

Avian and Exotic Animal Cytology

Abstract

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Cytology is a simple, rapid diagnostic procedure requiring little in terms of equipment and cost to the veterinarian. The basic equipment needed includes a microscope with good resolution (especially at 20X, 40X, and 100X, oil-immersion), clean microscope slides, coverslips, and cytologic stains (i.e. Wright-Giemsa and stat stains). The basic equipment needed for collection of cytologic samples includes syringes (6 to 12 cc), fine gauge needles (i.e. 23 to 20 gauge, 1 to 1 1/2 inch), sterile cotton swabs, sterile rubber or soft plastic tubes (i.e. feeding tubes), and sterile physiologic saline. A variety of methods can be used to concentrate cells from poorly cellular fluids and washes onto a microscope slide. A simple method is by marginating the cells using the spreader slide technique. Another method is the use of the sedimentation following centrifugation (as used in mammalian urine cytology) or use of a commercial cytocentrifuge. Cells can also be concentrated by allowing them to fall onto the slide via gravity using a sample column and filter paper firmly attached to a microscope slide.

Most clinical veterinarians are familiar with sample collection techniques for domestic mammals; which also apply to the small exotic mammals. Common cytological specimens used in avian and reptilian medicine include: aspirates, imprints of biopsy material, tracheal washes, crop (ingluvies) aspirates or washes in birds, gastric washes in reptiles, sinus aspirates, lung washes in reptiles, aspiration of coelomic fluid, and fecal smears.

CYTOLOGICAL RESPONSES

Many veterinarians are familiar with mammalian cytodagnosis, especially with the common domestic species. The cytological responses of lower vertebrates, especially birds and reptiles, are similar to those of mammals. The inflammatory response of mammals can be classified as either neutrophilic, eosinophilic, mixed cell, or macrophagic. Likewise, the inflammatory responses of birds and reptiles are either heterophilic, eosinophilic (may be difficult to detect with routine staining or is rare), mixed cell, or macrophagic. The type of inflammatory response may suggest a possible etiology and pathogenesis. Inflammatory responses of birds and reptiles are similar to those described for mammals, except the avian and reptilian heterophil replaces the mammalian neutrophil. The inflammatory cells of birds and reptiles include heterophils, eosinophils, lymphocytes, plasma cells, and macrophages. Heterophil granules in cytological specimens tend to lose their normal rod-shaped appearance and either appears more rounded or degranulated. Degenerate heterophils have similar characteristics to degenerate mammalian neutrophils (i.e. nuclear hyalinization, swelling, karyorrhexis, and karyolysis and cytoplasmic basophilia and vacuolization) and demonstrate varying degrees of degranulation. Degenerate heterophils suggest the presence of toxins, such as bacterial toxins, in the microenvironment.

Neutrophilic/ Heterophilic inflammation

As with neutrophilic inflammation of mammals, heterophilic inflammation of birds and reptiles is represented by a predominance of heterophils (greater than 80 percent of the inflammatory cells) in the cytologic sample. Heterophilic inflammation usually indicates an acute phase of the inflammatory response in birds and reptiles. It has been demonstrated that heterophilic inflammation can develop into a granuloma within one week. Apparently, the necrotic center of heterophilic inflammatory lesions produces necrotoxins that are chemotactic to macrophages and a granuloma quickly develops. Therefore, a granuloma formation in birds may be in response to necrotic tissue rather than an

infectious organism. Giant cell formation is a common occurrence in avian inflammatory lesions because the necrotic tissue stimulates a foreign body-like reaction. Thus, unlike mammalian giant cell formation, the presence of giant cells in avian inflammatory lesions does not necessarily suggest chronicity.

Mixed cell inflammation

Because of the rapid influx of macrophages and lymphocytes into inflammatory lesions, mixed cell inflammation is common to birds (and perhaps reptiles). Mixed cell inflammation is typically represented by a predominance of heterophils (greater than 50 percent of the inflammatory cells) with an increased number of mononuclear leukocytes. Lymphocytes and plasma cells can be associated with acute heterophilic granulomas, whereas the presence of epithelioid cells (macrophages that contain no vacuoles or phagocytized material) and connective tissue cells (i.e. fibroblasts) suggest chronic granulomas. Frequently, the epithelial and mesenchymal cells adjacent to inflammatory lesions proliferate resulting in the presence of these types of cells showing features of tissue hyperplasia. Heterophilic and mixed cell inflammation are associated with a variety of infectious (i.e. bacterial and fungal) and noninfectious (i.e. traumatic and foreign body) etiologies in birds and reptiles.

Macrophagic inflammation

Macrophagic inflammation may have a different pathogenesis than heterophilic and mixed cell inflammation in birds. Macrophagic inflammation is indicated by a predominance of macrophages (greater than 50 percent of the inflammatory cells) in the cytological sample. Large vacuolated macrophages that later develop into multinucleated giant cells, apparently responding to necrotic tissue, are a feature of this type of inflammation. Macrophagic inflammation is common in certain avian diseases, such as Mycobacteria and Chlamydia infections and cutaneous xanthomatosis. Areas of macrophagic inflammation and heterophilic inflammation can occur together as macrophages respond to necrotic materials. Therefore, depending upon where the sample is obtained from the inflammatory lesion, a macrophagic inflammatory response may predominate the cytology.

Eosinophilic inflammation

Eosinophilic inflammation appears to be rare in birds and reptiles. This may be either due to the difficulty in differentiating eosinophils from heterophils in cytologic samples using routine cytologic stains or avian eosinophils may behave differently from mammalian eosinophils. There is evidence that avian eosinophils do not act as modulators of immediate hypersensitivity reactions as they do in mammals, but may participate in delayed hypersensitivity reactions.

Hyperplasia and benign neoplasia

Based upon cytomorphology, tissue hyperplasia and benign neoplasia are indistinguishable. Tissue hyperplasia is a proliferative process of tissues responding to cellular injury or chronic stimulation (i.e. glandular hyperplasia). Cells representative of tissue hyperplasia or benign neoplasia have increased cytoplasmic basophilia and pale, vesicular nuclei. They have a uniform appearance with a uniform nucleus to cytoplasmic ratio (N:C). Cells suggestive of hyperplasia of epithelial and connective tissue often occur in cytologic specimens of long chronic inflammation. Other examples of tissue hyperplasia or benign neoplasia that are frequently identified by cytodiagnosis in birds include squamous cell hyperplasia or metaplasia associated with hypovitaminosis A, lipomas, and lymphoid hyperplasia. Plasma cell hyperplasia is commonly found in lymphoid tissues in association with avian chlamydia infection.

Malignant neoplasia

The cytologic criteria for the diagnosis of malignant neoplasia in domestic mammals also apply to avian and reptilian cytodiagnosis. The criteria for the cytologic diagnosis of malignant neoplasia can be divided into general cellular, nuclear, cytoplasmic, and structural features. General cellular features

include the presence on noninflammatory cells with an apparent common origin showing pleomorphism, increased cellularity in samples from tissues that normally provide low cellular samples, and the appearance of cells that are foreign to the tissue being sampled. The more frequently observed nuclear criteria for malignant neoplasia include anisokaryosis, variable N: C ratios, nuclear pleomorphism, abnormal mitoses, abnormal chromatin patterns, and large pleomorphic or multiple (greater than four) nucleoli. The two important cytoplasmic features of malignant neoplasia include increased basophilia and vacuolation. Increased cytoplasmic basophilia is suggestive of increased RNA activity typical of young, active cells. Increased cytoplasmic vacuolation could suggest cellular degeneration, especially if the vacuoles are small. Cells originating from secretory tissue, such as adenocarcinomas produce large secretory vacuoles. Finally, structural features of malignant neoplasia refer to those features that may suggest a possible origin of the neoplasm, such as epithelial neoplasia (carcinomas), mesenchymal neoplasia (sarcomas), or discrete cell neoplasia (round cell neoplasms). Epithelial cell neoplasms tend to provide highly cellular samples that contain round to polygonal cells with distinct cell margins and occurring in sheets or clusters. Mesenchymal cell neoplasms usually produce poorly cellular samples that contain spindle-shaped cells with indistinct cytoplasmic margins and generally do not occur in aggregates. Discrete cell neoplasms are composed of round to oval cells that exfoliate well as individual cells. A common discrete cell neoplasm of the lower vertebrates is lymphoid neoplasia.

EFFUSIONS

The coelomic cavity of normal birds and reptiles contains little, if any, fluid. Therefore, fluid aspirated from the coelomic cavity should be examined for specific gravity, protein content, and cellularity. As with mammals, fluids obtained from birds and reptiles can be classified as transudates, modified transudates, exudates, hemorrhagic effusions, or malignant effusions.

Transudates

Transudative effusions are characterized by low specific gravity (<1.020), low cellularity (<1,000 cells/ μ l), and low total protein (<3.0 gm/dl). Transudates are clear to straw color on gross inspection. The cells found in transudates are primarily macrophages with occasional mesothelial cells, lymphocytes, and nondegenerate heterophils. Transudative effusions in birds and reptiles most likely occur for the same reasons as those for mammals, such as oncotic pressure changes and other circulatory disorders.

Modified transudates resemble transudative effusions grossly but have higher protein content (3.0-3.5 gm/dl) and cellularity (1,000-5,000 cells/ μ l). The cells found in modified transudates are primarily macrophages and reactive mesothelial cells. Reactive mesothelial cells are round to oval cells that often have scalloped or villus-like eosinophilic margins, cytoplasmic vacuoles, multiple nuclei, and mitotic activity. Modified transudates occur from long-standing transudative effusions or as a result of hydrostatic pressure changes.

Exudates

Exudative effusions result from inflammatory processes in the coelomic cavity. They are characterized by a high cellularity (>5,000 cells/ μ l), protein content (>3.0 gm/dl), and specific gravity (>1.020). Exudative effusions vary in color and turbidity, may have a foul odor, and often clot during sample collection. Therefore, use of an anticoagulant (e.g. EDTA) is indicated with coelomic fluids suspected of clotting.

The cellular content of exudates varies with etiology, host response, and duration of time. Exudates demonstrating a heterophilic inflammation suggest an acute inflammatory response. Septic exudates may demonstrate intracellular bacteria and degenerate heterophils. Mononuclear leukocytes are characteristic of mild irritation to the coelomic cavity and nonseptic conditions.

Hemorrhagic effusions

Hemorrhagic effusions in the coelomic cavity of lower vertebrates often result from trauma or injury. Hemorrhagic effusions demonstrate a variable number of erythrocytes in the fluid sample. It is important to differentiate hemorrhagic effusions from peripheral blood contamination of the sample during collection. Because thrombocytes disappear quickly in hemorrhagic effusions, their presence usually suggests peripheral blood contamination of the sample. Chronic and resolving hemorrhagic effusions exhibit varying degrees of erythrophagocytosis that is indicated by leukocytic (usually macrophages) phagocytosis of intact erythrocytes or macrophages containing remnants of erythrocytes, such as red cell fragments and iron pigment. Iron pigment appears as blue-black to gray pigment in the cytoplasm of macrophages with Wright's stain.

Malignant effusions

Malignant effusions can have features of modified transudates, hemorrhagic effusions, or exudates and may demonstrate cells with features of malignant neoplasia. The cytologic features of the malignant cells may allow the cytologist to classify the malignancy involved, such as sarcoma, carcinoma, or lymphoid neoplasia.

Suggested reading:

Campbell TW. 2015. Exotic Animal Hematology and Cytology, 4th Edition. Ames IA, Wiley Blackwell.

Campbell, TW, Grant KR. 2010. Clinical Cases in Avian & Exotic Animal Hematology & Cytology, Ames, Iowa, Wiley-Blackwell.