

## VACCINE ASSOCIATED SARCOMAS IN CATS: EPIDEMIOLOGICAL AND HISTOPATHOLOGICAL FEATURES SARCOAMELE POSTVACCINALE LA PISICĂ: ASPECTE EPIDEMIOLOGICE ȘI HISTOPATOLOGICE

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### ABSTRACT | REZUMAT

Recently, postvaccinal sarcomas received an increased consideration from the veterinarians because the number of cats who suffer from this type of disorder is increasingly growing. A number of studies are dealing with the mechanisms that lead to the emergence of postvaccinal sarcomas. The aim of this study is to assess the incidence of the postvaccinal sarcomas in cats, in a period of 10 years (June 2004-June 2015) in the Department of Pathological Anatomy from Faculty of Veterinary Medicine Cluj-Napoca (Romania). This study describes aspects regarding the anatomic distribution of the postvaccinal sarcomas, their diagnostic mode and the histological types of these sarcomas. The assessed material consisted of corpses brought for necropsy examination and of feline biopsies brought for histopathology examination. The histological sections were stained with Hematoxylin-Eosin and Tricrom Masson methods. Histopathological examination was performed using Olympus BX51 microscope connected to a digital camera Olympus DP-25. The postvaccinal sarcomas occurred mostly in the European catbreed. The mean age of cats diagnosed with this condition was 7.91 years with limits ranging from 4 to 15 years. In the present study, PVSC (postvaccinal sarcomas in cats) was diagnosed more frequently in male subjects, and the most affected region was the supra scapular region, followed by the lumbar region. Of all postvaccinal sarcomas identified during the above mentioned time frame, around 33% of cases were diagnosed with postvaccinal fibrosarcoma, 27% of cases were diagnosed with osteosarcoma, some other histological types being diagnosed as well (e.g., rhabdomyosarcomas, sarcomas, a leiomyosarcoma and one malignant fibrous histiocytomas).

**Keywords:** vaccine-associated sarcoma, fibrosarcoma, vaccination, cat

Sarcoamele postvaccinale au primit tot mai multă atenție din partea medicilor veterinari, deoarece numărul pisicilor care suferă de această boală este în continuă creștere. Pe plan mondial există o serie de studii care au în vedere investigarea mecanismelor care duc la apariția sarcoamelor postvaccinale la pisică. Scopul studiului de față este acela de a evalua incidența sarcomului postvaccinal de la pisică pe o perioadă de 10 ani (iunie 2004-iunie 2015) la Disciplina de Anatomie Patologică de la Facultatea de Medicină Veterinară Cluj-Napoca (România). Acest studiu descrie aspecte privind distribuția anatomică, modul de diagnosticare și respectiv tipurile histologice de sarcom postvaccinal. Materialul luat în studiu a fost reprezentat fie de cadavre de pisică aduse pentru examen necropsic, fie de biopsii tumorale prelevate de la această specie în vederea realizării examenului histopatologic. Secțiunile histologice au fost colorate prin metodele clasice (hematoxilina-eozină și tricrom Masson). Examinarea histopatologică s-a realizat cu ajutorul microscopului Olympus BX51 conectat la camera foto digitală Olympus DP-25. Sarcomul postvaccinal a fost diagnosticat mai frecvent la subiecții din rasa Europeană. Vârsta medie de apariție a tumorii a fost de 7.91 ani, cu limite de la 4 ani la 15 ani. În studiul de față, sarcomul postvaccinal a fost mai frecvent diagnosticat la subiecții de sex masculin, iar zona cea mai afectată a fost regiunea suprascapulară, urmată de regiunea dorso-lombară. Din totalul sarcoamelor postvaccinale identificate în perioada menționată, în jur de 33% din cazuri au fost fibrosarcoame, 27% din cazuri au fost osteosarcoame, fiind diagnosticate și alte tipuri histologice de sarcoame postvaccinale (rabdomyosarcoame, sarcoame, un leiomyosarcom și un histiocitom fibros malign).

**Cuvinte cheie:** sarcom postvaccinal, fibrosarcom, vaccinare, pisică

Lately, postvaccinal sarcomas received more attention from veterinarians due to the increasing number of cats that were diagnosed with the disease.

Due to this fact, research groups were formed at a worldwide level, groups that study the vaccine associated sarcoma and its risk factors. At first, it was thought that only rabies vaccines, leukemic vaccines, and vaccines that contain aluminum induce sarcomas, but due to the occurrence of the sarcomas at interscapular level, recent research proved that both vaccines that contain adjuvants and the ones that

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don't contain adjuvants induce vaccine associated sarcomas in cats (VASC). It must be taken into consideration that differentiating between cutaneous sarcomas and vaccine associated sarcomas is often hard to be done, thus investigating the vaccine associated sarcomas may be sometimes a difficult task (1).

Pathologists Hendrick and Goldschmidt observed in 1980 a high incidence of fibrosarcomas in cats. The high incidence of vaccine associated sarcomas was associated with major events, like the utilization of aluminum in the rabies and leukemic vaccines in 1987 and the introduction of subcutaneous vaccines in cats.

The second event was in 1987 when Pennsylvania adopted the mandatory law for rabies vaccination for cats', therefore increasing the risk of developing vaccine associated sarcomas due to increasing vaccination (7, 2). The time of occurrence of the tumors varies between 1 month and 10 years after the administration of the vaccine. Due to this reason, in 1996, Vaccine-Associated Feline Sarcoma Task Force (VAFSTF) was formed and created the vaccination schemes that are used in the present day. Today, veterinarians weight between the prevention of infectious diseases and the risk of sarcomas occurrence, therefore vaccines recommendations are made individual for each animal (2).

Clinically, these tumors are manifested through local invasion. Subcutaneous tumors are not encapsulated like the sarcomas that occur in humans, therefore the relapse rate is higher after a conservatory surgery (60-70%) and radiotherapy is also recommended after such a surgery (3). Despite the histological aggressiveness of the tumors, the metastasis rate is very low, with less than 20% of tumor presenting metastases. The low rate of metastasis may be due to the fact that a lot of cats are early euthanized instead of dying from natural causes, therefore the metastasis rate it's subjective (3).

**Etiopathogenesis of PVSC:** for the postvaccinal sarcoma etiopathogenesis several theories were elaborated, among which the syringe reuse, gauge needle, and vaccine temperature at administration, which might favor cancer occurrence. Also, specific factors of vaccines were suspected due to the fact that local reactions were observed in 80-100% cats that were vaccinated with inactive rabies vaccine, FeLV vaccine containing aluminum, but also vaccines without adjuvants. Using Electron probe X-ray microanalysis, aluminum was found in cats that had vaccine associated sarcomas. Aluminum can be found as aluminum hydroxide or aluminum phosphate and may be a mar-

ker of a previous vaccination or it can have an important role in carcinogenesis (11, 12, 13, 16).

One theory related to this subject is that sarcomas occur due to exaggerated inflammations associated with the components of vaccines present at the site of inoculation. The reactions created lead to a fibroblasts and myofibroblasts uncontrolled proliferation that may result in malignant transformation in some cases. Similar reactions were observed in cats that developed intraocular sarcomas after suffering an ocular trauma or a chronic uveitis.

The relation between trauma, inflammation and healing are unique processes and the cellular and molecular events responsible for the carcinogenesis are intensely studied in cats (4, 5, 16).

The aim of this study is to evaluate the incidence of vaccine associated sarcomas in cats over a period of 10 years in the Pathological Anatomy Department from Faculty of Veterinary Medicine (FVM) Cluj-Napoca (Romania), and to describe aspects related to their anatomic distribution, the diagnosis and the histological features of vaccine associated sarcomas.

## MATERIAL AND METHODS

The assessed material was made of cat corpses brought for necropsy and of feline tumor biopsies for histopathological examination.

The considered aspects were: anatomical location of the tumor, case history, age, breed, histological and macroscopic aspects, dimension and consistency of the tumor, sectional appearance of the tumor. The collected fragments were immersed immediately after collection in 10% buffered formalin. The fixation was performed at room's temperature and the keeping period was 3 days, with changing the fixator at 3, 24 and 48 hours. The following processing consisted in dehydration with ethanol, clarifying with butyric alcohol, and the inclusion in paraffin.

The prepared tissues were cut at microtome (Leica microtome), the sections were made at a thickness of 5 micrometers, they were extended with hot distilled water (on a hot plate), and bonded on the port blades with Mayer glycerinated albumin.

After standing for 12 hours at 40 degrees thermostat for proper adherence to port blades, the sections were stained with Hematoxylin-Eosin and Trichromic Masson. Histopathological examination was performed using Olympus BX51 microscope connected to a digital camera Olympus DP-25 (in order to acquire histopathological images).

## RESULTS AND DISCUSSION

Between June 2004 and June 2015, in the Pathological Anatomy Department of FVM Cluj-Napoca, 176 cats were diagnosed with tumor formations, out of which 18 (10.23%; Fig. 1) had vaccine associated sarcomas of different histological types. It was to be mentioned that in the time period taken into account for the study, in the Pathological Anatomy Department, a number of 617 cats were examined, indicating that VASC represents 2.92% of all cases (Fig.2). The breed most affected by this neoplastic lesion was the European breed, only one cat being

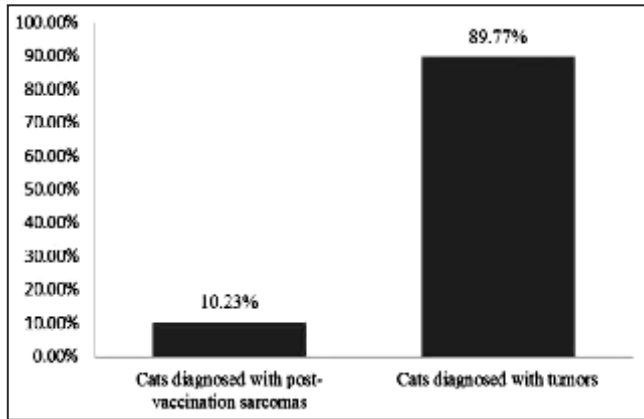
from the Russian Blue breed. The average age of tumor occurrence was 7.91 years with limits from 4 to 15 years. Ladlow (2015) argues that vaccine associated sarcomas usually occur in younger cats, with a first peak of occurrence between 6-7 and the second peak between 10-11 years old. Of the 18 cases (Table 1), a total of 9 cats were male (50% of all cases) and 4 were female (22.22%), the remaining cases (27.77%) did not have their sex specified in the records. As can be seen, males are more commonly affected by sarcoma after vaccination as compared to females. In most cases, sarcoma was diagnosed in the over scapular – inter-scapular – withers region (50% cases), but there

**Table 1**

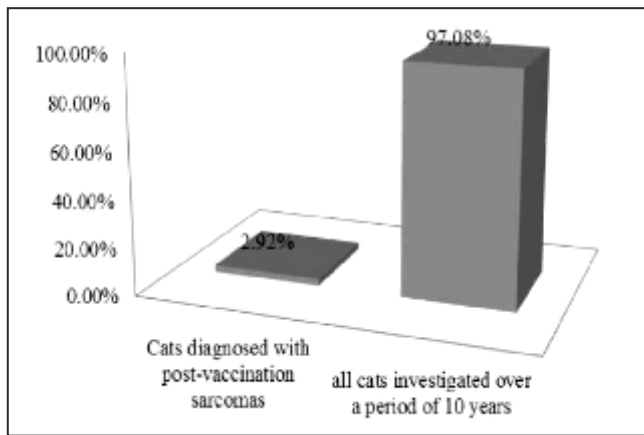
**PVSC cases between June 2004 and June 2015**

No crt	Nr. register	Breed	Age	Sex	Histopathological diagnosis	Anatomic location	Metastasis and recurrence
1	78368	European	8 years	F	Postvaccinal fibrosarcoma	Middle right chest	Relapse after one year as the withers and right chest
2	82395	European	10 years	-	Postvaccinal fibrosarcoma	The withers region	-
3	86410	European	4.5 years	M	Postvaccinal fibrosarcoma	Interscapular area	Relapsed after 3 months
4	84391	European	-	-	Postvaccinal fibrosarcoma	Lateral neck region	-
5	85060	European	7 years	M	Postvaccinal fibrosarcoma	Right hip area	-
6	86256	European	9 years	-	Postvaccinal fibrosarcoma	Dorsal region	-
7	81978	European	11 years	M	Postvaccinal osteosarcoma	The neck region	-
8	84311	European	9 years	-	Postvaccinal osteosarcoma	Rear member - left thigh	Pulmonary and hepatic metastasis
9	84766	European	10 years	M	Postvaccinal osteosarcoma	Scapulo-humeral area	Metastases to the neck muscles, lung, renal, mesenteric, orbit
10	86180	European	8 years	M	Postvaccinal osteosarcoma	Dorsal area (lumbar)	-
11	82236	European	15 years	M	Postvaccinal osteosarcoma	Scapular-humeral region	-
12	78366	European	5 years	F	Postvaccinal rhabdomyosarcom	Withers area	-
13	82181	European	8 years	F	Postvaccinal rhabdomyosarcom	Hindlimb right - thigh	-
14	87075	Russian Blue	6 years	F	Postvaccinal sarcoma	Interscapular region	-
15	87247	European	12 years	M	Postvaccinal sarcoma	Suprascapular region	-
16	84307	European	8 years	M	Postvaccinal sarcoma	Cervical region	Relapsed after 3 months
17	84131	European	8 years	-	Postvaccinal leiomyosarcoma	Dorsal cervical area	-
18	87436	European	4 years	M	Malignant fibrous histiocytoma	Loin area	Relapsed after 3 months

are other sites of this tumor such as: the cervical (i.e., dorsal side; Fig. 3, 5, 6) and dorsolateral regions (in 22.22% of cases), the lumbar region (in 27.77% of cases). Some other relatively atypical locations for this type of sarcoma were also involved, such as croup region and the middle third of the hip and chest.



**Fig. 1.** The prevalence of total neoplastic lesions of cats with postvaccinal sarcomas



**Fig. 2.** The prevalence of postvaccinal sarcoma of all cats investigated

It is known that VASC occurs more frequently in the area elected for vaccination, namely the over scapular area (Fig. 3), but some veterinarians choose other parts of the body for the vaccination, such as: the sides of the thorax region, the cervical area, the lumbar area, etc. These aspects were presented in other bibliographic reports (10, 18, 19). Furthermore, some authors suggest that sarcomas may occur as a result of the injection of other types of biological materials (not necessarily vaccines) such as antibiotics with sustained release or with corticosteroids. Thus, postvaccinal sarcomas were renamed as feline *injection site sarcomas (ISS)* since these may occur after injection of a variety of drugs, including antibiotics,

corticosteroids with extended release and chronically administered, microchips, insulin. Also, cases of PVSC as reaction to the non-absorbable sutures were cited in the literature (10, 18, 19).



**Fig. 3.** Macroscopic appearance of vaccination sarcoma



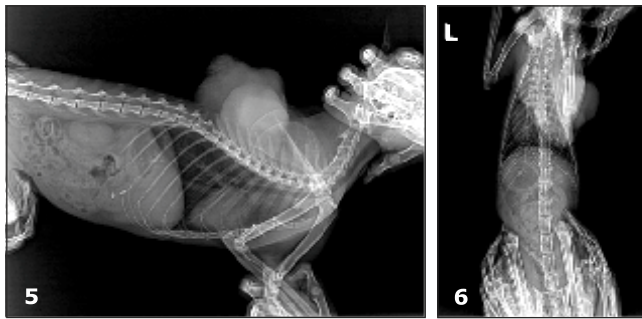
**Fig. 4.** Appearance on the section of vaccination sarcoma; presence of intratumoral necrosis and hemorrhage

Of the total number of vaccine associated sarcomas identified during that period, 6 cases were diagnosed with fibrosarcoma after vaccination (33.33%), 5 subjects were diagnosed with osteosarcoma (representing 27.77%), and other histological types were identified, as follows: 3 sarcomas (16.66%), 2 rhabdomyosarcoma (11.11%), 1 leiomyosarcoma and 1 malignant fibrous histiocytoma (each representing 5.55%). Regarding histology, one third of VASC were fibrosarcomas (33.33%), but another frequent histological type was osteosarcoma (27.77%).

The latter was manifested by the ability of tumo-

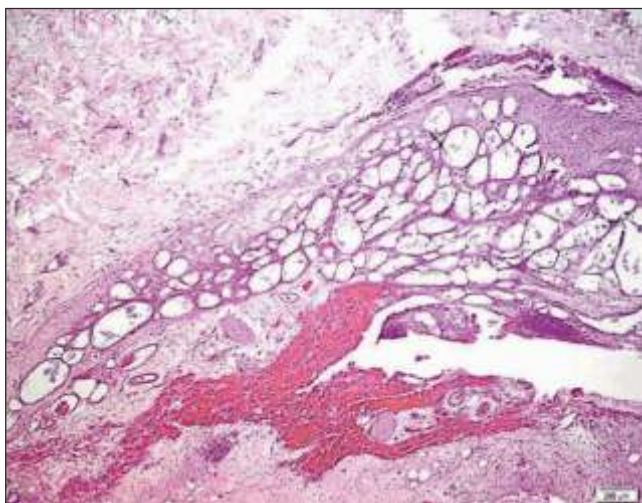


ral osteoblasts to produce varying amounts of partially mineralized or poorly mineralized osteoid in tumor mass. Hendrick and Brooks (6) diagnosed multiple tumors that are morphologically identical with vaccine associated fibrosarcomas and malignant fibrous histiocytomas (with few exceptions, where the tumor exhibits intratumoral bone structures suggesting an osteosarcoma). The authors noted that in one case, the tumor presented round or polygonal cells arranged in multilayer structures often limited by incomplete large structures, which suggest a cartilaginous tissue. As a result, the tumor was diagnosed as chondrosarcoma. They state that, the diagnosis of chondrosarcoma or osteosarcoma in an area with chronic inflammation should not be considered a surprising fact. Such progressive metaplasia to cartilage and bone structures has been described in areas with chronic irritation or in areas that have suffered previous trauma (6).



**Fig. 5.** Lateral-lateral radiographic appearance

**Fig. 6.** Dorsolateral radiographic appearance (L-Left)



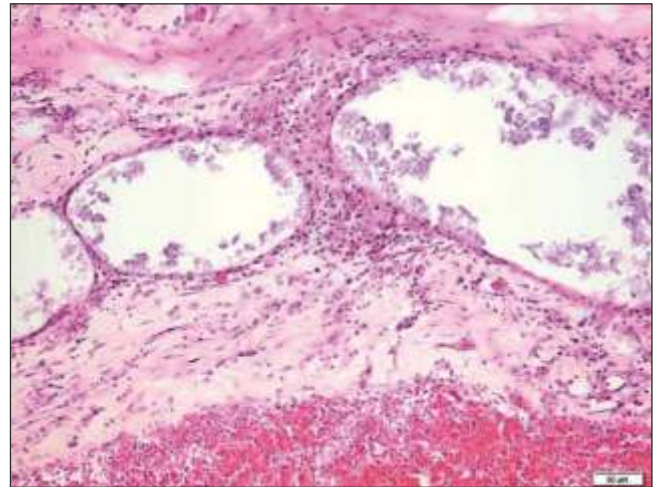
**Fig. 7.** The presence of abundant amorphous material on the periphery of tumors, intratumoral hemorrhage

Another PVSC histological type is rhabdomyosarcoma (11.11%) and leiomyosarcoma (5.55%).

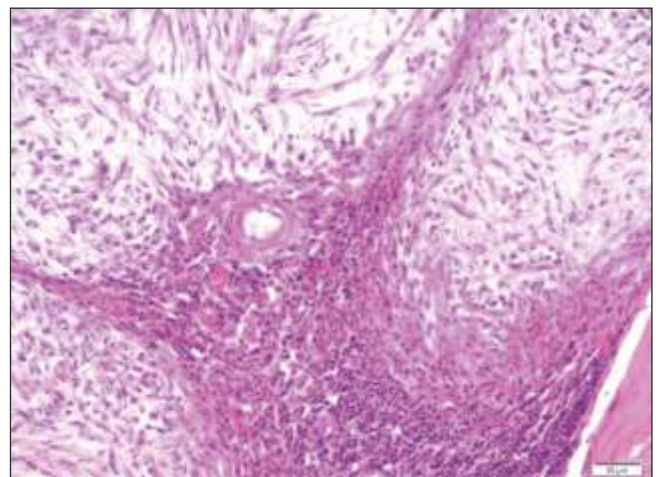
These tumors are rarely diagnosed in practical

veterinary oncology, but some authors mention and describe these types of sarcoma as PVSC.

Similarly, Hendrick and Brooks (1994) described two tumors that were very different from the rest of the tumors after vaccination, being made up of pockets of round cells with round nuclei and large cytoplasm with acidophil color, resembling with rhabdomyoblasts (hence the diagnosis of rhabdomyosarcoma at vaccination) (6).



**Fig. 8.** Abundant amorphous material with abundant macrophage reaction



**Fig. 9.** Sarcoma with poorly differentiated plasma cells and intratumoral lymphohistiocytic inflammation

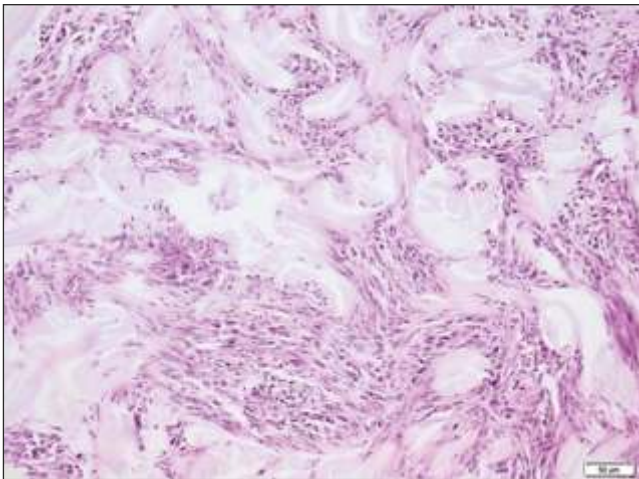
Briefly, histologic aspects that advocate for the vaccine associated sarcoma are:

- the presence of inflammation mainly at the periphery of the tumoral mass, finding quite frequently intratumoral inflammatory cells; leukocyte infiltration was often represented by lymphocyte-histiocytes, plasma cells and macrophages (Fig. 7, 8, 9, 10);
- identification of macrophages with foamy cyto-

plasm, often these are located around the hollow structures with a basophilic, finely granular material (perhaps the origin of the substance inoculated in the subcutaneous tissue by injection) (Fig. 8);

- the existence of histiocyte cells in certain sarcomatous tumoral lesions, cells that are encountered in malignant fibrous histiocytoma;

- detection of numerous foci of necrosis in the tumoral mass, aspect that is otherwise less common in most sarcomas, due to high degree of vascularization of these tumors; often these tumors showing necrotic foci may experience skin necrosis (Fig. 4).



**Fig. 10.** Fibrosarcoma invasion grade increased at tumor periphery

Similar to histological details presented above, Macy (2013) (30) and Madewell (2001) identified the following histological aspects: inflammation with lymphocytes, plasma and giant cells; the presence of a basophilic intracellular material, and the existence of some differentiated myofibroblasts and fibroblasts in a number of vaccine associated sarcomas (8).

McNeil (2001) mentions that in VASC, granulomatous inflammation often occurs at the periphery of the tumor, the center of tumor is often with necrosis; moreover, the author states that all tumors presented anaplasia and increased aggressiveness. As in the case of tumors in our study (Fig. 8), McNeil (2001) mentions that in most of the tumors it was observed a foreign material which was fine-granular to crystalline in tumor mass, this material having apparently in composition aluminum (3). Hendrick and Brooks (1994) indicate that most tumors after vaccination present: spindle cells, multi-nucleated giant cells, a variable number of polygonal cells or histiocyte cells. Most sarcomas are partially surrounded and infiltrated by lymphocytes and macrophages (6).

Axiak and Ladlow states that the inflammatory reaction, appeared after the injection, is due to the release of cytokines and growth factors (such as growth factor derived from platelets), thus resulting in the uncontrolled proliferation of fibroblasts and myofibroblasts, resulting to malignant transformation.

## CONCLUSIONS

1. Postvaccinal sarcoma is a neoplastic lesion frequently diagnosed in cats. In our study it was found in about 10% of all cases diagnosed with tumors (in the time period of 2004-2015).

2. Histopathological types of VASC most often diagnosed in the Pathological Anatomy Department (FVM Cluj-Napoca, Romania) were fibrosarcoma and osteosarcoma.

3. The most affected body regions were the over-scapular followed by the lumbar region.

4. The average age of the occurrence of post-vaccinal sarcoma was about 8 years.

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