

Comparative vaccine-specific and other injectable-specific risks of injection-site sarcomas in cats

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Objective—To compare associations between vaccine types and other injectable drugs with development of injection-site sarcomas in cats.

Design—Case-control study.

Animals—181 cats with soft tissue sarcomas (cases), 96 cats with tumors at non-vaccine regions (control group I), and 159 cats with basal cell tumors (control group II).

Procedures—Subjects were prospectively obtained from a large pathology database. Demographic, sarcoma location, basal cell tumor, and vaccine and other injectable history data were documented by use of a questionnaire and used to define case, control, and exposure status. Three control groups were included: cats with sarcomas at non-vaccine sites, cats with basal cell tumors, and a combined group of cats with sarcomas at non-vaccine sites and cats with basal cell tumors. χ^2 tests, marginal homogeneity tests, and exact logistic regression were performed.

Results—In the broad interscapular region, the frequency of administration of long-acting corticosteroid injections (dexamethasone, methylprednisolone, and triamcinolone) was significantly higher in cases than in controls. In the broad rear limb region, case cats were significantly less likely to have received recombinant vaccines than inactivated vaccines; ORs from logistic regression analyses equaled 0.1, with 95% confidence intervals ranging from 0 to 0.4 and 0 to 0.7, depending on control group and time period of exposure used.

Conclusions and Clinical Relevance—This case-control study measuring temporal and spatial exposures efficiently detected associations between administrations of various types of vaccines (recombinant vs inactivated rabies) and other injectable products (ie, long-acting corticosteroids) with sarcoma development without the need to directly measure incidence. These findings nevertheless also indicated that no vaccines were risk free. The study is informative in allowing practitioners to weigh the relative merits and risks of commonly used pharmaceutical products. (*J Am Vet Med Assoc* 2012;241:595–602)

Injection-site sarcomas are malignant tumors that were first reported to arise at vaccination sites in cats in 1991.¹ These tumors continue to manifest in cats in the United States and worldwide.^{2–18} Until recently, sarcoma development at vaccination sites was considered a phenomenon unique to cats,⁶ but reports^{19–24} also exist of similar occurrences in other species, including dogs, ferrets, and a dwarf rabbit.

Studies^{1,7,13,25} aimed at identifying the cause of IJS have implicated FeLV, rabies virus, and FVRCP vaccines as possible causes, but the possibility of other contributing factors or causative agents cannot be ignored. Injectable materials such as long-acting penicillin, lufenuron,

ABBREVIATIONS

CI	Confidence interval
FVRCP	Feline viral rhinotracheitis, calicivirus, and panleukopenia
IJS	Injection-site sarcoma
MLV	Modified-live virus

and methylprednisolone have also been implicated in the etiology of IJS in cats.^{26,27} Nonabsorbable suture material placed at a celiotomy site,²⁸ microchip implantation,²⁹ and a retained surgical sponge in the abdomen³⁰ have also been implicated as possible causes of sarcoma development.

Vaccine adjuvants have been proposed as a component cause in the etiology of sarcoma development by some researchers, although adjuvants alone are not sufficient to cause sarcoma development in cats.²⁶ However, the role of adjuvants in sarcoma causation is controversial. One study¹³ found an association between both adjuvanted (with or without aluminum salts) and nonadjuvanted vaccines and postvaccination sarcomas (recombinant vaccines were unavailable at that time). A later study²⁶ did not find any association between vaccine brand (within antigen class)

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or manufacturer and an elevated or diminished risk of IJS, although recombinant vaccines were rarely used during the study period. One report¹⁵ claimed that aluminum-containing FeLV vaccines induced a greater local inflammatory reaction than nonaluminum-containing FeLV vaccines, whereas rabies vaccination caused local postvaccinal reactions that were approximately twice the size of those caused by FeLV vaccines (no sarcomas formed during the study period). However, 3 epidemiological studies^{13,26,31} failed to provide evidence of increased risk of tumorigenesis for vaccines with or without aluminum. To date, there has been no published evidence indicating that commercial nonadjuvanted vaccines, which have become more available in recent years, are safer, with respect to tumorigenesis risk, than adjuvanted vaccines.³²

An improved understanding of the extrinsic risk factors associated with the development of IJS would enlighten clinical researchers regarding the risks for these sarcomas and could perhaps lead to practices that decrease the risk of IJS formation. As new products (eg, recombinant vaccines), which are purportedly safer than their predecessors, are gaining market share, epidemiological studies (ie, case-control studies) are the only realistic tool for corroborating such claims. With no feasible method to monitor incidence of IJS formation over time, and because novel vaccines have been introduced relatively recently into the market, their impact on the incidence of tumor formation is unknown. Studies are needed to examine the different vaccines and injectable drugs that are currently available to see whether there are significant differences in their risk for IJS development. Such information can guide veterinarians in their choice of products to offer clients as well as direct commercial manufacturers toward developing safer products. Vaccination and other injection practices have also changed after the Vaccine-Associated Feline Sarcoma Task Force recommendations³³ came into effect in 1998.

The purpose of the study reported here was to compare associations between vaccine types and other injectable drugs with the subsequent development of IJS in cats. This represents a refinement of previously published^{13,26} work by defining exposure to vaccines or other injectable drugs and definition of cases without requiring assumptions that potentially lead to misclassification, while allowing the estimation of the relative incidence of sarcoma formation among specific classes of vaccines and injectable drugs. Specifically, we compared recombinant to inactivated and MLV to inactivated vaccines as well as the use of other injectable products.

Materials and Methods

The present study collected vaccine, injection, and tumor data using temporal and spatial information provided by veterinarians. The approaches included using prospective (vs retrospective) patient (case and control) enrollment shortly after the pathological diagnosis of tumors; vaccine history was dependent upon the accuracy of the medical record (some owners may not have disclosed vaccines administered elsewhere)

and correct recall of unrecorded injection sites. Cases of the following types of soft tissue sarcoma were defined for inclusion: fibrosarcoma, malignant fibrous histiocytoma, myxofibrosarcoma, poorly differentiated sarcoma, spindle cell sarcoma, round cell sarcoma, myofibroblastic sarcoma, rhabdomyosarcoma, leiomyosarcoma, unspecified sarcoma, and undifferentiated sarcoma. Other tumors that have been associated with this phenomenon were also included, such as osteosarcoma, chondrosarcoma, and lymphoma. A topographic coordinate map with grids of ventral and dorsal views of a cat was used to better guide veterinarians in marking with accuracy the sites of vaccination and sarcoma development.

The case and control definitions were modified from those used in previous studies.^{5,11,34-36} For our study design, the traditional case definitions were modified into 2 new categories. All cases were restricted to sarcomas in cats arising no less than 30 days to no greater than 3 years after receiving a documented vaccine or injection within 1 grid (approx 7 cm/axis) of any side of the tumor site. Interscapular cases were defined as soft tissue sarcomas in cats that arose at the broad interscapular region, and broad rear limb region cases were defined as soft tissue sarcomas in cats that arose at the left hind limb region, right hind limb region, and gluteal or lumbar region.

A prospective case-control study design was used to select cases and controls from a pathology registry.^a Biannual computer searches of feline necropsy and biopsy case records were begun in 2005 to periodically identify feline sarcoma cases and controls reported from January 1, 2005, through December 31, 2008. Case and control information accompanying the biopsy samples from each accession were retrieved and included age, breed, sex, and referring veterinarian contact information.

Materials pertinent to this detailed data acquisition were then submitted to the referring veterinarian to collect information about sarcoma formation and the 3-year vaccination and injection history antecedent to the sarcoma development. These included a survey with detailed vaccination and injection and tumor history questions, a topographic coordinate map with grids of ventral and dorsal views of a cat, and a list of commercially available vaccines and other injectable products.

Controls from 2 clinical populations were defined for use in this design and were selected from the same calendar period as case accrual. The first control population (control I) consisted of cats with histologically confirmed soft tissue sarcomas at sites not commonly used for vaccination (including the head, ears, digits, and ventral aspect of the abdomen) under the assumption that these tumors were not caused by vaccination. The second control population (control II) consisted of cats in which a diagnosis of basal cell tumor was made on the basis of a histologic examination of the biopsy specimens. Cats with basal cell tumors were used as controls because the diagnosis of these tumors required a similar process of owner-initiated medical consultations, anesthesia, surgery, and submission of biopsy samples to that required for diagnosis of IJS. This re-

duced the potential for selection bias during control selection.²⁶ In addition, there is no published evidence indicating that basal cell tumors are associated with SC or IM administration of any vaccine or other chemical substance. A third control population (control III) was formed by combining control groups I and II.

Statistical analysis—Information from cases and controls was coded and entered into a commercially available software program.^b In a case-control analysis, the χ^2 test of homogeneity was used to evaluate 3 vaccine contrasts: the frequency of use of inactivated versus recombinant rabies, MLV versus inactivated FVRCP, and inactivated versus recombinant FeLV vaccines. For each contrast, the number of vaccines of each type administered within 1, 2, and 3 years was compared between cases and each of the 3 control groups for the 2 sites. In addition, case and control cats were separately assessed by means of marginal homogeneity tests to determine whether they were more or less likely to receive one vaccine type within a contrast than another type under the null hypothesis that either type was equally likely. The distribution of administered injectable non-vaccine products in cases and controls with basal cell tumors were compared with exact 1-tailed χ^2 tests of homogeneity (under the assumption that the injectable drugs studied were not protective against carcinogenesis). Results are presented separately for cats with sarcomas in the broad interscapular (including scapulo-humeral regions) and rear limb regions.

Exact logistic regression for small sample sizes was then used to model the risk of developing a vaccine-associated sarcoma as a function of the measured putative risk factors. For different models, these factors potentially included (for the most recent vaccines administered prior to tumor diagnosis) antigen type, recombinant versus inactivated, adjuvanted versus MLV, and the cumulative number of reported vaccines by type administered at the tumor site in the prior 1, 2, and 3 years. All models were adjusted for age. Results are presented as OR, 95% CI, and *P* values. Values of *P* ≤ 0.05 were considered significant. Calculations were performed with a commercially available software program.^c

Results

The response proportion for the surveys sent to veterinarians was 447 of 1,502 (30%). The mean ± SD age of cats with sarcomas in the broad interscapular region (*n* = 90) was 10.7 ± 3.2 years (median, 11.0 years [range, 2 to 17 years]), and of cats with sarcomas in the broad rear limb region (91) was 9.8 ± 3.9 years (median, 10.0 years [range, 4 to 15 years]). The mean age of cats in control I (tumors at non-vaccine regions; *n* = 96) was 10.5 ± 3.6 years (median, 10.2 [range, 2 to 20 years]), in control II (basal cell tumors; 159) was 11.1 ± 3.9 years (median, 11.0 years [range, 2 to 20 years]), and in control III (all control cats; 255) was 10.9 ± 3.7 years (median, 11.0 years [range, 2 to 20 years]). Among the 447 questionnaires that were returned by veterinarians, 243 (54.4%) had enough information on vaccination and injection histories to analyze, and 204 (45.6%) did not contain any vaccination and injection

information. Among 90 interscapular cases, 47 cases had vaccine and injection information provided, and among 91 rear limb region cases, 54 cases had vaccine and injection information provided. Among 96 controls with sarcomas at non-vaccine sites, 44 controls had vaccine and injection information provided, and among 159 basal cell tumor controls, 96 controls had vaccine and injection information provided.

χ^2 analyses of broad interscapular region cases (vaccines) versus controls—There were no significant differences in the frequency of use of inactivated versus recombinant rabies vaccines administered in the interscapular region for years 1 through 3 between cases with tumors in this region and using all control groups, although the total number of patients with complete rabies vaccine histories for this region was small. Four cats with sarcomas in this region were documented to have had inactivated rabies vaccines, and 3 cats were documented to have had recombinant rabies vaccines. Two cats received combined recombinant rabies vaccines that contained MLV FVRCP fractions, and 1 cat that received the recombinant rabies vaccine was also administered a separate MLV FVRCP vaccine at the same time on the same day; no other vaccines were documented to have been administered at this region in the 3 years prior to tumor development. Neither case cats nor control cats were more likely to have been given inactivated versus recombinant rabies vaccines within the 2 years preceding sarcoma or basal cell tumor formation, although cats with basal cell tumors were more likely to have received inactivated than recombinant vaccines over the prior 3 years (*P* = 0.020), perhaps reflecting a growing popularity of recombinant vaccines over this time period. There were no significant differences in the frequency of use of MLV versus inactivated FVRCP vaccines administered in this region over all times between cases and all control groups. Sarcomas were observed in this region following the use of both MLV and inactivated FVRCP vaccines. There were no significant differences in the frequency of use of inactivated versus recombinant FeLV vaccines administered in the interscapular region for years 1 through 3 between cases and all control groups.

Individual broad interscapular region case description—Among the 22 cats with sarcomas that were administered their most recent MLV FVRCP vaccines within 3 years prior to tumor diagnosis, 13 received only MLV FVRCP vaccines (4 contained live *Chlamydia* spp; 1 contained inactivated *Chlamydia* spp) within 1 (46%), 2 (23%), and 3 (31%) years, and 6 received MLV FVRCP vaccines in conjunction with at least 1 other inactivated vaccine component (*Chlamydia* spp, FeLV, or rabies) or inactivated vaccine (FVRCP, FeLV, or rabies) within 1 (50%) or 2 (50%) years. Three cats receiving MLV FVRCP (with or without live *Chlamydia* spp) also received recombinant rabies vaccines on the same day ranging from 0.5 to 2 years; no other vaccines were documented to have been administered at this region to these 3 cats in the 3 years prior to tumor diagnosis.

Marginal homogeneity test analyses of broad interscapular region cases and controls—Case cats

were more likely to have received MLV versus inactivated FVRCP vaccines prior to tumor formation ($P = 0.099$ for year 1, $P = 0.006$ for year 2, and $P = 0.001$ for year 3). Control II cats were also more likely to have received MLV versus inactivated FVRCP vaccines ($P = 0.023$ for year 1, $P = 0.062$ for year 2, and $P = 0.059$ for year 3; Table 1). Similarly, control III cats were more likely to have been given MLV versus inactivated FVRCP vaccines ($P = 0.006$ for year 1, $P = 0.021$ for year 2, and $P = 0.022$ for year 3).

No cats received recombinant FeLV vaccines in this region; there were case cats that received inactivated FeLV vaccines, although the number of cats was small ($n = 3$). All control cats were more likely to have received inactivated versus recombinant FeLV vaccines at this site in year 1 through 3 ($P = 0.003$, $P = 0.001$, and $P < 0.001$, respectively; Table 1).

Exact logistic regression analyses of broad interscapular region cases versus controls—Exact logistic regression analyses (adjusted for age) contrasting the use of the 3 major vaccine types (inactivated, re-

combinant, and MLV) across all antigen classes in the interscapular region between cases with tumors located there and controls did not reveal any compelling or significant differences in the use of the different vaccine types regardless of whether they were administered within 1, 2, or 3 years prior to tumor diagnosis (Table 2).

Broad interscapular region (non-vaccine injectable drugs)—From January 2005 to December 2008, 15 case cats received other injectable drugs at broad interscapular region. The age of the case cats ranged from 7 to 16 years. Among the 15 cases with sarcomas in this region, only 7 cases were administered other injectable drugs but no vaccines. Among the 7 cases, the injectable drugs included dexamethasone at the tumor site 1 month before sarcoma diagnosis; lactated Ringer's solution 6 months prior to sarcoma diagnosis; 5 depomedrol injections ranging from 5 months to 4 years before sarcoma diagnosis; cephalixin and vitamin B-complex 1.3 years prior to sarcoma diagnosis; triamcinolone 8 months prior to sarcoma diagnosis; depomedrol

Table 1—Results of marginal homogeneity test (P values) comparing 1, 2, or 3 years of cumulative vaccination with inactivated FVRCP versus MLV vaccines, inactivated rabies versus recombinant rabies vaccines, and inactivated FeLV versus recombinant FeLV vaccines with development of sarcomas in the broad interscapular and rear limb regions (case cats), compared with development of sarcomas in non-vaccine regions (control group I cats), basal cell tumors (control group II cats), and both (sarcomas in non-vaccine regions and basal cell tumors; control group III cats).

Region	Year	Inactivated FVRCP versus MLV FVRCP				Inactivated rabies versus recombinant rabies				Inactivated FeLV versus recombinant FeLV			
		Cases	Controls			Cases	Controls			Cases	Controls		
			I	II	III		I	II	III		I	II	III
Interscapular	1	0.099	0.16	0.023	0.006	0.69	1	0.53	0.53	0.22	0.099	0.02	0.003
	2	0.006	0.25	0.062	0.021	0.80	1	0.10	0.24	0.099	0.25	0.004	0.001
	3	0.001	0.26	0.059	0.022	0.80	1	0.02	0.052	0.25	0.13	0.001	< 0.001
Rear limb	1	0.73	0.39	0.45	0.17	0.001	1	0.84	1	0.016	0.016	< 0.001	< 0.001
	2	0.84	0.14	0.25	0.043	0.001	0.39	0.73	0.38	0.002	0.001	< 0.001	< 0.001
	3	0.33	0.13	0.32	0.056	< 0.001	0.22	0.39	0.14	0.001	< 0.001	< 0.001	< 0.001

Table 2—Results of exact logistic regression (ORs and 95% CIs) comparing development of sarcomas in the broad interscapular region (case cats) with development of sarcomas in non-vaccine regions (control group I cats), basal cell tumors (control group II cats), and sarcomas in non-vaccine regions or basal cell tumors (control group III cats) within 1, 2, or 3 years after vaccination with inactivated, recombinant, and MLV vaccines; all analyses were adjusted for age of the cat.

Year	Vaccine type	No. of case cats	Control group I			Control group II			Control group III		
			No. of cats	OR (95% CI)	P value	No. of cats	OR (95% CI)	P value	No. of cats	OR (95% CI)	P value
1	Inactivated	6	7	0.8 (0.3–2.1)	0.82	12	1.0 (0.4–2.23)	1.00	19	0.9 (0.4–2.0)	1.00
	Recombinant	1	2	0.5 (0.0–7.2)	0.63	1	1.9 (0.0–74.1)	1.00	3	0.8 (0.0–8.0)	0.86
	MLV	9	8	1.0 (0.3–3.5)	1.00	17	1.2 (0.4–3.4)	0.88	25	1.1 (0.4–2.9)	0.94
2	Inactivated	8	12	0.7 (0.3–1.5)	0.48	18	0.8 (0.5–1.3)	0.54	30	0.8 (0.5–1.3)	0.50
	Recombinant	3	2	0.9 (0.1–6.0)	1.00	2	2.1 (0.1–30.9)	0.79	4	1.2 (0.1–6.4)	1.00
	MLV	17	12	1.5 (0.7–3.3)	0.40	28	1.6 (0.8–3.2)	0.21	40	1.5 (0.8–2.9)	0.21
3	Inactivated	9	15	0.8 (0.4–1.4)	0.43	22	0.8 (0.5–1.2)	0.39	27	0.8 (0.5–1.2)	0.38
	Recombinant	3	3	0.6 (0.1–3.8)	0.89	2	2.1 (0.1–30.9)	0.79	5	1.0 (0.1–5.1)	1.00
	MLV	22	13	1.6 (0.9–3.2)	0.14	33	1.6 (0.9–2.8)	0.08	46	1.6 (1.0–2.7)	0.06

8 months prior to sarcoma diagnosis; and penicillin G benzathine 8.5 months prior to sarcoma diagnosis.

Eight of 15 cases in this region were administered other injectable drugs in conjunction with vaccines at the tumor site. The injectable products given to these case cats were enrofloxacin, meloxicam, lincocin, depomedrol, penicillin G benzathine, vitamin B complex, lactated Ringer's solution, prednisone, butorphanol, cephalixin, buprenorphine, amoxicillin, xylazine, acepromazine, saline (0.9% NaCl) solution, ketamine and praziquantel, and a microchip. The case cat had a microchip implanted at the interscapular region 1.2 years prior to sarcoma development at the site and was also administered an inactivated FVRCP vaccine 2.4 years before the tumor diagnosis.

The frequency of administration of long-acting corticosteroid injections (dexamethasone, methylprednisolone, and triamcinolone) was significantly ($P = 0.017$) higher in case cats than the controls. Two case cats received methylprednisolone injections without vaccines at the sarcoma site. One case cat received 5 methylprednisolone injections administered on 141, 538, 816, 975, and 1,400 days prior to the sarcoma diagnosis. The other case cat was administered methylprednisolone 237 days prior to the sarcoma diagnosis. One case cat received a dexamethasone injection 1 month prior to the sarcoma diagnosis, and 1 case cat received a triamcinolone injection 248 days before the sarcoma diagnosis; in neither case was a vaccine administered at the tumor site.

χ^2 analyses of broad rear limb region cases versus controls—There was statistical evidence of a higher frequency of use of inactivated rabies vaccines, compared with recombinant rabies vaccines, at the broad rear limb region. In year 1, cases were more likely to have received inactivated rabies vaccines ($n = 11$) than recombinant rabies vaccines (0) at this region than all controls ($P = 0.003$); in year 2, cases were more likely to have received inactivated rabies vaccines (14) than recombinant rabies vaccines (0) than all controls ($P = 0.003$); in year 3, cases were more likely to have received inactivated rabies vaccines (19) than recombinant rabies vaccines (1) than all controls ($P = 0.011$). There were no significant differences in the frequency of use of MLV versus inactivated FVRCP vaccines administered in this region over all times and comparison groups. Sarcomas were observed in this region following the use of both MLV and inactivated FVRCP vaccines. There were no significant differences in the frequency of use of inactivated versus recombinant FeLV vaccines administered in this region over all times and comparison groups. No case cats with vaccine histories received recombinant FeLV vaccine in this region; however, there were 5 case cats that received inactivated FeLV vaccines.

Individual broad rear limb region case description—The cat receiving the recombinant rabies vaccine also had an inactivated rabies vaccine administered at the same region, although it was given almost 5 years before tumor development and 2.67 years before the recombinant rabies vaccine. Seven case cats received MLV FVRCP vaccines, but only 1 cat received this vaccine (2.4 years prior to sarcoma diagnosis) without receiving any others at the same location. Four of the 7 cats also received inactivated FeLV, inactivated rabies vaccine, or both together with the MLV FVRCP vaccine within 1 year prior to tumor

development; 1 cat received a MLV FVRCP vaccine within 1 year prior to tumor development, but also received inactivated rabies and FeLV vaccines at the same site 1 and 2 years earlier, respectively; and 1 cat received an inactivated FeLV vaccine 2.3 years prior to tumor recognition.

Marginal homogeneity test analyses of broad rear limb region cases and controls—All case cats were more likely to have received inactivated versus recombinant rabies vaccines at the broad rear limb region in year 1 through 3 ($P = 0.001$, $P = 0.001$, and $P < 0.001$, respectively; Table 1). In contrast, the marginal homogeneity test results for controls showed no significant difference between use of inactivated versus recombinant rabies vaccine in the 3 control groups. Interestingly, case cats were not significantly more likely to have received either FVRCP vaccine, although control III (the combination of controls I and II) cats were more likely to have received MLV FVRCP vaccine in year 2 ($P = 0.043$) and year 3 ($P = 0.056$). Although case cats were more likely to have received inactivated versus recombinant FeLV vaccines prior to tumor formation ($P = 0.016$ for year 1, $P = 0.001$ for year 2, and $P < 0.001$ for year 3), all control cats were also more likely to have received inactivated versus recombinant FeLV vaccines at this site in year 1 through 3 ($P < 0.016$ to $P < 0.001$).

Exact logistic regression analyses of broad rear limb region cases versus controls—Exact logistic regression analyses (adjusted for age) results comparing cases with tumors located in the broad rear limb region and the 3 control groups are presented (Table 3). There was no compelling evidence of differences in the use of the inactivated and MLV vaccine types regardless of whether they were administered within 1, 2, or 3 years prior to tumor diagnosis. Conversely, there was evidence of a significantly lower frequency of use of recombinant rabies vaccines in case cats than controls. Using cats with non-vaccine site sarcomas as controls, in years 1, 2, and 3, the ORs were 0.1 (95% CI, 0.0 to 0.7; $P = 0.014$), 0.1 (95% CI, 0.0 to 0.4; $P = 0.001$), and 0.1 (95% CI, 0.0 to 0.6; $P = 0.005$), respectively. Using cats with basal cell tumors as controls, the ORs in years 1, 2, and 3 were 0.1 (95% CI, 0.0 to 0.4; $P = 0.001$), 0.1 (95% CI, 0.0 to 0.4; $P = 0.001$), and 0.1 (95% CI, 0.0 to 0.6; $P = 0.001$), respectively. Using the combined control group (control III), the ORs for years 1, 2, and 3 were 0.1 (95% CI, 0.0 to 0.4; $P = 0.001$), 0.1 (95% CI, 0.0 to 0.4; $P < 0.001$), and 0.1 (95% CI, 0.0 to 0.5; $P = 0.001$), respectively.

Broad rear limb region cases (non-vaccine injectable drugs)—From January 2005 to December 2008, 8 case cats received other injectable drugs at the broad rear limb site. The age of the case cats in this region ranged from 7 to 15 years. Among the 8 cases, only 4 cases received other injectable drugs with no vaccines administered. Among the 4 cases, the injections included butorphanol, medetomidine, and ketamine 2 months prior to tumor diagnosis, and enrofloxacin and butorphanol 6.5 months prior to sarcoma diagnosis; prednisolone 1 month prior and phenoxymethyl penicillin 1.1 years prior to sarcoma diagnosis; penicillin G benzathine injection 5 months before tumor diagnosis; and praziquantel 4 months prior to sarcoma diagnosis.

Four of 8 case cats were administered other injectable drugs and vaccines at the tumor site. The injectable drugs included acepromazine, saline solution, triamcinolone,

Table 3— Results of exact logistic regression (ORs and 95% CIs) comparing development of sarcomas in the broad rear limb regions (right hind limb, left hind limb, gluteal region, and lumbar region; case cats) with development of sarcomas in non-vaccine regions (control group I cats), basal cell tumors (control group II cats), and sarcomas in non-vaccine regions or basal cell tumors (control group III cats) within 1, 2, or 3 years after vaccination with inactivated, recombinant, and MLV vaccines; all analyses were adjusted for age of the cat.

Year	Vaccine type	No. of case cats	Control group I			Control group II			Control group III		
			No. of cats	OR (95% CI)	P value	No. of cats	OR (95% CI)	P value	No. of cats	OR (95% CI)	P value
1	Inactivated	15	10	1.2 (0.6–2.3)	0.74	27	1.0 (0.6–1.7)	1.00	37	1.0 (0.6–1.7)	0.93
	Recombinant	0	6	0.1 (0.0–0.7)	0.014	14	0.1 (0.0–0.4)	0.001	20	0.1 (0.0–0.4)	0.001
	MLV	5	4	0.9 (0.2–4.4)	1.00	10	0.8 (0.2–2.9)	0.98	14	0.8 (0.2–2.0)	0.96
2	Inactivated	20	21	0.8 (0.6–1.3)	0.43	38	1.0 (0.7–1.4)	1.00	59	0.9 (0.7–1.3)	0.78
	Recombinant	0	8	0.1 (0.0–0.4)	0.001	19	0.1 (0.0–0.4)	0.001	27	0.1 (0.0–0.4)	< 0.001
	MLV	5	8	0.6 (0.3–1.4)	0.30	14	0.8 (0.3–1.8)	0.72	22	0.7 (0.3–1.4)	0.42
3	Inactivated	25	25	0.9 (0.6–1.2)	0.49	48	0.9 (0.7–1.3)	0.79	73	0.9 (0.7–1.2)	0.65
	Recombinant	1	8	0.1 (0.0–0.6)	0.005	20	0.1 (0.0–0.6)	0.001	28	0.1 (0.0–0.5)	0.001
	MLV	7	8	0.9 (0.5–1.6)	0.74	15	1.0 (0.5–1.8)	1.00	23	0.9 (0.5–1.6)	0.96

amoxicillin, penicillin G benzathine, lufenuron, buprenorphine, and ketoprofen. The cat receiving the lufenuron injection 1 year prior to sarcoma diagnosis at the site was also administered inactivated rabies vaccine 1.6 years before the tumor development.

Discussion

The present study compared risks of IJS in cats arising from vaccines and other injectable drugs by temporally and spatially refining definitions of cases and vaccine and injection exposures. Obtaining detailed vaccine and injection histories in time and space (using a body map grid) obviated the need for highly precise location information that may not have been available in the medical record and allowed for more causally plausible case identification (eg, attributing a tumor to a vaccine given proximally and conserving a 30-day induction period prior to tumor identification), and validation of the control group I (ie, cats with sarcomas where vaccines were verified to have not been given in the time period studied).

In the present study, of the 181 sarcomas that arose in injection sites, only 101 (56%) of the cats reportedly received vaccines, and far fewer (23) reportedly received other injections. In the broad interscapular region, the absence of significant differences in risk between inactivated and MLV vaccines suggests that either vaccine is capable of sarcoma induction, although FVRCP vaccines were often not given alone. The finding of a greater frequency of MLV FVRCP vaccines, compared with inactivated FVRCP vaccines administered to cases in this region, must be interpreted in light of the similar finding among controls, reflecting a preference for use of MLV vaccines rather than a higher risk. The use of both rabies and FeLV vaccines in this region was too low to make meaningful inferences about comparative risk. Although 3 cats with sarcomas in this region received a recombinant rabies vaccine within 2 years prior to tumor diagnosis, they also concomitantly received MLV FVRCP vaccines at the same location.

In the broad rear limb region, a sufficient number of cats received recombinant and inactivated (predominantly rabies) vaccines in the present study to discern a significant difference in vaccine type distribution between case cats and controls. Results consistently showed that case cats were significantly less likely to have received recombinant vaccines than inactivated vaccines; ORs from logistic regression analyses equaled 0.1, with 95% CIs ranging from 0 to 0.4 and 0 to 0.7, depending on control group and time period used. The single cat with a sarcoma in this region that received a recombinant rabies vaccine had also received an inactivated rabies vaccine at the site approximately 2.7 years earlier, making a conclusive statement about which vaccine may have led to this cat's tumor impossible. Although MLV FVRCP vaccines were used more frequently than inactivated FVRCP vaccines in controls, no significant differences in the 2 types were observed in case cats, supporting earlier findings that inactivated FVRCP vaccines may carry more risk, although neither is risk free.^{25,37–41}

Day et al,³² in a comparative study of vaccine types (nonadjuvanted, lipid adjuvanted, aluminum adjuvanted), found that nonadjuvanted vaccines led to less tissue inflammation than adjuvanted vaccines. Although none of the 45 cats in their study³² developed sarcomas, they postulated that “if tissue inflammation can act as a potential trigger for neoplastic transformation of mesenchymal tissue, then this effect may have relevance to the risk of different vaccines inducing sarcoma. Epidemiological field studies might help to confirm whether this is true.” Using the temporal and spatial approaches as defined in the present study, this study provides results to support differential (although not zero) risk of sarcoma development by vaccine types.

Although the number of sarcoma cases associated with the injection of recombinant vaccines in our study was relatively small, these findings nevertheless also indicate that recombinant vaccines are not risk free. Similar IJS in cats purportedly caused by vectored (recombinant) vaccines were also reported in the United Kingdom for years 2008 and 2009.^{40,41}

Although vaccines remain the predominant cause of IJS, the results of the present study corroborate earlier findings that implicated other injectable drugs as potential initiators of tumors. Examples include reports^{26,27} of association between long-acting penicillin, lufenuron, and long-acting corticosteroids and sarcoma development in cats. The observation of sarcomas associated with microchips has been reported in dogs²⁴ and cats.²⁹ Careful evaluation of those reports suggests that immunization likely occurred in those sites also. One author (LDM) has noted this association in several cat sarcomas. The incidence in relation to the more than 25 million microchips sold by 1 company alone⁴² is extremely low.

This is the second epidemiological study that has identified long-acting corticosteroids such as dexamethasone, methylprednisolone, and triamcinolone as potentially involved in causing sarcoma at injection sites.²⁶ Despite the small number of case cats that were administered long-acting corticosteroids, no vaccines were documented to have been given to these cats at any time, further strengthening the claim that other injectable drugs may also be responsible for initiating sarcoma development in cats.

The association between sarcoma development and other injectable drugs is difficult to study because IJS are rare among cats not receiving vaccines, and most cats in American households that use veterinary care receive vaccines at some time in their lives. If vaccines are given at a young age and if their potential for tumorigenesis can last many years, it becomes formidably difficult to disentangle the potential effects of vaccines from the potential effects of other products in an observational study. Conversely, more desirable experimental studies would require enormous sample sizes because of the rarity of the outcome.

The most pragmatic way to evaluate non-vaccine injectable drugs is to focus on cats not having a history of vaccination at the tumor site. However, because this study relied on historic data, it is both possible that cats received vaccines earlier than 3 years at the site and that they received vaccines from different veterinarians that went unreported. In addition, veterinarians were expected to provide information from the medical record but may have depended on recall, which is subject to error, in the absence of recorded injection-site information. If errors in recall of vaccinations or other injectable products were nondifferential with respect to presence or absence of proximity to tumor site, then the impact of injections may be underestimated.

Although the present study provides the first epidemiological evidence for differential risk of vaccine brands, there are also limitations that must be acknowledged. The response proportion of the survey was lower than desired, leading to a lower than anticipated study size. In addition, veterinarians did not always provide a 3-year vaccination history (which could have arisen if owners sought some or all vaccines elsewhere); such missing data would have an adverse impact on statistical power, although a power calculation was not done for this study. Although there is no evidence that the data were not missing at random, differential responses on the basis of vaccination history (eg, MLV, inactivated, or recombinant vaccine usage) or lack thereof could affect study findings. Another study challenge was that multiple vaccines were often concomitantly given at the same site, making it impossible to isolate individual-level causal effects. The problem of vacci-

nation with multiple vaccines is virtually insurmountable, however, because a prospective experimental study would be both unrealistic given the rarity of the disease and ethically problematic.

The initial evidence provided by the present study that the recombinant class of vaccines may potentially be less likely to induce sarcomas than inactivated vaccines must be tempered by the finding that no vaccine can be assumed to be risk free in a susceptible individual. Further study is indicated to assess the relationship, if any, of the number of vaccine-associated sarcoma diagnoses and trends in vaccine class preference over time. Although the number of affected cases was small, the results of the present study provide corroborating evidence that long-acting corticosteroids remain a potential cause of IJS in cats. However, the findings of this study should be reassuring in that the use of medically indicated products does not appear to be associated with substantial morbidity of sarcomas, and their propensity to cause tumors appears to be small.

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- a. Animal Reference Pathology, ARUP Laboratories Inc, Salt Lake City, Utah.
 - b. Microsoft Excel, Microsoft Corp, Redmond, Wash.
 - c. LogXact-8 and StatXact-8, Cytel Software Corp, Cambridge, Mass.
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From this month's AJVR

Simplified procedure for the estimation of glomerular filtration rate following intravenous administration of iodixanol in cats

Rieko Katayama et al

Objective—To compare the use of a single-sample method involving IV administration of iodixanol with a multisample method involving inulin for the estimation of glomerular filtration rate (GFR) in cats.

Animals—24 cats, including 15 healthy cats and 9 cats with naturally occurring renal diseases.

Procedures—Each cat was coadministered iodixanol (a nonionic contrast medium; dose providing 40 mg of I/kg) and inulin (50 mg/kg), IV, and blood samples were collected 60, 90, and 120 minutes later. Serum iodixanol and inulin concentrations were determined by high-performance liquid chromatography and colorimetry, respectively. Serum urea nitrogen and creatinine concentrations were also measured.

Results—Analysis of the data from healthy cats and cats with naturally occurring renal diseases revealed an excellent correlation between GFR values estimated by the multisample and single-sample methods with iodixanol. Likewise, GFR values estimated from the single-sample method with iodixanol were closely correlated with those calculated from the multisample method with inulin.

Conclusions and Clinical Relevance—For estimation of GFR in cats, use of a single-sample method with iodixanol, instead of a multisample procedure, may be an expedient tool in both clinical and research settings because of its benefits to patient well-being as a result of reduced stress associated with blood sample collection. (*Am J Vet Res* 2012;73:1344–1349)



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