

Multicenter case-control study of risk factors associated with development of vaccine-associated sarcomas in cats

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Abstract

Objective—To determine whether particular vaccine brands, other injectable medications, customary vaccination practices, or various host factors were associated with the formation of vaccine-associated sarcomas in cats.

Design—Prospective multicenter case-control study.

Animals—Cats in the United States and Canada with soft tissue sarcomas or basal cell tumors.

Procedure—Veterinarians submitting biopsy specimens from cats with a confirmed diagnosis of soft tissue sarcoma or basal cell tumor were contacted for patient medical history. Time window statistical analyses were used in conjunction with various assumptions about case definitions.

Results—No single vaccine brand or manufacturer within antigen class was found to be associated with sarcoma formation. Factors related to vaccine administration were also not associated with sarcoma development, with the possible exception of vaccine temperature prior to injection. Two injectable medications (long-acting penicillin and methyl prednisolone acetate) were administered to case cats more frequently than to control cats.

Conclusions and Clinical Relevance—Findings do not support the hypotheses that specific brands or types of vaccine within antigen class, vaccine practices such as reuse of syringes, concomitant viral infection, history of trauma, or residence either increase or decrease the risk of vaccine-associated sarcoma formation in cats. There was evidence to suggest that certain long-acting injectable medications may also be associated with sarcoma formation. (J Am Vet Med Assoc 2003;223:1283–1292)

More than a decade since their first published description¹, feline vaccine-associated sarcomas (FVASs) remain an emerging epidemic problem in the United States. Even conservative estimates of disease incidence lead to projections that thousands of new cases will develop each year. Although research efforts are underway to determine the mechanism of oncogenesis and roles of the inflammatory and immune responses following vaccination, avoidance of vaccination altogether currently remains the only realistic way to prevent the development of FVASs.

That vaccines played a causal role in the increase in the number of soft tissue sarcomas identified in cats during the 1990s is no longer in question. An abundance of evidence

consistently points to a heightened risk among cats receiving FeLV, rabies virus, and possibly other vaccines.²⁻⁶ Nevertheless, avoiding using these vaccines can be even more dangerous and just as counterproductive. The diseases they are designed to prevent are not rare or without veterinary or human public health importance, and the agents that cause these diseases are themselves capable of resulting in epidemics. As veterinarians and cat owners strive toward an uneasy juxtaposition of the relative costs and benefits of vaccination, an improved understanding of exogenous (extrinsic) risk factors associated with the development of FVASs becomes even more vitally important.

A number of questions remain about putative extrinsic risk factors for FVASs. Initial suspicion focused on the possible etiologic role of aluminum salts, which are included in some vaccines as adjuvants and have been found in sarcomas from some patients.² Since 1985 when inactivated rabies and FeLV vaccines became commercially available, aluminum has frequently been incorporated in feline vaccines to enhance the host immune response,⁷ and aluminum-containing FeLV vaccines have been shown to induce a greater local inflammatory reaction than nonaluminum-containing FeLV vaccines, although the same is not true of rabies vaccines.⁷ However, 2 large epidemiologic studies^{4,5} failed to provide any evidence that aluminum-containing vaccines are associated with a higher risk of tumorigenesis than are nonaluminum-containing vaccines. Thus, it is not clear whether commercial nonadjuvanted vaccines, which have become more available in recent years, are safer to use than adjuvanted ones while still as effective in immunizing patients.

The issue of the safety of inactivated and adjuvanted vaccines was again raised in a study⁶ in which an unusually high incidence of sarcomas was identified in cats receiving a single manufacturer's feline viral rhinotracheitis-calicivirus-panleukopenia (FVRCP) vaccine. Earlier published epidemiologic studies did not statistically implicate any FVRCP vaccines in sarcoma development, possibly reflecting the relative lack of use of killed-virus FVRCP vaccines in the study areas. Nevertheless, findings of the more recent study⁶ underscore the importance of distinguishing the risks, if any, of the various commercially available vaccines. Identifying such risks, if they do exist, would be helpful in guiding manufacturers and veterinarians attempting to maximize product safety.

Other issues germane to the routine use of biologics in cats remain epidemiologically important. After accounting for known risk factors, such as age and vaccination history, all cats have been assumed to share essentially the same underlying risk of developing FVASs, with any observed variability attributable to random error. However, anecdotal reports^{6,7} of practices with unusually high or low incidence rates suggest that there may be other uncontrolled sources of variability and, hence, other determinants of risk. One source of such variability is genetic propensity, although there is no a priori reason to suspect a genetic propensity in unrelated cats treated at individual practices. There may, however, be specific vaccination policies within practices that potentially modify the risk of FVAS development. Such policies could include the frequency of vaccine administration, whether different vaccines are combined in a single syringe, and whether syringes are reused following chemical or steam sterilization. The frequency of routine vaccine administration could be important if the number of vaccines a cat receives over its lifetime somehow sensitizes the cat to the point that a final vaccine leads to tumorigenesis.

The present study was designed to prospectively examine potential risk factors that have been proposed to be associated with FVAS development ever since the association between vaccines and FVASs was documented. The goal of this study was to better understand whether there are additional practice strategies beyond simply avoiding vaccination that can be used to prevent FVASs or minimize the risk that they will develop. Specifically, the purpose of the study reported here was to determine whether specific vaccine brands, use of other injectable medications, specific vaccination practices, or various host factors were associated with the incidence of FVASs.

Materials and Methods

Case selection—The study was designed as a multicenter prospective case-control study. Participating study centers included the Central Laboratory for Veterinarians, Langley, BC, Canada; the University of California, Davis; IDEXX Veterinary Services Inc, Davis, Calif; the University of Pennsylvania, Philadelphia; Texas A&M University, College Station; Animal Reference Pathology Inc, Salt Lake City, Utah; and the U. S. Pharmacopeia, Rockville, Md. The U. S. Pharmacopeia maintains a national registry of voluntarily reported cases of FVAS.

For the present study, case subjects consisted of cats in which a soft tissue sarcoma arising at a site commonly used for vaccination was first diagnosed between January 1, 1998 and June 15, 1999, on the basis of histologic examination of biopsy specimens by 1 of the study centers. Veterinarians who submitted biopsy specimens were contacted to obtain medical histories for case subjects; whenever possible, owners were also contacted to obtain information on previous veterinarians. Additional medical information was collected on case subjects through December 2000.

Although most soft tissue sarcomas identified in case subjects were fibrosarcomas, other subcutaneous or intramuscular sarcomas that also fulfilled the case criteria included malignant fibrous histiocytomas, myxosarcomas, myofibrosarcomas, liposarcomas, leiomyosarcomas, chondrosarcomas, and osteosarcomas.

Control selection—Control subjects were typically selected from 2 clinical populations. The first consisted of cats in which a diagnosis of basal cell tumor had been made on the basis of histologic examination of biopsy specimens by 1 of the study centers. Cats with basal cell tumors were selected as control subjects because the diagnosis of such tumors required a similar amount of effort (ie, owner-initiated medical consultation, anesthesia, surgery, and submission of biopsy specimens) as did diagnosis of vaccine-associated sarcomas, reducing the potential for selection bias during control selection. In addition, there was no a priori evidence or compelling medical reason to believe that basal cell tumors were associated with SC or IM administration of any vaccine or other chemical substance.

The second control population consisted of cats with histologically confirmed soft tissue sarcomas at sites not commonly used for vaccination (including, but not limited to, the head, ears, digits, ventral aspect of the abdomen, and tail).

Control cats were selected during the same period in which case cats were identified. No fixed ratio of cases to controls was maintained because only a fraction of cats with sarcomas fulfilled the case criteria and which cats would fulfill these criteria was not known at the time case cats were identified. The U. S. Pharmacopeia did not provide information on any control cats because basal cell tumors are not considered by the veterinary medical community to be reportable as suspected adverse vaccination events.

Data analysis—Data analysis was complicated because although most vaccine-associated sarcomas have some distinctive histologic features, it is difficult to reliably distinguish morphologically or histologically a sarcoma that is vaccine-induced from a sarcoma that would have arisen at a vaccination site even if vaccines had never been administered. Sarcomas originate at a variety of body sites, and the reasons for sarcoma development remain largely unknown. Importantly, sarcomas were identified at sites typically used for vaccination long before higher-risk vaccines became available in medical practice.

Therefore, defining any cat with a sarcoma as a case of vaccine-associated sarcoma may lead to some degree of misclassification. For this reason, we used a variety of case and control definitions to determine whether potential risk factors were associated with the development of vaccine-associated sarcomas. In addition, because of the uncertain temporal connection between vaccine administration and sarcoma development, analyses were partitioned into time windows

ranging from 3 months to 3 years. Time windows were defined as the specified period of time prior to tumor diagnosis during which a suspected etiologic agent could have been delivered. Time windows were nested so that, for instance, the 3-month time window was nested in the 6-month time window, which in turn was nested in the 1-year time window, and so on. Time windows were selected to comport with claims that sarcomas can arise shortly after vaccine administration or an indeterminate number of years later.^{3,8}

Conservative case definition method 1—For this analysis, case cats were defined as cats that had a histologic diagnosis of a subcutaneous or intramuscular soft tissue sarcoma at a site on the body typically used for vaccination (sites included the interscapular, lateral thoracic, gluteal, and femoral regions) and that were known to have received at least 1 vaccination at that site. Control cats were defined as cats that had a histologic diagnosis of basal cell tumor or noninjection-site sarcoma. Factors that were examined included tumor location, country or state of residence, age, breed, sex, whether there was a history of trauma at the tumor site, concomitant viral (FeLV, FIV, and feline infectious peritonitis [FIP]) status, and whether any other cats with or without sarcomas shared the same residence.

Conservative case definition method 2—Case cats were defined as cats that had a histologic diagnosis of a subcutaneous or intramuscular soft tissue sarcoma at a site on the body typically used for vaccination (sites included the interscapular, lateral thoracic, gluteal, and femoral regions) but that had not received any vaccinations at this site during the time window for the analysis. Control cats were defined as cats that had a histologic diagnosis of basal cell tumor at a site on the body typically used for vaccination but that had not received any vaccinations at this site during the time window for the analysis. Factors that were examined included whether any nonvaccine injectable medication had been administered at the tumor site in case and control cats. We hypothesized that the temporal distribution of nonvaccine injectable medications should be different between case cats (in which no vaccines had been administered at the sarcoma site) and control cats (in which no vaccines had been administered at the tumor site) if nonvaccine injectable medications caused sarcomas.

Time window method 1—For this analysis, case cats were defined in 2 distinct ways: as cats that had a histologic diagnosis of a subcutaneous or intramuscular soft tissue sarcoma in the general dorsal thoracic region, including the interscapular, shoulder, neck, and thoracic regions, and as cats with a histologic diagnosis of a subcutaneous or intramuscular soft tissue sarcoma specifically in the interscapular region. Cats did not have to have a history of vaccine administration at the tumor site to qualify as case cats. Control cats were defined as cats with basal cell tumors or noninjection-site sarcomas. This analysis examined the distribution of vaccine administration specifically in the general dorsal thoracic and interscapular regions, as defined by the attending veterinarian.

Time window method 2—Case cats were defined in 2 distinct ways. The first group consisted of cats that had a histologic diagnosis of a subcutaneous or intramuscular soft tissue sarcoma in the general dorsal thoracic region (sites included the interscapular, shoulder, neck, and thoracic regions) and that had received at least 1 vaccination in this broad region during the time window for the analysis. The second group consisted of cats with soft tissue sarcomas strictly in the interscapular region that had received at least 1 vaccination in this specific region during the time window for the analysis.

Control cats for this analysis were defined in 2 distinct ways. The first group included cats with a histologically confirmed basal cell tumor or noninjection-site sarcoma that had received a vaccination anywhere on the body during the time window for the analysis. The second group included cats with a histologically confirmed basal cell tumor or noninjection-site sarcoma but that may or may not have received any vaccinations during the time window for the analysis. Thus, the second group of control cats contained the first group, along with cats that had not received any vaccinations during the time window.

For both groups of case cats, cats had received vaccinations during the time window of the analysis in the same regions (general dorsal thoracic region or strictly interscapular region) as the sites of the tumors. For both groups of control cats, cats that had received vaccinations were included regardless of where on the body those vaccines had been administered. This analysis compared the distribution of vaccinations, as well as veterinarians' customary vaccination practices, between case and control cats; a 1-year time window was used for all analyses. The 2 case definitions were used to examine the consistency of results under different assumptions about how to identify cats with sarcomas that were truly caused by a vaccine (which cannot be verified). The 2 control definitions were used to examine which control group better represented the distribution of vaccine practices in the underlying source population of cases.

Data collection—Following the commencement of the study, each study center was responsible for forwarding to the main coordinating center (University of California, Davis) information about case and control cats that qualified for entry into the study. This information included the name, address, and phone number of the veterinarian submitting the biopsy specimen; the histologic diagnosis for the tumor; the tumor site, if known; and any additional patient information that was known. In some instances, owner identification was provided. The study's coordinating center then contacted the referring veterinarian by first mailing an introductory letter and then following up with a telephone interview. In the event that the veterinarian was unable to provide a complete medical history, we requested the cat owner's contact information. After contacting the owner, we were sometimes directed to other veterinarians who provided health care to the cat. If a veterinarian declined to be interviewed by phone, copies of the patient's medical records were requested for abstraction.

Statistical analyses—2 Tests of homogeneity were used to evaluate associations between putative risk factors that had many levels (eg, vaccine manufacturers) and outcome (case vs control group). Potential differences in brands of vaccines from various manufacturers were assessed by use of the last administered vaccine in an antigen class prior to tumor diagnosis. When appropriate, results are presented as exact odds ratios (ORs) and exact 95% confidence intervals (95% CI); CIs were calculated with a mid-P correction. Retrospective time-to-tumor analyses were performed with the product-limit method of survival function estimation, and group differences were evaluated with a log-rank test. Calculations were performed with statistical software.^a Values of $P < 0.05$ were considered significant.

Results

During the study recruitment period, 1,598 cats were eligible for inclusion. Partial or complete information was obtained on 1,347 (84.3%) cats; however, because of the imposed restrictions on the constitution of the study groups, not all cats were included in all analyses. Cats included in the study lived in 42 states and 3 provinces. Information on 624 cats was provided by the University of California and IDEXX Veterinary Services, on 299 cats by Animal Reference Pathology, on 191 cats by the University of Pennsylvania, on 114 cats by the Central Laboratory for Veterinarians, on 71 cats by the U. S. Pharmacopeia, and on 48 cats by Texas A&M University.

CONSERVATIVE CASE DEFINITION METHOD 1

Demographic information—Cats were assigned to 3 groups. There were 662 case cats (cats with sarcomas at vaccination sites that were known to have received at least 1 vaccination at that site), 473 control cats (cats with basal cell tumors or noninjection-site sarcomas), and 212 cats that lacked adequate vaccination histories. Mean age of all cats was 11.0 years, with mean age of case cats being 10.8 years, mean age of control cats being 11.1 years, and mean age of cats lacking an adequate vaccination history being 11.0 years.

Most of the cats were of mixed breeding and described by their owners as domestic short-, medium-, or long-haired cats. Five hundred eighty (88%) case cats, 393 (83%) control cats, and

175 (83%) cats with an inadequate vaccination history were classified as mixed breeds. The most common breed represented was Siamese, constituting 31 (4.7%) cats in the case group, 34 (7.2%) cats in the control group, and 11 (5.2%) cats in the group without adequate vaccination histories.

Information on sex and neutering status was available for 1,319 (98%) cats. Female cats were significantly ($P < 0.001$) overrepresented in the case group ($n = 377$; 57%), compared with males (280; 43%), while males were significantly ($P < 0.001$) overrepresented in the control group (247; 53%), compared with females (221; 47%). Neutering status did not appear to be associated with sarcoma development. Of the 666 female cats for which neutering status was known, 652 (98%) had been spayed, and of the 607 male cats for which neutering status was known, 596 (98%) had been castrated. Information on neutering status was unavailable for 46 (3.4%) cats.

Tumor location—Sarcomas in 553 of the 662 (84%) case cats were located in the dorsal thoracic region, including the neck and interscapular regions. Other sites where sarcomas were located included the femoral (thigh) region ($n = 40$; 6%), flank (32; 5%), lumbar region (20; 3%), gluteal region (16; 2%), and distal portion of the hind limb (1; < 1%).

The distribution of sarcomas in cats with an inadequate vaccination history was similar to the distribution of sarcomas in the case cats, with minor differences. One hundred thirty-one of these 214 (61%) cats had a sarcoma in the dorsal thoracic region. Sarcomas in the remaining cats were located in the femoral region (24; 11%), flank (19; 9%), lumbar region (17; 8%), or gluteal region (11; 5%) or the region could not be determined (12; 6%). The similarity in site distributions for these 2 groups suggested that many of these 214 cats would have been classified as case cats if complete vaccination information had been available.

Concurrent infectious diseases—Veterinarians and owners of the cats were asked about results of testing for FIP, FIV infection, and FeLV infection. Few cats could be documented to have been tested for FIP, making it impossible to determine whether this disease could act as a cofactor in sarcoma tumorigenesis. Only 28 of the case cats had been tested, and only 1 was seropositive. Only 25 control cats were tested, and only 1 was seropositive.

Testing for FIV infection was more common, although most cats were not tested. In addition, only a small number of cats in each group had evidence of FIV infection. Of the 151 case cats tested, only 5 (3%) were seropositive. Of the 103 control cats tested, only 7 (7%) were seropositive. Of the 37 cats with an inadequate vaccination history that were tested, only 1 (3%) was seropositive. Results did not support the hypothesis that FIV acted as a cofactor in sarcoma tumorigenesis.

Overall, 43% of cats had been tested for FeLV infection. Of the 324 case cats tested, only 3 (1%) were seropositive. Of the 194 control cats tested, only 5 (3%) were seropositive. Of the 57 cats with an inadequate vaccination history that were tested, none were seropositive. Results did not support the hypothesis that FeLV infection enhanced the risk of sarcoma development.

Exposure to other cats in the same residence—Information about whether other cats shared the living environment with subject cats was available for 1,145 (85%) subject cats. The proportions of case cats ($n = 395$; 67%), control cats (268; 63%), and cats with unknown vaccination histories (85; 64%) that shared their living environment with another cat were not significantly different from each other. The number of other cats in the household was somewhat higher for case cats (mean, 2.1 cats) than for control cats (1.6 cats) but identical to the number of cats with unknown vaccination histories (2.1 cats).

Information about the frequency of cancer (any tumor type) in other household cats was available for 623 (46%) of the study cats. Although cancer was not rare, there were no significant differences in reported frequency of cancer among the 3 groups (case cats, 10%; control cats, 9%; and cats with unknown vaccination histories, 10%).

Traumatic injuries—Veterinarians and owners were asked whether there was any history of trauma (typically cat fight injuries) at tumor sites. Information was provided for 465 (70%) of the case cats, 324 (69%) of the control cats, and 95 (44%) of the cats with an unknown vaccination history. Seventy-six (16%) case cats had a history of trauma at the tumor site, compared with 29 (9%) control cats and 17 (18%) cats with unknown vaccination histories. There was a significant ($P = 0.003$) difference between case and control cats.

CONSERVATIVE CASE DEFINITION METHOD 2

Three-month time window—Seven hundred seventy-three case cats (cats that had a histologic diagnosis of a subcutaneous or intramuscular soft tissue sarcoma at a site typically used for vaccination but that had not received any vaccinations at this site during the time window) and 363 control cats (cats that had a histologic diagnosis of basal cell tumor at a site typically used for vaccination but that had not received any vaccinations at this site during the time window) were identified. Twenty-five of the case cats had received at least 1 nonvaccine injectable medication at the tumor site during the 3 months prior to tumor diagnosis. Medications included fluids, amoxicillin, penicillin, methyl prednisolone acetate, ketamine, praziquantel, acepromazine, and enrofloxacin. No particular medication was administered to > 4 case cats, except fluids. No significant difference in frequency of administration of nonvaccine injectable medications was observed between case and control cats.

Six-month time window—Seven hundred sixty-four case and 363 control cats were identified. Thirty-one case cats had received at least 1 nonvaccine injectable medication at the tumor site during the 6 months prior to tumor diagnosis. Medications included those listed in the preceding section, along with prednisolone, prednisone, diazepam, and lidocaine. No particular medication was administered to > 7 case cats, except fluids. No significant difference in frequency of administration of nonvaccine injectable medications was observed between case and control cats.

One-year time window—Seven hundred fifty-four case and 363 control cats were identified. Forty-four case cats had received at least 1 nonvaccine injectable medication at the tumor site during the year prior to tumor diagnosis. Medications included those listed in the preceding sections, along with dexamethasone, ampicillin trihydrate, penicillin G procaine, and ampicillin. No particular medication was administered to > 7 case cats, except fluids. No significant difference in frequency of administration of nonvaccine injectable medications was observed between case and control cats.

Two-year time window—Seven hundred thirty case and 263 control cats were identified. Sixty-one case cats had received at least 1 nonvaccine injectable medication at the tumor site during the 2 years prior to tumor diagnosis. Medications included those listed in the preceding sections, along with butorphanol. No particular medication was administered to > 13 case cats, except fluids. Methyl prednisolone acetate was administered to significantly ($P = 0.050$) more case ($n = 13$) than control (0) cats (95% CI of OR, 1.88 to infinity).

Three-year time window—Seven hundred thirteen case and 363 control cats were identified. Seventy-three case cats had received at least 1 nonvaccine injectable medication at the tumor site during the 3 years prior to tumor diagnosis. Medications included those listed in the preceding sections. No particular medication was administered to > 16 case cats, except fluids. Methyl prednisolone acetate was administered to significantly ($P = 0.006$) more case ($n = 16$) than control (0) cats (95% CI of OR, 3.35 to infinity). Long-acting penicillins ($P = 0.007$) were also administered to significantly more case ($n = 14$) than control (0) cats (95% CI of OR, 2.89 to infinity).

TIME WINDOW METHOD 1

Three-month time window—Ten rabies vaccine brands produced by 6 manufacturers were represented in this analysis. Regardless of whether the broader (cats that had a histologic diagnosis of a subcutaneous or intramuscular soft tissue sarcoma in the general dorsal thoracic region, including the interscapular, shoulder, neck, and thoracic regions) or narrower (cats with a histologic diagnosis of a subcutaneous or intramuscular soft tissue sarcoma specifically in the interscapular region) case definition was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.67$ and 0.83 , respectively; Table 1), specific brand ($P = 0.42$ and 0.57 , respectively), use of products with an adjuvant ($P = 0.48$ and 1.00 , respectively), although almost all vaccines administered contained an adjuvant, or use of multidose vials rather than single-use vials ($P = 0.51$ and 0.60 , respectively).

Thirteen FeLV vaccine brands produced by 9 manufacturers were represented in this analysis. Regardless of whether the broader or narrower case definition was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.76$ and 0.98 , respectively; Table 1), specific brand ($P = 0.73$ and 0.94 , respectively), use of products with an adjuvant ($P = 0.56$ and 1.00 , respectively), although almost all vaccines administered contained an adjuvant, or use of multidose vials ($P = 1.00$ and 0.23 , respectively).

Twenty-one FVRCP vaccine brands produced by 9 manufacturers were represented in this analysis. Regardless of whether the broader or narrower definition was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.57$ and 0.86 , respectively; Table 1), specific brand ($P = 0.98$ and 0.92 , respectively), use of products with an adjuvant ($P = 0.75$ and 0.83 , respectively), or use of multidose vials ($P = 0.62$ and 0.65 , respectively), although few multidose vials were used. There was also no significant difference in the frequency of administration of killed FVRCP vaccines between case and control cats ($P = 0.26$ and 1.00 , respectively).

Six-month time window—Ten rabies vaccine brands produced by 6 manufacturers were represented in this analysis. Regardless of whether the broader or narrower case definition was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.52$ and 0.89 , respectively; Table 1), specific brand ($P = 0.64$ and 1.00 , respectively), use of products with an adjuvant ($P = 1.00$ and 1.00 , respectively), although almost all vaccines administered contained an adjuvant, or use of multidose vials ($P = 1.00$ and 0.70 , respectively).

Thirteen FeLV vaccine brands produced by 9 manufacturers were represented in this analysis. Regardless of whether the broader or narrower case definition was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.78$ and 0.95 , respectively; Table 1), specific brand ($P = 0.45$ and 0.55 , respectively), use of products with an adjuvant ($P = 1.00$ and 1.00 , respectively), although almost all vaccines administered contained an adjuvant, or use of multidose vials ($P = 1.00$ and 0.15 , respectively).

Twenty-one FVRCP vaccine brands produced by 10 manufacturers were represented in this analysis. Regardless of whether the broader or narrower case definition was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.65$ and 0.40 , respectively; Table 1), specific brand ($P = 0.75$ and 0.66 , respectively), use of products with an adjuvant ($P = 0.59$ and 1.00 , respectively), or use of multidose vials ($P = 0.24$ and 0.37 , respectively), although few multidose vials were used. There was also no significant difference in the frequency of administration of killed FVRCP vaccines between case and control cats ($P = 0.091$ and 0.60 , respectively).

One-year time window—Seventeen rabies vaccine brands produced by 8 manufacturers were represented in this analysis. Regardless of whether the broader or narrower case definition

was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.67$ and 0.87 , respectively; Table 1), specific brand ($P = 0.18$ and 0.80 , respectively), use of products with an adjuvant ($P = 1.00$ and 1.00 , respectively), although almost all vaccines administered contained an adjuvant, or use of multidose vials ($P = 1.00$ and 0.78 , respectively).

Fourteen FeLV vaccine brands produced by 10 manufacturers were represented in this analysis. Regardless of whether the broader or narrower case definition was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.86$ and 0.84 , respectively; Table 1), specific brand ($P = 0.56$ and 0.75 , respectively), use of products with an adjuvant ($P = 1.00$ and 1.00 , respectively), although almost all vaccines administered contained an adjuvant, or use of multidose vials ($P = 0.77$ and 0.094 , respectively).

Twenty-six FVRCP vaccine brands produced by 11 manufacturers were represented in this analysis. Regardless of whether the broader or narrower case definition was used, there were no significant differences between case and control cats with respect to manufacturing company ($P = 0.73$ and 0.32 , respectively; Table 1), specific brand ($P = 0.57$ and 0.44 , respectively), use of products with an adjuvant ($P = 0.23$ and 1.00 , respectively), or use of multidose vials ($P = 0.057$ and 1.00 , respectively), although few multidose vials were used. There was also no significant difference in the frequency of administration of killed FVRCP vaccines between case and control cats ($P = 0.10$ and 0.64 , respectively).

TIME WINDOW METHOD 2

Mixing vaccines in a single syringe—Regardless of which of the 4 combinations of case ($n = 166$ to 285) and control (121 to 160) cats was evaluated, there was no significant association between the practice of mixing vaccines in a single syringe (yes vs no) and group designation (case vs control). Odds ratios for these analyses ranged from 0.84 (95% CI, 0.23 to 3.42) to 0.97 (95% CI, 0.20 to 5.29). No more than 6 (2.1%) case cats came from hospitals that mixed vaccines in a single syringe, making it too uncommon to be an important contributor to FVAS development.

Reuse of syringes following autoclaving or chemical sterilization—Regardless of which of the 4 combinations of case ($n = 167$ to 287) and control (123 to 162) cats was evaluated, there was no significant association between the practice of reusing disposable syringes (yes vs no) and group designation (case vs control). Odds ratios for these analyses ranged from 0.81 (95% CI, 0.43 to 1.58) to 1.02 (95% CI, 0.53 to 1.98). Reuse of syringes was relatively uncommon, with only 31 (12.1%) case cats coming from hospitals that reused syringes.

Syringe manufacturer—A large majority of practices used syringes from the same manufacturer; the remaining practices used syringes from a variety of other, less popular manufacturers. However, regardless of which combination of case ($n = 91$ to 153) and control (23 to 30) cats was used, no significant associations were found between use of syringes from the most popular manufacturer (vs use of syringes from any other manufacturer) and group designation (case vs control). Odds ratios ranged from 1.02 (95% CI, 0.40 to 2.49) to 1.32 (95% CI, 0.50 to 3.32).

Needle gauge—It has been hypothesized that larger-bore needles are more likely to induce trauma and, hence, a sarcoma. Veterinarians often used a variety of needle sizes in their practices, so this analysis compared practices that used needles that were 21 gauge or larger with practices that used needles that were 22 gauge or smaller. Regardless of which combination of case ($n = 169$ to 289) or control (123 to 161) cats was used, there was no association between needle gauge and group designation. Odds ratios ranged from 0.84 (95% CI, 0.40 to 1.85) to 1.30 (95% CI, 0.59 to 2.91).

Shaking multidose vials prior to vaccine withdrawal—Because vaccines are suspensions, it is possible that flocculent material may not be homogeneously distributed in a vial. If the flocculent material is capable of enhancing a vaccine's tumorigenic potential, then in theory, vaccine doses with larger-than-normal concentrations of the material would be more likely to induce tumors than doses with homogeneous concentrations of the material. Conversely, if some doses have higher-than-normal concentrations, then other doses drawn from the same vial must have lower-than-normal concentrations, which could reduce tumorigenic potential. It was not possible in this study to know which vaccines withdrawn from multidose vials were more or less concentrated. When responding to questions about handling of multidose vials prior to vaccine withdrawal, veterinarians sometimes provided equivocal information (eg, the vial is sometimes shaken). When comparing veterinarians who never shook multidose vials to veterinarians who at least sometimes shook multidose vials, no significant association between case (n = 168 to 288) and control (123 to 162) cats was found. Odds ratios ranged from 1.48 (95% CI, 0.55 to 4.58) to 2.18 (95% CI, 0.82 to 6.36). When comparing veterinarians who always shook multidose vials to veterinarians who sometimes shook multidose vials, no significant association between case (n = 168 to 288) and control (123 to 162) cats was found. Odds ratios ranged from 0.65 (95% CI, 0.35 to 1.20) to 0.67 (95% CI, 0.35 to 1.23). Finally, when comparing veterinarians who never shook multidose vials to veterinarians who always shook multidose vials, no significant association between case (n = 151 to 255) and control (112 to 148) cats was found. Odds ratios ranged from 1.53 (95% CI, 0.57 to 4.74) to 2.22 (95% CI, 0.83 to 6.51). Only 17 veterinarians reported that they never shook vaccine vials.

Temperature of vaccine at time of administration—Veterinarians were asked whether they administered vaccines at refrigerated or room temperatures. Regardless of which combination of case (n = 143 to 241) and control (108 to 137) cats was used, there was a significant association between temperature and group designation (case vs control). Administration of cold vaccines was associated with a higher risk of sarcoma development than was administration of room temperature vaccines, with ORs ranging from 2.04 (95% CI, 1.27 to 3.28) to 2.05 (95% CI, 1.20 to 3.54).

Massage of vaccination site—It has been hypothesized that massaging the site of vaccination immediately following injection may disperse the vaccine so that smaller concentrations reside at the injection site, possibly decreasing the risk of sarcoma development. Veterinarians often did not have a consistent approach to massaging vaccination sites, so this analysis examined massage in 3 ways: veterinarians who never massaged were compared with those who sometimes did, veterinarians who always massaged were compared with those who sometimes did not, and veterinarians who never massaged were compared with those who sometimes did. Regardless of which combination of case (n = 168 to 288) and control (122 to 161) cats was analyzed, there was no significant association between veterinarians who never massaged versus those who sometimes did and group designation; ORs ranged from 1.05 (95% CI, 0.69 to 1.61) to 1.21 (95% CI, 0.73 to 2.02). Similarly, regardless of which combination of case (n = 168 to 188) and control (122 to 168) cats was used, there was no significant association between veterinarians who always massaged versus those who sometimes did not and group designation; ORs ranged from 0.81 (95% CI, 0.50 to 1.32) to 0.84 (95% CI, 0.57 to 1.25). Finally, again regardless of which combination of case (n = 117 to 197) and control (86 to 115) cats was used, there was no significant association between veterinarians who never massaged versus those who always massaged and group designation; ORs ranged from 1.15 (95% CI, 0.72 to 1.84) to 1.29 (95% CI, 0.73 to 2.28).

Rabies vaccination—No significant differences were found among 8 manufacturers of rabies vaccines between case (n = 89 with the first case definition and 50 with the second) and control (73) cats (P = 0.63 and 0.64, respectively). Fifteen distinct brands of rabies vaccine were administered, with again no significant differences between case (n = 39 to 68) and control (62) cats (P = 0.29 and 0.54, respectively). The almost nonexistent use of nonadjuvanted rabies vaccine in the study population of case (n = 45 to 76) and control (67) cats precluded any meaningful evaluation of the effect of adjuvants (OR, 0 [95% CI, 0 to 10.21] and 0 [95% CI, 0 to

6.05], respectively). Multidose vials were also not used with much frequency, and no significant differences in their use were observed between case (n = 52 and 331, respectively) and control (44) cats, compared with single-dose vials (OR, 1.28 [95% CI, 0.54 to 3.04] and 1.39 [95% CI, 0.52 to 3.88], respectively).

FeLV vaccination—No significant differences were found among 10 manufacturers of FeLV vaccines between case (n = 136 with the first case definition and 84 with the second) and control (72) cats (P = 0.50 and 0.38, respectively). Fourteen distinct brands of FeLV vaccine were administered, with again no significant differences between case (n = 111 and 68) and control (62) cats (P = 0.44 and 0.28, respectively). The use of nonadjuvanted FeLV vaccines in this study population was still relatively uncommon, with approximately 20% of the case cats receiving nonadjuvanted FeLV vaccines. There was no significant difference in the use of nonadjuvanted vaccines between case (n = 125 and 75) and control (67) cats (OR, 1.39 [95% CI, 0.66 to 2.86] and 1.37 [95% CI, 0.60 to 3.11], respectively). Multidose vials were also not used with much frequency, with only approximately 10% of case cats receiving vaccines from multidose vials. There was no significant difference in the use of multidose vials between case (n = 111 and 71) and control (54) cats (OR, 0.70 [95% CI, 0.23 to 2.25] and 0.62 [95% CI, 0.16 to 2.24], respectively).

FVRCP vaccination—No significant differences were found among 13 manufacturers of FVRCP vaccine between case (n = 216 with the first case definition and 137 with the second) and control (104) cats (P = 0.41 and 0.36, respectively). Twenty-five distinct brands of FVRCP vaccine were administered, with again no significant differences between case (n = 183 and 107) and control (84) cats (P = 0.11 and 0.16, respectively). Evaluation of the effect of adjuvanted vaccines did not reveal significant differences between case (n = 218 and 124) and control (99) cats (OR, 0.77 [95% CI, 0.48 to 1.25] and 0.98 [95% CI, 0.58 to 1.67], respectively). Multidose vials were rarely used, with only approximately 1% of the case cats receiving vaccines from multidose vials. There was no significant difference in the use of multidose vials between case (n = 190 and 114) and control (81) cats (OR, 0.21 [95% CI, 0.026 to 1.19] and 0.17 [95% CI, 0.0068 to 1.40], respectively). No significant differences were observed between case (n = 184 and 105) and control (76) cats with respect to the use of killed versus modified-live FVRCP vaccines (P = 0.32 and 0.88, respectively). There were many FVRCP preparations that included combinations of other viral antigens with killed and modified-live components. Although these were used with less frequency than noncombination products, significant differences were not observed between case and control cats.

RETROSPECTIVE TIME-TO-TUMOR ANALYSIS

Time intervals between the most recent administration (if any) of FVRCP, FeLV, and rabies vaccines and the date of diagnosis of tumor were analyzed, with stratification on tumors found in the following locations: sarcomas in the interscapular and femoral regions; sarcomas in areas less often used for vaccination (eg, thoracic, shoulder, lumbar, gluteal, and flank regions); sarcomas at sites not generally used for vaccination (eg, ears, head, toes, and tail); and basal cell tumors. Under the null hypothesis of no effect, we hypothesized that the (unspecified) distribution of time between last antigen-specific vaccine administration and diagnosis of tumor should be approximately equal among these 4 groups.

No significant differences among the 4 groups were established for FVRCP (P = 0.69), FeLV (P = 0.32), and rabies (P = 0.71) vaccines (Figure 1, Figure 2, and Figure 3). Furthermore, on examination of strictly modified-live or killed FVRCP vaccines, neither significant nor meaningful differences were observed among the 4 groups (P = 0.79 and 0.056, respectively).

A sensitivity analysis that excluded all vaccinations administered within 30, 90, and 120 days of tumor diagnosis was conducted under the restrictive assumption that it was impossible for a sarcoma to develop from a vaccine until at least 30, 90, or 120 days had transpired. Again,

however, no significant differences ($P > 0.05$) among the 4 groups were established regardless of vaccine antigen or formulation examined.

Discussion

The goal of this study was to examine the distribution of putative determinants of FVAs between groups of cats with various probabilities of having tumors caused by vaccines and, potentially, other injectable agents. Such determinants included not only the vaccines but also the medical practices of veterinarians who oversaw the medical care of cats enrolled in the study. Given the uncertainty about knowing which cats' sarcomas were truly caused by vaccines, instead of being coincident with them, we used a series of temporal sensitivity analyses to identify associations under a wide variety of case, control, and exposure definitions.

We did not find that any single manufacturer or vaccine brand (conditional on vaccine antigen) had a significantly higher or lower association with group (case vs control), compared with any other manufacturer or brand. This finding was consistent for all vaccine antigen groups studied. We were similarly unable to find a higher average likelihood of sarcomas among cats that received adjuvanted vaccine brands, compared with non-adjuvanted ones. Our results remained invariant under a variety of case and control definitions and assumptions. Therefore, the superiority of any single vaccine brand for any preventive medicine purpose cannot be supported by results of this study. Although newer and purportedly less inflammatory vaccines (eg, recombinant vaccines) reached the market after this research began, they were not used frequently enough in our study population to allow us to make any claims about their relative safety.

Our findings are consistent with the contention that the vaccine itself is not sufficient to cause development of FVAs but is instead a component cause. Because the cats in this study resided throughout the United States and Canada, this leaves little doubt of the role of genetic predisposition in developing such tumors as an additional and important component cause. Such a postulated predisposition currently remains undefined, although future research will unquestionably better characterize why only a small fraction of vaccinated cats develop these tumors.

This study also refutes a number of contentions that have been made about how other factors involved in preventive medicine practice may predispose cats to sarcoma development. The roles of needle gauge, use and shaking of multidose vials, mixing vaccines in a single syringe, and syringe type in tumorigenesis appear to be nonexistent or inestimable because of the rarity of the practice. The 1 exception to this may be the temperature of the vaccines immediately prior to administration. This study found a higher risk when cold vaccines were administered, compared with vaccines at room temperature. However, this finding is unconfirmed by others and should be regarded as tentative and subject to verification.

This study cannot rule out the possibility that other injectable medications beside vaccines play a role in tumorigenesis, but merely inducing an inflammatory response appears to be insufficient to inevitably lead to neoplasia, despite a higher prevalence of a history of trauma at tumor sites in case cats, compared with control cats. In many cases, the frequency of administration of other injectables was low, precluding meaningful conclusions about their roles, if any, in tumor formation. However, 2 classes of injectable medications did appear to be found more frequently among cats with sarcomas: methyl prednisolone acetate (a long-acting corticosteroid) and penicillin (often injected in a long-acting form). The association between these drugs and tumor formation only began to become apparent after at least 2 years following administration, suggesting that their tumorigenic potential is somewhat more limited, compared with vaccines. However, this finding should also be regarded as tentative until verified by others.

The direction in which future epidemiologic endeavors on causes of FVAs should proceed remains uncertain. Ideally, 2 conditions should be met for future studies to yield reliable results.

First, a more sensitive and specific definition of vaccine-site (or injection-site) sarcomas is needed to decrease nondifferential misclassification of cats as cases versus controls. Second, substantially better medical record keeping, particularly with regard to manufacturer and injection site, will decrease nondifferential misclassification of exposure status, again improving study validity. Such misclassification can otherwise lead to bias towards the null hypothesis of no association or causal effect, a source of error that may have figured in this research.

The difficulty in achieving the first condition should not be underestimated. It is difficult to routinely distinguish, on the basis of morphologic or histologic characteristics, vaccine-independent sarcomas from vaccine-associated and vaccine-caused sarcomas. Nevertheless, such distinctions are critical for correct classification when examining causal inference. Identification of foreign material found in vaccines is not in itself sufficient to determine causation, any more than the absence of foreign material is sufficient to absolve vaccines in tumor etiology. Current research underway to better characterize sarcomas with vaccines as a component cause may yet help pathologists in this endeavor.

The practical difficulty of achieving the second condition is no less problematic. Accuracy of medical records is a perennial problem, and because no single set of standards yet exists for vaccine administration sites, investigators must often rely on subjective recall by veterinarians, which is potentially subject to error. Veterinarians may justifiably question the necessity of recording such details because of the small likelihood of ever retrieving them.

In retrospect, expending considerable expense and effort in attempting to gain lifetime vaccination histories in the present study was ill-advised. While such information could be potentially useful in estimating cumulative exposure effects, it was in fact not readily available for most subjects in this study. The reasons for this varied. Owners frequently patronized numerous veterinarians over the course of their pets' lives, veterinarians sometimes discarded medical records of patients no longer seen at their practices, and detailed vaccine history information (eg, site of administration, manufacturer and brand, and needle gauge) was frequently not recorded. Future epidemiologic endeavors would be better restricted to acquiring medical histories within a few years prior to case or control ascertainment to enhance the availability and validity of the data. The resources that would otherwise be expended in gaining more historical medical information could then be redirected into acquiring more study subjects.

Despite these and other obstacles, however formidable, it is inevitable that additional population-based studies in cats will eventually be completed. Given the still low incidence of FVAs, product safety testing of new vaccine products will fail to detect this disease and other rare, adverse health outcomes. Passive surveillance methods are notoriously poor in detecting extraordinary changes in disease incidence because of generally poor voluntary participation. As new products, perhaps purporting to be safer than their predecessors, achieve market penetration, thorough and exhaustive epidemiologic studies (ie, case-control studies) will remain the only pragmatic strategy in verifying that such biologics will ultimately do more good than harm.

^aStatXact, Cytel Software Corp, Boston, Mass.

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