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主旨：函轉行政院農業委員會動植物防疫檢疫局函，為避免貓隻注射部位肉瘤(Feline Injection Site Sarcoma, 簡稱FISS)狀況之發生，請依說明事項辦理，請查照。

說明：

- 一、依據行政院農業委員會動植物防疫檢疫局107年10月29日防檢一字第1071472673號函辦理。
- 二、據相關資料顯示，注射廣泛種類之藥物(包括抗生素、長效型類固醇與胰島素或疫苗等)均有發生FISS之疑慮，其確實致病機轉及危險因子仍未完全明瞭，且根據美國獸醫協會(American Veterinary Medical Association, AVMA)指出，發生FISS之機率不高，僅為0.003%至0.01%。同時，世界小動物獸醫師協會出版之犬貓疫苗指南亦建議，任何FISS風險均不宜重於疫苗保護之效果。惟為降低FISS發生疑慮，世界小動物獸醫師協會建議為貓隻施打疫苗時應避免施打於肩胛間，施打處以側腹為宜，疫苗注射部位應輪替、並以圖示記錄注射部位，以利後續追蹤。
- 三、因FISS確實致病機轉及危險因子仍未完全明瞭，而根據疫苗供應業者之說明，施打無佐劑疫苗亦不能完全排除FISS發生之可能性，故為貓隻施打狂犬病疫苗前，獸醫師應向

飼主說明防疫之重要性及可能發生FISS之風險，並說明目前國內有含佐劑及不含佐劑之狂犬病疫苗可供民眾選擇。

四、檢附原函及參考資料2份。

正本：桃園市獸醫診療機構、桃園市獸醫師公會

副本：本處動物保護課、本處動物管制課

處長陳英豪



行政院農業委員會動植物防疫檢疫局 函

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附件：如文1071472673-a1~a2(2015FISS(ABCDguidelines).pdf、2018FISS.pdf)

主旨：為避免貓隻注射部位肉瘤(Feline Injection Site Sarcoma, 簡稱FISS)狀況之發生，請轉知所轄動物醫院依說明事項辦理，請查照。

說明：

- 一、依據本局105年11月17日防檢一字第1051472915號函送「研商供應無佐劑狂犬病疫苗避免貓注射部位肉瘤產官學會」紀錄(諒達)及監察院107年10月5日院台財字第1072230479號函副本辦理。
- 二、據相關資料顯示，注射廣泛種類之藥物(包括抗生素、長效型類固醇與胰島素或疫苗等)均有發生FISS之疑慮，其實致病機轉及危險因子仍未完全明瞭，且根據美國獸醫協會(American Veterinary Medical Association, AVMA)指出，發生FISS之機率不高，僅為0.003%至0.01%。同時，世界小動物獸醫師協會出版之犬貓疫苗指南亦建議，任何FISS風險均不宜重於疫苗保護之效果。惟為降低FISS發生疑慮，世界小動物獸醫師協會建議為貓隻施打疫苗時應



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有附件

避免施打於肩胛間，施打處以側腹為宜，疫苗注射部位應輪替、並以圖示記錄注射部位，以利後續追蹤。

三、因FISS確實致病機轉及危險因子仍未完全明瞭，而根據疫苗供應業者之說明，施打無佐劑疫苗亦不能完全排除FISS發生之可能性，故為貓隻施打狂犬病疫苗前，獸醫師應向飼主說明防疫之重要性及可能發生FISS之風險，並說明目前國內有含佐劑及不含佐劑之狂犬病疫苗可供民眾選擇。請惠予轉知所轄動物醫院應參考相關研究之建議盡可能排除或降低可能導致貓隻產生FISS之風險因子。

四、檢送參考資料共2份如附。

正本：臺北市動物保護處、新北市政府動物保護防疫處、基隆市動物保護防疫所、桃園市政府動物保護處、新竹市動物保護及防疫所、新竹縣家畜疾病防治所、苗栗縣動物保護防疫所、臺中市動物保護防疫處、彰化縣動物防疫所、南投縣家畜疾病防治所、雲林縣動植物防疫所、嘉義市政府建設處、嘉義縣家畜疾病防治所、臺南市動物防疫保護處、高雄市動物保護處、屏東縣動物防疫所、宜蘭縣動植物防疫所、花蓮縣動植物防疫所、臺東縣動物防疫所、澎湖縣家畜疾病防治所、金門縣動植物防疫所、連江縣政府

副本：動植物防疫檢疫局基隆分局、動植物防疫檢疫局新竹分局、動植物防疫檢疫局臺中分局、動植物防疫檢疫局高雄分局、本局動物防疫組(均含附件)

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SPECIAL ARTICLE

FELINE INJECTION-SITE SARCOMA

ABCD guidelines on prevention and management

ABCD

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Overview: In cats, the most serious of adverse effects following vaccination is the occurrence of invasive sarcomas (mostly fibrosarcomas): so-called 'feline injection-site sarcomas' (FISSs). These develop at sites of previous vaccination or injection. They have characteristics that are distinct from those of fibrosarcomas in other areas and behave more aggressively. The rate of metastasis ranges from 10–28%.

Pathogenesis: The pathogenesis of these sarcomas is not yet definitively explained. However, chronic inflammatory reactions are considered the trigger for subsequent malignant transformation. Injections of long-acting drugs (such as glucocorticoids, and others) have been associated with sarcoma formation. Adjuvanted vaccines induce intense local inflammation and seem therefore to be particularly linked to the development of FISS. The risk is lower for modified-live and recombinant vaccines, but no vaccine is risk-free.

Treatment and prevention: Aggressive, radical excision is required to avoid tumour recurrence. The prognosis improves if additional radiotherapy and/or immunotherapy (such as recombinant feline IL-2) are used. For prevention, administration of any irritating substance should be avoided. Vaccination should be performed as often as necessary, but as infrequently as possible. Non-adjuvanted, modified-live or recombinant vaccines should be selected in preference to adjuvanted vaccines. Injections should be given at sites at which surgery would likely lead to a complete cure; the interscapular region should generally be avoided. Post-vaccination monitoring should be performed.

*The ABCD is grateful to Professor Michael Day, of the School of Veterinary Sciences, University of Bristol, UK, who, though not a member of the Board, contributed to this article.

Introduction

Recently, vaccination of cats has received scientific and public attention linked to the supposition that a range of rare adverse effects can arise following vaccination. In cats, the most serious of these adverse consequences is the occurrence of invasive sarcomas (mostly fibrosarcomas), so-called 'feline injection-site sarcomas' (FISSs), that can develop within the skin at sites of previous vaccination. Despite extensive research on the pathogenesis of these sarcomas, there is no definitive causal relationship that explains their occurrence and the direct link to vaccination. The most accepted hypothesis suggests that a chronic inflammatory reaction at the site of injection provides a trigger for subsequent malignant transformation.

Epidemiology and characterisation

In 1991, an increased incidence of tumours in cats that developed at injection sites was first reported in the United States.¹ This observation was connected to an increased use of rabies and feline leukaemia virus (FeLV) vaccinations.^{2,3} As a consequence, these tumours were first called feline 'vaccine-associated sarcomas'. However, the subsequent finding that other, non-vaccinal injectables can also cause this type of tumour has led to reclassification of these neoplasms as 'feline injection-site sarcomas' (FISSs). These tumours seem to be unique to cats,⁴ although comparable tumours have been reported in ferrets⁵ and very occasionally in dogs.⁶

FISSs occur at sites typically used for vaccination and injections, such as the interscapular region (Figure 1), the lateral thoracic or abdominal wall, the lumbar region, and the area of the semimembranosus and semitendinosus muscles. FISSs are most commonly located in the subcutis, but also can occur intramuscularly.^{7,8}

FISSs can occur as early as 4 months and up to 3 years after an injection. They are characterised by invasive local growth in the subcutis, often with spread along fascial planes.⁹ Most FISSs are fibrosarcomas,¹⁰ but other malignancies, such as osteosarcomas,¹¹ chondrosarcomas,⁷



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rhabdomyosarcomas,⁷ malignant fibrous histiocytomas,^{7,11} and myofibroblastic sarcomas⁸ have also been described.

FISSs have histological characteristics that are distinct from those of fibrosarcomas in other areas. Typically there is perivascular infiltration of lymphocytes and macrophages at the tumour periphery, a central area of necrosis, inflammation and local infiltration of tumour cells (Figure 2).^{10,12} FISSs behave more aggressively than sarcomas at other sites.¹³ The rate of metastasis ranges from 10–28%.^{14,15} The lung is the most common site of metastasis, followed by regional lymph nodes and abdominal organs, such as the kidney, spleen, intestine and liver.^{16,17}

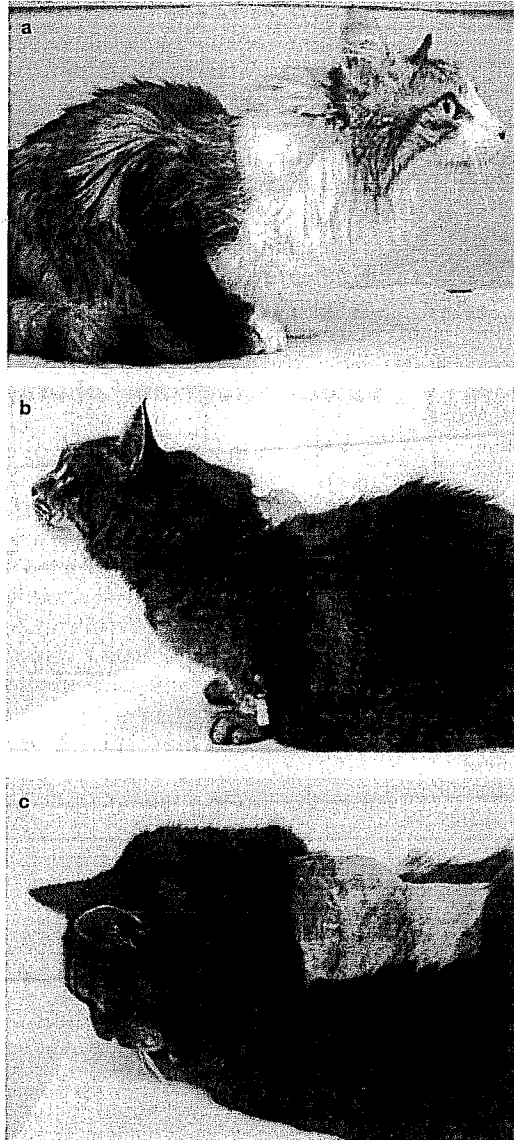
In the past 20 years, an epidemiological association has been demonstrated between vaccination and the later development of FISS.^{3,13,18–21} The incidence of FISS has been estimated at 1–4 in every 10,000 vaccinated cats in the USA,^{22,23} and the ratio of injection-site to non-injection-site sarcomas increased from 0.5 in 1989 to 4.3 in 1994.¹⁰ In one study in the USA, reported rates of reaction were 0.3 FISSs per 10,000 vaccinations and 11.8 postvaccinal inflammatory reactions per 10,000 vaccinations in cats.²² If inflammatory reactions are a necessary prelude to FISS, then these rates suggest that 1 in 35–40 inflammatory reactions develop into FISS. In the UK, the incidence of FISSs seems to be relatively low (incidence risk of FISS per year was estimated to be 1 per 16,000–50,000 cats registered by practices, 1 per 10,000–20,000 cat consultations, and 1 per 5000–12,500 vaccination visits).²⁴ One reason for the low rate might be that rabies vaccination is not a routine procedure for cats in the UK. One study in Canada investigated the annual prevalence of feline postvaccinal sarcomas among 11,609 feline skin mass submissions from 1992 to 2010 and revealed no decrease in disease prevalence or increase in age of affected cats in response to change in vaccination formulation or recommended changes in feline vaccination protocols.²⁵

Pathogenesis

Despite extensive research, there is no definitive proof of the pathogenesis of FISS. The most widely accepted hypothesis suggests that a chronic inflammatory

FISSs are usually firm, indolent, seemingly well-circumscribed, subcutaneous masses that are often not freely moveable.

Figure 1 (a–c) Cats with feline injection-site sarcoma. Courtesy of Johannes Hirschberger, Ludwig Maximilians University, Munich, Germany



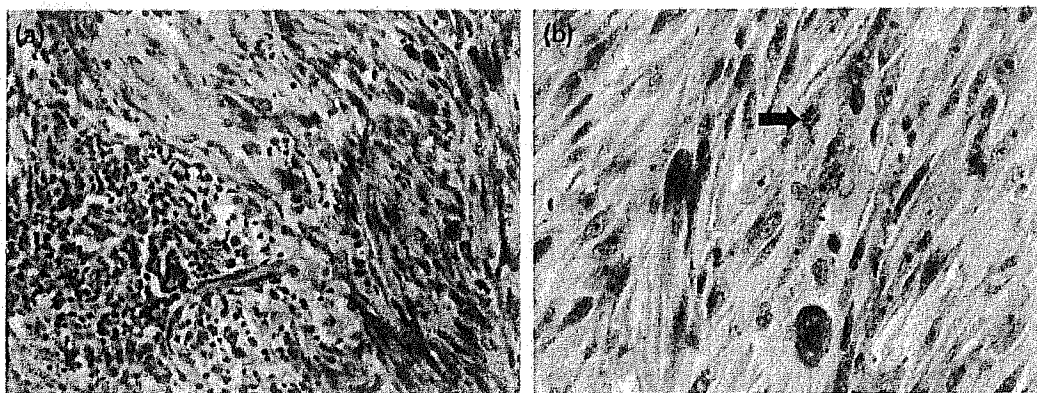
reaction at the site of an injection acts as a trigger for subsequent malignant transformation. Adjuvanted vaccines seem to be particularly linked to the development of FISS due to the more intense local inflammation associated with such products. This idea is supported by frequent identification of adjuvants in histological or ultrastructural investigations of these sarcomas.^{12,18}

Many data suggest an association between vaccination and FISS in cats. Aluminium, a vaccine adjuvant, has been found in biopsy samples of FISS.²⁶ In most inactivated vaccines, an adjuvant is added to enhance the inflammation at the site of injection, which is intended and necessary when applying a killed agent in order to trigger the necessary immune response. However, this inflammation might potentially lead to malignant transformation. Traces of adjuvants can be seen in the inflammatory reaction, specifically accumulated within macrophages or multinucleate giant

cells, and later in histological sections of FISS in the transformed fibroblast.¹⁸ Intracellular crystalline particulate material was found in an ultrastructural study in 5 of 20 FISSs investigated, and in one of the five cases was identified as aluminium-based.¹² Although no specific vaccine or adjuvant has been incriminated,²⁷ local irritation from adjuvant is thought to stimulate mainly fibroblasts to the point that malignant transformation occurs.

At first, only rabies and FeLV vaccines were identified as risk factors,^{3,13,23} but subsequently other vaccines, including vaccines against feline panleukopenia virus (FPV), feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV), were also found to be involved in the development of FISS in some cases.^{13,23,28–30} In addition to vaccines, injections such as long-acting glucocorticoids, penicillin, lufenuron,^{27,31,32} cisplatin³³ and meloxicam³⁴ have been associated with sarcoma formation. One study found that the frequency of administration of long-acting glucocorticoid injections (dexamethasone, methylprednisolone and triamcinolone) was significantly higher in cats with FISS in the interscapular region than in control cats.³⁵ Fibrosarcomas were also reported at the site of

Figure 2 Histological sections of a 2 cm diameter mass removed from the lateral thorax of a 13-year-old domestic shorthair cat. A similar interscapular mass had been removed from this cat 2 months previously. (a) A focus of lymphoplasmacytic inflammation is contained within the surrounding sarcoma. (b) Higher magnification of the neoplastic tissue reveals a pleomorphic population of neoplastic spindle cells with occasional giant nuclei and irregular mitotic activity (arrow). Haematoxylin and eosin stain. Courtesy of Michael Day, School of Veterinary Sciences, University of Bristol, UK



a deep, non-absorbable suture in one cat;³⁶ around a surgical swab in the abdomen of one cat;³⁷ adjacent to the site of microchip implantation in two cats;^{38,39} and associated with a subcutaneous fluid port device.^{38,39} This suggests that all inflammatory reactions, theoretically, have the potential to lead to the development of FISS by triggering uncontrolled proliferation of fibroblasts and myofibroblasts, which, in some cases, results in malignant transformation.

Although many causes of inflammation are associated with FISS development, the risk seems to be higher for vaccines compared with other injections; among vaccines, the risk seems to be higher when adjuvanted vaccines are used. Srivastav et al³⁵ compared associations between vaccine types and other injectable drugs with the development of FISS in a case-control study of 181 cats with soft tissue sarcomas (cases), 96 cats with tumours at non-vaccine regions (control group 1), and 159 cats with basal cell tumours (control group 2). There was a significant association between the administration of various types of vaccines and other injectable products (eg, long-acting corticosteroids) and FISS development. Of 192 cats with sarcoma, 101 had vaccinations at the site of tumour development during the preceding 3 years, and 23 had received other injections.³⁵ This study also showed that adjuvanted inactivated vaccines were significantly more commonly associated with FISS development than other vaccines; of 35 vaccinated cats with sarcoma on the hindlimb, 25 cats had received adjuvanted vaccines, seven cats had received modified-live virus (MLV) vaccines (FPV, FHV-1 and FCV), and only one cat had received a recombinant vaccine. These findings also indicated that no vaccines are risk-free.³⁵

The mechanism by which the inflammatory reaction causes tumour formation is not fully understood. Growth factors promote proliferation, can induce malignant transformation, and also can be involved in the regulation of

angiogenesis. Overexpression of growth factors and oncogene activation have been demonstrated in cats with FISS and are suspected to play a role in tumour development.^{40–42}

As vaccination against FeLV is associated with a higher risk of FISS, some studies looked at a possible role of FeLV and its mutant feline sarcoma virus (FeSV) in the development of FISS, but could not detect either FeLV or FeSV in the tumours.⁴³ Furthermore, no other viruses, including feline immunodeficiency virus, feline foamy virus, polyomaviruses or papillomaviruses were detected in tumour tissues.^{44–47} No evidence has been found to implicate replication or expression of endogenous retroviruses in FISS formation.^{45,46}

The observation that not all cats develop FISS after vaccination suggests that there might be a genetic predisposition. It has been suggested that there is a higher incidence of FISS in siblings of affected cats, and that some cats tend to develop more than one FISS. Alterations with unknown relevance such as hyperploidy,⁴⁸ translocations⁴⁹ and triploidy⁵⁰ of oncogene and tumour suppressor loci have been found on extra chromosomes and monosomic chromosomes in affected cats. Mutations have been identified in the tumour suppressor gene p53, which is implicated in cancer initiation and progression in sarcoma tissue of cats with FISS.^{51–55} A case-control study (50 domestic shorthair cats with a confirmed diagnosis of FISS and 100 disease-free matched controls) investigating a possible association between polymorphisms in the genomic sequence of the feline p53 gene and a predisposition to FISS, found a strong association between FISS and the presence of specific nucleotides at two of the polymorphic sites.⁵⁶ However, another study, conducted in Munich, Germany, could not reproduce these findings and observed no association with the polymorphisms described.⁵⁷

European Advisory Board on Cat Diseases

The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

The latest version of the guidance provided in this article is available at www.abcdcatsvets.org and www.abcd-vets.org

Management

Appropriate treatment should first include staging and careful planning of the surgery, because aggressive, radical excision is crucial to avoid tumour recurrence. The prognosis improves if, in addition to radical surgery, adjunctive treatments such as radiotherapy or immunotherapy are used. Preoperatively, (contrast-enhanced) computed tomography (CT) or magnetic resonance imaging (MRI) should be obtained for staging, and to determine the extent of the tumour and the size of the radiation field required to maximise the chance of a successful outcome.⁵⁸ It was shown that the actual size of tumours determined by CT could be twice that estimated at physical examination.^{59,60} Surgeons should attempt to achieve complete, en bloc, surgical tumour resection with at least 3 cm (ideally, 5 cm) margins⁶¹ [EBM grade III] and the removal of one fascial plane underlying the tumour, because incomplete resection can result in recurrence as early as 2 weeks after surgery [EBM grade III].^{28,62} Treatment using surgical excision alone has a recurrence rate of up to 70%, with tumour regrowth usually occurring in the first 6 months after surgery [EBM grade III].¹³ Tumour-free margins are very important for a longer disease-free interval, which was 700 days when complete tumour excision was accomplished, but only 112 days for incomplete resection [EBM grade III].⁶³ However, even with clean surgical margins, the recurrence rate can be as high as 50% [EBM grade III].⁶⁴

Preoperative or postoperative radiation therapy significantly decreases recurrence rates and prolongs remission times,^{16,63,65} while the benefit of chemotherapy is not proven as large prospective randomised controlled trials are lacking. One non-randomised study found no significant difference between control cats (surgery alone) and cats treated with surgery and doxorubicin [EBM grade III],⁶⁶ while a recent study demonstrated chemotherapy benefits compared with historical controls using a combination of neoadjuvant and adjuvant chemotherapy (three epirubicin doses before and after surgery) [EBM grade III].⁶⁷ Chemotherapy mainly remains an option for palliative treatment in cats with non-resectable FISS, when radiation therapy is not available.

Additional immunotherapy appears to be promising.⁶⁸⁻⁷⁰ Results of prospective randomised controlled studies of cytokine gene transfer techniques for adjuvant-immunological treatment of FISS showed reduced recurrence rates. In cats receiving gene therapy by the peritumoural administration of histo-incompatible Vero cells expressing human interleukin-2 (hIL-2) in addition to surgery and

EBM grades

The ranking system for grading the level of evidence of various statements within the management and prevention sections of this article is described on page 574 of this Special Issue.

radiation therapy, only 5/16 (31%) had FISS recurrence, while 11/16 control cats (69%) that had surgery and radiation therapy, but no immunotherapy, had FISS recurrence within 16 months [EBM grade I].⁷¹ Use of neoadjuvant gene therapy using a non-viral vector that expresses feline granulocyte-macrophage colony-stimulating factor (GM-CSF) or a combination of the feline genes GM-CSF, interleukin (IL)-2 and interferon- γ (IFN- γ) was well tolerated by cats [EBM grade I]^{68,69} and showed promising results. Recombinant feline IL-2 is now commercially available in Europe for the treatment of FISS in combination with surgical excision and radiation therapy. In a randomised controlled clinical trial, administration of a recombinant canarypox virus expressing feline IL-2 was well tolerated and resulted in a significantly longer median time to relapse and a significant reduction in the risk of relapse at 1 year and 2 years [EBM grade I].⁷⁰

Prevention

Prevention consists of three general considerations:

Key considerations in the prevention of FISS

- ✦ Injections in cats should always be given at sites at which surgery (such as amputation of a limb or excision of lateral abdominal skin) would likely lead to a complete cure with the least complicated surgical procedure
- ✦ General recommendations to reduce the inflammatory reaction at injection sites should be followed, such as avoiding the administration of irritating substances
- ✦ It is advised to vaccinate only as often as necessary and as infrequently as possible (eg, according to the principles of current vaccination guidelines, avoiding FeLV vaccination in FeLV antigen-positive, FeLV PCR-positive or FeLV antibody-positive cats)

Choice of injection site

In general, injecting distally in a leg aids, where necessary, in the subsequent treatment of sarcoma by amputation of the leg (because these tumours are very difficult to excise completely and often recur after resection).²⁰ Administration of vaccines (or other injections) between the scapulae is generally contraindicated because tumour resection is almost impossible in this location.

To assess the acceptance of the recommendations of the Vaccine-Associated Feline Sarcoma Task Force (VAFSTF), published in 1996, a study involving 392 cats with FISSs compared the anatomical locations of tumours between cases with FISS diagnosed before and after publication of these recommendations.⁷² The proportions of FISS significantly decreased in the interscapular (53% to 40%) and right and left thoracic (10% to 4% and 9% to 1%, respectively) regions, whereas

the proportions of FISS significantly increased in the right thoracic limb (1% to 10%) and the combined regions of the right pelvic limb with the right lateral aspect of the abdomen (13% to 25%) and the left pelvic limb with the left lateral aspect of the abdomen (11% to 14%). Thus, while veterinarians are complying with vaccination recommendations to some extent, a high proportion of tumours still developed in the interscapular region. There was also an increase in lateral abdominal FISSs, which could be attributable to aberrant placement of injections intended for the pelvic limbs. It remains the case that only administration of vaccines as distally as possible on a limb allows for complete surgical margins if limb amputation is required [EBM grade III].⁷³ Current data in Europe shows a similar situation. In a study examining the location of FISSs in cats presented to the oncology service at the University teaching hospital in Munich, most still occurred between the scapulae (40%), followed by the right (19%) and left thoracic walls (13%).⁷⁴

Unfortunately, there is still insufficient clinical information to enable evidence-based vaccine site recommendations. The majority of safety and efficacy data comes from licensing studies in which vaccines are administered subcutaneously in the interscapular region (which should not be used for any injection in the clinical setting). Current research indicates that radical surgical resection of injection-site sarcomas including margins of at least 3 cm, but preferably 5 cm [EBM grade III],⁶¹ is associated with the highest response rate and long-term survival [EBM grade III].¹⁵ With this in mind, the Feline Vaccination Advisory Panel of the American Association of Feline Practitioners (AAFP) conducted an informal survey of veterinarians whose practices focused on radiation (12), surgical (36), and medical (44) oncology for opinions on what the preferred vaccination sites should be.⁶² These experts agreed that distal to the stifle, followed by distal to the elbow, were their preferred sites. Nearly as popular was the tail. Respondents frequently commented that vaccines should be administered as low on the leg as possible. They added that vaccination of cats resting in a crouched position often resulted in inadvertent injection of the skin fold of the flank, leading to tumours that were difficult to resect.⁶² This is reflected in a recent paper that found an increase in lateral abdominal injection-site sarcomas since the publication of the VAFSTF's vaccination recommendations in 1996.⁶¹

Based on these expert opinions, the AAFP now recommends in its new guidelines,⁶² consistent with the earlier (2006) guidelines,⁷⁵ that vaccines against FPV, FHV-1 and FCV should be administered below the right elbow; FeLV vaccines should be administered below the

Although many causes of inflammation are associated with FISS development, the risk seems to be higher for vaccines (particularly adjuvanted vaccines) compared with other injections.



left stifle; and rabies vaccines should be administered below the right stifle.⁶² So far, vaccination in the tail has not been considered a practical option. However, a recent pilot study demonstrated that vaccination in the tail was well tolerated and that tail-vaccinated cats developed an antibody response comparable to that observed following injection of the vaccine distally in the leg [EBM grade II].⁷⁶ Further studies are warranted to confirm whether this would be an alternative option leading to equal protection rates.

Alternative recommendations are made by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association, which recognises the practical difficulties often faced by veterinarians attempting vaccination into limbs or the tail. The advice of the VGG is that a preferred site for vaccine delivery (and surgical resection of a FISS that might arise) is the skin over the lateral abdomen. This is a procedure that appears well tolerated in the majority of cats.

As a general recommendation, recording the sites of injections in the patient's medical records is important. In addition, post-vaccination monitoring plays a vital role (see box).

Recommendations for reducing inflammatory reactions

In terms of preventing inflammatory reactions at injection sites, there are a few recommendations to follow. Cats should receive as few subcutaneous injections as possible. Intramuscular injections in cats should be avoided because intramuscular tumours develop with a similar frequency, but are more difficult to detect early. Whenever feasible, cats should receive drugs orally or intravenously. The subcutaneous injection of long-acting irritating substances (such as long-acting glucocorticoids) should be avoided.

One study examined potential risk factors when administering vaccines²⁷ and few factors

Post-vaccination monitoring

Veterinarians should instruct their clients to monitor vaccination (and other injection) sites for swelling or lumps in order to detect potential sarcomas early and at a time when they still can be removed successfully.

Practitioners and owners should follow the '3-2-1' rule.

Incisional wedge biopsies or total removal and histological examination of any mass is warranted if the mass

is still present 3 months after vaccination (and if the mass becomes larger than 2 cm in diameter, or is increasing in size 1 month after vaccination).

Practitioners and owners should follow the '3-2-1' rule.

In general, a diagnostic work-up is warranted when any cutaneous mass is noted in a cat. FISSs are usually firm, indolent, seemingly well-circumscribed, subcutaneous masses that are often not freely moveable.

were associated with the development of FISS. It was observed that the size of the needle and the syringe, the velocity of injection, and whether manual pressure was applied after injection or not, played no role. In contrast, the temperature of the vaccine made a significant difference, with cold vaccines being associated with a higher risk of FISS development than vaccines at room temperature.²⁷ Thus, vaccines should be taken out of the refrigerator about 15 minutes before injection, but not much longer, to avoid reduction in vaccinal efficacy.

If available, intranasal or oral vaccines would be preferable over injectable vaccines in cats. However, in most countries only injectable vaccines are available. Therefore, vaccines are preferred that cause the least subcutaneous inflammatory reaction. Vaccines without adjuvants should be used rather than adjuvant-containing vaccines, which means that MLV or recombinant vaccines (eg, canarypox-vectored vaccine) without adjuvant are preferred over inactivated vaccines with adjuvants.

It has been shown that recombinant canarypox-vectored vaccines cause less inflammation at the injection site. This was demonstrated in rats,⁷⁷ and in a study in cats, in which the typical granulomatous inflammation did not develop at the injection site when using these particular vaccines.⁷⁸ An extensive study investigating the subcutaneous tissue response following administration of a single dose of multi-component vaccines confirmed these findings.⁷⁹ Three groups of 15 cats were injected with one of three vaccines or saline as a negative control; cats in group A received a non-adjuvanted recombinant canarypox-vectored FeLV vac-

Recording the sites of injections in the patient's medical records is important.



cine; cats in group B received an FeLV vaccine with a lipid-based adjuvant; and cats in group C were vaccinated with an FeLV vaccine adjuvanted with an alum-Quil A mixture. On days 7, 21 and 62 post-vaccination, significantly less inflammation was associated with administration of the non-adjuvanted recombinant canarypox-vectored vaccine. The inflammation was most severe in the cats receiving the aluminium-based adjuvant. Cats receiving adjuvanted vaccines had evidence of residual adjuvant material accumulated within macrophages even at 62 days post-vaccination.⁷⁹ In a case-control study investigating associations between vaccine types and development of FISS, adjuvanted inactivated vaccines were significantly more commonly associated with sarcoma development than other vaccines; of 35 vaccinated cats with sarcoma on the hindlimb, 25 cats had received adjuvanted vaccines, seven cats had received MLV vaccines (FPV, FHV-1 and FCV), while only one cat had received a recombinant canarypox-vectored vaccine [EBM grade III].³⁵

Vaccination schedules

Finally, to prevent development of FISS, cats should be vaccinated no more than necessary. Therefore, long vaccination intervals should be applied in adult animals; vaccines (such as rabies vaccines and FPV vaccines) that are licensed for 3 year or even 4 year boosters should be preferred; no FeLV or rabies vaccinations should be administered to indoor-only cats; and immune cats should not be vaccinated (eg, if antibodies are detected). This confirms the necessity of individual vaccination schedules.

KEY POINTS

- ✦ Vaccination of cats provides essential protection and should not be stopped because of the risk of feline injection-site sarcoma (FISS).
- ✦ Vaccines are not the only injectable medical products associated with FISS.
- ✦ An individual vaccination schedule is important. Cats should be vaccinated no more than necessary, in accordance with current guidelines.
- ✦ Appropriate sites for injection should be selected. The interscapular region should generally be avoided. Vaccines should be injected at a site from which a mass can easily be surgically removed, such as distally on a leg or in the skin of the lateral abdomen.
- ✦ Vaccines should be brought to room temperature prior to administration, but should not be kept unrefrigerated for hours.
- ✦ Whenever possible, subcutaneous, rather than intramuscular, injection should be performed.
- ✦ The preference is for non-adjuvanted vaccines over those containing adjuvant, modified-live vaccines or recombinant vaccines over inactivated vaccines, and vaccines with a long duration of immunity.
- ✦ Post-vaccination monitoring should be performed. Any lump at the site of injection that is still present 3 months after vaccination, that is larger than 2 cm in diameter, or that it is increasing in size 1 month after vaccination should be surgically removed.

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Conflict of interest

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Prevention of Feline Injection-Site Sarcomas

Is There a Scientific Foundation for Vaccine Recommendations at This Time?



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KEYWORDS

• Injection-site sarcoma • Vaccines • Adverse reactions • Cat

KEY POINTS

- Authority figures have made vaccine recommendations to reduce the incidence of feline injection-site sarcomas.
- The evidence supporting these vaccine recommendations is surprisingly weak.
- Until additional research is performed, there is little evidence supporting the recommendation that use of certain vaccines will prevent sarcoma formation.

Over 25 years have passed since the initial report of vaccine-site sarcomas (FISS) appeared in the veterinary medical literature.¹ Almost from the point of recognition of these iatrogenic tumors, the veterinary medical profession and its allied professional communities have valiantly struggled to promulgate recommendations to mitigate, if not eliminate, the risks associated with vaccinations. Examples of such recommendations have included avoidance of multidose vaccine vials, distributing vaccines over different parts of the body, using vaccines less likely to induce local inflammation, restricting vaccines to cats with potential exposure to other animals with communicable diseases, and even not vaccinating at all.

One article, "Feline Injection-site sarcoma: ABCD guidelines on prevention and management"² encapsulates considerable thought to date, and perhaps even mainstream credence on strategies for treating and preventing these iatrogenic tumors, products of the veterinary medical profession's well-intentioned and largely successful attempt to eliminate the incidence of rabies and, to a lesser extent, other mostly species-specific infectious diseases in domestic cat populations. Given the widespread market penetration of vaccines in the United States, Canada, and many

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countries of Europe, together with the large number of owned cats, there are now more than 20 years of experience managing afflicted patients, providing a plethora of information about current standards of practice as well as emerging state-of-the-art therapies. The veterinary medical professional manifestly benefits from such reflection, as do owners and their feline companions.

I am less sanguine, however, that these authors' recommendations for prevention share the same evidence-based scientific standing that their management recommendations have. For there to be standing to justify recommendations there must be foundation. For there to be foundation there must be evidence; for there to be evidence there must be research. The latter presents in many forms, and I have become increasingly concerned that the findings from preliminary or tenuous research have, over time, taken on a quasi-mythical standing through a disciplinary support network that places more weight on belief than on the weight of the evidence itself. Opinion is, of course, the natural evolution of the assimilation of information, and is the provenance of assertions by decision makers occupying positions of leadership, influence, and change. In the proper setting, and in the appropriate context, such expressions contribute to a healthy exchange and dialogue (eg, the Vaccine-Associated Feline Sarcoma Task Force).³ For an article focusing on prevention of this disease in a peer-reviewed scientific journal, far more circumspection is not only warranted, but arguably essential. In this article, I hope to underscore this contention by illustrating that not only do I judge that such recommendations are premature (although not necessarily incorrect), but that others absorbing the same body of evidence could be impelled to reach entirely different conclusions.

The key statement in that article, and hence the most provocative, is the following from the abstract: "Non-adjuvanted, modified-live or recombinant vaccines should be selected in preference to adjuvanted vaccines." This is manifestly similar to a principle expressed in the World Small Animal Veterinary Association's (WSAVA) Guidelines for the Vaccination of Dog and Cats⁴: "Non-adjuvanted vaccines should be administered to cats wherever possible." Indeed, the WSAVA⁴ and Hartmann and colleagues² articles share authors in common. However, these prescriptions go well beyond the recommendations of the 2013 American Association of Feline Practitioners Advisory Panel Report, which judiciously exercised considerably more restraint in writing: "Overall, however, the Advisory Panel concluded that, at the current time, there is insufficient information to make definitive recommendations to use particular vaccine types to reduce the risk of FISS [feline injection-site sarcomas]."⁵

What is the evidence to support the Hartmann and colleagues² recommendation, as indicated in the abstract and on page 611: "Vaccines without adjuvants should be used rather than adjuvant-containing vaccines, which means that MLV or recombinant vaccines (eg, canarypox-vectored vaccine) without adjuvant are preferred over inactivated vaccines with adjuvants?" The section "Recommendations for reducing inflammatory reactions" (pages 610–611) provides some guidance. Three articles cited found that recombinant canarypox-vectored vaccines caused less inflammation when injected into rats and cats.^{6–8}

The use of such experimental studies to measure postvaccinal tissue inflammation is enigmatic and can be faulted on several grounds. Using rodents as models of adjuvant-induced inflammation or carcinogenesis in the cat remains notional, and its validity has previously been called into question.⁹ Given the near-certain differences between species in immunologic and tissue-based responses to vaccine adjuvants, it should be difficult to ascribe more than a passing interest in these results. As for the use of cats in experimental studies, the goal should not be to measure relative

inflammatory responses, which would inevitably be expected under different vaccine formulations, but rather to estimate neoplastic incidence. None of these experimental studies, however, had anything close to the statistical power necessary to detect differences in vaccine risk. Suppose, for example, that the incidence of sarcomas following vaccination is 5 cases in 10,000 cat-doses, and the incidence in the absence of vaccines is 1 case in 10,000 cat-doses (ie, the relative risk is 5). A prospective 2-armed randomized study analyzed with a 2-sided Pearson's chi-square test, with equal allocation between arms and Type I and Type II error proportions of 5%, would require almost 100,000 cats. In contrast, the experimental studies in rats and cats cited previously had sample sizes in the double-digits.

Although they employed different methods to arrive at their respective conclusions, the experimental studies, not unexpectedly, shared a key critical feature: none of the study subjects developed injection site sarcomas. Although their findings may have implications for the study of postvaccinal inflammatory responses, they fail to provide a rational basis for making causal inferences about vaccine propensity to induce tumorigenesis. Such a causal connection between postvaccinal inflammation and tumorigenesis remains to this day one entirely of conjecture, speculation, and hypothesis, and until that connection can be firmly established, such articles may be useful in understanding vaccine-specific inflammation, but have unproven and hence questionable value in understanding vaccine formulation-specific risk of sarcoma development. They and others (eg, the vaccine manufacturer-funded experimental Grosenbaugh and colleagues¹⁰ study) emphatically do not rise to the level of research upon which policy supported by science about reducing the incidence of injection-site sarcomas should be promulgated and distributed.

Nevertheless, this has not prevented several authors from doing exactly that,¹¹⁻¹⁴ a practice that at this time I consider to be imprudent. Of considerable concern is that these articles include unpublished data, personal communications, citations of review articles, reliance on science by authority, or engagement in associational speculation. It is also sometimes the case that authors have financial ties, as collaborators, consultants, or employees, to the very industries that are impacted by their recommendations. It must be incumbent on all authors (and presenters) in the field of FISS to fully disclose such relationships to their readers (and audiences) to further transparency and scientific integrity.

The final article the authors invoked to support their recommendation is from my own research group at the University of California, Davis.¹⁵ And although this study did include cats with injection-site sarcomas, and indeed found supportive evidence for differential tumorigenic propensity between vaccine types, I would firmly contend that it alone (discounting the previously mentioned articles about inflammation but not sarcomas) remains insufficient to this day to be the basis for the recommendations in Hartmann and colleagues². In fact, we attempted to temper our findings within the article itself by citing the study's shortcomings that could pose a threat to validity:

- A low response rate from veterinarians, which could have been differential with respect to the types of vaccines administered

- A small sample size, which makes a single study far more susceptible to biased and imprecise estimates

- Missing data

- The use of multiple vaccines at the same site either at one or multiple times

The tendency to report a single number or conclusion from a single study is unfortunately all too common, and I have witnessed individual odds ratios (ORs) from this study presented outside of their proper statistical context. For example, Srivastav

and colleagues¹⁵ reported that "... there was evidence of a significantly lower frequency of use of recombinant rabies vaccines in case cats than controls. Using cats with nonvaccine site sarcomas as controls, in years 1, 2, and 3, the ORs were 0.1 (95% CI, 0.0–0.7; $P = .014$), 0.1 (95% CI, 0.0–0.4; $P = .001$), and 0.1 (95% CI, 0.0–0.6; $P = .005$), respectively." At a recent international meeting, I heard these findings communicated as: the odds of cases receiving a nonrecombinant vaccine was tenfold greater than receiving a recombinant vaccine (ie, $1/0.1 = 10$). Although literally correct in an algebraic sense, such statements entirely ignore a key purpose of statistical inference in the first place: the analysis of variance. Focusing solely on point estimates fails to convey important information (eg, an OR of 10 from a sample size of 50 should naturally be given far less credibility than an odds ratio of 10 from a sample size of 500). A more recent presentation at least pointed out that the CI around the communicated value of 10 would have been (using, for example, the year 2 value) 2.5 to infinity.¹⁶ But the real story about the quantitative relationship between vaccine type and sarcoma incidence, which from this article is profoundly imprecise, is how little we still really understand even after this study's publication. Moreover, it is often unappreciated that the P -values associated with such tests are only correct insofar as the assumptions underlying them are accurate, including the absence of bias, under the probability distribution model utilized in the analysis. In other words, if any of the biases noted previously were present, then regardless of the statistical significance, the P -values would be incorrect, as would the point estimates (ORs) and CIs. It is a scientific disservice to recapitulate potentially headline-grabbing findings from articles without concomitantly and fully assessing and disclosing those features that could adversely impact study accuracy. Far too often excessive credence is placed on statistical significance, and far too little weight on the myriad subtleties of observational study design and analysis that can lead to invalid inferences:

- Errors of comparisons (confounding bias)
- Errors in selection of study subjects (selection bias)
- Errors in (often historical) measurements (information bias)
- Errors in statistical modeling (specification bias)

In the case of the Srivastav and colleagues article,¹⁵ such threats to validity could include (but are not limited to):

- The low veterinarian participation rate (eg, participation could be related to veterinarian preference for vaccine type)
- Diagnostic work-up being related to type of vaccine administered
- Missing data that could have been related to type of vaccine administered

I contend that the authors' avidity for their prevention recommendations² exceeds the weight of foundational scientific evidence to support them at this time. Although they apparently consider them to be accurate, and they may be correct, the insertion of such recommendations into such an authoritative report strikes me as premature, fails to convey the paucity of evidence supporting them, and omits a critical analysis of research upon which they are based. It is evocative of the admonitions of author/journalist Christopher Hitchens: "Forgotten were the elementary rules of logic, that extraordinary claims require extraordinary evidence and that what can be asserted without evidence can also be dismissed without evidence."¹⁷ A similar forewarning about the shortcomings of published research was forcefully made by Ioannides,¹⁸ who wrote: "Several methodologists have pointed out that the high rate of nonreplication (lack of confirmation) of research discoveries is a consequence of the convenient, yet ill-founded strategy of claiming conclusive research findings solely based on a

single study assessed by formal statistical significance, typically for a *P*-value less than 0.05." All too often, human medicine has been forced to disavow widely disseminated public health recommendations founded on nonexperimental studies when later, more robust evidence failed to support their establishment.¹⁹ I only hope that history does not repeat itself here.

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