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Cytodiagnosis of Common Skin Lesions in Dog and Cat: A Review

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Abstract: Diagnostic cytology has recently been employed for diagnosis of some of the diseases in dogs and cats, more commonly for the diagnosis of skin lesions or masses. Valuable additional information has been provided by cytological features of fine needle aspirates (FNA), skin scrapings, tape preparations, swabs or by imprints of tissue cells. Cytology is a reliable and minimally invasive method of obtaining tissues for diagnosis. It is a rapid method to be used and it can often differentiate non-neoplastic lesions (follicular cysts and inflammatory lesions) from proliferative lesions of the skin in dogs and cats. Inflammation is easily differentiated from neoplasia based on the presence of inflammatory cells and/or the etiological agents. For example, the presence of neutrophils with intra-cytoplasmic phagocytosed cocci confirms a pyoderma. Acetate tape impression while squeezing the skin has been found to be a sensitive method for the diagnosis of Demodex canis in dogs and often detects more adult and larval mites than deep skin scraping. Cytological evaluation of skin lesions or masses is an effective means for diagnosing many cutaneous and subcutaneous tumors in dogs and cats. For instance, based on cytologic category, mast cell tumors are in the group of round cell tumors. These tumors are composed of individual round cells that are fairly abundant on cytologic preparations and in many cases cytology was found more accurate in diagnosis of neoplastic skin lesions than the non-neoplastic ones. Finally, the interpretation of skin origin cytology relies on the understanding of the most common skin diseases that occur in these animals and on the familiarity with the most typical cytological features that characterize each of these conditions.

Key words: Cytology · Diagnosis · Inflammation · Neoplasia · Skin lesions

INTRODUCTION

The skin is the largest organ of the body with many different functions as thermoregulation, immune protection, sensory perception, vitamin D production and it acts as a barrier between the animal and the environment. Besides all of these important functions, there are many diseases that affect directly the skin, it may also share or reflect pathologic processes from other tissues [1]. Skin and subcutaneous lesions are readily discovered by veterinarians during routine physical examination and may be detected by owners, prompting a visit to the veterinarian. It is often difficult from physical examination alone to determine the cause. Cytology is a non-invasive technique that can often provide useful diagnostic information [2].

Cytology is the analysis of tissue cells and it is a highly efficient and valuable examination to evaluate a lesion, after which it is possible to establish the next step in the diagnostic approach [3]. Cytology, the microscopic examination of tissue samples spread onto slides, is a powerful tool for evaluation of skin lesions in small animal patients. In many cases, cytology can provide a definitive diagnosis for cutaneous masses. In others, sample evaluation can rule out some differential diagnoses and help the clinician choose the best next diagnostic test to perform. More recently, valuable additional information has been provided by cytological features of fine needle aspirates, skin scrapings or from imprints of tissue sections [4].

Cytology can often differentiate non-neoplastic cysts, inflammatory lesions secondary to tissue damage/infection, or proliferative lesions. Proliferative lesions can then be characterized as benign (hyperplasia or benign neoplasia) or malignant neoplasia. Neoplasia is easiest to recognize when cytology reveals a monomorphic or monotypic population of non-inflammatory cells. Although it is sometimes difficult to

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determine specific types of neoplasia using cytology, morphologic criteria can be used to differentiate benign and malignant proliferations and to classify tumors into general categories [2]. Cytologically, neoplastic cells are divided into three groups, namely round or discrete cell, epithelial and mesenchymal tumour cells [5]. Overall, cytology was found most accurate in diagnosis of neoplastic lesions followed by inflammatory lesions and hyperplastic lesions [6]. Therefore, based on the above facts, to the main goal is to review the common skin lesions and their cytologic features in dogs and cats.

General Approach For Cytological Diagnosis

Cytological Sample Collection Techniques and Sample Preparation: Fine-needle aspiration (FNA) cytology is a useful technique for the investigation of soft tissue masses (Cutaneous lesions, lymph nodes, intra-thoracic or intra-abdominal masses) and effusions from body cavities. The technique can be easily performed in a practice setting. Acquisition of a fine-needle aspirate for cytological examination is a fast and easy, minimally invasive technique which can be performed in every practice or clinic [3, 7].

The advantages are that generally no anaesthesia or sedation is required and the risk of haemorrhage is minimal while the technique provides an excellent evaluation of single cell morphology. Impression smears and scrapings can be made directly from ulcerated skin lesions or from the cut surface of excised tissue/biopsy specimens. Imprints can be made from wet surfaces (e.g. biopsies, ulcerated or exudative skin lesions) as well as from dry skin lesions using Sellotape [7].

The disadvantages of impression smears are that they only collect cells from the surface of the lesion and therefore may not be representative of underlying pathology, fewer cells are collected and bacterial contamination is more likely. Scrapings may be useful for sampling extremely firm lesions which are less likely to exfoliate cells with the aspiration technique. This technique can be used for cutaneous lesions and excised tissue specimens. This is a particularly useful technique for collecting cells from masses which do not readily exfoliate e.g. mesenchymal tumors [2]. The scraped material is then transferred to a slide and cytological smears can be prepared using the blood smear or squash preparation technique. Several stains are available which can be used alone or in combination. The routinely used stains for cytology are Romanowsky-type stains [8].

Cytological Evaluation and Interpretation: Microscopic evaluation of the smears should always be performed in the same sequence: After a macroscopic (eye) evaluation of the smear to detect potential areas of interest, the smear is scrutinised at low magnification with a 10× or 20× objective lens to detect areas in which cells are arranged in a monolayer and also areas which are hypercellular or have different staining characteristics. These areas are then examined under higher magnification (With a 100× oil objective lens). The first step in making interpretation of a cytological diagnosis is to classify the lesion into one of five general categories of disease processes: 1) inflammation, 2) cyst formation, 3) hemorrhagic lesion, 4) neoplasia, or 5) mixed cell population. In some cases, more than one pathologic process may be occurring simultaneously in a single lesion. For instance, there may be hemorrhage within a neoplasm or inflammation within a cyst. However, when the population is not mixed and they are in their purist form, the categories are easily distinguished by the cell population present [9].

Major Non-Neoplastic Skin Problems and Their Cytological Features

Pyoderma: Pyoderma is a bacterial skin infection and it is among the most common causes of skin diseases in dogs, however, it is less common in cats [10]. Lesions may be superficial and involve only the epidermis or they may affect deeper structures in the dermis or subcutaneous tissue and it is therefore divided into surface, superficial and deep pyoderma. *Staphylococcus pseudintermedius* was the most commonly isolated bacteria from dog's skin [11].

Superficial pyodermas are the most common causes of cutaneous bacterial infection in dogs. They affect the superficial portion of the hair follicles (bacterial folliculitis) or the epidermis (impetigo), causing pustules. The most common lesions are crusted papules due to the transient nature of canine pustules. Pruritus, epidermal collarettes, hyperpigmentation and alopecia are also common findings. Deep pyoderma does not occur spontaneously, often starting as superficial pyoderma. Other organisms such as *Proteus spp., Pseudomonas spp.* and *E. coli* may be involved [12].

Clinical Signs: The affected skin appears erythematous, hyperpigmented, with the presence of seropurulent debris from the ruptured pustules; variable pruritus, swelling, skin stiffness and evident pain are also noted [13].

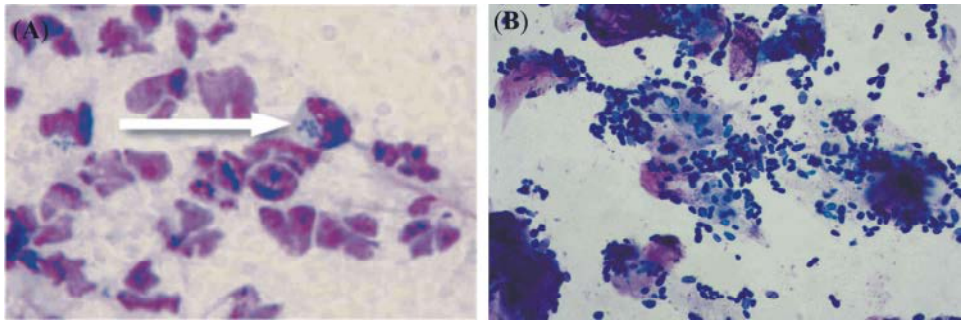

Fig. 1: The presence of intracytoplasmic cocci phagocytosed by neutrophils (arrow) confirms a pyoderma (1000x magnification) (A) [14]. Malessezia organisms of skin scraping of dog's skin (B) [18].

Cytologic Appearance: Cytology is a reliable, fast and minimally invasive in-house test to confirm the presence of a bacterial infection. The presence of neutrophils with intracytoplasmic phagocytosed cocci confirms a pyoderma (Figure 1A). Where there is deep pyoderma, the inflammatory pattern is characterized by the presence of degenerated neutrophils, macrophages and sometimes eosinophils. Rods can also appear in rare cases. Lack of micro-organisms in skin cytology does not rule out an infection and whilst cytology is the first diagnostic test to be performed, it cannot replace bacterial culture or histopathology [14].

Malassezia Dermatitis: *Malassezia pachydermatis* is a commensal skin yeast, commonly isolated from lips, interdigital skin, anal mucosa and external auditory canal. It is an opportunistic yeast, which usually manifests itself after the installation of other diseases. It is very common in dogs and less frequent in cats [15]. Previous antibiotic therapy is associated with the development of cutaneous *M. pachydermatis* over growth in dogs, as well as disorders of keratinization and hypersensitivity diseases. Dog breeds of Basset Hounds, Cocker Spaniels and West Highland White Terriers are more predisposed to this type of infection [16].

Clinical Manifestations: *Malassezia* dermatitis is the presence of moderate to intense pruritus, erythema, lichenification, oily skin, malodor, alopecia and erosions [16].

Cytologic Appearance: Cytology is the diagnostic method of choice and allows microscopic identification of the increased number of yeasts (Figure 1B). Samples can be obtained by skin scrapings, swabs, direct imprint or by acetate (clear) tape preparation [17, 18].

Sporotrichosis: Sporotrichosis is a subcutaneous mycosis caused by a dimorphic fungus, *Sporothrix schenckii*, which can infect animals and humans. It is a zoonotic disease and transmission to humans occurs through bites or scratches and contact with cats ulcers. *Sporothrix schenckii* is present in the decaying vegetation and soil and animal contamination occurs by skin open lesions such as perforations, bites and scratches. Once the organism is in the host, the fungus may cause local lesions and possibly systemic signs [19].

Clinical Forms and Signs: Sporotrichosis has three forms: cutaneous, lymphocutaneous and generalized and more than one form can occur simultaneously in the same animal. The cutaneous form is usually confined to the area of fungus inoculation and manifests after an incubation period of one month. If this lesion is not treated, the progression to lymphocutaneous form can occur. The lymphocutaneous form is characterized by the development of nodules that evolve into ulcers, affecting skin, subcutaneous tissue, lymph vessels and regional lymph nodes [18, 19].

Cytologic Appearance: Cytological evaluation usually reveals oval to elongate yeast cells consistent with *S. schenckii* form and inflammatory cells may also be present [20].

Cryptococcosis: *Cryptococcus spp.* is a saprophytic fungus present in the environment and in the feces of pigeons, capable of causing systemic infection in dogs and cats, with a higher incidence in felines. The species of interest in veterinary medicine are *C. neoformans*, which has a global distribution and *C. gattii* that has a limited distribution [21]. *Cryptococcus neoformans* typically infects animals by inhalation and may cause ophthalmic,

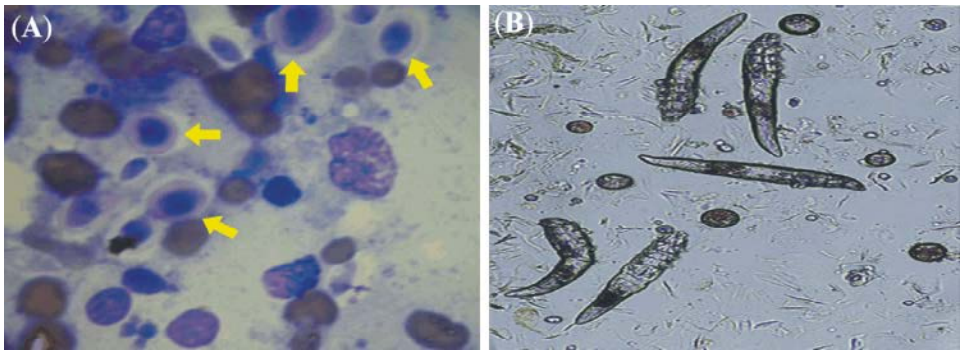

Fig. 2: Feline Cryptococcosis: Cytology by fine needle aspirate of the nasal masse showing several encapsulated structures (arrows) compatible with *C. neoformans* (100x oil objective) (A) [23]. Demodex: Unstained skin preparation (B) [26].

upper respiratory tract and central nervous system lesions. In cats with positive serology for feline immunodeficiency virus (FIV) cryptococcosis tends to manifest itself in a disseminated or advanced form [22].

Clinical Signs: Ulcerative lesions in the nasal, oral or pharyngeal mucosae, or a nasal masse may be present. Mycotic rhinitis and cutaneous nasal bridge and nasal plan involvement are the most frequent findings [15].

Cytologic Appearance: Cytological examination may reveal the presence of leukocytes, macrophages and numerous encapsulated structures (Yeasts) of different sizes (Figure 2A) [23].

Demodicosis: Demodicosis is a very common skin disease in dogs but rare in cats. It is an inflammatory disease, in which large amounts of *Demodex* mites are found in the skin. Mites proliferate in the hair follicles and sebaceous glands causing the disease. It is a common condition and it is often serious in dogs. Besides *Demodex canis*, two less common species were reported, *Demodex cornei* and *Demodex injai*. Feline demodicosis may be caused by two different kinds of mites, *Demodex cati* and *Demodex gatoi*. The mites are transferred from the mother to the offspring in the early life. *Demodex canis* is considered a commensal in canine skin. It is believed that this disease is a consequence of a specific immunosuppression, which allows the proliferation of the mites [24].

Clinical Manifestations: Erythema, comedones, scaling, partial or complete alopecia, papules, follicular casts, pustules and in severe cases, furunculosis, crusting, exudation and ulceration with focal draining tracts can be clinical sings and lesions. Generally, the lesions begin on the face and limbs, but they may become generalized. Demodicosis can be classified into generalized or localized. The involvement of one complete body region, five or more focal areas, or the involvement of the legs is considered generalized demodicosis. The diagnosis is made by acetate tape impression, deep skin scrapings or trichogram. In some rare cases, in the legs or certain breeds such as Sharpeis, these tests may be negative, requiring biopsies for mite detection [25].

Cytologic Appearance: Acetate tape impression while squeezing the skin has been found to be a sensitive method for the diagnosis of *Demodex canis* in dogs and often detects more adult and larval mites than deep skin scraping (Figure 2B). The tape method may be better tolerated by patients and easier to perform than deep scrapings, depending on location [26].

Pemphigus Foliaceus: The most common autoimmune skin disorder in the dog, pemphigus foliaceus (PF) is a pustular to crusting autoimmune dermatitis. It affects the epidermis, targeting various adhesion molecules, especially desmosomes, which hold keratinocytes together. In human PF, the desmoglein-1 glycoprotein (DSG1) in the desmosome is the primary target of autoantibodies and the same glycoprotein was previously suspected to be the primary target in dogs; however, it is now believed to be a minor autoantigen, with current evidence suggesting that desmocollin-1 is a major autoantigen in canine PF. Genetic factors appear to play a role in the development of PF, with Akitas and Chows the breeds considered most at risk. Triggers include

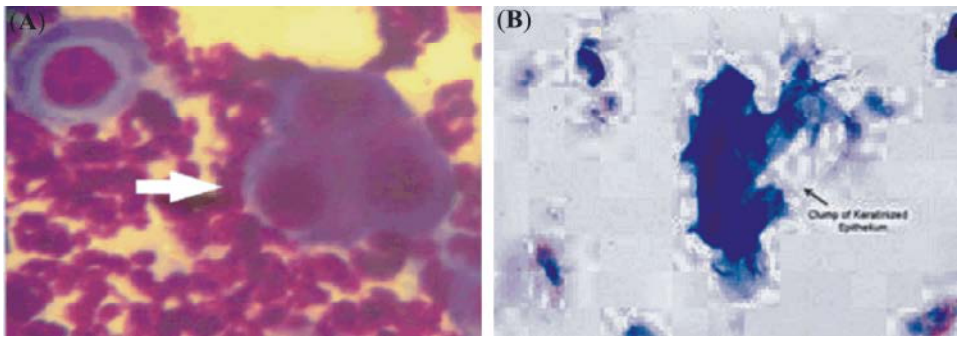

Fig. 3: Impression cytology obtained below a crust on the nasal planum of a dog diagnosed with pemphigus foliaceus. Note the number of neutrophils surrounding clusters of large, basophilic acantholytic keratinocytes (arrow) (100x magnification) (A) [29]. A follicular cyst cytology revealed that the epithelial cells degenerate leaving an amorphous, basophilic cellular debris with cholesterol crystals (arrow) (B) [9].

chronic allergic skin disease and drugs (Antibiotics, NSAIDs, topical flea spot-ons), but the most important one is ultraviolet light [27].

The initial lesion is a macule that rapidly progresses to pustules, which are often large and coalesce. The pustules are frequently fragile and easily ruptured, resulting in crusting [27].

Clinical Observations: Crusts are the most common clinical signs and lesions. Erosions may be noted; ulcerations are rare but can be present in cases complicated with a deep pyoderma. Canine PF is often characterized by crusting, initially involving the face (Especially dorsal muzzle and nasal planum, periocular region and pinnae) and subsequently progressing to a generalized form [28].

Cytologic Appearance: Cytology of an intact pustule or of the skin below a crust will often reveal the presence of numerous non-degenerate neutrophils surrounding individual or rafts of acantholytic keratinocytes, which appear as large, rounded basophilic nucleated keratinocytes (Figure 3A) [29].

Follicular Cyst: This non-neoplastic lesion may also be termed epidermal inclusion cyst or epidermoid cyst. These cysts are found in a third to a half of the non-neoplastic non-inflammatory tumorlike lesions removed in dogs and cats, respectively. The cyst occurs most frequently in middle to older aged dogs.

Clinical Signs: Single or multiple, firm to fluctuant, with a smooth, round, well circumscribed cyst appearance. These are often located on the dorsum and extremities. Degradation of cells within the cyst may lead to the formation of cholesterol crystals which appear as negative stained, irregularly notched, rectangular plates best seen against the amorphous basophilic cellular debris of the background [30].

Cytologic Appearance: Cytologically, large numbers of mature, keratinized squamous epithelial cells are seen. Eventually, the epithelial cells degenerate leaving anamorphous, basophilic cellular debris with cholesterol crystals (Figure 3B). If the cystic structure ruptures, a neutrophilic inflammatory response occurs [9].

The Common Neoplastic Skin Lesions and Their Cytological Features

Mast Cell Tumor: Mast cell tumors (MCTs) arise from malignantly transformed mast cells. In dogs, most of these tumors arise as primary tumors in the skin. They are the most common skin tumor in dogs, accounting for roughly 20% of all reported skin tumors. Any breed may be affected with MCTs, but certain breeds are predisposed, including golden retrievers, Labrador retrievers, Boston terriers, boxers and pugs. These tumors demonstrate more benign behavior and rarely lead to death. Canine MCTs can grossly look similar to many different lesions, including lipomas and mammary tumors or infections [31].

In the cat, the cutaneous/subcutaneous and the visceral form are the two distinct forms of MCTs. The two forms rarely co-exist. The vast majority of cutaneous mast cell tumors in the cat are located on the head, neck and trunk. Very few cutaneous tumors are located on the limbs. There are two clinical presentations of cutaneous MCTs in cats. The solitary form occurs as a single, well-circumscribed mass. These often occur on cats greater

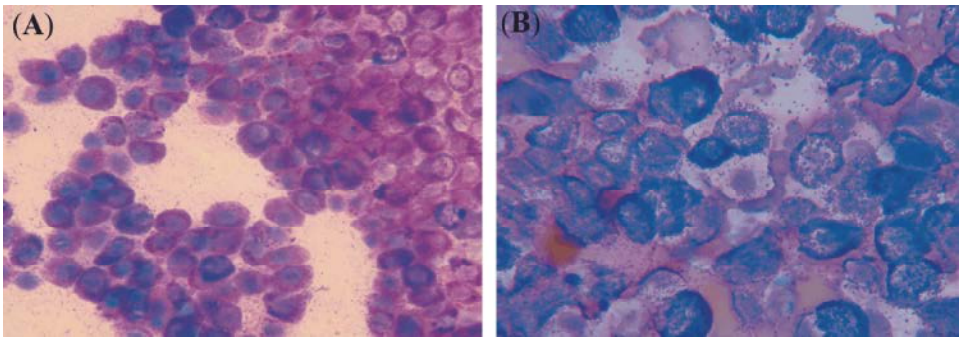

Fig. 4: Cytology of a feline MCT; note the numerous round cells with purple staining granules (Diff Quick stain; magnification, 20×) (A). Cytology of a canine MCT; similar to the feline MCT, this slide has a large number of round cells with varying amounts of purple staining granules (Diff Quick stain; magnification, 50×) (B) [31].

than 4 years of age and there is no breed predisposition. The multiple form, or histiocytic form, occurs as multiple, raised nodules. These have reportedly been seen more commonly in younger cats, less than 4 years of age, with a breed predisposition in the Siamese [9].

Cytologic Appearance: Most MCTs are easily diagnosed via cytology. Mast cell tumors are in the cytologic category of round cell tumors. The tumors in this category are composed of individually arranged, round cells that are fairly abundant on cytologic preparations (Figure 4) [31]. The background of such preparations often contains numerous small, purple granules from ruptured cells. The cytoplasm of intact cells frequently contains numerous purple (Metachromatic) granules which can obstruct visualization of the nuclei. Nuclei are round if visible. Anaplastic cells from undifferentiated mast cell tumors may contain few to no granules. Inflammation with eosinophils is often present in canine MCTs but is rare in feline MCTs [9].

Squamous Cell Carcinoma: Squamous cell carcinoma (SCC) occurs primarily in older dogs and cats. Animals with lightly pigmented skin that spend time in the sun are predisposed to solar (actinic) induced SCC in their thinly haired areas. Multiple lesions may be present in these cases, particularly in white cats. In dogs, cutaneous SCC unrelated to sun exposure is the most commonly reported digital tumor and most often affects dark-haired dogs. On physical examination, cats present with facial lesions more frequently than dogs, usually at thinly haired areas, such as the ear tips, eyelids and nasal planum. These lesions are often crusty and ulcerated. In dogs, digital SCC is usually single but may affect multiple digits in giant schnauzers and other large, black-haired breeds. Squamous cell carcinoma of the digit appears as a swollen digit with an abnormal nail; it is often diagnosed initially as a fractured nail with a nailbed infection (Figure 5A) [9].

Cytologic Appearance: Cytology can be diagnostic for SCC of the digit (Figure 5B). Direct the needle deep into the midpoint of the digit; the bone is often involved and lytic; therefore, a good sample may be obtained from the bone itself. Occasionally, dogs will have so much pain that sedation is needed for FNA [14]. Squamous cell carcinomas have some distinguishing characteristics that aid in the identification of this tumor. Unlike other epithelial tumors, cells from these tumors may be more individually arranged, with angular cytoplasmic borders. Most tumors are composed of anaplastic appearing squamous cells. The cells are usually very pleomorphic. They contain moderate amounts of lightly basophilic to aqua-blue cytoplasm (Keratinized). Some cells contain small, clear, punctate perinuclear vacuoles called keratohyalin granules. One prominent feature of squamous cell carcinomas is the presence of dysplasia and dyskeratosis, both of which indicate an abnormal cell development [9, 14].

Basal Cell Tumor: Basal cell tumors are common on the head and neck of the cat. They commonly occur in the dog as well, but rare in other domestic species. They are one of the most commonly reported cutaneous tumors in the feline. They have also been called basal cell carcinomas [9] but since these typical masses are benign, well-circumscribed and previously called *basal cell tumor* and now it is recognized as a *trichoblastoma* (Figure 6A) [32]. The term *basal cell tumor* was also used as an umbrella term for a large heterogeneous group that included tumors from epidermal, trichofollicular and

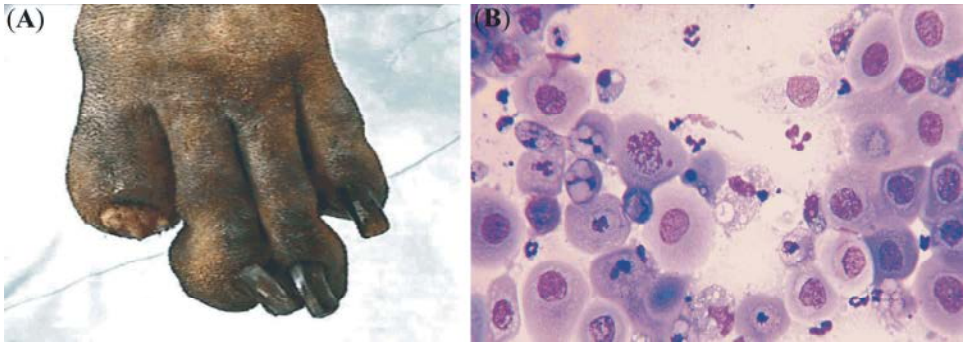

Fig. 5: The shaved foot of a Gordon setter with a digital SCC, showing severe swelling of digit: a broken toe nail and some soft tissue proliferation with discharge are seen at the nail bed (A) [9]. Cytology of a SCC; note the large angular cells, some of which appear as individuals, while others appear to be joined. The cytoplasm ranges in color, with many showing deep blue consistent with keratinization. Features of malignancy, including anisocytosis and anisokaryosis, are prominent. Scattered neutrophils are present in the background (B) [14].

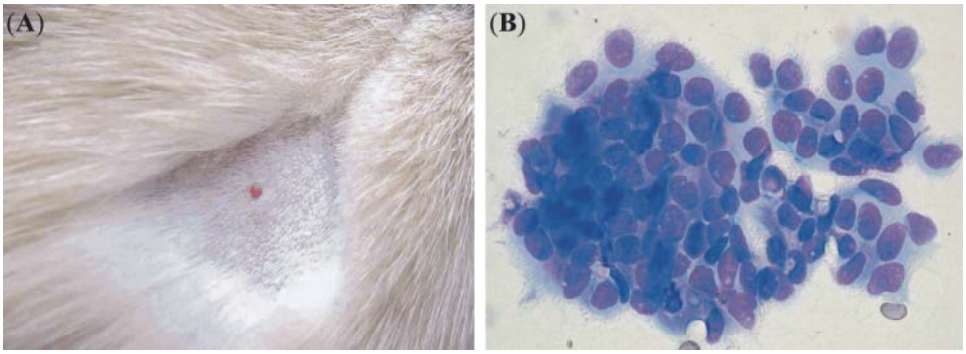

Fig. 6: A 4-mm raised hairless lesion on the lateral thorax of a 10-year-old spayed female Siamese cat, diagnosed as a trichoblastoma (A). Cytology of a trichoblastoma: note the cluster of small round to cuboidal cells with small amounts of cytoplasm and relatively uniform nuclei (Diff Quick stain; magnification, 50×) (B) [32].

adnexal tissues demonstrating basal cell characteristics. This is particularly relevant in cats, in which benign and malignant apocrine ductular sweat gland tumors and basal cell carcinomas are now recognized as having been lumped into the basal cell tumor category. Basal cell tumors arise in middle-aged to older dogs and cats, with some dog breeds, such as Poodles, being overrepresented [18].

Cytologic Appearance: The cytologic diagnosis of a tumor of basal cell origin is based on small, round to cuboidal cohesive cells arranged in tight clumps or ribbons (Figure 6B) [32]. Specifically, these tumors contain tight clumps of epithelium with deep blue cytoplasm. Mitotic figures may occasionally be observed. There may be low numbers of mature sebaceous gland epithelium mixed within the clumps of basal cells. Sebaceous differentiation is associated with some basal cell tumors. Some basal cell tumors, especially in the cat, contain variable amounts of melanin pigment and must be distinguished from true melanocytic tumors [9].

Histiocytoma: Histiocytomas affect primarily young dogs, but they may arise at any age. These tumors have not been identified in cats. These are benign, cutaneous tumors, typically seen in dogs less than 3 years of age. Grossly, histiocytomas have a distinctive appearance (Figure 7A): they are raised, rounded and alopecic and have small indentations all over ("pock marked"), which create an appearance similar to the surface of a strawberry. The tumors are also described as "button-like" because of their shape. These benign tumors will regress on their own but often after several months. As they do so, the surface may become ulcerated and intermittently bleed. Experienced practitioners may diagnose these tumors on the basis of gross appearance alone, although cytologic confirmation is suggested [7].

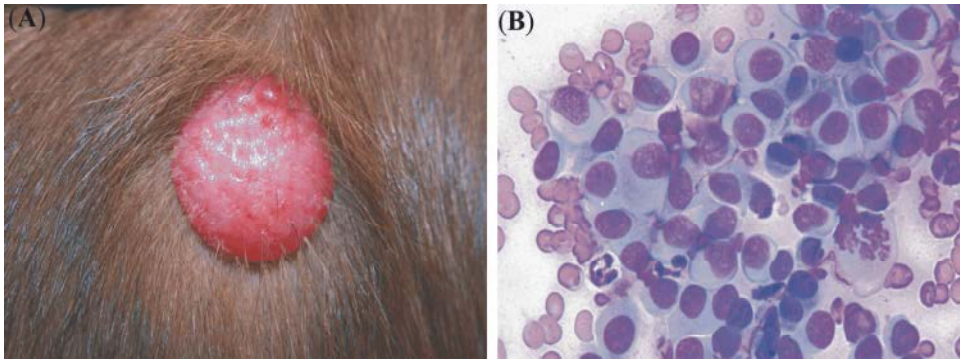

Fig. 7: A 2-cm diameter, round, raised, mostly hairless lesion on the antebrachium of a 7-month-old castrated male cocker spaniel, consistent with the classic clinical appearance of a histiocytoma (A). Cytology of the histiocytoma: note the population of round cells with moderate to large amounts of cytoplasm and distinct cytoplasmic margins (the cells look "outlined" due to clearing of the cytoplasm at the periphery). Prominent anisocytosis can be seen, as well as one mitotic figure (Diff Quick stain; magnification, 50×) (B) [7].

Cytologic Appearance: Cytologically, histiocytomas are in the round cell tumor category. They may be confused with other round cell tumors, such as plasma cell tumors or poorly granulated MCTs. Despite their benign behavior, histiocytomas often exhibit cytologic features of malignancy, such as numerous mitotic figures and marked anisocytosis (Figure 7B). Histiocytomas can be routinely diagnosed using a combination of cytologic and gross appearance. As they begin to regress, cytology will reveal lymphocytes scattered among the tumor cells [7, 9].

CONCLUSION

Cytology refers to microscopic evaluation of cells. Diagnosis can be made with cytology. Often the common skin lesions or masses have very distinct cytologic features that can be differentiated from one another since cutaneous masses are easily accessible for fine needle aspiration (FNA), skin scrapings, tape preparations, swabs or for imprints of tissue cells and this kind of cytology is quick, minimally invasive and useful technique for assessing any mass within the skin. Overall, cytology is a reliable and minimally invasive method of obtaining tissues for diagnosis. It is a rapid method to be used for differentiating non-neoplastic from neoplastic skin lesions of dogs and cats. Inflammation is easily differentiated from neoplasia based on the presence of inflammatory cells and neoplasia is easily distinguished from inflammation based on the presence and types of neoplastic cell. Finally, the interpretation of skin origin cytology relies on the understanding of the most common skin diseases that occur in these animals and on the familiarity with the most typical cytological features that characterize each of these conditions.

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