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Cytopathology of cutaneous and subcutaneous neoplasms in feline species: a retrospective study

Citopatologia das neoplasias cutâneas e subcutâneas na espécie felina: estudo retrospectivo

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ABSTRACT: It is estimated that a quarter of all feline neoplasms affect the integumentary system. Cytological examination is of admirable importance for detection of neoplasms in companion animals, being considered a simple and non-invasive method. To date, there are limited information regarding the prevalence of feline skin cancer as well as the use of cytopathology as a diagnostic tool. In this sense, the study aimed to characterize cutaneous and subcutaneous tumors in 192 cats from the archives of a private clinic in São Paulo-SP, Brazil, specialized in the care of felines. Data regarding the animal (breed definition, sex, and age group) and tumor characteristics (biological behavior, cytomorphological category of neoplasm, and cytological suggestion for neoplasm) were obtained in a five-year period. A 95% confidence interval (CI) was estimated to verify the statistical difference between the animal and tumor characteristics. The chi-square test was carried out to verify the association between the tumor biological behavior and animal variables, as well as other tumor characteristics. Most cats were mixed breed and aged 11 to 15 years. Malignant tumors were predominant. A statistical association was observed between cytomorphological category of neoplasm and biological behavior ($p<0.001$). Mesenchymal (24.5%) and epithelial tumors (25%) presented the highest frequency among those benign and malignant, respectively. The cytological suggestion for neoplasm was also statistically associated with biological behavior ($p<0.001$). The highest percentage of occurrence of benign and malignant tumors consisted of lipomas (22.9%) and sarcomas (19.3%), respectively.

KEYWORDS: Cytological examination; domestic cats; oncology; dermatology.

RESUMO: Estima-se que um quarto de todas as neoplasias felinas afetam o sistema tegumentar. O exame citológico é importante para detecção de neoplasias em animais de companhia, sendo considerado um método simples e não invasivo. Até o momento, há informações limitadas sobre a prevalência das neoplasias cutâneas felinas, bem como o uso da citopatologia como ferramenta diagnóstica. Nesse sentido, o estudo teve como objetivo caracterizar neoplasias cutâneas e subcutâneas em 192 gatos do arquivo de uma clínica veterinária privada da cidade de São Paulo-SP, Brasil, especializada no atendimento de felinos. Dados referentes ao animal (raça, sexo e faixa etária) e características das neoplasias (comportamento biológico, categoria citomorfológica e sugestão citológica) foram obtidos em um período de cinco anos. Foi estimado um intervalo de confiança (IC) de 95% para verificar a diferença estatística entre as características do animal e da neoplasia. O teste do qui-quadrado foi realizado para verificar a associação entre o comportamento biológico da neoplasia e as variáveis dos animais, além de outras características neoplásicas. A maioria dos gatos era sem definição racial e com idade entre 11 e 15 anos. As neoplasias malignas foram predominantes. Foi observada associação estatística entre a categoria citomorfológica e o comportamento biológico da neoplasia ($p<0,001$). Neoplasias mesenquimais (24,5%) e epiteliais (25%) apresentaram a maior frequência entre os benignos e malignos, respectivamente. A sugestão citológica para neoplasia também foi estatisticamente associada ao comportamento biológico ($p<0,001$). O maior percentual de ocorrência de tumores benignos e malignos consistiu em lipomas (22,9%) e sarcomas (19,3%), respectivamente.

PALAVRAS-CHAVE: Exame citológico; gato doméstico; oncologia; dermatologia.

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INTRODUCTION

Skin is the most common anatomical region for the occurrence of tumors in felines. The integument is composed of different layers and structures subject to risks of neoplastic transformation, thus reflecting on a variety of cutaneous tumors. The skin is particularly susceptible to a variety of external damage, as it is the largest and most exposed organ in the body (DALECK; DE NARDI, 2016; HO; SMITH; DOBROMYLSKYJ, 2018). Moreover, the increased life expectancy of domestic cats due to the evolution of management, diagnosis, and treatment techniques has led to an increase in the incidence of chronic diseases, such as tumors (PRIEBE et al., 2011; CHALITA, 2020). A quarter of all feline tumors is estimated to affect the integumentary system (SHELLY, 2003). However, the occurrence of skin tumors in domestic cats can reach 41 to 56% of the total number of tumors in this species (PRIEBE et al., 2011; HO; SMITH; DOBROMYLSKYJ, 2018; MANUALI et al., 2020).

The early observation of cutaneous lesions, which are externally perceptible solid formations, easily visible and palpated both by the owner and by the veterinarian, enables higher efficiency in the control and treatment of these conditions. Premature diagnosis is directly related to a favorable prognosis. Cytological evaluation of lesions, even if small, can contribute to prolonging the patient life, as there is a greater chance of cure when a malignant neoplasm is diagnosed early (HO; SMITH; DOBROMYLSKYJ, 2018; CHALITA, 2020). The cytological examination is mainly intended for the previous diagnosis of tumors and consists of the morphological study of free or isolated cells, showing a high diagnostic value (ROSSETO et al., 2009).

The cytological examination is of paramount importance among the means of investigation of tumors in companion animals, as it is relatively simple and less invasive, minimizing the risk of complications. It has an excellent attractive cost-benefit ratio, being considered an affordable approach. Also, cytology may be the main or even the only basis for the adoption of crucial medical procedures in financial situations that limit additional diagnostic procedures (SHARKEY; WELLMAN, 2011). It is a method whose material is easy to obtain and requires minimal or no physical or chemical restraint. It emerges with more and more frequent use for the detection of papulonodular or tumoral lesions, as it provides speed in determining the result, facilitating timely clinical decisions and becoming an extension of the physical examination (ROSSETO et al., 2009; SHARKEY; WELLMAN, 2011; ROSOLEM et al., 2013; DALECK; DE NARDI, 2016). It is also useful as a screening resource in the distinction between neoplastic and non- neoplastic processes (ROSOLEM et al., 2013).

Epidemiological studies to determine the prevalence of cutaneous tumors in felines, based on a cytological examination, have high relevance. This information is important for the treatment and prevention of tumors, assisting veterinarians in the diagnosis of oncodermatoses in felines. However, little is known about the real prevalence of cutaneous tumors in felines involving cytopathology, as few studies have used this model for this species (PRIEBE et al., 2011; HO; SMITH; DOBROMYLSKYJ, 2018). Little relevant information on this subject is available in the national and international literature. Thus, this study aimed to systematize the data collected from the archives of a private clinic in São Paulo, Brazil, a reference in specialized care in feline medicine, to identify the most expressive cutaneous and subcutaneous tumors in cats through cytological diagnosis.

MATERIAL AND METHODS

The information was obtained of the Veterinary Clinic Vetmasters (private clinic, specialized in the care of felines, São Paulo, Brazil). From the archived database of that clinic, cytological reports of skin tumors of 192 felines were obtained. The study corresponded to a retrospective evaluation from January 2014 to December 2018. Data regarding the characteristics of the animals (breed, sex, and age group) and anatomical location of the tumor and its characteristics (biological behavior, cytomorphological category of neoplasm and cytological suggestion for neoplasm) were obtained. The collection techniques were carried out mainly by non-aspirating puncture or fine-needle aspiration puncture and affixing or decaling (printing). The Romanowsky staining was used (Rosenfeld or May-Grünwald and Giemsa).

A descriptive analysis, with a simple and relative frequency estimate, considering the characteristics of animals and tumors separately, was carried out. A 95% confidence interval (CI) was estimated for the perception of statistical difference between the simple frequencies of animal and tumor characteristics. The interpretation of the statistically significant difference was performed by the absence of a cross between the confidence intervals. Subsequently, the chi-square test was performed to verify the association between the neoplastic biological behavior and animal variables, as well as other tumor characteristics. The tests were considered significant when $p<0.05$. The analyses were performed using SPSS 21.0 (IBM, 2012).

RESULTS AND DISCUSSION

The table 1 shows the patient variables (breed definition, sex, and age group). The statistically significant majority of the individuals were mixed breed, with a percentage frequency of 74.5% (95% CI: 67.2–80.3%). There was no statistically significant difference in terms of breed or sex. Age group showed a predominance for animals aged 11 to 15 years (52.1%), considering the statistical significance (95% CI: 44.7–58.4%).

The growing raising of mixed-breed cats could justify its preponderance for the study in question. In this sense, studies that sought to correlate the predisposition of feline breeds with the occurrence of cutaneous tumors are always hampered by the hegemony of mixed-breed cats, with little representation

of purebred animals in the feline population (HO; SMITH; DOBROMYLSKYJ, 2018). Manuali et al. (2020) also realized that mixed-breed cats in European countries were the most predisposed to neoplastic processes because of their common and hegemonic breeding. In the present study, the predominant breed was the Persian. Moreover, the relative homogeneity between the number of males and females explained the absence of statistical difference for sex. Similarly, Rossetto et al. (2009) observed that the proportion between males and females of canines affected by cutaneous tumors was similar (51 and 49%, respectively), with no significant disagreement. According to Ho; Smith; Dobromylskyj (2018), the median age of cats at the time of diagnosis of cutaneous tumors was 11 years. Most of the neoplastic processes occur in older animals, probably due to the prolonged exposure to carcinogenic agents and the decrease in the immune response inherent to aging (VENTURA; COLODEL; ROCHA, 2012). These studies revealed agreement and also justified the occurrence of tumors for the most compromised age group. There is a trend to increase the number of tumors due to advancing age, with a decrease in extremely elderly animals (PRIEBE et al., 2011). Corroborating the reflections of these authors, the present study showed that the occurrence of cutaneous tumors in cats for the age group that integrated the most advanced ages was not statistically significant.

The anatomical sites for the distribution of cutaneous and soft tissue tumors are shown in table 2. There was a high-frequency variability, with no statistical difference. Thus, the data were subdivided only descriptively into absolute and percentage frequency.

Rossetto et al. (2009) analyzed the frequency of cutaneous tumors by cytological examination in dogs and found that the anatomical locations were also different, as observed in the present study. Although no statistical analysis was carried out regarding the body regions in the present retrospective analysis, the highest percentages of involvement were related to the location in the thoracic and abdominal area (10.4% each). In the present study, this distribution may be associated with

Table 1. Animal characteristics according to breed definition, sex, and age group.

Animal characteristic		N	%	95% CI	
				Lower	Upper
Breed definition	Purebred	49	25.5%	19.7%	32.8%
	Mixed breed	143	74.5%	67.2%	80.3%
Sex	Female	90	46.9%	40.0%	53.1%
	Male	102	53.1%	46.9%	60.0%
Age group	1 to 6 years	19	9.9%	6.3%	15.1%
	7 to 10 years	50	26.0%	20.3%	32.3%
	11 to 15 years	100	52.1%	44.7%	58.4%
	16 to 19 years	23	12.0%	7.7%	15.7%
Total		**192**	**100.0%**		

Table 2. Anatomical distribution of cutaneous and subctaneous tumors in felines.

Anatomical region	N	%	Anatomical region	N	%
Antebrachial	1	0.5%	Masseteric	9	4.7%
Axillary	5	2.6%	Pelvic limb	12	6.3%
Carpal	1	0.5%	Thoracic limb	13	6.7%
Cervical	12	6.3%	Mentual	7	3.6%
Auditory canal (vertical branch)	1	0.5%	Patellar	1	0.5%
Costal	20	10.4%	Outer ear	10	5.2%
Muzzle	1	0.5%	Perineal	2	1.0%
Sternal	8	4.2%	Nasal plane	6	3.1%
Supraorbital fossa	2	1.0%	Popliteal region	2	1.0%
Frontal	7	3.6%	Sacral	1	0.5%
Interscapular	12	6.2%	Tarsal	2	1.0%
Labial	4	2.0%	Thoracic vertebral	11	5.7%

the occurrence of sarcomas, which were frequently observed (Table 3). According to Cecco et al. (2019), most feline sarcomas are located in the subcutaneous costal and abdominal spaces, which correspond to predilection zones for the administration of vaccines and parenteral drugs.

The table 3 shows the neoplastic characteristics consistent with biological behavior, distribution according to the cytomorphological category of neoplasm and cytological suggestion for neoplasm. The statistically significant majority of the tumors was malignant, reaching 67.2% (95% CI: 60.4–73.5%). The lowest frequency with statistical significance consisted of mesenchymal and/or epithelial tumors (4/2.1%; 95% CI: 0.0–4.2%), with the highest values being observed for the same types separately, but with no statistical difference between the frequencies of both. No statistically significant differences were observed between the percentages of cytological findings for tumors. The lowest percentages were found for melanoma, adenocarcinoma, and trichoblastoma (1/0.5%, 4/2.1%, and 7/3.6%, respectively), and the highest percentages were associated with sarcoma and lipoma (37/19.3% and 44/22.9%, respectively).

The highest number of malignant tumors was due to the inherent biological behavior of cutaneous tumors in felines, as 60–80% of the tumors are malignant (CHALITA, 2020). Additionally, the delay of the owners in taking their animals to the veterinary service may be responsible for the higher rate of malignancy, as it hinders early diagnosis, providing a long evolution and increasing the possibility of transforming benign into malignant tumors (ROSSETTO et al., 2009; PRIEBE et al., 2011). According to Ho; Smith; Dobromylskyj (2018), cutaneous tumors in felines with malignant biological behavior had a frequency of 53%, while benign tumors reached 47%. However, Manuali et al. (2020) observed 68.3% of malignant cutaneous tumors and 31.7% benign tumors, showing a higher similarity with the results now obtained.

Regarding the cytomorphological category of neoplasm , epithelial tumors accounted for 46% of all cutaneous tumors in cats, followed by mesenchymal tumors (37%), and round-cell tumors, with a frequency of 17% (LIMA et al., 2018). These values diverged numerically (and not necessarily statistically) from the present survey, which presented an inversion in frequency for the tumors, with a predominance of those of mesenchymal origin, followed by epithelial tumors. This variation between studies may be due to certain factors, such as regional diversification for the most frequent cytomorphological category of neoplasm, quality of the collected sample, experience in the analysis of the cytological evaluation and the degree of communication between the clinician and the veterinarian cytologist. The round-cell tumors showed a similarity between the obtained frequency and that extracted from the literature.

When comparing our findings with the study of Schmidt et al. (2010), there were some differences in the percentage of tumors type. These divergences in the prevalence of tumors type could be mostly related to environmental and behavioral factors (JOHNSON; MYERS, 2017; HO; SMITH; DOBROMYLSKYJ, 2018; MANUALI et al., 2020) since these studies were performed in different geographical areas.

Table 3. Neoplastic characteristics related to biological behavior, cytomorphological category of neoplasm, and cytological suggestion for neoplasm.

Tumor characteristics		N	%	95% CI	
				Lower	Upper
Neoplastic biological behavior	Benign	62	32%	26.5%	39.6%
	Malignant	130	68%	60.4%	73.5%
Distribution of tumor according to cytomorphological category of neoplasm	Round cells	38	19.8%	13.9%	25.5%
	Epithelial	64	33.3%	26.5%	40.1%
	Mesenchymal	86	44.8%	37.9%	51.0%
Cytological suggestion for neoplasm	Adenocarcinoma*	4	2.1%	0.4%	4.2%
	Carcinoma*	19	10%	6.2%	14.1%
	Squamous cell carcinoma	10	5.2%	2.1%	8.3%
	Lymphoma	11	5.7%	2.5%	9.4%
	Lipoma	44	22.9%	18.1%	28.7%
	Mastocytoma	25	13.0%	8.8%	18.2%
	Melanoma	1	0.5%	0.0%	1.6%
	Trichoblastoma	7	3.6%	1%	6.8%
	Sarcoma	37	19.3%	14.0%	24.6%
	Inconclusive	38	19.8%	12.9%	23.5%

* Undetermined origin

According to Rosolem et al. (2013), inconclusive or not defined cytological examinations totaled 22%, being similar to the findings of the present analysis. It may be related to the low tissue exfoliative capacity during the collection procedure or the low cell differentiation index of tumors (AL-ABBADI, 2011). Similar to that already mentioned regarding the cytomorphological category of neoplasm, this discussion became relevant to increase the knowledge on the subject despite the lack of statistical significance between the cytological conclusions.

The table 4 shows the statistically significant inequality between benign and malignant tumors according to the animal characteristics and other aspects related to the tumors (cytomorphological category of neoplasm and cytological suggestion for neoplasm). No statistically significant association was observed between the neoplastic biological behavior and breed definition, sex, and age of the animals (p=0.423, p=0.175, and p=0.409, respectively). However, the distribution of tumors according to the cytomorphological category of neoplasm was statistically associated with the biological behavior (p<0.001). In other words, mesenchymal (24.5%) and epithelial tumors (25%) presented the highest proportion among those benign and malignant, respectively. The cytological suggestion for neoplasm of the tumor was also statistically associated with the biological behavior (p<0.001). Therefore, the highest percentage of occurrence of benign and malignant tumors consisted of lipomas (22.9%) and sarcomas (19.3%), respectively.

According to Ho; Smith; Dobromylskyj (2018), when considering all cutaneous tumors (benign and malignant), certain breeds of cats showed an increase, with statistical significance, in the development of cutaneous tumors compared to the population of mixed-breed animals. No statistically significant increase in the neoplastic genesis was observed for malignant tumors when comparing purebred and mixed-breed felines. Malignant cutaneous tumors have

Table 4. Distribution of benign and malignant tumors according to breed definition, sex, age group, cytomorphological category of neoplasm and cytological suggestion for neoplasm.

Animal characteristics, cytomorphological category of neoplasm and cytological suggestion for neoplasm		Neoplastic biological behavior				p-value
		Benign*		Malignant*		
		N	%	N	%	
Breed definition	Purebreed	15	7.8%	34	17.7%	0.423
	Mixed breed	48	25.0%	95	49.5%	
Sex	Female	26	13.5%	64	33.3%	0.175
	Male	37	19.3%	65	33.9%	
Age group	1 to 6 years	9	4.7%	10	5.2%	0.409
	7 to 10 years	14	7.3%	36	18.7%	
	11 to 15 years	34	17.7%	66	34.4%	
	16 to 19 years	6	3.1%	17	8.9%	
Distribution of tumors according to the cytomorphological category of neoplasm	Round cells	0	0.0%	38	19.8%	<0.001
	Epithelial	16	8.3%	48	25.0%	
	Mesenchymal	47	24.5%	39	20.3%	
	Mesenchymal and/or epithelial	0	0.0%	4	2.1%	
Cytological suggestion for neoplasm	Adenocarcinoma	0	0.0%	4	2.1%	<0.001
	Squamous cell carcinoma	0	0.0%	10	5.2%	
	Carcinoma	0	0.0%	19	10%	
	Lymphoma	0	0.0%	11	5.7%	
	Lipoma	44	22.9%	0	0.0%	
	Mastocytoma	0	0.0%	25	13.0%	
	Melanoma	0	0.0%	1	0.5%	
	Trichoblastoma	7	3.6 %	0	0.0%	
	Sarcoma	0	0.0%	37	19.3%	
	Inconclusive	11	5,7%	27	12.0%	

* Definition of biological behavior (benign or malignant) according to cytomorphological findings.

been observed in a more senile population of animals (mean age of 12 years) compared to benign tumors. This inequality has been more pronounced in male cats (HO; SMITH; DOBROMYLSKYJ, 2018). The results of these studies are important despite the lack of statistical significance between the variables.

Some cases with genesis in the keratinocytes of the spinous layer of the epidermis may be included among the tumors of epithelial origin with malignant biological behavior classified as carcinomas, which would increase the frequency of squamous cell carcinoma, thus corroborating with other studies, which have cited this malignant tumor as one of the most common of the feline integument, reaching 50% of all cutaneous tumors of cats or even up to 73 to 95% (MURPHY, 2013). In the present study, analyzing the percentage of squamous cell carcinomas together with carcinomas of undefined origin (possibly originated from the spinous layer of the epidermis), we found a low prevalence. A possible explanation may be related to the cats' environment. In this regard, all animals were from São Paulo, the most populated city in Brazil, where there is a high prevalence of cats living in apartments without access to external environments. Consequently, the animals are restricted to the action of solar incidence. As a predisposing factor for the appearance of squamous cell carcinomas in the skin, the prolonged exposure to ultraviolet solar radiation is well-documented. (DALECK; DE NARDI, 2016). The highest prevalence for sarcoma among tumors of mesenchymal origin was similar to other studies (SCHMIDT et al., 2010; HO; SMITH; DOBROMYLSKYJ, 2018; LIMA et al., 2018). Subcutaneous sarcomas in felines are usually related to the application and most are consistent with fibrosarcomas, but they can also receive other morphological classifications, such as osteosarcomas, rhabdomyosarcomas, chondrosarcomas, neurofibrosarcomas, and liposarcomas (GRAF et al., 2018). Thus, the term soft-tissue sarcoma can be used for several of these tumors (SHELLY, 2003). Cytology alone may not be able to distinguish the multiple types with consistent precision (SHELLY, 2003). Also, tumors classified in this category generally have no variation for prognosis regardless of the histological distinction (JOHNSON; MYERS, 2017; GRAF et al., 2018). Therefore, a specific tumor diagnosis other than soft-tissue sarcoma is not clinically necessary (JOHNSON; MYERS, 2017).

Different results from those found in the literature were observed when the tumor had a mesenchymal origin with benign biological behavior. According to Shelly (2003) and Johnson; Myers (2017), lipomas are uncommon in cats. Thus, these studies are divergent from the data observed in the retrospective evaluation of the present study since lipoma was the most frequent among all tumors. The explanation for this difference may be related to the current body condition score of cats, as lipoma may be related especially to obese animals (SCHMIDT et al., 2010; JOHNSON; MYERS, 2017). The prevalence of obesity in felines has increased in recent years (estimated between 30 and 40%), and the species is very likely to become obese due to its unique characteristics in the metabolism of glucose and lipids (OKADA et al., 2019).

Technological advances and technical improvement that have occurred in recent years have made cytological examination an increasingly accurate diagnostic method, with lower rates of false negatives or false positives (VENTURA; COLODEL; ROCHA, 2012). A sensitivity of 89% and specificity of 100% of the cytological method were observed relative to the histopathological technique for skin tumors in dogs. The sensitivity and specificity of the cytology reached 40 and 100%, respectively, among epithelial tumors, differently from what happened in the other groups (mesenchymal and round cells), with results of 100% (SHARKEY; DIAL; MATZ, 2007). The correlation for cutaneous tumors in cats can reach up to 90% (SHARKEY; WELLMAN, 2011). Cytology effectiveness can reach 85 to 97% when comparing the results from cytopathological and histopathological processing (MAGALHÃES et al., 2001; CHALITA, 2020). Cytology agreed with the histopathological examination in 91% of the cases regarding the presence or absence of tumors, with all cutaneous tumors correctly classified (SHARKEY; DIAL; MATZ, 2007). Thus, there is a good assertiveness when comparing the results obtained by cytopathology and histopathology (MASSERDOTTI, 2006). Cytology is satisfactory in fulfilling the role of discerning benign or malignant tumor growths (BRAZ et al., 2016). The margin of error of the cytological examination is equivalent to only 8%, standing out for its great usefulness to diagnose tumors in general (MAGALHÃES et al., 2001). The small number of national and international retrospective studies directed exclusively to the cytopathology of cutaneous tumors in felines and the information of the studies mentioned in the presented study make it reliable to use some histopathological results for comparison with those obtained by cytology. Although cytopathology can be considered a good screening method with potential prognostic value, the definitive diagnosis for certain situations depends on the histopathological result (MASSERDOTTI, 2006; BRAZ et al., 2016).

The present study characterized cutaneous tumors in cats treated at a specialized veterinary clinic during the period of five years. The cytological examination allowed the identification of different tumor types, showing that this technique can assist veterinary clinicians in the diagnosis of cutaneous and subcutaneous tumors in the feline species. The limitation of this research refers to the absence of histopathological reports, which would allow a comparative diagnosis between the techniques. Further studies are needed to establish the prevalence of cutaneous tumors in felines on a national and international scale, such as the use of cytology for their diagnosis.

CONCLUSIONS

Most cats with cutaneous and subcutaneous tumors had no breed definition and were classified in the age group from 11 to 15 years. Tumors malignant were the most common. The highest proportion of benign tumors was found for those of mesenchymal origin, while the highest frequency for malignant tumors was observed when the genesis was epithelial. Lipomas and sarcomas predominated among benign and malignant tumors, respectively. The cytological examination emerges as an excellent alternative for the diagnosis of screening, being sometimes definitive for cutaneous and subctaneous tumors of the feline species. However, an increment in research related to the subject is necessary for a better understanding and validation, such as a higher sample size, with the interaction between establishments with specialized care in feline medicine, providing the generation of multicenter studies.

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