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CYTOLOGICAL INVESTIGATIONS IN VETERINARY MEDICINE: A RETROSPECTIVE STUDY WITH A DEBATE ON THE IMPORTANCE OF ASSOCIATED CYTOGENETIC DIAGNOSTIC

INVESTIGAȚII CITOLOGICE IN MEDICINA VETERINARĂ: UN STUDIU RETROSPECTIV CU O DEZBATERE ASUPRA IMPORTANȚEI DIAGNOSTICULUI CITOGENETIC ASOCIAT

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ABSTRACT | REZUMAT

The present study reveals the importance of cytological investigations in veterinary medicine, based on their use in the diagnosis of various inflammatory, neoplastic, or circulatory disorders. 66 samples were cytologically investigated in a period of two years (collected from 60 individuals, of which 40 dogs and 20 cats), including 10 cerebrospinal fluid samples (15.2%), 8 punctures from enlarged lymph nodes in volume (12.1%), 9 punctures from hard or soft skin formations (13.6%), 7 breast punctures (10.6%), 9 thoracentesis - collections of pleural fluid, pericardial fluid, and fine needle punctures from lung masses (13.6%), and 22 abdominocentesis - ascitic fluid collection, fine needle punctures from liver tissue, spleen tissue, prostate, and bladder wall (33.3%). Microscopic analysis of cytological samples led to the diagnosis of 28 inflammatory processes (46.6%), some with a definite etiological classification (infectious, parasitic, and mycotic), 21 neoplastic processes (35.1%), and some with a certain cellular classification (lymph sarcoma, adenocarcinoma, histiocytoma, and mesothelioma). Only 11 cases were uncertainly cytologically diagnosed: oligocytosis, hypo cellularity, reactive hyperplasia, and circulatory disorders (18.3%). It can be concluded that the cytological examination continues to remain a valuable, non-invasive, rapid, and inexpensive tool for the diagnosis of inflammatory and neoplastic diseases in veterinary medicine. In addition, the cytological examination can constitute a screening for the proposal of more accurate investigations, such as cell block-immunocytochemical or immunohistochemical tests (CB-ICC, CB-IHC), cytogenetic tests (FISH), and the determination of tumour markers with a view to targeted antitumour therapies.

Keywords: cytology, cytogenetics, veterinary medicine, inflammatory, neoplastic diagnosis

Studiul de față relevă importanța investigațiilor citologice în medicina veterinară, pe baza utilizării lor în diagnosticul diferitelor afecțiuni inflamatorii, neoplazice sau circulatorii. Au fost investigate citologic 66 de probe într-o perioadă de doi ani (recoltate de la 60 de indivizi, dintre care 40 de câini și 20 de pisici), incluzând 10 probe de lichid cefalorahidian (15,2%), 8 puncții din limfonoduri mărite în volum (12,1%), 9 puncții din formațiuni cutanate dure sau moi (13,6%), 7 puncții mamare (10,6%), 9 toracocenteze - colecții de lichid pleural, lichid pericardic și puncție cu ac fin din mase pulmonare (13,6%), 22 abdominocenteze - colectare de lichid ascitic, puncție cu ac fin din țesutul hepatic, splină, prostată, peretele vezicii urinare (33,3%). Analiza microscopică a probelor citologice a condus la diagnosticarea a 28 de procese inflamatorii (46,6%), unele cu clasificare etiologică certă (infecțioase, parazitare, micotice), 21 de procese neoplazice (35,1%), unele cu clasificare celulară certă (sarcom limfatic, adenocarcinom, histiocitom, mezoteliom), doar 11 cazuri fiind diagnosticate citologic incert - oligocitoză, hipocelularitate, hiperplazie reactivă, tulburări circulatorii (18,3%). Se poate concluziona că examenul citologic continuă să rămână un instrument valoros, neinvaziv, rapid și ieftin pentru diagnosticul bolilor inflamatorii și neoplazice în medicina veterinară. În plus, examenul citologic poate constitui un screening pentru propunerea unor investigații mai precise, precum teste imunocitochimice (CB-ICC), imunohistochimice (CB-IHC), citogenetice (FISH), determinarea markerilor tumorali, în vederea unor terapii antitumorale țintite.

Cuvinte cheie: citologie, citogenetică, medicină veterinară, diagnosticul inflamațiilor, diagnosticul neoplaziilor

Cytology deals with the study of cells in terms of their structure, function, and development. It is used in human and veterinary medicine to differentiate normal cells from inflammatory and neoplastic ones. The use of cytological examination as a routine protocol or in critical situations has increasingly made the final diagnosis easier by limiting the number of diseases in the differential diagnosis. The recognition of the cell as the fundamental structural and functional unit of living organisms is related to Theodor Schwann's (1810-1882) Cell Theory (1838-1839). A special credit in this regard is also offered to Matthias Jacob Schleiden (1804-1881),who correctly assumed, together with Schwann, the fundamental role of the cell in the structure of a multicellular organism.

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In 1855, Rudolph Virchow (1821-1902) published his aphorism "omnis cellula e cellula" ("every cell stems from another cell") in the context of cellular pathology, stating that all diseases involve changes in normal cells, cellular damages being the foundation of various pathologies' clinical expression (36, 37). "Omnia nucleus e nucleo" is another aphorism known to the scientific world to have been authored by Walther Flemming (1843-1905) when mitotic division was described in salamander (1882) (11). The development of cancerous tumours that start from a single cell, with chromosomal changes that cause aberrant mitoses and uncontrolled growth/division, was described as a process of "carcinogenesis". This idea was first debated in the first Chromosomal Theory of Inheritance formulated independently by Theodor Boveri (1865-1915) in 1902 and Walter Sutton (1877-1916) in 1903, chromosomes being entities carrying genetic information but whose structural damage could be related to an uncontrolled cellular division (5, 11, 23). As early as 1838, Johannes Peter Müller (1801-1858) highlighted on the microscope the presence of neoplastic cells of breast carcinoma and osteosarcoma on the section surface of surgically removed tumours (14, 19). In the first half of the last century, Victor Babeș (1854-1926) in Romania and George Papanicolaou (1883-1962) in America distinguished themselves with valuable publications on cervical cancer cytology (41). In the 1960s, cytology became a specialty of medical pathology and was accepted as a diagnostic method because it was non-invasive, fast, and inexpensive. Initially, techniques were used to take cytological samples from areas accessible by scraping, swabbing, or cavity puncture. As a result of the development of imaging techniques, especially ultrasonography, fine needle aspiration cytology (FNAC) from deep tissues and internal organs successfully replaced the histopathological examination of the biopsy sample. However, there are limits to the cytological examination regarding the differentiation and classification of tumours because some inflammatory processes are accompanied by dysplastic phenomena and even mimic the malignant characteristics of neoplasia (mesothelioma versus hyperplastic mesothelial reaction), while some tumours are discrete, hypo cellular, and do not reveal malignant characteristics. In addition, the exfoliated cells undergo changes that make it impossible to recognise their origin and even differentiate between carcinoma and sarcoma. For these reasons, the histopathological examination is considered definitive and certain for the diagnosis of a tumour disease and its staging, while the immunohistochemical examination and the cytogenetic examination are important for establishing the therapeutic method and for prognosis (2, 25).

The analysis of the genetic material in cytological investigations is at a general level and not in-depth, although its qualitative and quantitative reorganisation in various types of neoplastic processes is recognized. However, a cytogenetic diagnosis is more time- and financial-consuming than cytological investigations and is especially suitable for identifying genetic material reorganisations at the chromosomal level, constituted in primary lesions that lead to neoplastic processes with clinical expression. One of the most well-known links between a chromosomal structural mutation and cancer was made in 1960 by Peter Nowell (1928-2016) and David Hungerford (1927-1993). They reported the absence of the normal Y chromosome and the presence of a small Y chromosome in the leukocytes of two out of four patients with acute or chronic granulocytic leukaemia. This abnormal Y chromosome was below the average size of the smallest autosomal chromosomes and was considered by the authors to result either from the loss of an important segment of the Y chromosome or from the replacement of the Y chromosome with an autosomal fragment (28). In 1973, Janet Rowley (1925-2013) analyses chromosomes from bone marrow cells of nine patients with chronic myeloid leukaemia and determined that the abnormal chromosome reported by Nowell and Hungerford is chromosome 22 that has lost its long arm ("the Philadelphia chromosome"), a segment that is found at the end of the long arm of one of the chromosomes of the ninth pair. So, the genetic substrate in chronic myeloid leukaemia was a chromosomal structural mutation, a rearrangement in the form of a translocation (t 9;22) (32). Until now, various types of chromosomal rearrangements have been recognised as substrates of malignant processes, their identification evolving from classic cytogenetic to modern cytogenetic techniques. Each of these offers numerous advantages to tumour diagnosis, having particular value in assessing the prognosis and choosing the therapeutic protocol. Although they are important in the context of tumour diagnosis, cytogenetic techniques remain relatively laborious and expensive, while modern variants require high-precision equipment. Therefore, cytogenetic diagnosis remains a specialised one, accessible to specialised researchers in the field and within laboratories designed for this purpose. On the other hand, the use of cytological examination in routine and emergency protocols has brought benefits to veterinarians by limiting the number of diseases in the differential diagnosis. The cytological examination provides accurate information to clinicians and to perform it, laboratory equipment and supplies are relatively common.

The purpose of this paper is to provide an overview of the importance of cytological diagnosis in veterinary medicine, focusing on the presentation of retrospective investigations of the cytological diagnosis of various inflammatory and neoplastic processes.

MATERIAL AND METHODS

The cytological investigations presented in this paper were carried out in the Clinical Laboratory of the Faculty of Veterinary Medicine of Iasi during a period of two years, using cytological samples collected from 60 cases, including 40 dogs and 20 cats. A total of 66 cytological examinations were performed on smears stained with one of the Romanowsky-type methods (May-Grünwald-Giemsa stain or Diff-Quick stain). The investigated samples were obtained by cytopuncture, either from intracavitary fluid collections (CSF - Cerebrospinal Fluid, pericardial, pleural, and peritoneal fluid), or from lymph nodes, skin, breast, or deep masses by aspiration with a fine needle under ultrasound guidance (FNAC – Fine Needle Aspiration Cytology).

RESULTS AND DISCUSSIONS

A cytological diagnosis was established for each individual, including the description of the general cellular and extracellular background of the smear, highlighting the inflammatory cells and possible phagocytosed microorganisms, and the presence of atypical cells with malignant characteristics, isolated or organized in cellular plaques.

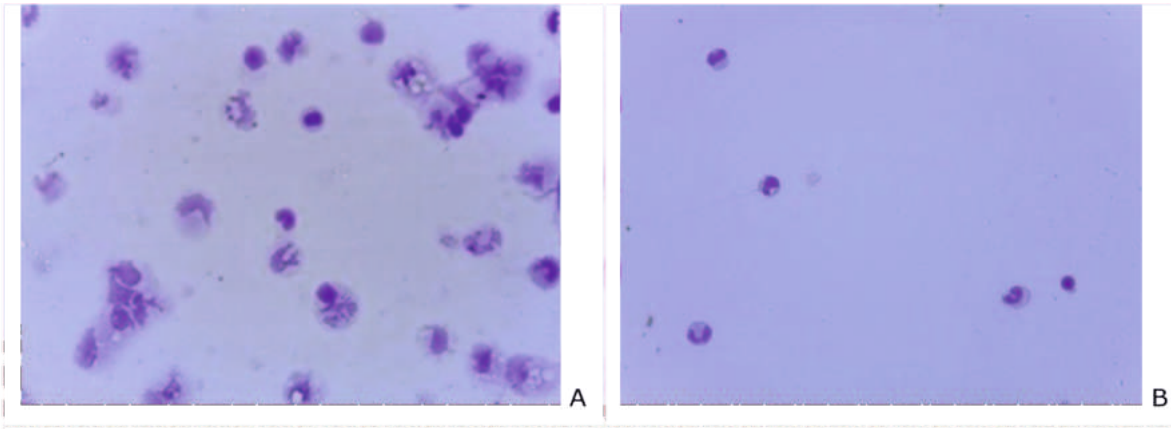

Fig. 1. Mixed accentuated pleiocytosis – predominantly neutrophils in the first stage of the disease (A) and predominantly activated Mo in the advanced stage of the disease (B). CSF. MGG x400 (Dog, F, 10 years old)

The cytological examination of the cerebrospinal fluid (CSF) was performed on eight samples (15.2 % of the total cytological samples examined), collected from five dogs and two cats (in one of the cases, the cytological examination was repeated 10 days after the treatment). Pleiocytosis (the increase of the total number of nucleated cells above the physiological limit of 5 cells/µl, NCC-Nucleated Cells Count) was evident in five of the CSF samples investigated (over 50%). In two cases, we found a mixed pleiocytosis (neutrophilic and monocytic pleiocytosis) (Fig. 1), and in three of them, a mononuclear pleiocytosis (with mononuclear blood cells or tissue macrophages). Thus, the cellular suspicion of meningitis or meningoencephalitis was confirmed in 71.4% of diagnosed cases by highlighting inflammatory cells (Nf - neutrophils, Mo - monocytes, Mfg - macrophages, Lf - lymphocytes) in the CSF samples. Oligocytosis (NCC/µl below 5 in CSF samples), the presence of red blood cells, yeast spores, and a higher percentage of mononuclear cells led us to an uncertain diagnosis for the other two individuals in this group of seven. Cerebrospinal fluid analysis provides clinicians with information about the type of inflammation of the central nervous system and allows, to some extent, a differential diagnosis. Neutrophilic pleocytosis (Nf in CSF greater than 10%) is associated with acute inflammatory diseases, including trauma, bacterial or fungal infections, and immune-mediated diseases. A number of studies have shown that 98% of dogs with bacterial or fungal meningitis had marked neutrophilic pleocytosis, but this was replaced by monocytic pleocytosis after antibiotic therapy. In steroid-responsive meningitis-arteritis (SRMA) in dogs, in feline infectious peritonitis (FIP), and in tumour necrosis, a neutrophilic CSF reaction has also been shown. Monocytic pleocytosis (with an increase in Lf above 70%, with or without a concomitant increase of monocytes or macrophages) has been described in viral diseases such as Canine Distemper Virus (CDV) infection in dogs, parasitic encephalomyelitis (toxoplasmosis, neosporosis), fungal encephalomyelitis (cryptococcosis), or necrotizing meningoencephalitis (6, 8, 30). A major diagnostic dilemma of neurological disease in the dog remains meningoencephalitis of unknown origin (MUO), which has several subtypes: granulomatous meningoencephalitis (GME), and necrotizing meningoencephalitis (NME) (27). In these two subtypes of MUO, diagnostic imaging reveals focal or multifocal intracranial lesions, and CSF examination indicates mononuclear (lymphocytes 60-90%, Mo 10-20%) or mixed pleocytosis, with a mixed population of Lf, Mo, Mfg, Nf, and rare Eo and plasma cells, as well as increased proteins, but none of the known methods show infectious causes (8,

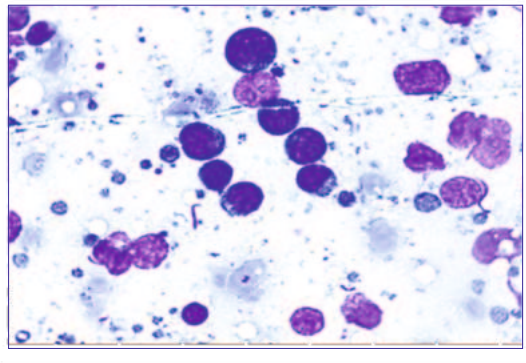

Fig. 2. Lymphosarcoma, Lymph node puncture. Background with free cytoplasmic bodies; predominant cells: lymphoblasts, stromal reticular cells. MGGx400 (Dog, Rottweiler, 5 years old)

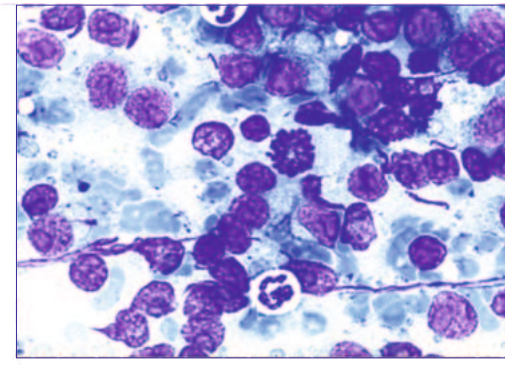

Fig. 3. Lymphosarcoma. Lymph node puncture. Background with RBC - red blood cells, rare neutrophils; predominant cells: lymphoblasts, stromal reticular cells; a mitosis is present. MGGx400 (Dog, Bull Terrier, 3-4 years old)

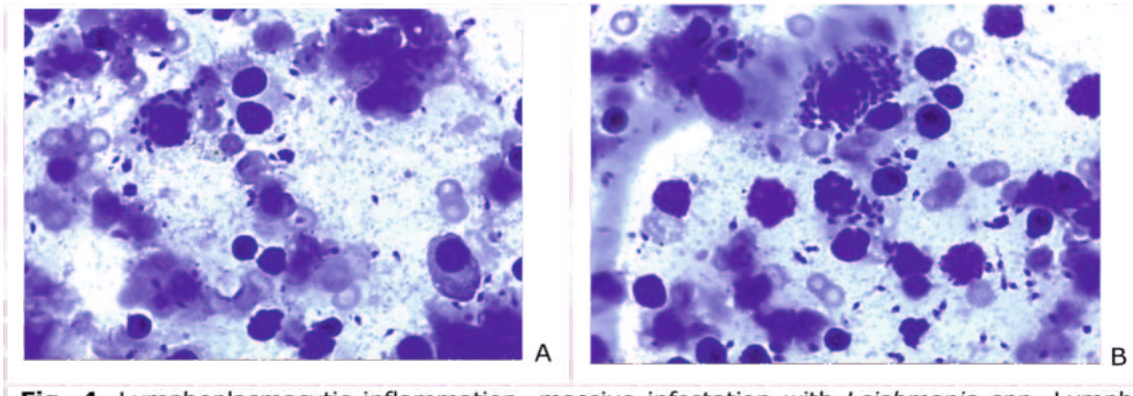

Fig. 4. Lymphoplasmacytic inflammation, massive infestation with *Leishmania* spp. Lymph node puncture. Inflammatory cells: lymphocytes, plasma cells (A), macrophages (B); amastigotes inside the cells and extracellular amastigotes. DQ x 400 (Dog, Bull Terrier, M, 5 y.o.)

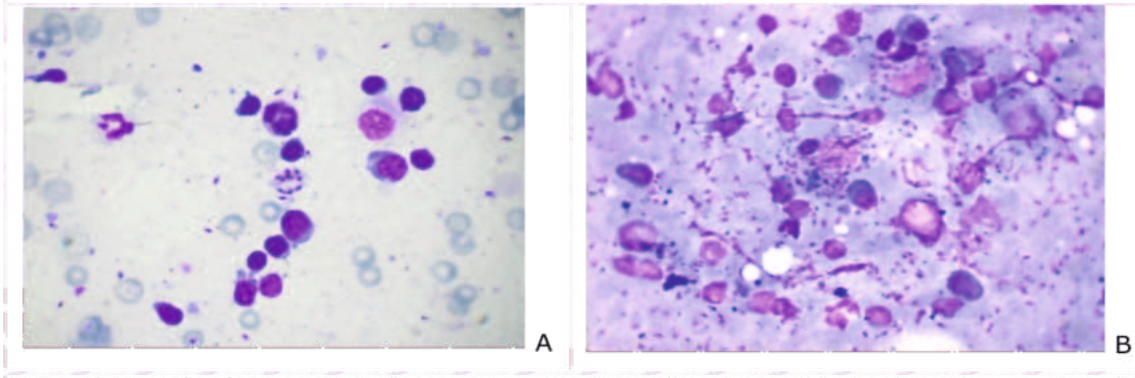

Fig. 5. Lymphoplasmacytic inflammation, massive infestation with *Leishmania* spp. Lymph node puncture. Reactive hyperplasia – mitosis of lymphoid cells (A); extracellular *Leishmania* spp. amastigotes (A and B), plasma cells (B). MGGx400 (same case as in Fig. 4)

30). In this context, Nessler et al. (2021) attempted to use a novel technology, next-generation sequencing (NGS), that has revolutionised the rate and breadth of virus discovery, but without success, with CSF samples being free of viral RNA or DNA. There are also studies (6, 8) that have detected anti-astrocytic autoantibodies in canine CSF that are highly specific for necrotizing meningoencephalitis (NME) and granulomatous meningoencephalitis (GME). The conclusion reached by the researchers is that MUO has a multifactorial aetiology, including a genetic susceptibility (Pug Dog, Maltese, and Chihuahua breeds are known to have a genetic defect in DLA-II, which increases the risk of developing MUO), and also involving an external trigger, not yet known (27, 35). Negative NGS results cannot exclude an infectious pathogen that initiated the pathological process, but this pathogen was eliminated by the immune system without blocking the inflammatory response ("hit and run" theory, supported by several authors) (27).

The eight cytological samples obtained by lymph-nodal puncture from seven dogs with generalised superficial lymphadenomegaly ensured the diagnosis of lymphosarcoma in three of the cases (Figures 2 and 3) and of lymphoplasmacytic inflammation associated with *Leishmania spp.* infection (Figures 4 and 5) in one of the cases. Thus, four cases were certainly diagnosed through the cytological examination of the lymph nodes (57.1%), with the rest of the cases being reactive lymph node hyperplasia or neoplasms of uncertain cellular origin.

In a 4-year retrospective study of 30 dogs in a Mediterranean area, it was found that the most common cause of lymphadenopathy was lymphoproliferative disease, represented by lymphoma (36%), followed by dermatological diseases (18.4%) and vector-borne diseases (16.5%), with 19 dogs being positive *for Leishmania infantum* and 2 dogs being positive for *Dirofilaria imitis* (34). The lymphocyte population is similar in normal and reactive lymph nodes (90% small lymphocytes), but plasma cells occur in greater percentage in lymph node reactivity caused by prolonged antigen stimulation. Conversely, if the percentage of large lymphocytes and lymphoblasts increases by more than 50% in the cytological sample, lymphoma is diagnosed. In dogs, generalised peripheral lymphadenomegaly is the most common sign of lymphoma, and in cats, enlarged internal lymph nodes occur more commonly (20). If Mott cells (plasma cells containing round packets of immunoglobulins called Russell bodies) and numerous macrophages with phagocytosed parasitic or mycotic organisms (as in the present study, amastigotes of *Leishmania* spp.) appear in the cytological sample, cytology allows not only the diagnosis of inflammation (granulomatous/lymphoplasmacytic lymphadenitis) but also the etiological diagnosis (leishmaniosis in this study), subsequently confirmed by PCR test to identify the species involved in the pathological process. In a study of 50 dogs naturally infested with *Leishmania infantum*, included as positive by conventional PCR and ELISA, Guerra et al. (2019) suggest that cell block immunocytochemistry (CB-

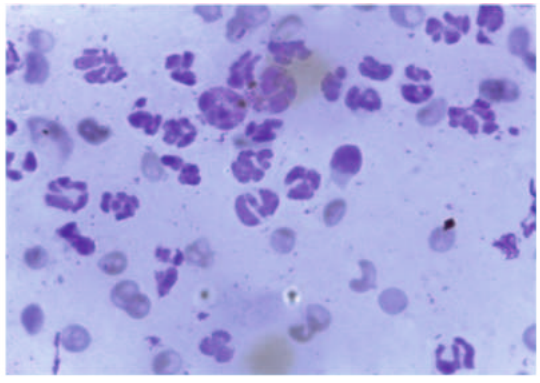

Fig. 6. Neutrophilic inflammation and skin eosinophilic reactivity. Skin imprint. MGG x 400 (Dog, Shar Pei, 6 years old)

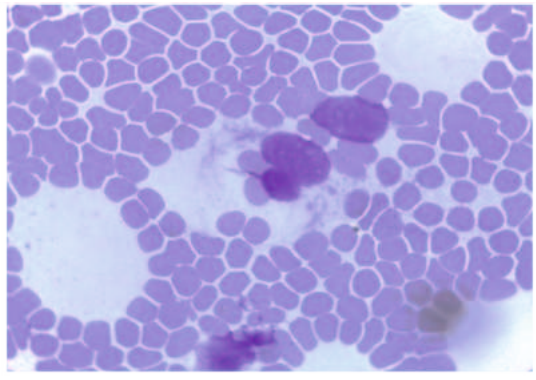

Fig. 7. Haemangioma/ hemangiopericytoma. Skin mass FNA. MGG x 400 (Dog, Metis, 3 years old)

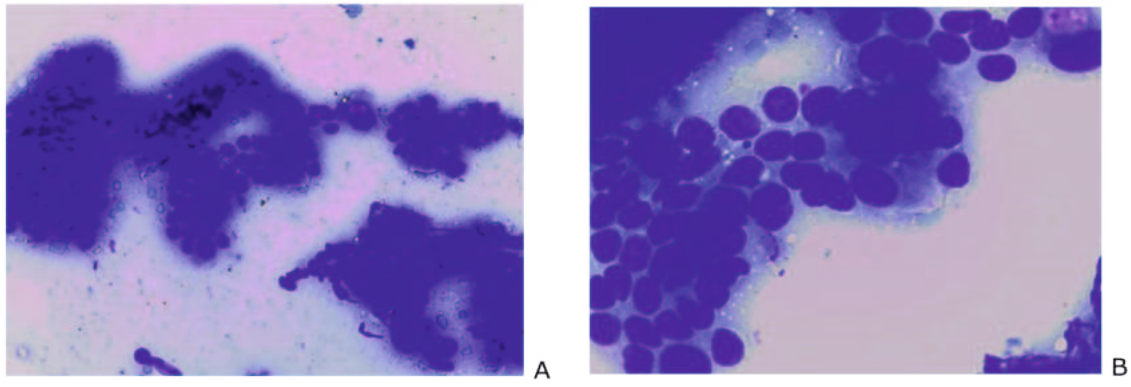

Fig. 8. Mammary gland adenocarcinoma. FNA from nodular mass of mammary gland. MGG x 400 (A), x1000 (B) (Cat, Burmese, 11 years old)

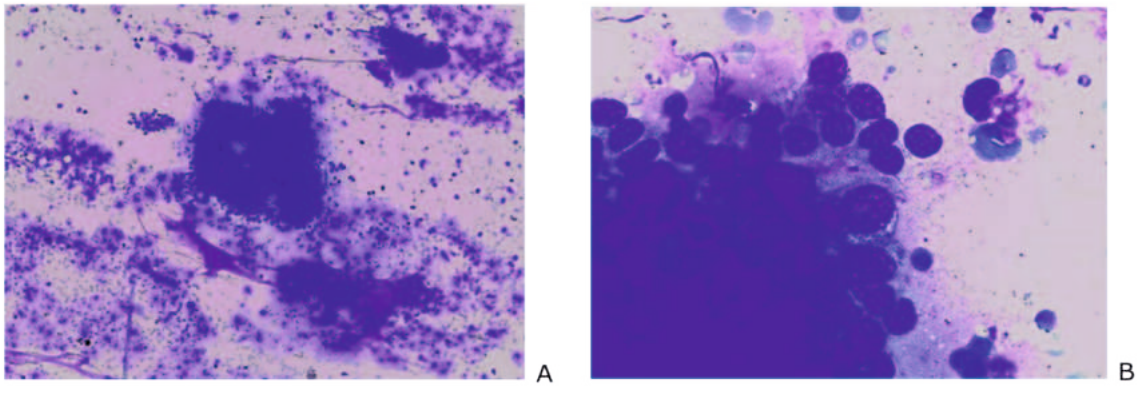

Fig. 9. Mammary gland adenocarcinoma with associated inflammatory processes. FNA from the nodular mass of the mammary gland. DQ x400 (A), x1000 (B) (Cat, Siberian Metis, 11 years old)

ICC) obtained from lymph node FNA is a promising tool for the routine epidemiological screening of canine visceral leishmaniosis (CVL)because this method has a higher sensitivity (70%) than conventional smear cytology (34%), but also a specificity and accuracy comparable to those obtained by serological and molecular methods.

Immunophenotyping methods are required to differentiate T and B lymphoma in dogs or sometimes to confirm or exclude the diagnosis of lymphoma in cats. In human medicine, immunohistochemistry, flow cytometry, and PARR (PCR for Antigen Receptor Rearrangements) techniques are currently used, but for veterinary medicine, the technique of immunolabelling on CB (cell block) is more advantageous, being in fact a technique to maximise the information obtained by FNAC, a less invasive method accepted by most pet owners, instead of invasive and costly procedures (biopsy for histopathological and immunohistochemical examinations). A recent study of 30 dogs and 27 cats suspected of lymphoma proved the usefulness of the CB ICC of lymph node FNA using CD3 and PAX-5 markers in lymphoma cell type diagnosis (33).

Numerous comparative human and veterinary clinical cytogenetic studies show genetic determinism of hematopoietic cancers, including lymphoma or lymphosarcoma in

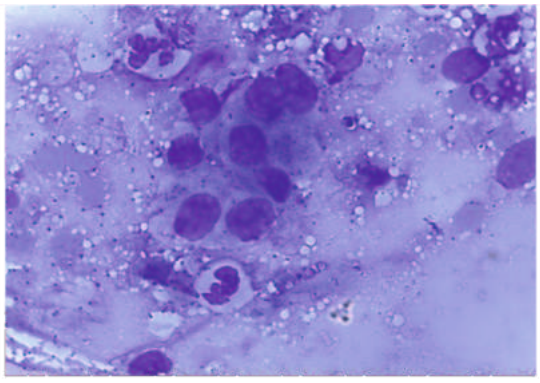

Fig. 10. Mammary gland adenoma with associated inflammatory processes. FNA from the nodular mass of the mammary gland. MGGx400 (Dog, Pekinese, 12 years old)

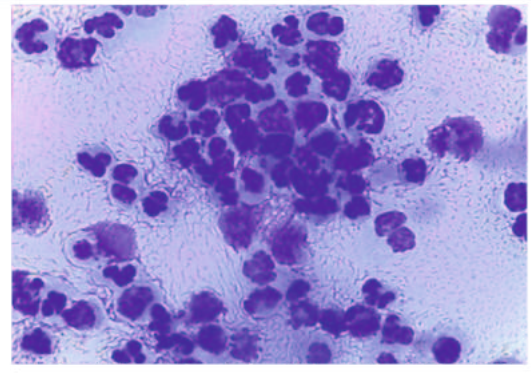

Fig. 11. Septic (bacterial) mastitis. FNA from the lump of the mammary gland. MGGx400 (Dog, Pug, 9 years old)

humans and dogs. Comparative genomic hybridization (CGH) techniques, in classical or molecular versions, are used to detect complex chromosomal rearrangements causing the loss or gain of genetic material in lymphoma cancer cells. The most common alteration in dogs with lymphoma or lymphosarcoma was trisomy 13, considered homologous to trisomy 8 in human lymphoma and myeloid leukaemia (40). Furthermore, increased Rb (retinoblastoma) phosphorylation and CDK4 activation were identified in high-grade canine T-cell lymphoma, resulting from p16 deletion/loss of chromosome 11, while increased Rb phosphorylation in high-grade canine B-cell lymphoma was correlated with c-Myc overexpression and chromosome 13 trisomy (43). The genetic background of canine lymphoma is supported by its more frequent occurrence in middle-sized to larger dog breeds (bullmastiff, Rottweiler, Scottish terrier, golden retriever, etc.), and it is also known that certain breeds are more likely to develop a particular immunophenotype of canine lymphoma (43).

The cytological examination of skin lesions or nodular formations obtained from nine investigated cases (6 dogs and 3 cats, representing 13.6% of the total) allowed a reliable diagnosis of inflammatory processes (in eight cases, 88.9%) of the lymphhistiocytic, eosinophilic (Fig. 6), pyogranulomatous, or septic types. One of the cases had a guiding diagnosis of neoplasm with mesenchymal cells (most likely haemangioma) (Fig. 7).

Cytology of the mammary gland from seven mammary nodular masses (10.6 %), belonging to four cats and three bitches, showed in all cats' neoplastic changes consistent with mammary adenocarcinoma (Figures 8A, 8B, 9A, and 9B), while in bitches a mammary adenoma (Fig. 10), an adenocarcinoma, and a septic inflammatory process were diagnosed (Fig. 11). In all tumour cases, the neoplastic process was associated with necrosis and/or inflammatory processes.

In other reported results, cytological diagnosis in cutaneous and subcutaneous masses from dogs and cats was in agreement with histopathological diagnosis in 90.9% of cases; sensitivity and specificity of cytological examination were 89.3% and 97.9%, respectively, while positive and negative predictive values were 99.4% and 68.7%, respectively, indicating the utility of FNAC in all types of cutaneous and subcutaneous lesions in dogs and cats (20).

Cutaneous and subcutaneous lesions can have four cytological aspects: non-cellular (with cyst significance or a non-diagnosable sample); predominantly cellular debris (from degenerated or necrotic areas, always non-diagnosable, requiring repeat cellular aspiration); cellular, with inflammatory cells; cellular, with non-inflammatory cells. Inflammatory skin lesions are considered purulent and aseptic when non-degenerated neutrophils predominate in the cytological sample and are caused by immune-mediated diseases (pemphigus foliaceus) or trauma, without completely excluding infections. The presence of degenerated neutrophils in cytological slides (karyolysis by hydropic swelling of nuclei with pale chromatin) is always associated with bacterial or fungal infections. If the neutrophils become karyorrhectic or pycnotic (with a degenerated nucleus by hyper segmentation, chromatin condensation, or fragmentation) and a few macrophages and lymphocytes appear alongside the neutrophils, the dermatitis is considered chronic. There are several pathological conditions associated with eosinophilic dermatitis (eosinophils more than 10 %), such as parasitic infections, allergies, type I hypersensitivity reactions, immune-mediated diseases, paraneoplastic conditions (canine mastocytoma, canine or feline carcinoma), and feline eosinophilic granuloma - extensive alopecic, erythematous masses. Granulomatous inflammation, characterised by an increased number of macrophages and the presence of multinucleated giant cells in the cytological smear, is caused by foreign body reactions, fungal infections, and atypical bacterial (mycobacterial) infections. If a mixed population of neutrophils and macrophages is present in the skin lesion, pyogranulomatous dermatitis is diagnosed, which is frequently caused by fungi (such as *Blastomyces dermatidis*, dermatophytes, etc.) and filamentous bacteria (such as *Actinomyces* spp.). Cutaneous lympho-plasmacyte inflammation, characterised by the predominance of small lymphocytes and plasma cells in the cytological preparation, could be caused by a type IV hypersensitivity reaction following insect stings, tick bites, or vaccines (1, 20).

Skin lesions and mammary mass with few or no inflammatory cells but with clustered or isolated tissue cells are classified as benign or malignant tumour processes. In this case, identification of the cell type and malignant features in the cytological sample determines the diagnosis of the morphological type of tumour: benign/malignant *epithelial type* - sebaceous adenoma, basal cell epithelioma/ squamous cell carcinoma; benign/malignant *mesenchymal type* - lipoma, fibroma, myxoma, haemangioma, he-

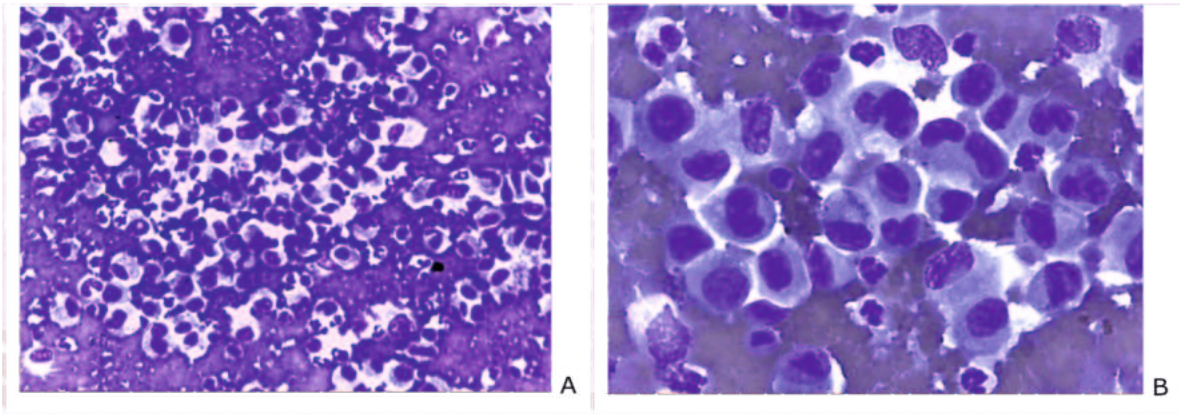

Fig. 12. Serohaemorrhagic pleural effusion. Systemic histiocytosis (cells of histiocytic type, pleiomorphic, with vesicular, spherical, oval nuclei, usually eccentric, with an obvious nucleolus, with a basophilic, vacuolar cytoplasm; sometimes binucleated or multinucleated cells are present). DQ, x 400 (A), x 1000 (B) (Cat, 16 years old)

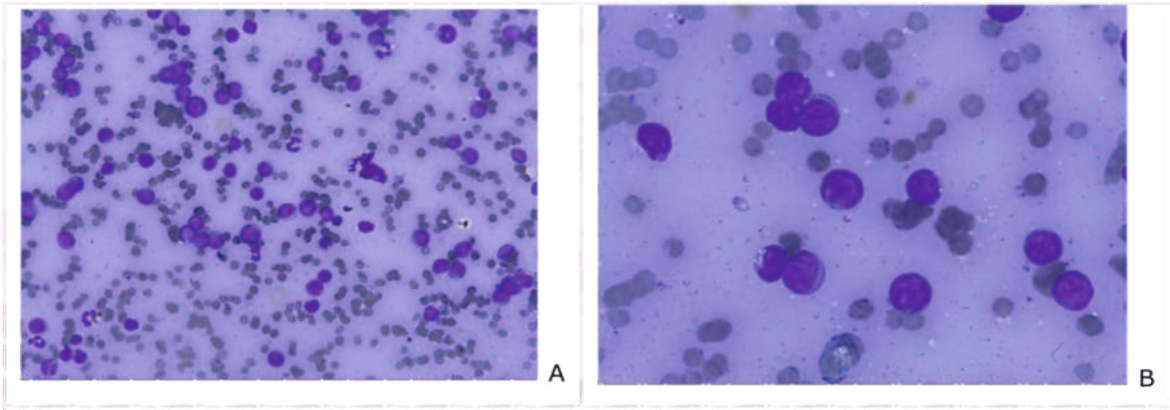

Fig. 13. Chylohaemorrhagic pleural effusion. Suspected lymphoma or thymoma (haemorrhagic and inflammatory background; lymphoblasts and atypical lymphocytes are present). DQ, x 400 (A), x 1000 (B) (Persian cat, 6 years old)

mangiopericytoma/liposarcoma, fibrosarcoma, hemangiosarcoma, histiocytic sarcoma; *discrete (round) cell tumour* - lymphoma, plasmacytoma, histiocytoma, mastocytoma, transmissible venereal tumour. If the neoplastic cells are epidermal, they appear in the cytological sample isolated or in small groups and have some distinctive malignant features: aberrant keratinization, abnormal vacuolization giving the neoplastic cell a signet ring appearance, atypical phagocytosis - emperipolesis. If the cells are of glandular origin, they form large three-dimensional nests and may have a pseudoacinar appearance (20). In addition to the 3 types of skin tumours, some books on skin cytology and dermatology include a fourth type under the heading of *melanocytic tumours*, because neoplastic melanocytes are characterised by a marked polymorphism (round or fusiform shape or epithelioid arrangement) (1).

Previous studies in dogs and cats with mammary tumours have estimated that 30% of canine mammary tumours and 80-90% of feline mammary tumours are malignant, and the sensitivity and specificity of cytology in the diagnosis of mammary tumours were 88% and 96%, respectively (20).

The predisposition of certain breeds of dogs to certain types of cancer has been documented by various studies. Based on molecular genetics and cytogenetic research, the susceptibility of canine breeds to certain types of tumours have been established, e.g., the Bernese Mountain dog has the highest susceptibility to histiocytic sarcoma, the Boxer to mastocytoma, and the Golden Retriever to several types (haemangiosarcoma, lymphoma, mastocytoma). Cytogenetic profiling of histiocytic sarcoma diagnosed in the Bernese and Golden Retriever revealed chromosomal aberrations, the majority being deletions of the tumour suppressor genes *CDKN2A/B*, *RB1*, and *PTEN* located on canine chromosomes 2, 11, 22, and 31. Several molecular genetic studies of canine hemangiosarcoma have revealed mutations of the tumour suppressor gene *TP53*, and other studies have suggested the involvement in the pathogenesis of canine hemangiosarcoma of alterations in the p16-cyclin D1-Rb pathway, the same pathway also being dysregulated in the same type of cancer in humans. Some investigators have also found overexpression of angiogenesis-related growth factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), with the VEGF receptor preferentially enriched in tumours of Golden Retrievers compared to other breeds. Dysregulation of the c-KIT proto-oncogene encoding stem cell factor receptor (KIT) has been shown to play an important role in the aetiology of canine mastocytoma. In canine melanoma, loss of tumour suppressor function due to reduced *p16, TP53, Rb*, and *p21* gene expression are the most common genetic abnorma-

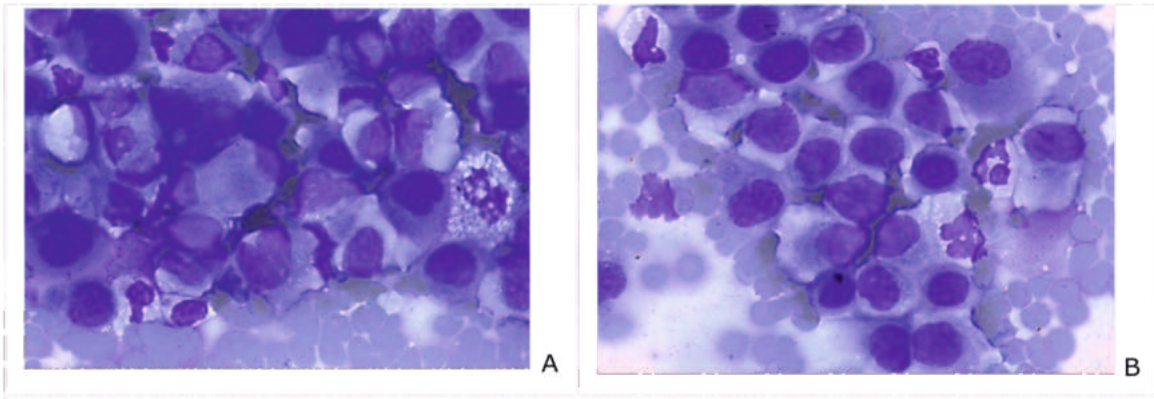

Fig. 14. Haemorrhagic pericardial effusion. Metastatic carcinoma. Cells with malignant characteristics: bi- or multinucleate cells, anisocytosis, anisokaryosis (A), obvious nucleoli, sometimes macronucleoli (B). MGG, x 1000 (Dog, Rottweiler, M, 12 years old)

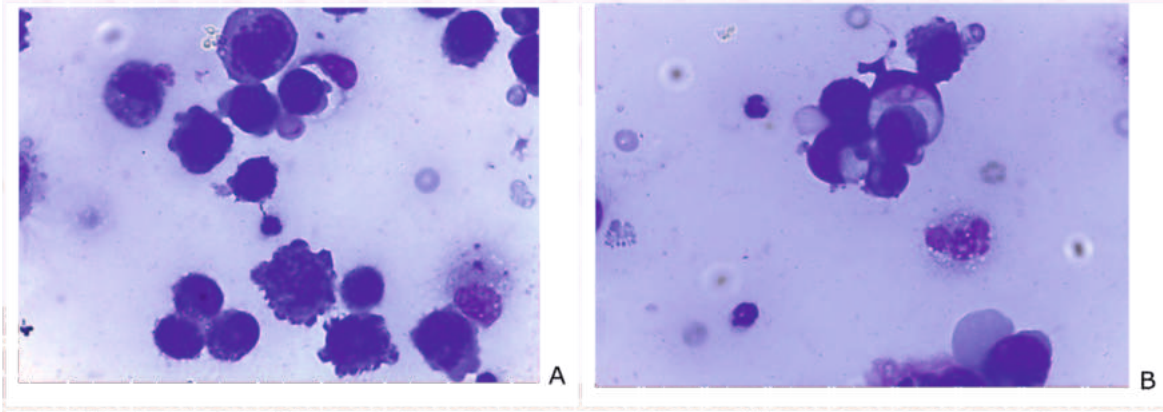

Fig. 15. Pericardial effusion. Metastatic carcinoma. Mitoses (A), groups of carcinomatous cells-signet ring aspect (B). MGG, x 1000 (Dog, Rottweiler, M, 12 years old)

lities. Comparative gene expression studies in human breast cancer and canine mammary tumours have shown an overlap of dysregulated genes (*TP53, BRCA1, BRCA2, P13*) (9, 7). It is known that TVT cells have a karyotype composed of only 59 chromosomes, different from the 78 chromosomes of normal canine diploid cells. This tumour can be transmitted from one dog to another, via the direct implantation of tumour cells (1).

The cytological examination of fluids and organ tissues of the thoracic cavity was performed for nine cytological samples (13.6% of the total samples), collected from three cats and five dogs. Out of five pleural fluids cytologically examined, three were associated with inflammatory processes (pleuritis, pleuropneumonia), of which one was suggested as feline infectious peritonitis (FIP); the other was classified as a tumour (malignant systemic histiocytosis) (Fig. 12); and the third was considered lymphoma or thymoma (Fig. 13). The two pericardial fluids examined were classified as neoplastic, either being associated with neutrophilic or mesothelial inflammatory reactions (Figures 14 and 15). The only fine needle aspiration cytological sample from the lung mass examined in this study highlighted a process of tissue necrosis without excluding necrosis from a tumour. For the eight cases with changes in the cytology of the fluids or organs of the thoracic cavity, a definite cytological diagnosis was established for six cases (three inflammations and three neoplastic processes), representing 75%.

The cytological examination of the peritoneal fluid and the fine needle aspirate from the abdominal and pelvic cavity organs were performed on 22 samples (representing 33.3% of the total samples), collected from 14 dogs and six cats. Of these, 13 cases received a clear diagnosis (61.9%), including six neoplastic tissues (four carcinomatous types, one histiocytoma, and one mesothelioma) (Figures 16 and 17), five inflammatory processes, pyogranulomatous with viral, parasitic or mycotic aetiology - feline infectious peritonitis (Fig. 18), ehrlichiosis (Fig. 19), suspicion of histoplasmosis (Fig. 20), a case of hepatic steatosis, and a case of intracavitary haemorrhage. The rest of the cases were uncertainly diagnosed: no exfoliative neoplasms, hypo cellular transudates, or tissue reactions caused by the migration of *Dirofilaria imitis* larvae.

According to our results and those of other authors, cytology of effusions is a very useful method for the diagnosis of organ diseases associated with fluid accumulation in the pericardial, pleural, or peritoneal cavities, sometimes allowing a definitive diagnosis by identifying specific etiological agents or by highlighting characteristic neoplastic cells. Three simple investigations of effusions - total protein, nucleated cell count, and cell morphology of the smear - are sufficient to classify effusions into the following types: pure transudate, modified transudate, septic or non-septic exudate, haemorrhagic effusion, or neoplastic effusion (4, 39).

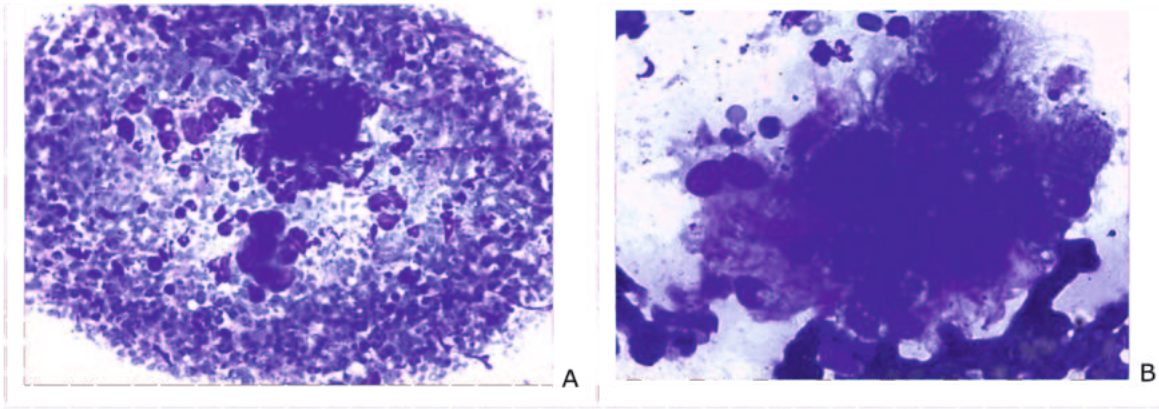

Fig. 16. Cholangiocellular carcinoma and associated inflammatory process (haemorrhagic background, cluster of canalicular epithelial cells with malignant characteristics – anisocytosis, anisokaryosis, multiple obvious nucleoli; inflammatory cells are present. MGG, x 400 (A), x 1000 (B). Liver FNA (Cat, 13 years old, common breed)

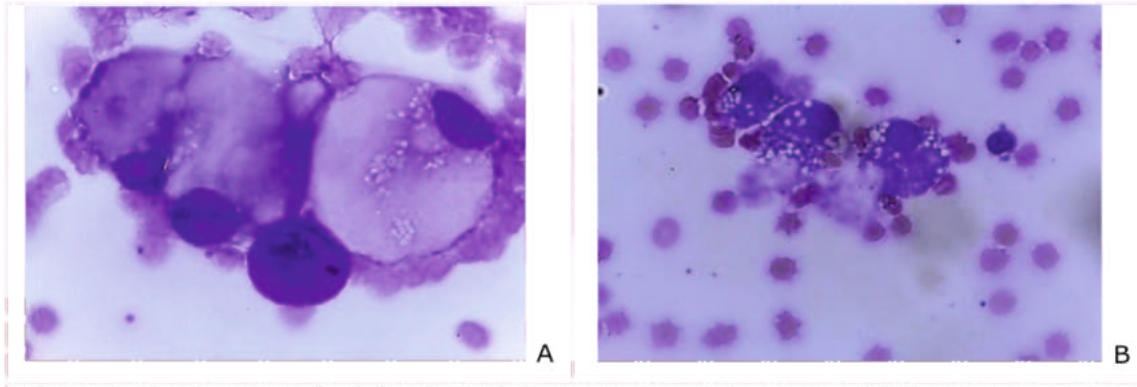

Fig. 17. Bloody ascitic fluid (NCC 164/µl). Carcinoma and associated inflammatory process (group of giant epithelial cells, with a carcinomatous appearance - fine perinuclear vacuolization or large vacuoles push the eccentric nucleus giving it a signet ring appearance (A). Inflammatory cells are also present, mainly macrophages (B). MGG, x 1000 (Dog, German Shepherd, 7 y. old)

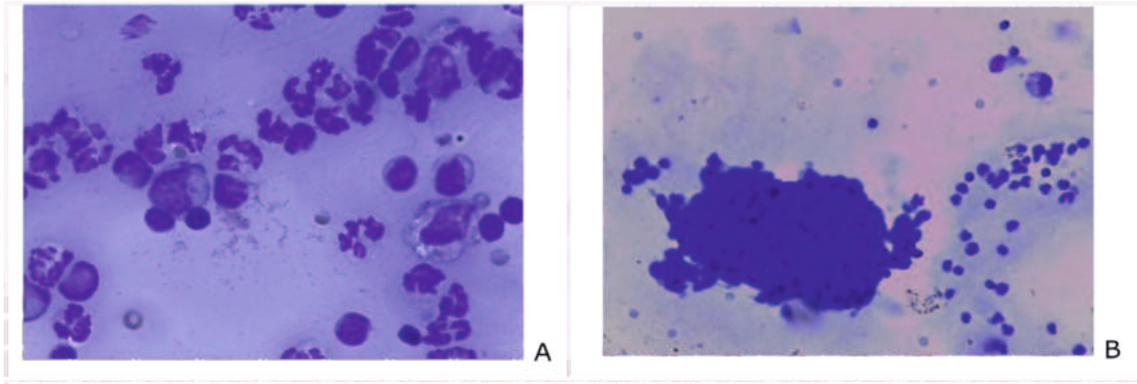

Fig. 18. Ascitic fluid (NCC 5760/µl). Pyogranulomatous inflammation with hyperplastic mesothelial reaction from feline infectious peritonitis (FIP). Proteinaceous granular background, numerous non-degenerate neutrophils, some lymphocytes, monocytes, macrophages (A), plaques of exfoliated mesothelial cells (B). DQ,x400 (A), x1000 (B) (Cat, common breed, 7 m.)

Pure transudates are passive processes of fluid accumulation in the serous cavities without alteration of capillary permeability (with few blood cells and proteins), most often caused by hypoalbuminemia (liver failure, cirrhosis, nephropathies, and enteropathies with albumin loss). Altered transudates are due to venous and lymphatic obstruction or vascular abnormalities such as lymphangiectasis, effusions being rich in lymphocytes (chylous effusion), few non-degenerate neutrophils, and mesothelial cells; they occur in the following clinical conditions: congestive heart failure, mediastinal tumours (lymphoma, thymoma), abdominal neoplasms, diaphragmatic hernia, or any disease with intrahepatic portal hypertension. Exudates are caused by increased vascular permeability and

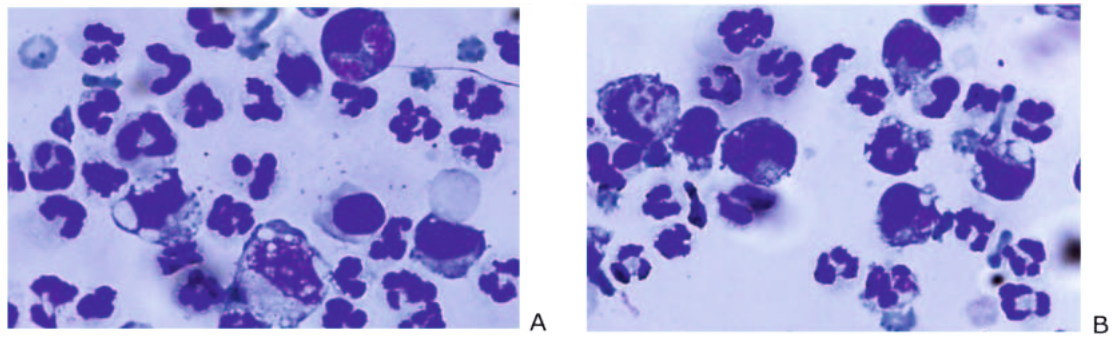

Fig. 19. Ascitic fluid (NCC 30050/µl). Pyogranulomatous inflammation. Inflammatory cells: predominantly non-degenerate neutrophils, monocytes, macrophages, lymphocytes (A and B), cytophagocytosis (B), Monocyte with 3 *Erlichia canis morulae* (A), 2 monocytes with *Erlichia canis morulae*. DQ x 1000, (Argentine Dog, F, 1.3 years)

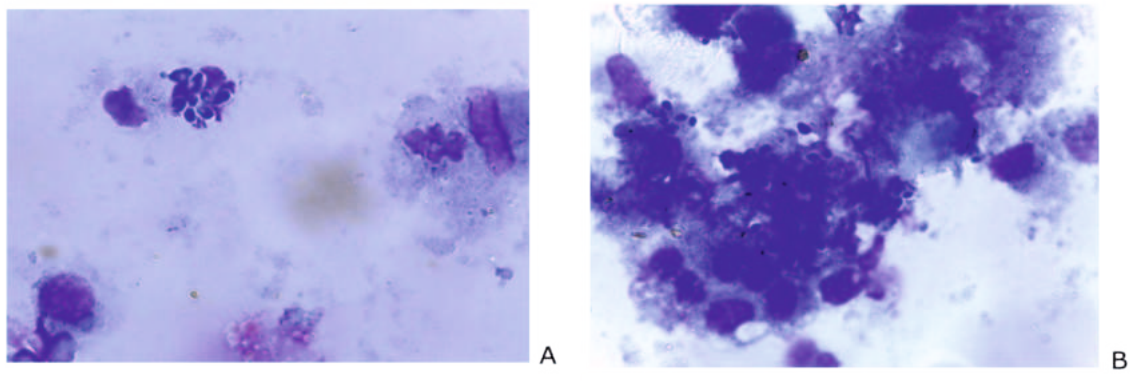

Fig. 20. Ascitic fluid (NCC 70/ µl, proteins 4 g/dl). Inflammatory process associated with a systemic mycosis *(Histoplasma capsulatum).* Protein background, inflammatory cells (macrophages with oval or round yeast-like organisms), eosinophilic granules (A), groups of macrophages that include fungal organisms (B), DQ x 1000 (Dog, Pekinese, 4 years old)

inflammation, with the predominant cell population being neutrophils, which may also be accompanied by a variable number of lymphocytes (more in cats), macrophages, plasma cells, and mesothelial cells. Non-septic exudates characterised by the presence of non-degenerate neutrophils are the result of long-standing modified transudates but are also caused by some irritating pathological conditions inducing an inflammatory response, such as biliary peritonitis or pleuritis characterised cytologically by mesothelial reactivity and the presence of bile on the smear; feline infectious peritonitis (FIP) with a granular protein background and a mixed cell population on the slide. Septic exudates, characterized cytologically by the presence of a large number of degenerated neutrophils (swollen pal nucleus or nuclear fragmentation), are caused by the penetration of bacteria into body cavities as a result of intestinal perforations, migration of foreign bodies, or pleuropulmonary infections (classic pyothorax in cats) and peritoneal infections (pyometra in bitches, prostate abscess in dogs). Identification of phagocytized, intracellular bacterial or fungal organisms on the smear clarifies the aetiology of the disease. Neoplastic effusion, resulting from the exfoliation of neoplastic cells in the cavity fluids, may or may not be accompanied by a significant haemorrhage or inflammatory process. Exfoliative tumours generating effusions in dogs and cats are lymphoma/lymphosarcoma (pleural), carcinoma/adenocarcinoma (pleural and peritoneal), haemangiosarcoma (pleural and peritoneal), and less commonly, mesothelioma (24, 38).

In a study of dogs and cats with pyothorax (2010-2020), cytological examinations and bacterial cultures of fluid extracted by thoracocentesis identified intracellular bacteria in 93% of cats and 73% of dogs, with isolated bacteria being aerobic in 60% of dogs (more *Actinomyces spp*. and *Escherichia coli*) and 66% of cats (more *Pasteurella spp.* and *Actinomyces spp.*), but anaerobic bacteria were also isolated, more in cats (79%) than in dogs (45%), represented in both species by *Bacteroides spp.* and *Fusobacterium spp.* (17).

Feline infectious peritonitis caused by feline coronavirus (FCoV) has an effusive form, characterised by the production of peritoneal, pleural, and pericardial effusions, with a physicochemical and cytological profile considered characteristic but also partially mimicked by other pathological conditions. For this reason, a group of researchers initiated a study to highlight the utility of the lactate dehydrogenase (LDH)/total nucleated cell count (TNCC) ratio in the effusion (sensitivity 82.1%; specificity 83%) and concluded that an increased LDH/TNCC ratio is consistent with the diagnosis of FIP (31).

Disseminated histoplasmosis is a fungal infection prevalent in dogs and cats worldwide in temperate and subtropical areas. Cases with thoracic or abdominal effusions due to nodular granulomatous inflammation in the organs have been reported. Cytological examination of the effusion sediment alone, as in our reported case, allowed the

identification in macrophages of oval or round organisms, 3-5 µm in diameter, with a spherical central body and a clear halo, compatible with *Histoplasma capsulatum* (3, 22, 42). Neoplasms are common causes of effusions in dogs (57% pericardial effusions and 11% peritoneal and pleural effusions) and cats (37% pleural effusions) (29). Cytological evaluation for the diagnosis of neoplasia has a sensitivity of 64% in dogs and 61% in cats. Approximately half of the neoplastic effusions reported in dogs and cats were carcinomas (pancreatic adenocarcinomas and ovarian tumours), and more than half of the neoplastic effusions reported in cats were lymphomas. Splenic and atrial haemangiosarcoma are the most common in dogs, resulting in haemorrhagic effusions. Mesotheliomas have a controversial diagnosis because mesothelial reactivity can mimic mesothelioma and mesothelial cell changes can generate confusion with adenocarcinoma (24, 26).

Some studies have turned to alternative techniques already known in human medicine to differentiate neoplastic cell populations from effusions in dogs and cats. A human study on paraffin-embedded cell block fluid samples and biopsies from cases with mesothelioma and mesothelial reactions showed that in determining mesothelial malignancy, BAP1 immunohistochemistry and *p16* FISH analysis have increased sensitivity in cell block as well as in biopsies (16). FISH analysis quantifies chromosomal aberrations for one or more chromosomes and is a specific technique for discriminating malignant from benign effusions (15). Marcos et al. (2019) used the CTB-IHC (cell tube block immunohistochemistry) technique for 25 cavity effusions collected from dogs and cats, cytologically diagnosed as reactive, neoplastic, suspected neoplastic, and chylous. CD3 and PAX5 immunostaining allowed phenotyping of lymphomas; carcinomas were CK positive, and normal, reactive, and neoplastic mesothelial cells were WT1 positive. Milne et al. (2021) compared conventional effusion cytology and IHC results for effusion cell blocks and biopsies taken from dogs with mesothelioma (M), mesothelial hyperplasia (MH), and carcinoma (C) and concluded that cytology alone had a diagnostic sensitivity of only 56% and IHC results for cell blocks and biopsies were comparable for potentially useful markers that helped discriminate between M, MH, and C. Another example of the utility of the cell block IHC technique is the study performed by Kita et al. (2022) on seven dogs diagnosed with papillary ovarian adenocarcinoma causing peritoneal and/or pleural effusion. Immunohistochemistry was performed on cell blocks from effusions using the same markers as in human ovarian carcinoma, and the results showed that canine ovarian adenocarcinomas are consistently positive for PR (progesterone receptor); PR expression could be predictive of the outcome of anti-progesterone therapy in dog ovarian adenocarcinomas.

CONCLUSIONS

Carrying out a retrospective of 60 cases investigated by cytological examinations, it can be concluded that the microscopic examination of cytopunctures from cavity fluids or from superficial or deep solid masses has the greatest diagnostic value in differentiating between inflammatory and neoplastic processes. Thus, 28 inflammatory processes (46.6%) and 21 neoplastic processes (35.1%) were certainty diagnosed, with the rest of the cases (18.3%) being uncertain due to hypo cellularity, reactive hyperplasia, and circulatory disorders. Some of the inflammatory processes benefited from an etiological diagnosis without other complementary examinations, either due to some cytological characteristics (three cases of FIP, one case of eosinophilic dermatitis) or due to the evidence of intracellular or extracellular pathogens (one case of *Staphylococcal* infection, one case of Leishmaniosis, one case of systemic histoplasmosis, and one case of canine ehrlichiosis).Among the neoplasms diagnosed,15 cases (75%) benefited from accurate cellular classification by direct cytological examination (three cases of lymphosarcoma, two cases of histiocytoma, four cases of mammary gland adenocarcinoma, one mammary gland adenoma, one mesothelioma, and four cases of hepatic and cholangiocellular carcinoma). It can be concluded that the cytological examination continues to remain a valuable, non-invasive, rapid, and inexpensive tool for the diagnosis of inflammatory and neoplastic diseases in veterinary medicine. In addition, the cytological examination can constitute a screening for the proposal of more accurate investigations, such as cell-block immunocytochemistry (CB-ICC), cell-tube block immunohistochemistry (CTB- IHC), quick cytogenetic tests, and the determination of tumour markers with a view to targeted antitumour therapies.

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