Feline Health Topics

for veterinarians

November 1997

Volume 12, Number 3

Vaccine-Associated Feline Sarcoma Task Force Awards Research Grants

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Veterinarians have recently noticed an increase in sarcomas appearing in cats at body sites commonly used for vaccine administration. The Vaccine-Associated Feline Sarcoma Task Force (VAFSTF) has been formed to address this emerging feline health issue (see the Journal of the American Veterinary Medical Association, Vol. 210, No. 3, pp. 310-311). The task force met in November to review research proposals and designate the recipients of support. Available financial resources permitted the funding of four projects for the upcoming year; other studies were approved but are awaiting additional funds. Parties interested in supporting studies to examine the epidemiology, etiology, and treatment of these rare but aggressive tumors should contact Dr. Robin Starr, VAFSTF Chairperson, American Animal Hospital Association, PO Box 150899, Denver, CO, 80215.

Inside this issue ...

The VAFSTF would like to thank the following donors for their generous support of this year's studies:

- American Animal Hospital Association Foundation - $50,000
- American Association of Feline Practitioners- $25,000
- Fort Dodge Animal Health - $25,000
- Cornell Feline Health Center - $10,000
- Intervet Inc. - $10,000
- Veterinary Cancer Society - $5,000
- Synbiotics Corporation - $2,500

Epidemiologic Study of Vaccine-Specific Risk and Vaccination Protocols in the Incidence of Vaccine-Associated Sarcomas in Cat. This study focuses on some of the remaining controversial and unsolved issues regarding the association between the administration of some vaccines and the development of soft-tissue sarcomas in cats. The investigators will compare cats with sarcomas diagnosed at vaccine sites with cats having a histologic diagnosis of basal cell tumor. This prospective case control study will examine the relationship between putative risk factors and the conditional probability of development of vaccine-associated feline sarcomas (VAFS), thus seeking to measure relative risk and incidence. This

multicenter study will involve six major collaborating centers in the United States and Canada, and will be the most exhaustive vaccine-associated sarcoma study ever undertaken.

Some questions to be addressed include:

1. Is the risk associated with the use of non-adjuvanted vaccines equivalent to that of adjuvanted vaccines?

2. Is the risk associated with the use of attenuated vaccines equivalent to that of inactivated vaccines?

3. Within antigen classes, is there homogeneity of risk?

4. Do other factors, for example, injections administered at the same location over the cat's lifetime, re-use of syringes, or mixing of vaccines in the same syringe have any effect on sarcoma incidence?

Principle investigator: P.H. Kass, DVM, PhD.

Co-investigators: M.J. Hendrick, VMD; S. Lester, DVM, MVSc; D.G. Esplin, DVM, PhD; L.D. McGill, DVM, PhD; M. Slater, DVM, PhD; W.L. Spangler, DVM, PhD.

Feline Health Topics

A publication for veterinary professionals

The ultimate purpose of the Cornell Feline Health Center is to improve the health of cats everywhere by developing methods to prevent or cure feline diseases, and by providing continuing education to veterinarians and cat owners. All contributions are tax-deductible.

Director: **James R. Richards, D.V.M.**
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Molecular Biomarkers of Vaccine-Associated Feline Sarcomas. One of the features of vaccine-associated feline sarcomas is either the presence or the subsequent development of multiple synchronous tumors at the vaccination site or at the margins of previous excisions. The specific reasons for regrowth of tumors at the vaccination site are unknown. It is possible that the multiple tumors represent field cancerization at the vaccination site. During field cancerization, distinct genetic alterations occur in numerous cells throughout the exposed area or "field" (in this case, the vaccination site). Some of these cells undergo clonal expansion and the tissue becomes predisposed to growth of multiple primary tumors and premalignant lesions. The importance of determining the occurrence of field cancerization lies in the fact that the risk of developing cancer in the affected area remains high for several years, requiring extended follow-up and repeated surgical intervention. The prognosis for such patients is often poor. Therefore, it is important to develop molecular biomarkers for defining disease-free margins and predicting clinical outcomes.

The study is designed to achieve the following objectives:

1. Establish a comprehensive tissue bank and database at the University of Minnesota for future VAFS-related molecular and molecular epidemiologic analyses. The tissue bank will not only include primary sarcomas, subsequent tumors collected on follow-up, and metastases (if any), but also blood samples, and biopsies of the histologically normal margins taken at specified distances and orientations from the tumors.

2. Investigate if patients with VAFS harbor alterations in two potential molecular biomarkers: the p53 tumor suppressor gene and the c-myc proto oncogene. These genes are known to play important roles in the pathogenesis of soft tissue sarcomas in humans. Moreover, results of preliminary studies indicate a loss of heterozygosity at the p53 locus in VAFS.

3. Test the hypothesis of field cancerization in VAFS. All cases will be followed-up for subsequent tumor growth in the region of the primary tumor. The presence of a p53 mutation in the primary tumor that is distinct from any p53 mutations detected in subsequent tumors will be considered as molecular evidence of field cancerization.

4. Categorize the types of p53 mutations detected in VAFS for preliminary indications of "signature" mutations. Such mutations are likely to provide important clues on disease etiology.

5. Record clinical outcome and initiate studies on the prognostic significance of general or specific alterations in p53 and c-myc from tumor and histologically normal tissues. Maps of the site of tumor development indicating locations of genetic alterations and subsequent tumors may help in providing guidelines for reliable identification of disease-free margins.

Principal investigator: S. Kanjilal, PhD.

Co-investigators: J S. Klausner, DVM, DACVIM; V. Kapur, BVSc, MSc, PhD; C. Khanna, DVM, PhD, DACVIM; C. Wood, DVM.

Growth Factor Expression and Vaccine-Associated Sarcoma Tumorgenicity. The molecular events leading to the development of vaccine-associated feline sarcomas is unknown. However, there is accumulating evidence that the activation of genetically-altered growth factor signaling pathways contributes to the development and progression of many forms of cancer. The major goal of this study is to provide direct evidence that autocrine/paracrine growth factors and their receptors regulate or influence neoplastically-transformed mesenchymal cells and play an important role in the formation of vaccine-associated feline sarcomas. Overexpression of growth factor receptors in cancer cells may provide an important target for therapeutic intervention; it is hoped that the results of this study will guide in the designing of effective therapeutic strategies.

This study will pursue the following specific aims:

1. Develop new feline sarcoma cell lines from cats with VAFS and those from non-vaccine sites such as the oral cavity or distal extremities.

2. Determine the gene expression of insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and platelet derived growth factor (PDGF) in tumor tissue or cells grown in culture, and adjacent non-neoplastic tissue from cats with sarcomas.

3. Determine the level of gene expression of IGF-1 receptor, HGF receptor, and PDGF receptor on tumor cells.

4. Determine the functional (growth and invasion) significance of IGF-1, HGF, and PDGF on sarcoma cells growing in tissue culture.

5. Determine the tumorigenic and metastatic potential of feline sarcoma cells.

Principal investigator: E.G. MacEwen, VMD.

Co-investigator: R. Radinsky, PhD.

Comparable Efficacy of Doxorubicin Versus Stealth Liposomal Doxorubicin in Cats with Vaccine-Associated Sarcomas: A Multicenter Randomized Clinical Trial. Vaccine-associated feline sarcomas (VAFS) are associated with unacceptably high recurrence rates following surgical excision and relatively low response rates to standard chemotherapeutic approaches instituted in either an adjuvant or primary setting. While radiation therapy may be a viable option for VAFS, availability makes this modality unrealistic for a significant proportion of patients. Several institutions are presently recommending doxorubicin adjuvant or primary chemotherapy for the management of these tumors. However, only anecdotal evidence exists regarding efficacy of this approach.

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The dose limiting toxicities of doxorubicin includes myelosuppression, cardiotoxicity, and in the feline species in particular, anorexia. Recently, various liposome formulations have been utilized as doxorubicin drug carrier systems to reduce toxicity. A commercially available doxorubicin-entrapped stealth liposome formulation (Doxil®, Sequus Pharmaceuticals Inc., Menlo Park, CA) has proven to be effective in enhancing tumoricidal effects when compared to free doxorubicin in a variety of tumor models, and to decrease systemic toxicity. A dose escalation trial is currently underway to determine the maximally tolerated dose of Doxil in cats. Preliminary results suggest that cats can safely receive at least 40% more doxorubicin when it is delivered in this encapsulated form; even higher dosage rates may be possible. In a phase I clinical trial, a 36% response rate (partial or complete) to Doxil has been observed in cats with advanced, unresectable VAFS. Fifty-six percent of these responding cats have achieved at least a 50% reduction in tumor volume.

The purpose of this study is to prospectively evaluate, in the context of a randomized multicenter clinical trial, the comparable efficacy of doxorubicin versus stealth liposomal doxorubicin in cats with VAFS in two settings:

1. Adjuvant therapy in cats with microscopic disease subsequent to cytoreductive surgery.

2. Primary therapy in cats with measurable macroscopic disease.

Principal investigator: D.M. Vail, DVM, MS, DACVIM.

Co-investigators: P.A. Ciekot; R. Chun; J.E. Obradovich; M. O'Brien; R.M. Fred III; K.A. Jeglum.■

Editor's Note

In the article entitled "Part 1: Hypokalemia in Cats" which appeared in the September, 1997, issue (Vol. 12, No. 1) of *Feline Health Topics for Veterinarians*, a statement was made that could potentially lead to erroneous conclusions. The observation regarding hypokalemia in cats fed Hill's® Prescription Diet® Feline c/d® dry or Hill's® Science Diet® Feline Maintenance® dry referred to a 1989 paper in JAVMA entitled, "Hypokalemia in cats: 186 cases (1984-1987)" by Dow, et. al. In that original paper, the authors noted that these diets contained sufficient potassium for healthy cats. However, in fact, most of the hypokalemic cats (91%) were not healthy. They also stressed that the diets named in the study were formulations made prior to July, 1987, and that additional potassium had been included in subsequent formulas. The potassium levels in current formulations of the aforementioned diets are above the Association of American Feed Control Officials (AAFCO) Nutrient Profiles for Cat Foods recommendation of no less than 0.6% (dry matter basis) for dietary potassium intake. For complete nutrient profiles of these diets, please refer to the document available from the manufacturer entitled *Hill's Key to Clinical Nutrition* published in January, 1998.■

Research Briefs

Evaluation of different techniques for washing cats: Quantitation of allergen removed from the cat and the effect on airborne Fel d 1

(Authors: D.B. Avner, M.S. Perzanowski, T.A. Platts, T.A. Mills, and J.A. Woodfolk) —The purpose of this study was to examine the quantity and distribution of the major cat allergen, Fel d 1, on cats and to evaluate the efficacy of washing, both in removing allergen from the cat and reducing airborne allergen levels. Airborne samples were collected before and 3 hours after serial washing of eight cats. Aliquots of hair and bath water were also collected and assayed for Fel d 1 content.

Extracting cat hair with tap water or pet shampoo for 3 minutes removed mean levels of 191 and 245 mu g of Fel d 1 per gram of hair, respectively; the quantity of allergen on samples of cat hair ranged from 1 to more than 1770 micrograms/gm. The highest concentration of allergen was found on hair from the neck. Estimates of the total Fel d 1 on the cat, based on shaving the whole cat, ranged from 3 to 142 mg (mean = 67 mg). Washing three cats at weekly intervals for 5 weeks in a veterinarian's office produced a mean decrease of 44% in airborne Fel d 1. Washing three cats by immersion for 3 minutes at weekly intervals for a 1-month period produced a mean decrease in airborne allergen of 79%. However, after repeated washing, the airborne levels before the next wash were not consistently decreased. The quantity of Fel d 1 removed by immersion varied from 1 to 35 mg.

Conclusion: Cats carry large quantities of Fel d 1, only a small proportion of which (approximately 0.002%/hr) becomes airborne. Washing cats by immersion will remove significant allergen from the cat and can reduce the quantity of Fel d 1 becoming airborne. However, the decrease is not maintained at 1 week. *(Resource: J. Allergy Clin. Immunol. 100, 307-312, 1997.)*

Diabetic ketosis and ketoacidosis in cats: 42 cases (1980-1995)

(Authors: K.A. Bruskiewicz, R.W. Nelson, E.C. Feldman, and S.M. Griffey)—The objective of this retrospective study was to determine the clinical signs, clinicopathologic abnormalities, prevalence of concurrent disease, treatment, complications of treatment, and outcome in cats with diabetic ketosis (DK) or diabetic ketoacidosis (DKA). The medical records of 42 diabetic cats with ketonuria were reviewed. In 26 cats, diabetes was newly diagnosed; in 16, diabetes had been diagnosed previously and cats had been treated with insulin (n = 14) or sulfonylurea drugs (2). Common clinical findings were lethargy, anorexia, polyuria, polydipsia, and weight loss. Common laboratory findings were hyperglycemia, hyponatremia, hypochloremia, hypokalemia, hypocalcemia, hypophosphatemia, low total CO_2 content, hyperosmolality, high serum alanine transaminase activity, azotemia, glycosuria, and ketonuria. Concurrent disorders were identified in 39 cats and included hepatic lipidosis, cholangiohepatitis, pancreatitis, chronic renal failure, urinary tract infection, and neoplasia.

Complications during treatment included abnormalities in serum electrolyte concentrations (27 cats), hemolytic anemia (4), hypoglycemia (3), and neurologic abnormalities unrelated to hypoglycemia (2). Eleven cats died or were euthanatized during the initial hospitalization period for treatment of DK or DKA. Azotemia, metabolic acidosis, and hyperosmolality were more severe in cats that died than in cats that survived.

Differences in regard to treatment or complications were not apparent between cats that died and

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cats that survived. The results of this study imply that a thorough diagnostic evaluation should be performed on cats with DK or DKA to identify concurrent disorders, formulate an appropriate treatment plan, and provide prognostic information to the owner. *(Resource: J. Am. Vet. Med. Assoc. 211, 188-192, 1997.)*

Dynamics of two feline retroviruses (FIV and FeLV) within one population of cats

(Authors: F. Courchamp, C. Suppo, E. Fromont, and C. Bouloux.)—The authors present a deterministic model of the dynamics of two microparasites simultaneously infecting a single host population. Both microparasites are feline retroviruses: namely feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV). The host is the domestic cat *Felis catus*. The model has been tested with data generated by a long-term study of several natural cat populations. Stability analysis and simulations show that, once introduced in a population, FIV spreads and is maintained, while FeLV can either disappear or persist. Moreover, introduction of both viruses into the population induces an equilibrium state for individuals of each different pathological class. The viruses never induce the extinction of the population. Furthermore, whatever the outcome for the host population (persistence of FIV only, or of both viruses), the global population size at the equilibrium state is only slightly lower than it would have been in the absence of the infections (i.e. at the carrying capacity), indicating a low impact of the viruses on the population. Finally, the impact of the diseases examined simultaneously is higher than the sum of the impact of the two diseases examined separately. This seems to be due to a higher mortality rate when both viruses infect a single individual. *(Resource: Proc.R.Soc.Lond.[Biol.]264,785-794, 1997.)*

Primary hyperparathyroidism in two cats

(Authors: E. Den Hertog, M.M. Goossens, J.S. Van der Linde-Sipman, and H.S. Kooistra)—Primary hyperparathyroidism (PHP) is an infrequently diagnosed disorder in cats. In this report, the signs and symptoms of two cats with hypercalcemia due to PHP are described, together with the diagnostic approach, results of treatment, and immunohistochemical findings.

A 9-year-old and a 13-year-old neutered male domestic shorthair cat were presented with signs of lethargy, anorexia, and vomiting. Both cats had persistent hypercalcemia and normo- to hypophosphatemia. Cytological examination of a fine-needle aspiration biopsy sample of a palpable cervical mass revealed groups of benign glandular-epithelial cells in one cat. In the other cat no cervical mass was palpable. In this cat plasma parathyroid hormone (PTH) levels were measured repeatedly, and exceeded the maximum reference value on two occasions. Following exclusion of other causes of hypercalcemia, both cats were subjected to neck surgery; in both, a solitary parathyroid adenoma was removed. The adenomas contained an abundance of PTH, as demonstrated by immunohistochemical techniques. Plasma calcium and phosphate concentrations returned to within reference ranges postoperatively. Recovery was uncomplicated and there were no signs of recurrence on follow-up examinations. *(Resource: Vet. Q. 19, 81-84, 1997.)*

Feline colostrum - friend or foe: maternal antibodies in queens and kittens

(Authors: U. Giger and M.L. Casal)—The transfer of immunoglobulins (Ig) by colostrum from the queen to the neonatal kitten not only provides protection from infection, but may also cause serious illness. Neonatal isoerythrolysis may occur when kittens of blood type A or AB receive colostral anti-A alloantibodies from a type B queen. In contrast to other species, Ig concentrations in milk and colostrum did not differ markedly. Gastrointestinal absorption of IgG was limited to the first day of life. The half-lifes of maternally derived IgG and IgA in kit-

tens were shorter than in puppies. In conclusion, milk from another queen may be given as a replacement for colostrum to neonatal kittens. Kittens at risk of neonatal isoerythrolysis must be removed from their type B queen during the first day of life and may safely receive milk or colostrum from a type A queen. *(Resource: J. Reprod. Fertil. 313-316, 1997.)*

Factor X deficiency in a cat

(Authors: J.L. Gookin, M.B. Brooks, J.L. Catalfamo, S.E. Bunch, and K.R. Muñana)—Severe congenital deficiency of factor X was diagnosed in a 3-year-old castrated male domestic shorthair cat with clinical signs of generalized seizures and prolonged bleeding after venipuncture. Heritability of factor X deficiency was suspected because of a prolonged Russell's viper venom time in the dam and reductions in factor X activity in the dam and 1 sibling. To our knowledge, factor X deficiency in cats has not been reported previously. Definitive diagnosis for animals with clinical signs of coagulopathy may require repetition of coagulation screening tests using different assay methods or specific coagulation factor analyses. *(Resource: J.Am.Vet.Med.Assoc. 211, 576-579, 1997.)*

Primary lung tumors in cats: 86 cases (1979-1994)

(Authors: K.A. Hahn and M.F. McEntee)—The objective of this study was to classify histologic type and morphology of primary lung tumors in cats, to describe clinical findings in these cats, and to determine whether clinical findings were associated with histologic type or morphology. Medical records for 86 cats treated between 1979 and 1994 at any of 14 participating veterinary referral hospitals were reviewed. Weight loss, lethargy, and dyspnea were the most common clinical signs. Solitary or multiple pulmonary masses were seen on radiographs from 53 of 79 cats; effusion was seen on radiographs from the other 26. In 45 cats, tumors involved a single lung lobe. Caudal lung lobes were more commonly affected than were cranial lung lobes. Sixty-five cats had metastases. Tumors were classified as bronchial (n = 65), bronchiolar-alveolar (9), or other (12) and as poorly differentiated (59), moderately differentiated (20), or well differentiated (7). Breed, age, sex, weight, clinical signs, duration of clinical signs, and radiographic findings were not associated with histologic type or morphology. The results of this study indicate that to identify possible occult primary lung tumors, thoracic radiography should be performed on cats with clinical signs of long duration, including weight loss, lethargy, and dyspnea. *(Resource: J. Am. Vet. Med. Assoc. 211, 1257-1260, 1997.)*

Management of osteoarthritis in cats

(Author: E.M. Hardie)—Osteoarthritis is a condition most frequently recognized in the geriatric cat but may occur in any cat suffering from joint abnormality or injury. Clinical signs include weight loss, anorexia, depression, urinating outside the litter box, poor grooming, and lameness. Radiographs and synovial fluid analysis are used to distinguish this disease from the various forms of inflammatory arthritis that affect the cat. Management consists mainly of environmental manipulation and medical management. Agents used for the medical management of painful osteoarthritis in cats include aspirin, butorphanol, corticosteroids, and oral nutritional supplements. *(Resource: Vet. Clin. N.A. Small Anim. Pract. 27, 945-953, 1997.)*

Systemic hypertension and its management

(Author: R.A. Henik)—The pathophysiology of hypertension in dogs and cats, the methods available to monitor blood pressure, and the signs and treatment of hypertension are reviewed. Clinical signs of hypertension are usually referable to target organ damage, most notably in ophthalmic, renal, and cardiovascular tissues, which have a rich arteriolar supply. Blood pressure should be measured in any animal with renal disease, hyperthyroidism, hyperadrenocorticism, retinal detachment or hemorrhage,

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hyphema, or echocardiographically determined cardiac hypertrophy. All cats with acquired cardiac murmur should also be evaluated for hypertension. Antihypertensive medication should be administered if the indirect blood pressure in cats is consistently over 170/100 mmHg, or if the indirect blood pressure in dogs is greater than 180/100 mmHg. *(Resource: Vet. Clin. N.A. Small Anim. Pract. 27, 1355-1372, 1997.)*

Persistence and evolution of feline coronavirus in a closed cat-breeding colony

(Authors: A.A. Herrewegh, M. Mahler, H.J. Hedrich, B.L. Haagmans, H.F. Egberink, M.C. Horzinek, P.J. Rottier, and R.J. De Groot)—The persistence and evolution of feline coronavirus (FCoV) were studied in a closed cat-breeding colony known to be endemically infected with serotype 1 FCoV. By utilizing reverse transcriptase polymerase chain reaction (RT-PCR), coronaviral RNA was detected in the feces and/or plasma of 36 of 42 cats (86%) tested. Four of five cats identified as FCoV shedders during the initial survey were found to have viral RNA in the feces when tested 111 days later. Two of these cats were placed in strict isolation to determine whether the presence of viral RNA was due to reinfection or to viral persistence. Feces was monitored every 2 to 4 days; virus shedding continued for up to 7 months in one cat. After 124 days of continuous virus shedding, the other cat was euthanatized to allow identification of sites of viral replication. Viral mRNA was detected only in the ileum, colon, and rectum; FCoV-infected cells were also identified in these tissues by immunohistochemistry. These findings provide the first formal evidence that FCoV causes chronic enteric infections.

To assess FCoV heterogeneity in the breeding facility and to study viral evolution during chronic infection, FCoV quasispecies sampled from individual cats were characterized by RT-PCR amplification of selected regions of the viral genome followed by sequence analysis. Phylogenetic comparison of specific nucleotide sequences from independent European and American isolates indicated that the viruses in the breeding facility are likely to have originated from a single founder infection. Comparative consensus sequence analysis of a more variable region of the FCoV S gene revealed that each cat harbored a distinct viral quasispecies. Additionally, FCoV appeared to be subject to immune selection during chronic infection. This data suggests that endemic FCoV infection is maintained by chronically infected carriers. Almost every cat born to the breeding facility becomes infected, indicating that FCoV is spread very efficiently. *(Resource: Virology 234, 349-363, 1997.*■

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