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Prevalence of pathological lesions diagnosed by cytology in cats, with association of diagnosis to age, breed and gender

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ABSTRACT

Cytology is the diagnostic procedure of the microscopic evaluation of cells. It is becoming increasingly important and more frequently used in veterinary diagnostics, having many advantages including simplicity, speed and low cost. To determine the pathological changes diagnosed by cytology in cats, as well as the distribution of age, breed and gender in the diagnosed changes, we performed a retrospective study on slides submitted to the Department of Veterinary Pathology for routine cytological examination. The archive was searched for all feline cytology slides submitted from 2009 to 2018. All the slides were re-evaluated and classified into one of the four pathological processes: 'neoplasia', 'inflammation', 'other condition' or 'non-diagnostic sample'. Breed, age, gender and the tissue from which the lesion was sampled were noted from the submission form, and statistically analyzed. The most frequent type of pathological process diagnosed was neoplasia, which reflects the high prevalence of neoplastic diseases in cats reported in literature data. Pathological changes were mostly diagnosed in domestic shorthaired cats of both sexes, with an average age of 8.4 years, but no breed, age or gender predisposition was found. The most evaluated tissue was skin, probably due to its accessibility and the ease of obtaining a sample from skin lesions. The most frequent neoplasia were malignant and the most frequent diagnosis was round cell neoplasia. Cats affected with round cell neoplasia had a significantly lower average age (7.3 years) than cats diagnosed with epithelial and mesenchymal neoplasia (9.9 and 10.3 years, respectively), probably reflecting the common retroviral infection in Croatian cats.

Key words: diagnostic cytology; cat; neoplasia; inflammation; epidemiology

Introduction

Cytology is the diagnostic procedure of the microscopic evaluation of cells and it is becoming an increasingly important and frequently used tool in veterinary practitioners' diagnostics (RADIN and WELLMAN, 2001). One of the many advantages of cytology as a diagnostic aid is that samples can be easily obtained in every practice or clinic, requiring no special equipment (WYPIJ,

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2011). The procedure can often be performed without the need for anesthesia, and sometimes without sedation (STONE and REPPAS, 2010). This method is less costly and faster than biopsy and subsequent histopathology, both in sample collection and laboratory analysis (STONE and REPPAS, 2010). In-house interpretations can be made the same day, while interpretations from reference laboratories are frequently available within 24 hours (RADIN and WELLMAN, 2001). The procedure of acquiring cytological samples is minimally invasive, presenting little or no risk to the patients and is less likely to result in complications when compared to tissue biopsy (STONE and REPPAS, 2010). Cytological findings are valuable in establishing a diagnosis, identifying the disease process (neoplastic vs. inflammatory), and forming a prognosis (WYPIJ, 2011). All these benefits of cytology make it an attractive diagnostic method for clients and professionals (WYPIJ, 2011).

Although some research has been performed regarding pathology of cats, systematic research is still lacking as most Croatian reports have only focused on a single etiology or pathological process, such as neoplasia or vector-borne disease. Neoplasia is a common health burden in cats in Croatia, with lymphoma being the most common tumors in Croatia (ANDREIĆ, 2012). Other tumors diagnosed in Croatian cats include mammary gland (GUDAN KURILJ et al., 2015), skin, endocrine and exocrine gland tumors, as well as lung tumors (GRABAREVIĆ et al., 2009; MEDVEN ZAGRADIŠNIK et al., 2015). Infections in Croatian cats have only been investigated in the lungs and the diagnoses have included bacterial or verminous pneumonia (GAGOVIĆ et al., 2015; GRABAREVIĆ et al., 2015). Other notable diseases that may be diagnosed with cytology include vector-borne diseases with hemoplasmas, which are the most common infections in Croatian cats (HUBER, 2018).

Due to the lack of systematic research, as well as a rising usage of cytology in diagnostics and a constant increase in samples submitted to the Department of Veterinary Pathology, we performed a retrospective study on slides submitted to the Department for routine cytological examination. The aim was to determine the pathological processes in feline patients diagnosed by cytology, with determination of age, breed and gender distribution in the diagnosed pathological processes.

Materials and methods

The archive of the Department of Veterinary Pathology was searched for all cytological samples originating from cats, submitted for routine evaluation from 1st January 2009 to 31st December 2018. All cytological samples had been previously stained with May-Grünwald Giemsa stain and archived in a dry and dark place protected from dust. All slides were re-evaluated by light microscopy using standard keys for diagnostic cytology (RASKIN and MEYER, 2016). The final diagnosis was classified into one of four pathological processes: 'neoplasia', 'inflammation', 'other condition' or 'non-diagnostic sample'. 'Neoplasia' was further classified into types by origin such as round cell neoplasms, epithelial neoplasms, mesenchymal neoplasms, neuroendocrine neoplasms or undifferentiated neoplasms. If possible, the biological behavior was determined as benign, malignant or not possible to determine. All round cell and undifferentiated neoplasms were treated as malignant neoplasms. Inflammatory lesions were further classified by type as purulent, pyogranulomatous, granulomatous, eosinophilic, lymphocytic or mixed. 'Other condition' included lesions which were not neoplastic or inflammatory by cause. 'Non-diagnostic samples' were diagnosed when the cellularity or sample quality was not adequate to set a diagnosis.

From the submission form accompanying the cytology slide, history and clinical data were noted, including the breed, age and gender of the animal, as well as the tissue from which the lesion was sampled (body cavities, cardiovascular system, digestive tract, endocrine system, lymphoid tissues, musculoskeletal system, neurological system, reproductive system, respiratory system, skin, special senses or urinary system). Samples from the mammary gland were classified as skin (SHARKEY and SEELIG, 2014). Animals with

multiple sampled lesions were treated as the same case if the diagnosis was the same from all the sampled lesions. If the diagnosis differed between lesions in cats with multiple sampled tissue, they were treated as different cases.

Statistical analyses were performed using Statistica v.13 (TIBCO Software Inc., 2017). Normality of distribution was tested with the Kolmogorov-Smirnov Test for Normality. Analysis of variance (ANOVA) and post-hoc Tukey test were applied in order to determine statistical differences between the group means (association of age) and: i) pathological processes; ii) type of neoplasm by origin, and iii) type of neoplasm by biological behavior. Results were presented as median and range (minimum - maximum). For determination of associations between categorical variables (gender, pathological process and breed) the $\chi 2$ test was used. Significance was determined at $P<0.05$.

Results

In the investigated period, cytological samples originating from 306 feline patients were analyzed. Samples originated from 149 females (48.69%) and 157 males (51.31%). The average age of the investigated animals was 8.4 years (n = 290 cats, range: 6 months to 20 years; in 16 cats age was unknown). No statistically significant difference was found between the ages of cats diagnosed with different pathological processes (P = 0.17).

The details of the breeds of all the investigated cats and for each pathological process are presented in Table 1. The most frequent breed was domestic short-haired cat, while pure-breed cats were less frequent (Table 1). The difference between breeds of cats diagnosed with different pathological process was not statistically significant (P = 0.26).

Cytological slides were prepared from 11 tissues: body cavities, cardiovascular system, digestive tract, endocrine system, lymphoid tissues, musculoskeletal system, neurological system, respiratory system, skin, special senses and urinary system, with the skin being the predominant anatomic location of tissue sampling (Table 2). The anatomical locations of the sampled tissues overall, and for each pathological process are presented in Table 2.

The most frequently diagnosed pathological process was 'neoplasia' (131 cats; 42.81%), followed by 'inflammation' (61; 19.93%), 'other condition' (57; 18.63%) and 'non-diagnostic sample' (57; 18.63%). The four most common diagnoses for each pathological process are presented in Figs 1 and 2.

Neoplasia (n = 131). Neoplasia was diagnosed in 71 females (54.20%) and 60 males (45.80%). The average age of the affected cats was 9.2 years (range: 6 months to 20 years; n = 128; in three cats age was unknown). Neoplasia was most frequently diagnosed in domestic short-haired cats (Table 1), with the skin being the predominant location of sampling (Table 2).

The most frequently diagnosed neoplasms were round cell neoplasms (n = 44; 33.60%; Fig. 1A), followed by mesenchymal neoplasms (n = 41, 31.30%; Fig. 1B), epithelial neoplasms (n = 38, 29.00%; Fig. 1C), neuroendocrine neoplasms (n = 7, 5.34%) and undifferentiated neoplasms (n = 1, 0.76%; Fig. 1D). Round cell neoplasia was diagnosed in 20 female (45.45%) and 24 male cats (54.55%) with an average age of 7.3 years (range 11 months to 14 years). The most frequent diagnosis in this type of neoplasm was lymphoma (31 samples). Mesenchymal neoplasms were diagnosed in 21 females (51.22%) and 20 males (48.78%), with an average age of 10.3 years (2 to 20 years). The most common diagnosis was malignant mesenchymal neoplasia (23 samples). Epithelial neoplasms were diagnosed in 24 females (63.16%) and 14 males (36.84%) with an average age of 9.9 years (6 months to 19 years). The most common diagnosis was squamous cell carcinoma (6 samples). Undifferentiated neoplasms were diagnosed in 4 females (57.14%) and 3 males (42.86%) with an average age of 11 years (7 to 18 years). The only neuroendocrine neoplasm was diagnosed in a 10 year old female cat. The difference in average age was statistically significant between: i) cats diagnosed with round cell neoplasia vs. cats diagnosed with epithelial neoplasms (P = 0.048), and ii) cats diagnosed with round cell neoplasms vs. cats with mesenchymal neoplasms (P = 0.01).

Table 1. Number of investigated cats of each breed in relation to pathological process

Breed	Overall (n = 306)	Neoplasia (n = 131)	Inflammation (n = 61)	Other condition (n = 57)	Non-diagnostic sample (n = 57)
Birman	1 (0.33%)	0	1 (1.63%)	0	0
British Shorthair	2 (0.65%)	2 (1.53%)	0	0	0
Chartreux	1 (0.33%)	1 (0.76%)	0	0	0
Domestic short-haired	211 (68.95%)	90 (68.70%)	45 (73.77%)	40 (70.18%)	36 (63.16%)
European shorthair	39 (12.74%)	17 (12.99%)	5 (8.20%)	6 (10.53%)	11 (19.30%)
Exotic Shorthair	5 (1.63%)	1 (0.76%)	2 (3.29%)	2 (3.51%)	0
Maine Coon	4 (1.31%)	0	0	3 (5.26%)	1 (1.75%)
Norwegian Forest	3 (0.98%)	3 (2.29%)	0	0	0
Oriental Shorthair	2 (0.65%)	1 (0.76%)	0	1 (1.75%)	0
Persian	9 (2.94%)	5 (3.82%)	1 (1.63%)	0	3 (5.26%)
Ragdoll	1 (0.33%)	1 (0.76%)	0	0	0
Russian Blue	1 (0.33%)	1 (0.76%)	0	0	0
Siamese	7 (2.29%)	4 (3.05%)	0	1 (1.75%)	2 (3.51%)
Turkish Angora	1 (0.33%)	0	0	0	1 (1.75%)
Unknown	19 (6.21%)	5 (3.82%)	7 (11.48%)	4 (7.02%)	3 (5.26%)

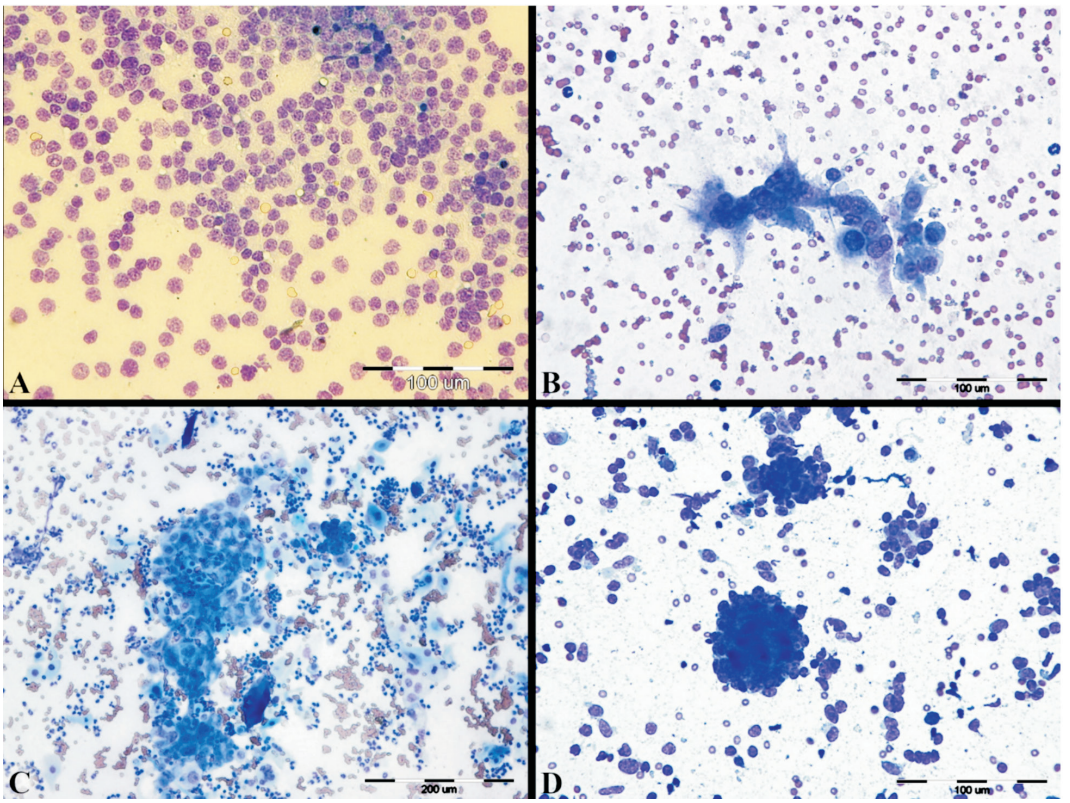

Fig. 1. Neoplasia. A - Round cell neoplasia. May-Grünwald Giemsa stain (MGG), objective magnification ×40. B - Malignant mesenchymal neoplasm, MGG, ×40. C - Malignant epithelial neoplasm, MGG, ×20. D - Undifferentiated neoplasm, MGG, ×40.

Table 2. Anatomic location of sampled tissue

Anatomic location		Overall (n = 306)	Neoplasia (n = 131)	Inflammation (n = 61)	Other condition (n = 57)	Non-diagnostic sample (n = 57)
Body cavity		35 (11.44%)	7 (5.34%)	13 (21.31%)	7 (12.28%)	8 (14.04%)
Cardiovascular system		4 (1.31%)	0	0	3 (5.26%)	1 (1.75%)
Digestive tract		43 (14.05%)	18 (13.74%)	15 (24.59%)	3 (5.26%)	7 (12.28%)
Endocrine system		1 (0.33%)	0	0	0	1 (1.75%)
Lymphoid tissues		50 (16.34%)	19 (14.50%)	2 (3.28%)	23 (40.36%)	6 (10.53%)
Musculoskeletal system		9 (2.94%)	2 (1.53%)	0	1 (1.75%)	6 (10.53%)
Neurological system		0	0	0	0	0
Reproductive system		0	0	0	0	0
Respiratory system		9 (2.94%)	3 (2.29%)	6 (9.84%)	0	0
Skin		118 (38.55%)	66 (50.38%)	14 (22.95%)	16 (28.08%)	22 (38.60%)
Special senses		24 (7.84%)	6 (4.58%)	10 (16.39%)	3 (5.26%)	5 (8.77%)
Urinary system		3 (0.98%)	1 (0.77%)	0	1 (1.75%)	1 (1.75%)
Multiple locations		8 (2.63%)	7 (5.34%)	1 (1.64%)	0	0
	Body cavity and cardiovascular system	1 (0.33%)	0	1 (1.64%)	0	0
	Body cavity and lymphoid tissues	1 (0.33%)	1 (0.77%)	0	0	0
	Digestive tract and lymphoid tissues	1 (0.33%)	1 (0.77%)	0	0	0
	Digestive tract, lymphoid tissue and respiratory tract	1 (0,33%)	1 (0.77%)	0	0	0
	Digestive tract, lymphoid tissue, neurological tissue, special senses and urinary system	1 (0.33%)	1 (0.77%)	0	0	0
	Digestive tract, lymphoid tissues and skin	1 (0.33%)	1 (0.77%)	0	0	0
	Lymphoid tissues and skin	2 (0.65%)	2 (1,53%)	0	0	0
Unknown location		2 (0.65%)	2 (1.53%)	0	0	0

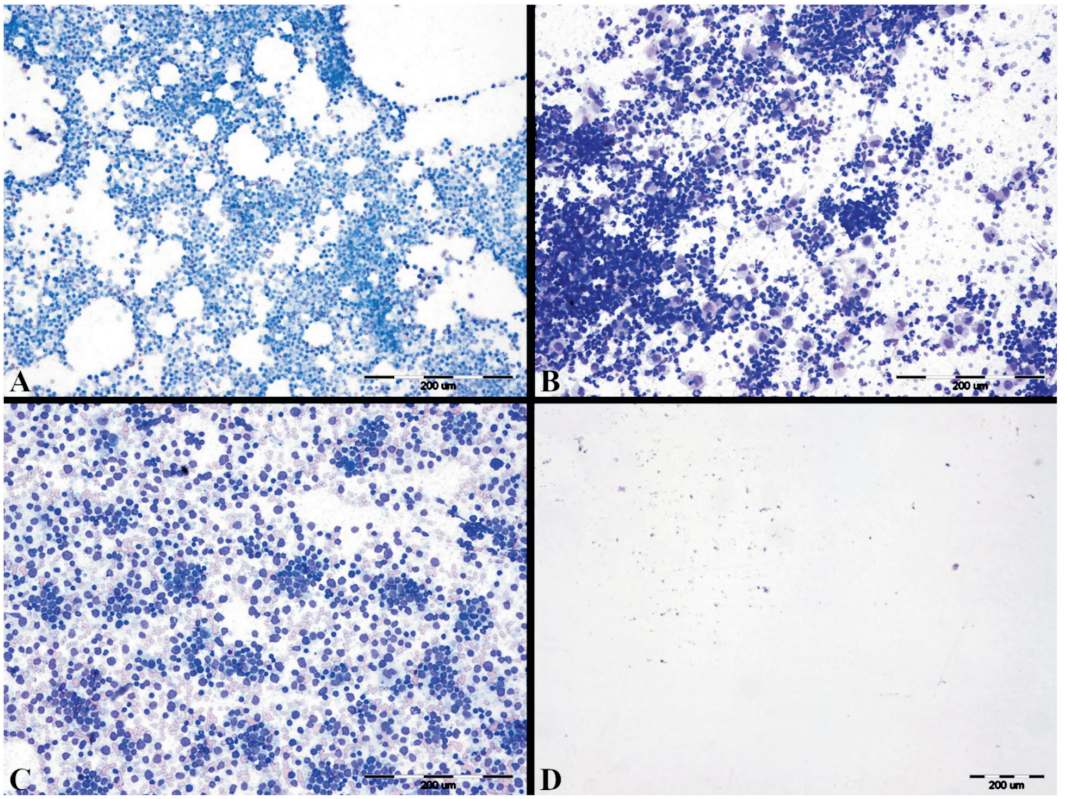

Fig. 2. MGG, ×20. A - Purulent inflammation. B - Pyogranulomatous inflammation. C - Reactive hyperplasia of lymph node. D - Non-diagnostic, acellular sample.

Other differences were not statistically significant in the average age of: i) cats diagnosed with epithelial neoplasms vs. cats diagnosed with mesenchymal neoplasms (P = 0.98); ii) cats diagnosed with neuroendocrine neoplasm vs. cats diagnosed with mesenchymal neoplasms (P = 0.99); iii) cats diagnosed with neuroendocrine neoplasm vs. cats diagnosed with epithelial neoplasms (P = 0.97) and iv) cats diagnosed with neuroendocrine neoplasm vs. cats diagnosed with round cell neoplasia (P = 0.39).

In terms of biological behavior, the most frequent were malignant neoplasms (96 cats; 73.28%). Benign neoplasms were diagnosed in 6 cats (4.58%), while biological behavior could not be determined in 29 cats (22.14%). The average age of cats with malignant neoplasms was 8.7 years (11 months to 19 years, in two cats age was unknown), 10.9 years in cats with benign neoplasia (6 months to 20 years; age of one cat was unknown) and 9 years (2 to 18 years) in cats with neoplasms whose biological behavior could not be determined. Statistically, no significant difference was found between the average age of cats and the biological behavior of the tumor (P = 0.08).

Inflammation (n = 61). Inflammation was diagnosed in 32 females (52.46%) and 29 males (47.54%). The average age of the affected cats was 8 years (n = 55, 10 months to 20 years; in 6 cats age was unknown). This pathological condition was most often diagnosed in domestic short-haired cats (Table 1) and in body cavities, the digestive tract and skin (Table 2). The most common type of inflammation was purulent inflammation, diagnosed in 26 cats (42.62%; Fig. 2A), followed by pyogranulomatous inflammation (20 cats; 32.78%; Fig. 2B), eosinophilic inflammation (8; 13.12%), mixed inflammation (3; 4.92%), granulomatous (2; 3.28%) and lymphocytic inflammation (2, 3.28%).

Other condition (n = 57). "Other conditions" were diagnosed in 23 females (40.35%) and 34 males (59.65%). The average age of the affected cats was 7.7 years (n = 56, 7 months to 19 years; in one cat age was unknown). The most frequent diagnosis was reactive hyperplasia of the lymph nodes (22; 38.60%; Fig. 2C), followed by bleeding (5; 8.77%), follicular cysts in the skin (5; 8.77%), necrosis (5; 8.77%), hyperplasia of epithelial cells (3; 5.26%), accumulation of modified transudate within a body cavity (2; 3.51%), a cyst filled with clear fluid (1; 1.75%), degeneration of epithelial cells (1; 1.75%), chylothorax (1; 1.75%), jaundice (1; 1.75%), melanosis (1, 1.75%), necrosis with bleeding (1; 1.75%), neutrophilia (1; 1.75%) and sialokele (1; 1.75%). The sampled tissue did not show any pathology, but corresponded to normal findings for sampled tissue in seven cats (12.28%). This pathological process was mostly diagnosed in domestic short-haired cats (Table 1), and mostly from the lymphoid tissues and skin (Table 2).

Non-diagnostic sample (n = 57). Non-diagnostic samples (Fig. 2D) were diagnosed in 31 females (54.39%) and 26 males (45.61%) with an average age of 8.6 years (n = 51, 7 months to 18 years; in 6 cats age was unknown). Non-diagnostic samples were mostly submitted from domestic short-haired cats (Table 1), with skin as the most frequently sampled tissue (Table 2).

Discussion

Cytological analysis is a fast, cheap and minimally invasive method often used in the diagnosis of pathological changes in cats (RASKIN and MEYER, 2016). As cytological samples are often analyzed in Croatia, we performed an epidemiological study to evaluate the cat population which had been subjected to this investigation with the determination of the most common tissues sampled.

Cytological analysis was performed in an almost equal number of male and female cats. Gender predisposition was not found in any pathological process, indicating that cats of both sexes are affected by the same diseases. The most common breed of the investigated cats was domestic short-haired breed, which was the most common overall, and in all the determined pathological processes. This is probably a consequence of the predominance of this breed of cats in Croatia.

The most frequent pathological process was neoplasia, indicating that cytology is most often used to analyze suspected neoplastic changes in patients. Also, neoplasia is one of the four most common causes of the death of feline patients (O'NEILL et al., 2015; HUANG et al., 2017; TOGNI et al., 2018; WITHOEFT et al., 2019), hence the large amount of this pathological process is expected. Malignant neoplasia was more often diagnosed than benign neoplasms in the current study. This corresponds to the literature data which states that the most common neoplasms in cats are malignant neoplasms, including lymphomas and malignant skin tumors (including mammary gland tumors) (WITHROW et al., 2013). Further, cats are predisposed to develop postvaccinal sarcomas, another malignant tumor (WITHROW et al., 2013).

Statistical analysis showed that cats diagnosed with round cell neoplasia were younger than cats diagnosed with epithelial and mesenchymal neoplasms. This may be a consequence of retroviral infection, which is associated with the development of lymphoma, and is a malignant, round cell neoplasm (WITHROW et al., 2013). Retroviral infection is common in Croatian cats, affecting up to 20% of stray and client-owned cats (PERHARIĆ et al., 2018). The infection results in the development of lymphoma, mostly in juvenile and young adult cats (WITHROW et al., 2013), which is in concordance with the results of the current study.

The most frequently diagnosed inflammation was purulent and pyogranulomatous, while other types of inflammation were less often diagnosed. These results may show that cats are more susceptible to purulent and pyogranulomatous types of inflammation, mostly associated with bacteria (RADIN and WELLMAN, 2001). However, it may also show that cytology is more frequently used for diagnosis of infections associated with bacteria, due to their exudative nature, which makes them easy to sample for cytology, while other types of inflammation are diagnosed by other laboratory procedures. Further research is needed to elucidate these assumptions fully.

The skin was the most commonly investigated tissue overall as well as in 'neoplasia' and 'non-diagnostic samples'. The accessibility of this tissue makes it easy to note pathological changes both for the owner and the veterinarian examining the animal. Further, all skin lesions are easy to sample due to their vicinity to the body surface (RADIN and WELLMAN, 2001). It is therefore understandable that this tissue was the most frequently investigated tissue in the current research.

As seen in this study, cytological analysis is useful in diagnosing neoplasia, inflammatory lesions and other lesions not characterized by a neoplastic or inflammatory process. Although it has numerous advantages, assessment of cellular organization or architecture is not possible by cytological evaluation, but requires histology as an additional diagnostic method (STONE and REPPAS, 2010). Even if the final diagnosis cannot sometimes be set based solely on cytology, cytological findings are of value in setting a preliminary diagnosis, identifying sites of tumor metastasis, determining additional diagnostic procedures, or directing therapy (STONE and REPPAS, 2010; WYPIJ, 2011).

Conclusions

The most frequent type of pathological process diagnosed by cytology was neoplasia, with malignant neoplasms being more frequent than benign. Cats affected with round cell neoplasia had a significantly lower average age than cats diagnosed with epithelial and mesenchymal neoplasia. All the diagnosed processes were found in an approximately equal number of male and female cats, and domestic shorthaired cats were the most prevalent breed, but no gender or breed predisposition was found by statistics. The tissue that was most frequently evaluated by cytology was the skin.

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SAŽETAK

Citologija je sve važniji i sve češće primjenjivan dijagnostički postupak u kojemu se mikroskopski analiziraju stanice, a ima i brojne prednosti, kao što su jednostavnost izvedbe, brzina i niska cijena. Kako bismo odredili patološke promjene dijagnosticirane citologijom u mačaka kao i raspodjelu prema dobi, pasmini i spolu, proveli smo retrospektivno istraživanje na uzorcima dostavljenima Zavodu za veterinarsku patologiju Veterinarskoga fakulteta Sveučilišta u Zagrebu za rutinsku citološku analizu. Pretraženi su arhivski preparati za sve mačje citološke nalaze od 2009. do 2018. godine. Svi su preparati ponovno analizirani i razvrstani u jedan od četiri patološka procesa: neoplazija, upala, druga stanja i nedijagnostički uzorak. Pasmina, dob, spol i tkivo iz kojega su uzorci uzeti zabilježeni su u odgovarajućem obrascu i statistički analizirani. Najčešće dijagnosticiran patološki proces bio je neoplazija, što govori o visokoj prevalenciji neoplastične bolesti u mačaka opisanoj u literaturi. Patološke promjene najčešće su dijagnosticirane u domaće kratkodlake mačke oba spola, prosječne dobi 8,4 godina. Pasminska, dobna i spolna predispozicija međutim nisu utvrđene. Najčešće analizirano tkivo bila je koža, vjerojatno zbog najlakše dostupnosti i lakog dobivanja uzorka iz kožnih lezija. Najčešća je neoplazija bila zloćudna tvorevina, a najčešća dijagnoza neoplazija okruglih stanica. Mačke oboljele od ove neoplazije imale su znakovito nižu prosječnu dob (7,3 godina) od mačaka kojima je dijagnosticirana epitelna i mezenhimna neoplazija (9,9 i 10,3 godina), što je vjerojatno pokazatelj da se radi o čestoj retrovirusnoj infekciji u mačaka u Hrvatskoj.

Ključne riječi: dijagnostička citologija; mačka; neoplazija, upala; epidemiologija

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