–425.

Withrow, S. J., and E. G. MacEwen, 2001: Small animal clinical oncology. W.B. Saunders Company, Philadelphia.

Yager, J. A., and B. P. Wilcock, 1994: Color atlas and text of surgical pathology of the dog and cat. Mosby Year Book Europe Limited, London.