PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 41:e06927, 2021
DOI: 10.1590/1678-5150-PVB-6927

Topic of General Interest
Small Animal Diseases

Down the rabbit hole: a quick guide for histopathology description[1]

Claudio S.L. Barros[2]* and Daniel R. Rissi[3]

ABSTRACT.- Barros C.S.L. & Rissi D.R. 2021. **Down the rabbit hole: a quick guide for histopathology description**. *Pesquisa Veterinária Brasileira 41:e06927, 2021*. Laboratório de Anatomia Patológica, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Av. Senador Filinto Müller 2443, Vila Ipiranga, Campo Grande, MS 79074-460, Brazil. E-mail: claudioslbarros@uol.com.br

Histopathology is an old science that is still currently utilized for disease diagnosis and research. The routinely processed histologic slides stained with hematoxylin and eosin are still used worldwide in most if not every histopathology laboratory. The technique is inexpensive, quick to perform, and allows the diagnosis of a fantastic variety of tissue changes and diseases. Skills in description and interpretation in histopathology are a craft that can be learned by repeatedly and systematically observing simple rules. In this article, we offer a few advices to help trainees in veterinary pathology at the start of their careers. Those advices are drawn from our experience in the diagnostic pathology routine and from the veterinary pathology literature. To enhance the understanding of these important steps in the histopathologic description of tissues, we decided to illustrate most concepts expressed here. We hope that our effort can add a bit to the development of future pathologists. Just like Alice, let us follow the White Rabbit into his burrow for this challenging experience!

INDEX TERMS: Pathology, histopathology, description, interpretation.

RESUMO.- [**Descendo a toca do coelho: um guia rápido para descrição histopatológica.**] A histopatologia é uma ciência antiga, mas ainda usada para diagnosticar e investigar a patogênese de doenças. As lâminas histológicas processadas rotineiramente e coradas por hematoxilina e eosina ainda são utilizadas em virtualmente todos os laboratórios de histopatologia do mundo. A técnica não é cara, é de execução rápida e permite o diagnóstico de uma fantástica variedade de doenças. As habilidades em descrever e interpretar os achados histopatológicos é um ofício que pode ser aprendido pela observação repetida e sistemática de regras simples. Para ajudar os estudantes de patologia no início de suas carreiras, abordamos aqui algumas dessas regras, extraídas tanto de nossa experiência quanto da literatura relacionada à patologia veterinária. Para aumentar a compreensão dos tópicos, decidimos ilustrar praticamente todos os conceitos expressados neste manuscrito. Esperamos que nosso esforço possa contribuir um pouco para o desenvolvimento de aspirantes a patologistas. Assim como Alice no País das Maravilhas, vamos seguir o coelho branco até a sua toca para essa aventura desafiadora.

TERMOS DE INDEXAÇÃO: Patologia, histopatologia, descrição, interpretação.

INTRODUCTION

Histopathology is an ancient science that has been used to diagnose and study diseases for more than a century. As a specialized field, histopathology dates back to 1838, when Johannes Miller published "On the nature and structure characteristics of cancer", the first text on the subject (Titford 2006). Formalin as a tissue fixative (Bracegirdle 1986), paraffin embedding of tissues (Titford 2006), and the hematoxylin and eosin (HE) method for staining tissues (Cook 2000) all became available about the same time, in the last decades of the 19th century.

The HE is the routine method widely utilized to stain tissue specimens processed in a histology or histopathology laboratory. The technique is non-expensive, quick to perform, and allows for the diagnosis and interpretation of a wide variety of tissue changes (Titford 2009). The widespread use of HE in the diagnostic routine prompted the pathologist Hans

[1] Received on April 20, 2021.
Accepted on May 5, 2021.
[2] Laboratório de Anatomia Patológica, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul (UFMS), Av. Senador Filinto Müller 2443, Vila Ipiranga, Campo Grande, MS 79074-460, Brazil.*Corresponding author: claudioslbarros@uol.com.br

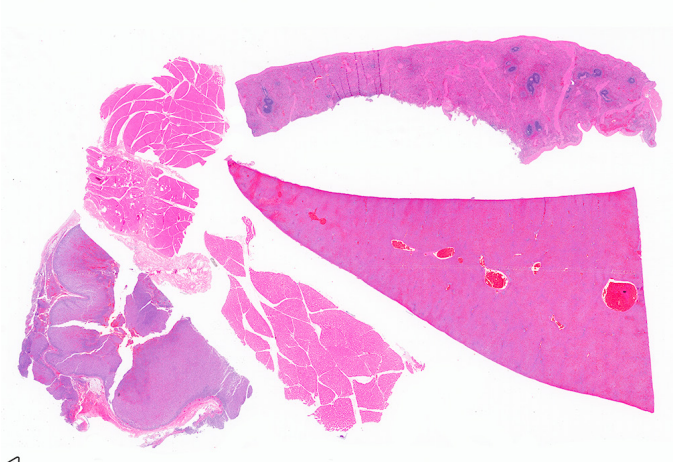
[3] Athens Veterinary Diagnostic Laboratory, Department of Pathology, University of Georgia, College of Veterinary Medicine, Athens, GA 30602, USA.

Popper (1903-1988), considered by many to be the founder of hepatology, to state that the best tool in pathology is "a hematoxylin and eosin-stained slide connected to the brain" (Gerber & Thung 1988). Although pathologists have, over the years, introduced supportive and complementary new techniques to the study of diseases, including histochemistry (Kiernan 2008, Titford 2009), immunohistochemistry (IHC) (Klopfleisch & Bauer 2016), *in situ* hybridization, and polymerase chain reaction (PCR) (Henrich 2016, Waugh et al. 2016), histopathology remains at the epicenter of diagnostic pathology. The description and interpretation of tissue changes or lesions under the microscope, and more recently via digital pathology, are skills that we can learn and master by means of repetitive and systematic practice performed in the diagnostic pathology routine. Pathology is repetition and "repetition brings familiarity" (Ham & Cormack 1979), so our idea is that these systematic approaches to histopathology should be taught to pathology trainees right at the start of their training.

Here we provide general guidelines for histopathologic description and interpretation of tissue changes, aiming to contribute to the education and training of anatomic pathology students. These are not original ideas but rather a compilation of what we think are the more basic, succinct and yet comprehensive ways to approach the morphologic description and interpretation of a lesion. Similar methods have been utilized by human and veterinary pathologists for as many years as pathology has existed as a science.

TEN SIMPLE RULES TO START WITH

1. Be attentive. Before placing the slide under the microscope, verify how many tissue sections are under the coverslip. Sometimes multiple tissue sections are placed onto one slide (Fig.1), and you may easily miss important tissues and even the diagnosis if you do not examine all samples. Looking at the slide against the light using the inverted objective lenses as a magnifying glass will help you to get a subgross view of the represented tissues on the slide. The same careful preliminary examination of all tissue sections should also be performed in case digitized slides are being utilized (in which case the whole process is much easier).



1

Fig.1. Slide with multiple tissue sections, dog. Make sure all tissue sections are examined. In this case, only one tissue had pathologic changes. HE.

2. Be direct and brief when starting the description. There is no need to state "we have a triangular section of a kidney measuring 2 x 2 x 3 centimeters. The tubular epithelium is..." Just write "Kidney: The tubular epithelium is…" It will save you time and make the reading flow more easily.

3. Be concise and careful. Almost all tissue changes can and should be described in a few sentences. No one likes to go through two or three pages of tedious details. We easily lose focus. Avoid tautologies such as "pathologic lesions" (Hadlow 1994) or "the liver has a severe hepatic injury". Redundancies such as "blue in color", "oval in shape", and "firm consistency", among others, should also be avoided. In other words, blue will always be a color, oval will always be a shape, and firm will always be a consistency. Speaking of color, be simple when describing it. For example, you do not need to be familiar with all the existing shades of green or blue or any color when describing a tissue change. Describe it as light green or dark green, and so on. Further, avoid vague and useless adjectives such as greenish or bluish or yellowish. If something is greenish, it should be described simply as green; if it is bluish or yellowish, it should be described as blue or yellow. At the end of a description you do not need to write "no other noteworthy lesions were found." The reader will be intrigued about the "unworthy" lesions you did not care to describe. Importantly, be aware of spelling and grammatical errors. A report with many such errors will look sloppy and will likely lose credibility. Correct spelling and grammar help the description flow. Spelling errors and language vices frequently make their way into necropsy and biopsy reports; they are too many to list here, but a good tutor can guide their trainees accordingly during their training.

4. Try to describe an organ systematically. If you regularly break down specific organs into their essential components, you will never neglect a lesion. For example, when describing a lung section (Fig.2-4), always examine the conductive airways (bronchi, bronchioles), followed by the alveolar spaces, alveolar septa, blood vessels, interlobular septa, and pleura. If it is a kidney section (Fig.5-7), examine the cortex (outermost part), corticomedullary junction, medulla (innermost area), and renal pelvis. In the cortex, examine the glomeruli, proximal tubules, medullary rays, and blood vessels. In the medulla, examine the nephron loops, collecting ducts, and blood vessels. Observe the pelvis and the transitional epithelium. Repeat this procedure with any organ.

5. Describe tissue changes in a decreasing order of importance, from the most important to the least important lesions. Describe first the main lesion and only then the secondary changes (Fig.8 and 9). It is not necessary to describe each cell or cell pattern seen on a slide. Describe the most abundant population of cells followed by the least abundant populations. For instance, an area of granulomatous inflammation can be described as "consisting of sheets of epithelioid macrophages and neutrophils admixed with fewer multinucleated giant cells and scattered lymphocytes and plasma cells" (Fig.10). Maybe there are a couple of mast cells within that lesion, but they are probably not important for the description and diagnosis.

6. Do not describe normal features unless strictly necessary. Likewise, avoid negative comments, such as "there is no

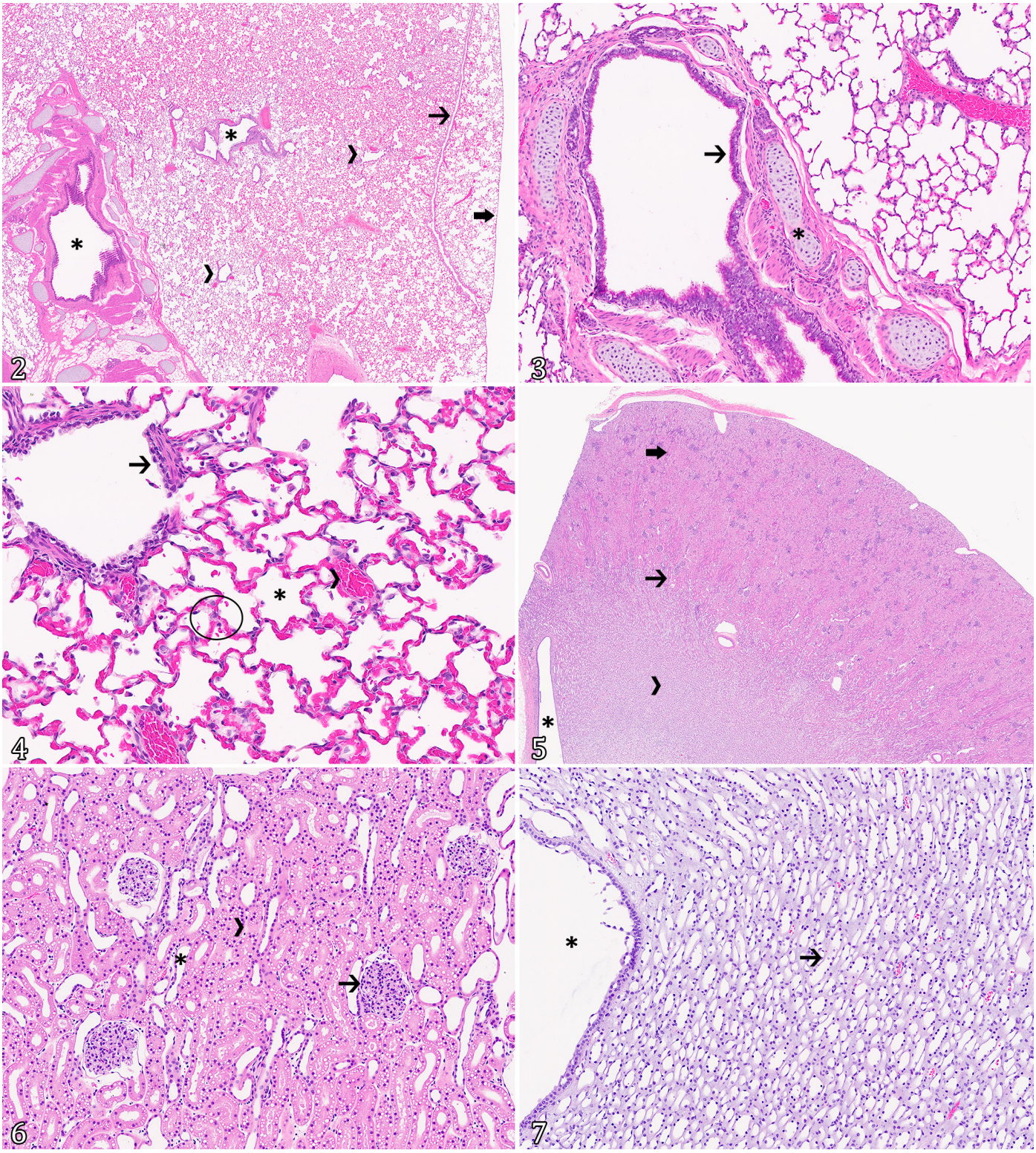


Fig.2-7. (**2**) Normal lung, dog. Tissue section of lung evidencing the pleura (thick arrow), interlobular septa (thin arrow), two bronchi (asterisks), and multiple bronchioles (arrowheads). HE, obj.2x. (**3**) Normal lung, dog. Closer view of the lung shown in Figure 2 detailing a bronchus lined by ciliated epithelium (arrow) and surrounded by cartilage (asterisk). HE, obj.8x. (**4**). Normal lung, dog. Closer view of the lung shown in Figure 2 highlighting a bronchiole lined by ciliated epithelium (arrow), alveolar septa (circle), alveolar spaces (asterisks), and blood vessels (arrowhead). HE, obj.20x. (**5**) Normal kidney, cat. Subgross view of the kidney highlighting the cortex (thick arrow), corticomedullary junction (thin arrow), medulla (arrowhead), and pelvis (asterisk). HE, subgross view. (**6**) Normal kidney, cat. Closer view of the renal cortex evidencing multiple glomeruli (arrow), proximal tubules (arrowhead), and distal tubules (asterisk). HE, obj.8x. (**7**) Normal kidney, cat. Closer view of the renal medulla and its many collecting ducts (arrow) and the renal pelvis (asterisk). HE, obj.8x.

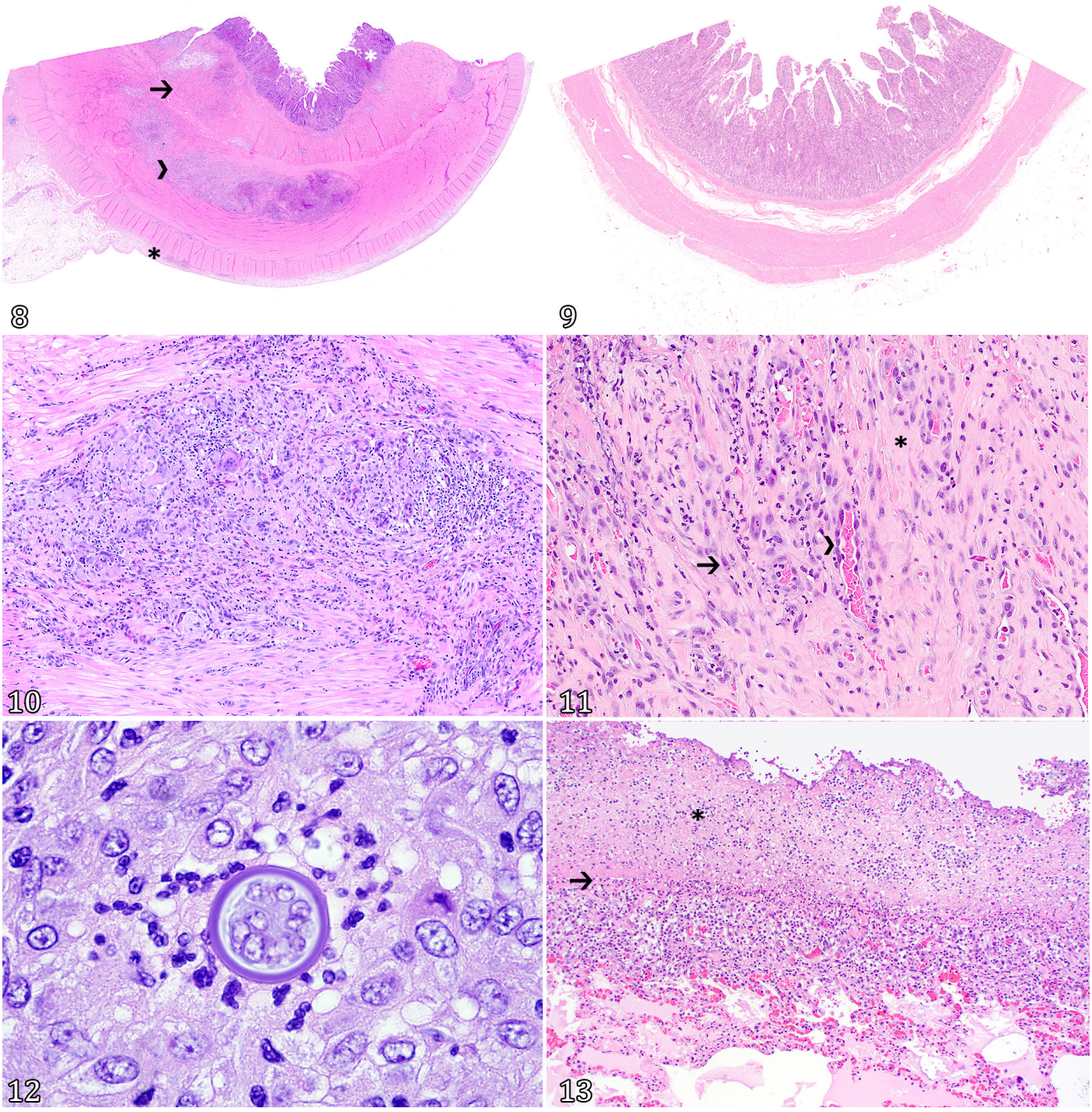


Fig.8-13. (**8**) Pythiosis, small intestine, dog. The main inflammatory changes expand the intestinal submucosa (arrow) and inner muscular layer (arrowhead). Secondary inflammatory changes are present in the mucosa (white asterisk) and serosa (black asterisk), and should be described after the main lesion. HE, subgross view. (**9**) Normal small intestine, dog. Compare the histology of the normal intestine with the affected intestine in Figure 8. HE, subgross view. (**10**) Pythiosis, small intestine, dog. The majority of the inflammatory cells consists of multinucleated giant cells with fewer epithelioid macrophages and fewer lymphocytes and plasma cells. HE, obj.10x. (**11**) Fibrovascular tissue, skin, dog. There are numerous fibroblasts (arrow) and newly formed blood vessels (arrowhead) with abundant collagen (asterisk) in an area of chronic inflammation and healing. HE, obj.20x. (**12**). Coccidioidomycosis, skin, dog. Fungal spherules (center) are 60µm in diameter and have a 5µm thick, double contoured basophilic wall that surrounds flocculent basophilic material and 8µm in diameter round endospores. HE, obj.60x. (**13**) Feline infectious peritonitis, lung, cat. The pleura (arrow) is covered with abundant, eosinophilic lacy material that consists of a web of loosely arranged strands of fibrin admixed with neutrophils (asterisk). HE, obj.8x.

evidence of hepatocellular necrosis." If there were hepatocellular necrosis, you would have described it (hopefully). Exceptions to this rule occur when the clinician or surgeon has specific questions or requests such as "please check spinal cord injury at T2-T3". In that case, you can state, "there were no spinal cord lesions at T2-T3."

7. Do not imply movement when describing a tissue change. For example, it can be tempting to write that "numerous fibroblasts are producing a large amount of collagen" for chronic lesions such as those observed at sites of fibrovascular tissue (Fig.11). Avoid doing that. You are technically unable to appreciate that active process. A more realistic descriptive approach would be "there is a large number of fibroblasts amidst a moderate amount of eosinophilic extracellular matrix (collagen)". The formalin-fixed, paraffin-embedded tissue you are examining is a snapshot of a once dynamic but currently static process no longer capable of movement. The active production of collagen in that tissue section can only be inferred.

8. Whenever possible, mention the size and shape of important structures in your description. For example, "there are 60µm in diameter fungal spherules with a 5µm thick, double contoured basophilic wall that surrounds flocculent basophilic material and 8µm in diameter round endospores'' (Fig.12). Knowing the size of adjacent cells such as red blood cells or neutrophils (in mammals, roughly 6 and 12µm in diameter, respectively) can help you to determine the size of organisms of other structures you may see.

9. Do not hesitate to interpret lesions, but make sure that a parenthesis separates your description from your interpretation. For example, "abundant eosinophilic strands of lacy material (fibrin) cover the pleura" (Fig.13).

10. Know the specific terminology to use in your descriptions, including the names of anatomical sites, specific descriptive terms for the various types of necrosis, circulatory disturbances, inflammation, and growth disturbances (see ahead), and morphologic features of different infectious organisms. The specificity and accuracy of the terminology convey to the reader the confidence that you know your trade.

HISTOPATHOLOGIC DESCRIPTION AND INTERPRETATION IN A NUTSHELL

Once you describe all the tissue changes found in the tissue section you are examining, you will be able to interpret those findings and formulate a morphologic diagnosis. Using additional information such as signalment, clinical history, and other clinical data, you will likely be able to think of an etiologic diagnosis, establish the cause of that specific tissue change (if applicable), and determine the disease diagnosis in that particular case. For instance, think of a tissue sample (myocardium) from a 1-month-old puppy that died after respiratory distress (Fig.14). After linking the clinical history with the cardiac lesions, you can establish a morphologic diagnosis (lymphoplasmacytic myocarditis with intranuclear viral inclusions), an etiologic diagnosis (parvoviral myocarditis), an etiology (canine parvovirus-2 or CPV-2), and the disease name (canine parvovirosis). Myocarditis in young dogs can occur in systemic viral infections, such as canine distemper, canine herpesviral infection, and CPV-2 infection. Lymphoplasmacytic CPV-2 myocarditis with intranuclear viral inclusions in puppies typically leads to acute or chronic heart failure and respiratory distress (Robinson et al. 1980). Thus, a routine diagnosis in this case can be perfectly reached by histopathology. Depending on the circumstances, ancillary testing, such as IHC or PCR, can be used for diagnostic support and confirmation.

HOW TO GET FROM AN ABNORMAL TISSUE SECTION TO A DIAGNOSIS

Once you start examining any tissue section and writing your morphologic description, think of the following basic points you need to address:

1. Location of the lesions: Which organ or part of an organ is affected? (refer to items 4 and 5 above, under "Ten simple rules to start with"). Be as specific as you can. For example, it is much better to list "jejunum" than "small intestine" or "intestine".

2. Distribution of the lesions: How are the lesions spatially distributed within a tissue section? The basic descriptive terms include focal, multifocal, multifocal to coalescing, focally extensive, segmental, and diffuse (Fig.15). You do not need to be redundant with these terms. For instance, rather than reporting that "the dermis is multifocally expanded by distinct areas of granulomatous inflammation", you can just state that "the dermis is expanded by distinct areas of granulomatous inflammation". The fact that there are distinct "areas" implies that the lesions are multifocal.

3. Category of lesions: We can accommodate tissue changes into four large groups, namely 1) degeneration, necrosis, and cell adaptations; 2) circulatory disorders; 3) inflammation; and 4) growth disorders.

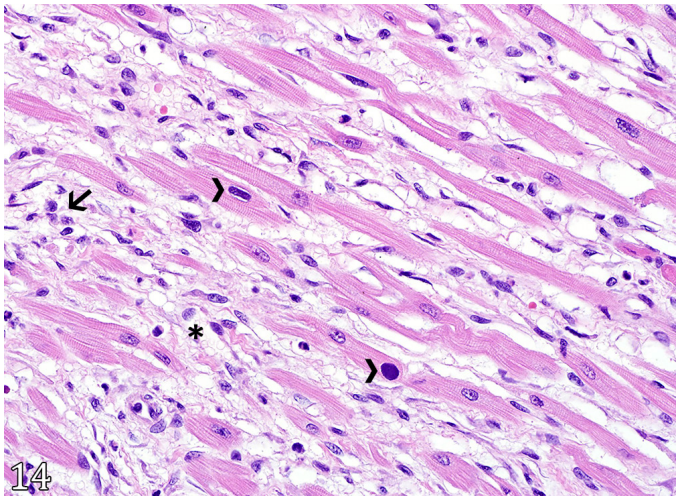
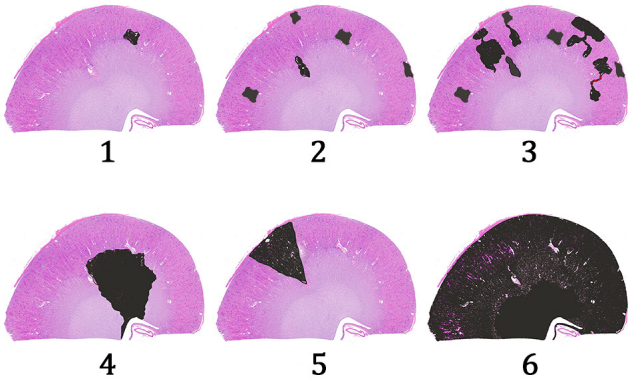


Fig.14. Parvoviral myocarditis, myocardium, dog. The interstitium (asterisk) is the area more markedly affected, and cardiomyocytes are dissociated due to interstitial accumulations of lymphocytes and plasma cells (arrow). A few cardiomyocytes contain intranuclear viral inclusions (arrowheads). A suitable morphologic diagnosis is "lymphoplasmacytic myocarditis with intranuclear viral inclusions." Using additional information such as signalment and clinical history, you can make an etiologic diagnosis, establish a cause, and determine a specific condition, which in this case would be "parvoviral myocarditis", "canine parvovirus-2", and "canine parvovirosis", respectively. HE, obj.40x.

DENEGERATION, NECROSIS, AND CELL ADAPTATIONS

The most common changes in the cytoplasm leading to cell degeneration include vacuolation associated with hydropic or ballooning degeneration (Fig.16) and accumulation of glycogen (Fig.17) or lipid (Fig.18). Changes in the nucleus typically characterize irreversible cell injury and necrosis (Fig.19-21). These changes include pyknosis (condensation of chromatin rendering the nucleus smaller and darker), karyorrhexis (nuclear fragmentation), and karyolysis (chromatin dissolution by endonucleases, leading to nuclear vanishing). Necrosis can be qualified into five categories that are typically associated with the causative agent (Table 1), including coagulative necrosis (Fig.22), caseous necrosis (Fig.23), liquefactive necrosis (Fig.24), fat necrosis (Fig.25), and fibrinoid necrosis (Fig.26). Apoptosis (Fig.27) is a programed form of cell death resulting from the sequential activation of "death genes" and "suicidal enzymatic pathways" (Reisner 2015). During apoptosis, there is nuclear pyknosis and fragmentation. Subsequently, these nuclear debris and parts of the cytoplasm are enclosed in apoptotic bodies by a membrane and are released in the interstitial spaces to be phagocytosed by macrophages or other cells.



15

Fig.15. Kidney, cat. Tissue sections depicting the main distribution patterns of lesions: 1) focal lesion (one lesion separated by unaffected tissue); 2) multifocal lesion (multiple lesions distributed throughout the organ separated by unaffected tissue); 3) multifocal to coalescent lesion (multiple lesions that coalesce); 4) focally extensive lesion (lesions extend over approximately one-third of the reference area); 5) segmental lesion (a well-defined segment of the tissue is affected, usually with a distinct geometric shape corresponding to an affected vascular bed); and 6) diffuse lesion (widespread involvement of an organ or tissue). HE, subgross view.

Cells under chronic, sublethal injury can adapt and undergo hyperplasia, hypertrophy, atrophy, hypoplasia, dysplasia, and metaplasia (Fig.28-30). Hyperplasia is an increase in the number of cells in a tissue (Thomson 1978a, Miller & Zachary 2017). It can be physiologic (e.g., erythroid bone marrow expansion) or pathologic (Fig.31). Hypertrophy is the increase in cell size (Fig.32) without an increase in the number of cells (simple hypertrophy) or associated with hyperplasia (compound hypertrophy). Simple hypertrophy occurs in the cardiac and skeletal muscles, whereas compound hypertrophy occurs in populations of cells capable of multiplication, such as the prostate epithelium (Thomson 1978a). Atrophy (Fig.33) and hypoplasia (Fig.34) occur when there is a decrease in cell size and/or number in a tissue or organ after or before it has reached its mature size, respectively. Affected tissues have a reduced mass and volume.

Dysplasia means cell disorientation. It occurs mainly in epithelia and consists of a set of changes in which cells lose individual uniformity and structural orientation (Fig.35). Dysplasia can be a precancerous lesion, but it does not always progress to cancer (Kumar et al. 2015). Metaplasia (Fig.36) is a reversible change in which there is a replacement of a mature cell population with another differentiated cell population of the same germ line (Reisner 2015). For example, the change from glandular prostatic epithelium in dogs with a Sertoli cell tumor to stratified squamous epithelium is referred to as squamous prostatic metaplasia (Agnew & MacLachlan 2017). In addition, the transformation of the squamous esophageal epithelium in humans with gastric reflux into glandular (gastric epithelium) is another form of metaplasia (Kemp et al. 2008). Although metaplasia is common in epithelial cells (Brooks & Perosio 2007), it can occur virtually in any adult tissue (Reisner 2015).

Endogenous or exogenous intracellular accumulations can also occur when cells are injured. These accumulations typically result from metabolic changes, genetic mutations, and exposure to inert particles. While some of these substances are innocuous, others can lead to cell death. We have already discussed the most common intracellular accumulations, including glycogen and lipid. However, other endogenous substances commonly observed in the diagnostic routine include melanin, produced by normal or neoplastic melanocytes

Table 1. Main types of necrosis in diagnostic histopathology

Type	Comments
Coagulative necrosis	Denaturation of cytoplasmic proteins with preservation of the general outlines of cells. Affected cells have hypereosinophilic cytoplasm and pyknotic or karyorrhectic nucleus. Examples: hypoxia, ischemia (infarcts), toxic insults.
Caseous necrosis	Extensive loss of cell and tissue architecture. Affected cells are hypereosinophilic, granular, and amorphous. Examples: tuberculous, mycotic granulomas.
Liquefactive necrosis	Extensive loss of cell and tissue architecture due to lysis by inflammatory cells (neutrophils and macrophages). Affected cells and tissues are liquefied and converted to an amorphous suppurative (purulent) exudate. Examples: abscesses, malacia of the central nervous tissue.
Fat necrosis	Caused by lipolysis that occurs when lipases and other enzymes act upon adipocytes. Released of cytoplasmic calcium gives adipocytes a basophilic hue when necrotic. Examples: fat necrosis of the omentum, mesentery, or subcutaneous tissue.
Fibrinoid necrosis	Affects the wall of blood vessels, which appear impregnated with fibrin and homogeneously hypereosinophilic. Examples: feline infectious peritonitis, malignant catarrhal fever.

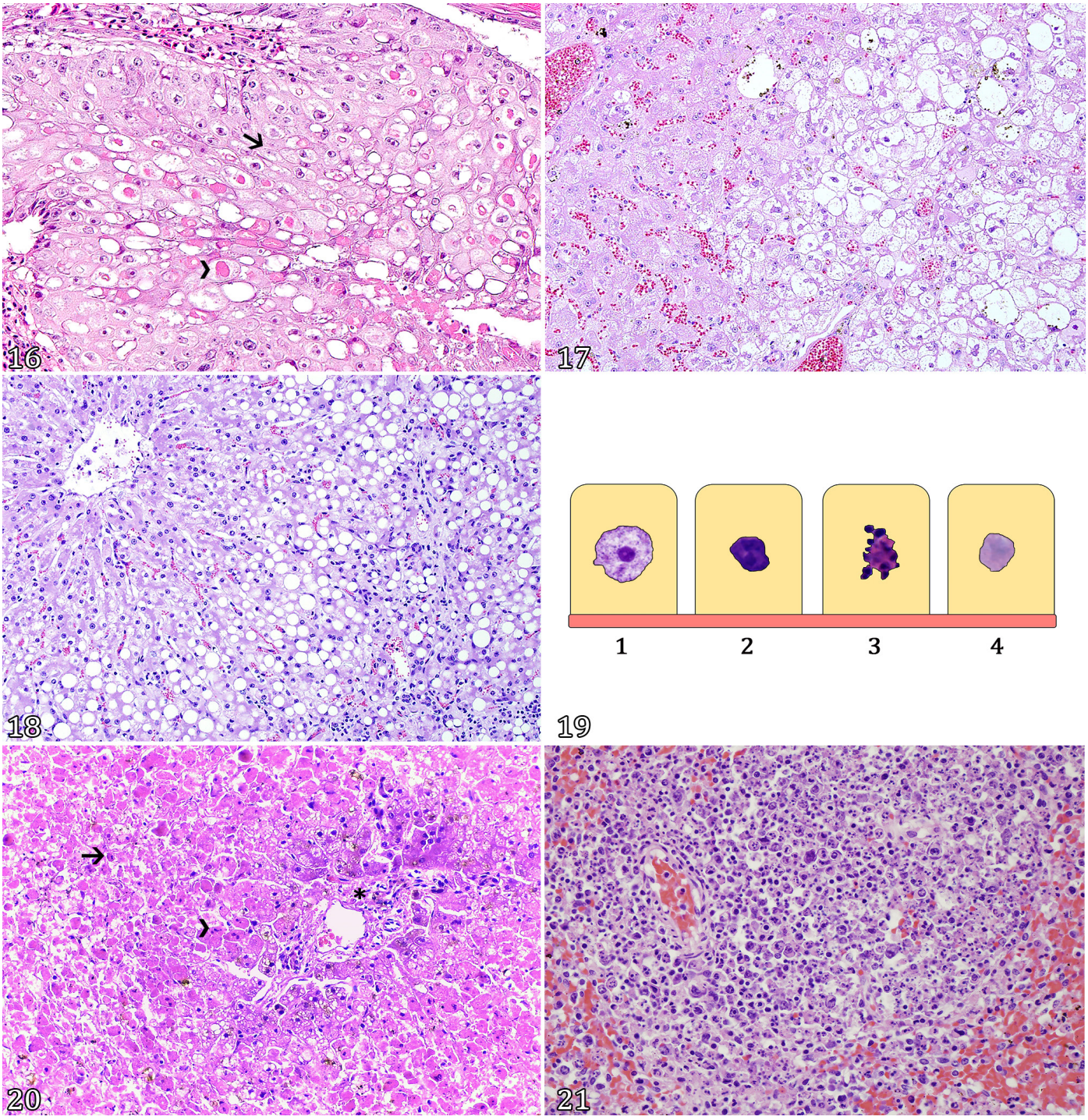


Fig.16-21. (**16**) Avian poxvirus infection, skin, canary. Hydropic or ballooning degeneration is characterized by swelling of keratinocytes due to cytoplasmic edema (arrow). Intracytoplasmic viral inclusions (arrowhead) are a hallmark of poxviral infections. HE, obj.20x. (**17**) Steroid-associated hepatocellular degeneration, liver, dog. Hepatocytes are distended with fine cytoplasmic vacuoles that often give the cells a foamy or lacy appearance typical of glycogen accumulation. HE, obj.20x. (**18**) Hepatic lipidosis, liver, goat. Hepatocytes are distended with single or multiple cytoplasmic vacuoles that often displace the nuclei to the periphery of the cell. HE, obj.20x. (**19**) Nuclear changes associated with necrosis (2-4) compared to a normal cell (1). Pyknosis (2) is the condensation of chromatin that makes the nucleus smaller and darker. Karyorrhexis (3) is the fragmentation of nuclear material. Karyolysis (4) is the dissolution of the chromatin by endonucleases leading to nuclear vanishing. (**20**) Acute hepatotoxicosis, liver, ox. Coagulative necrosis due to acute hepatotoxicosis. A few vacuolated hepatocytes around portal triads (asterisk) appear spared from necrosis. Pyknosis (arrow) and karyorrhexis (arrowhead) are observed throughout. In several hepatocytes, no nucleus is apparent. The eosinophilic hepatocellular cytoplasm is another feature of coagulative necrosis. HE, obj.20x. Image: Service of Veterinary Pathology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. (**21**) Splenic lymphoid necrosis, spleen, dog. Necrotic lymphocytes exhibit multiple areas of nuclear pyknosis and karyorrhexis throughout. HE, obj.20x.

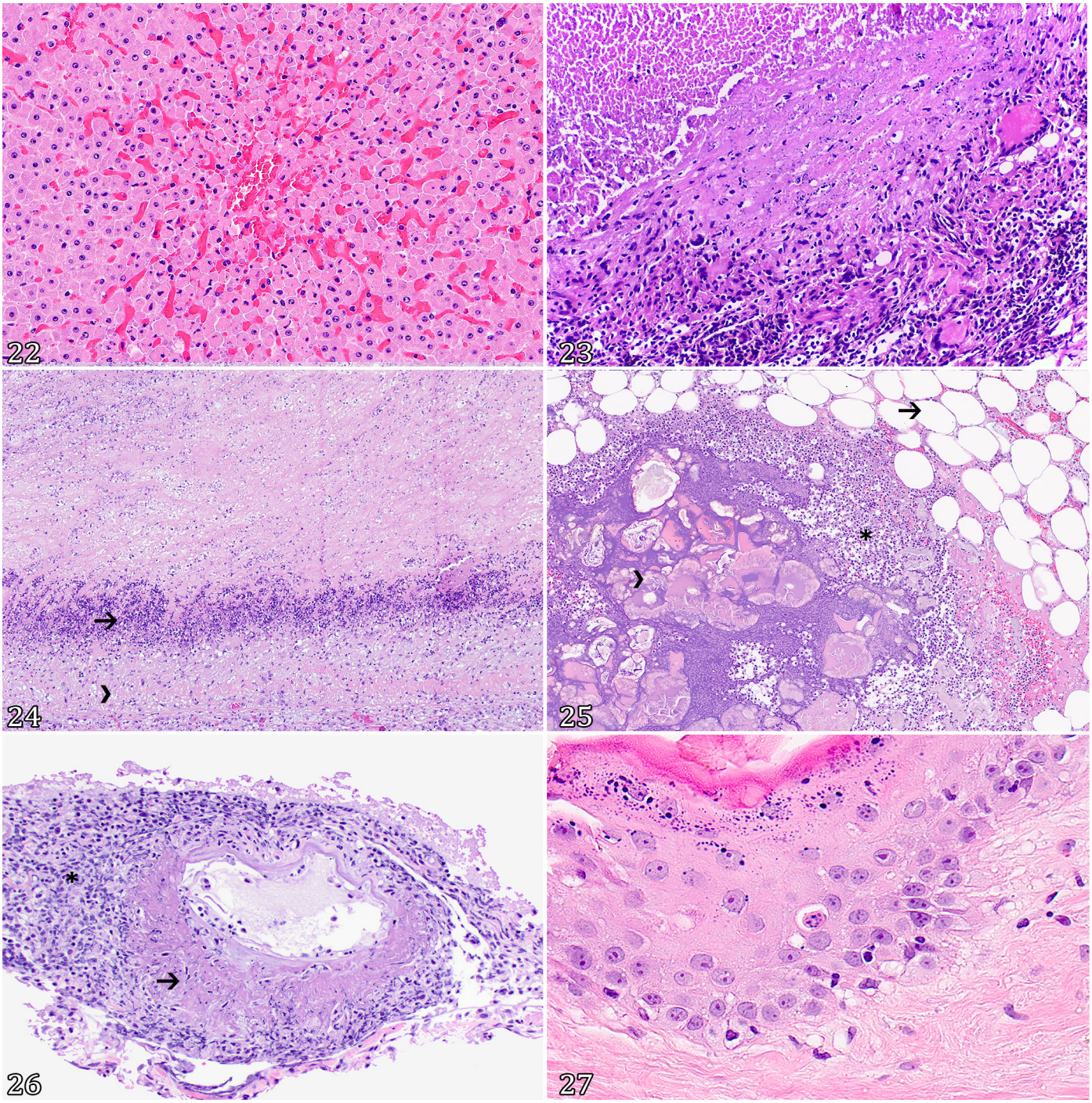


Fig. 22-27. (**22**) Acute hepatocellular necrosis, liver, dog. Centrilobular hepatocytes have undergone coagulative necrosis and exhibit hypereosinophilic and shrunken cytoplasm with pyknotic or karyorrhectic nuclei. HE, obj.20x. (**23**) Tuberculosis, lymph node, cow. *Mycobacterium bovis* typically leads to an extensive central area of caseous necrosis with mineralization (upper left) that is surrounded by multinucleated giant cells, epithelioid macrophages, and fewer lymphocytes and plasma cells (bottom right). HE, obj.20x. Image: Service of Veterinary Pathology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. (**24**) Abscess, pulmonary artery, goat. The area of liquefactive necrosis (top) consists of abundant cell and nuclear debris admixed with scattered neutrophils. The abscess is surrounded by a layer of inflammatory cells (arrow) and fibrovascular tissue (arrowhead). HE, obj.10x. (**25**). Fat necrosis secondary to acute pancreatitis, abdominal fat, cat. Fat necrosis occurs due to the action of triacylglycerol-lipase on triacylglycerol, which releases fatty acids and glycerol. Necrotic adipocytes are basophilic due to deposition of mineral. Typically, an inflammatory reaction to the necrotic adipose tissue is present (hence the alternative term steatitis). Regular adipocytes (arrow) and a large cluster of basophilic necrotic adipocytes (arrowhead) are present. Inflammation (asterisk) is also visible. HE, obj.10x. (**26**) Feline infectious peritonitis, meningeal vein, cat. The vascular wall is expanded and effaced by fibrillar eosinophilic material (fibrin) (arrow) and clusters of inflammatory cells (asterisk). HE, obj.20x. (**27**) Lupus erythematosus, skin, dog. An isolated apoptotic keratinocyte (center) exhibits hypereosinophilic cytoplasm and nuclear fragmentation. HE, obj.40x.

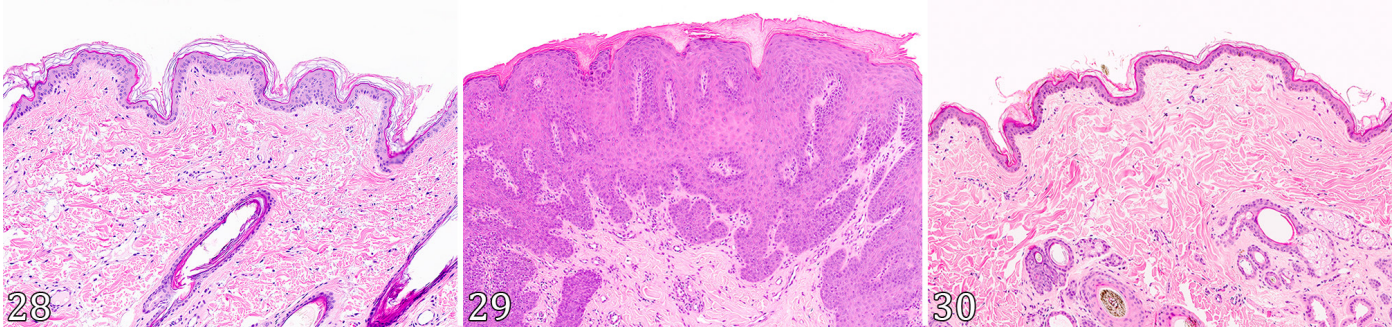


Fig.28-30. (**28**) Normal skin (abdomen), dog. The epidermis consists of 4-5 layers of keratinocytes covered by thin layers of keratin. HE, obj.10x. (**29**) Epidermal hyperplasia (abdomen), skin, dog. Chronic injury has led to marked thickening of the epidermis (epidermal hyperplasia) and orthokeratotic hyperkeratosis. Compare the affected skin with the normal skin in Figure 28. HE, obj.10x. (**30**) Epidermal atrophy (abdomen), skin, dog. Hyperadrenocorticism has caused thinning of the epidermis (atrophy), which consists of two and occasionally three layers of keratinocytes. Compare the lesions with the normal skin in Figure 28. HE, obj.10x.

(Fig.37); hemosiderin in areas of red blood cell degradation (Fig.38); bile (Fig.39), which can be observed engorging hepatic canaliculi in cases of hemolysis and intra-hepatic or obstructive biliary disease; proteins, including the cytoplasmic hyaline droplets in the proximal renal epithelium in cases of protein-losing nephropathy or the immunoglobulin globules in the cytoplasm of plasma cells (Fig.40); and lipofuscin (Fig.41), a by-product of cell aging that is often observed in neurons and hepatocytes of aged animals. Viral inclusions (Fig.42) (or other inclusions such as lead inclusions) are examples of exogenous intracytoplasmic or intranuclear accumulations that can be useful for the diagnosis of many diseases.

An endogenous extracellular pigment commonly observed includes hemoglobin in the renal tubules in cases of hemolytic diseases (Fig.43). Exogenous carbon particles (anthracosis) are typically observed as an incidental finding in the cytoplasm of macrophages in the lung or thoracic lymph nodes of dogs repeatedly exposed to air pollution or smoke or coal dust (Fig.44). Accumulation of calcium in soft tissues can occur due to elevated serum calcium levels (metastatic calcification) or as result of cell death (dystrophic calcification). The mineral appears as clusters of irregular basophilic material within affected tissues (Fig.45). Cholesterol clefts appear as acicular (needle-shaped) clefts in tissue sections (Fig.46), and are associated with areas of hemorrhage and necrosis.

Amyloid is an amorphous pathological material that accumulates in the extracellular matrix resulting in significant function compromise of the organ. It is perceived as an eosinophilic material in HE preparations. This proteinaceous fibrillar deposit exhibits b-pleated sheet secondary structure and can be by the apple-green birefringence when stained with the Congo red technique under polarized light (Sipe & Cohen 2000). In domestic animals, it can occur in many organs but is frequently seen in the renal glomeruli (Fig.47), pancreatic islets, hepatic sinusoids, around lymphoid follicles in the spleen, and in or around blood vessels in several tissues (Thomson 1978a). More recently, amyloid protein has been associated with brain lesions in cases of transmissible spongiform encephalopathy in many animal species (Yam 2003).

CIRCULATORY DISORDERS

Edema is the accumulation of fluid within the interstitial spaces (Mosier 2017). The main causes of edema include 1) increased vascular permeability, 2) increased vascular hydrostatic pressure, 3) decreased vascular osmotic pressure, and 4) decreased lymphatic drainage. Histologically, edema fluid appears as a brightly eosinophilic amorphous material (Fig.48) that typically expands the affected tissues.

The engorgement of blood vessels with excessive blood in a tissue is a consequence of hyperemia or congestion (Fig.49). Hyperemia is an active process that results from increased metabolic activity or inflammation, among other causes. In contrast, congestion is a passive process resulting mainly from decreased blood outflow with a normal blood inflow. Hyperemic or congested tissues will have distended capillaries filled with blood. While it may be relatively easy to observe hyperemia when associated with inflammation, other forms of hyperemia and, particularly congestion, can be subjective or difficult to be interpreted (how much blood is too much blood?). In fact, congestion is one of the most overdiagnosed processes in the diagnostic routine, often utilized as a "filler" in cases which have no other pathologic changes.

Just like you can see too much blood in a tissue, decreased tissue perfusion due to cardiac disease, chronic congestion, or vascular obstruction can cause tissues to inadequately meet their metabolic needs. For instance, vascular obstruction due to any cause (thrombosis or compression by adjacent lesions) can lead to tissue ischemia and well demarcated areas of coagulative necrosis surrounded by a rim of hemorrhage and inflammatory cells (infarcts). These infarcts will occur in the areas of the tissue that are supplied by the affected blood vessel (Fig.50-55).

Hemostasis allows blood to be constantly flowing within the blood vessels and heart. It also inhibits blood loss during injury by the formation of a clot at the affected site. When hemostasis fails, blood escapes the circulation and hemorrhage occurs. The most common causes of hemorrhage include 1) damage to blood vessels, 2) decreased platelet number (thrombocytopenia) or altered platelet function, and 3) coagulation disorders. Histologically, hemorrhage is characterized by the presence of red blood cells outside blood

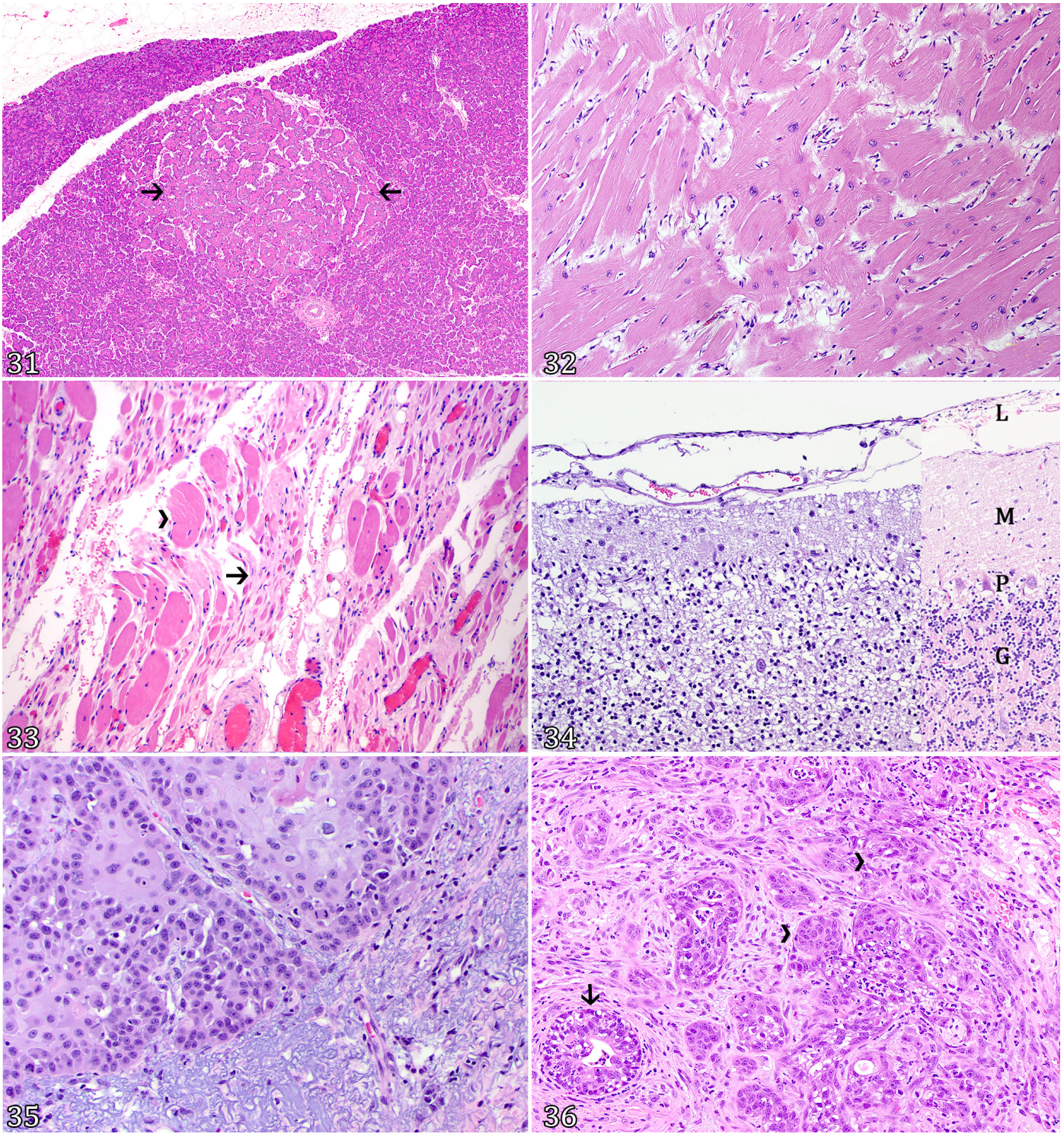


Fig.31-36. (**31**) Pancreatic nodular hyperplasia, pancreas, cat. These are common lesions in older cats, and consist of well demarcated, nodular areas of hypercellularity composed of normal exocrine cells (arrows). HE, obj.4x. (**32**) Cardiac hypertrophy, heart, cat. Cardiomyocytes are disorganized and exhibit large, hypertrophied cytoplasm and round nuclei. HE, obj.20x. (**33**) Skeletal muscle atrophy, cricoarytenoid muscle, horse. Compare the irregularly thinned, atrophied affected myofibers (arrow) with the normal adjacent myofibers (arrowheads). Chronic denervation has led to myofiber atrophy and replacement with fibrous connective tissue. HE, obj.10x. (**34**) Cerebellar hypoplasia, cerebellum, goat. There is overall hypocellularity across the three cerebellar layers. Compare the affected tissue with the normal cerebellum (right). Leptomeninges (L), molecular layer (M), Purkinje cell layer (P), granular layer (G). HE, obj.20x. (**35**) Ultraviolet light damage, skin, horse. Keratinocytes are disorganized and form cords and nests throughout (upper left). The superficial dermis (bottom right) is expanded and effaced by densely aggregated, coiled, irregular basophilic elastin fibers (solar elastosis). HE, obj.40x. (**36**) Necrotizing sialometaplasia, salivary gland, dog. Many salivary ducts have undergone hyperplasia and still have a central lumen (arrow), whereas others have undergone squamous metaplasia (arrowhead) as part of the disease process. HE, obj.20x.

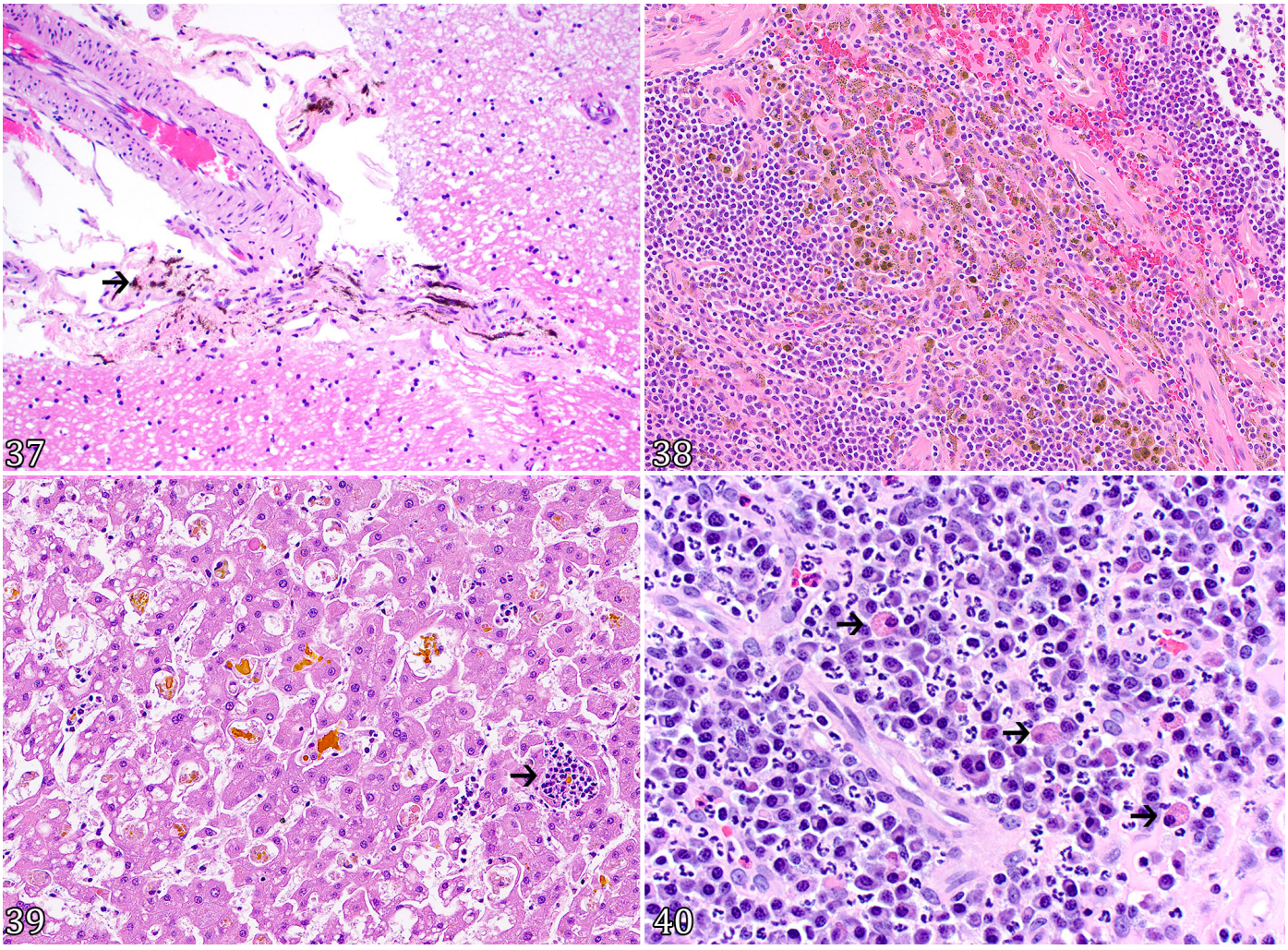


Fig.37-40. (**37**) Leptomeningeal melanocytes, brain, cow. Leptomeningeal melanocytes have brown cytoplasmic pigment (arrow). HE, obj.20x. (**38**). Hemosiderin, spleen, dog. Macrophages throughout the spleen contain distinct, bright to dark brown cytoplasmic pigment (hemosiderin). HE, obj.20x. (**39**) Bile stasis, liver, sheep. Multiple hepatocytes are disrupted due to canalicular and cytoplasmic accumulation of bile (orange). Necrotic hepatocytes have been replaced by clusters of neutrophils (arrow). HE, obj.20x. (**40**) Plasmacytic and neutrophilic pododermatitis, skin, cat. Plasma cells contain distinct eosinophilic globules of protein (immunoglobulin) in the cytoplasm (arrows). HE, obj.40x.

vessels. The clinical significance of hemorrhage depends on the amount of blood that has been lost to the extravascular spaces (Fig.56).

Another problem that can occur when hemostasis fails is the formation of an intravascular solid mass (thrombus) that can persist and cause partial or complete vascular occlusion and tissue ischemia. The three mechanisms which lead to vascular thrombosis include 1) endothelial cell injury, 2) changes in blood flow (stasis or turbulence), and 3) hypercoagulable states. Histologically, a thrombus appears as a collection of eosinophilic fibrin strands admixed with a mixed population of platelets, red blood cells, and inflammatory cells that are usually attached to the endothelial surface of a blood vessels or heart (Fig.57). A fragment of a thrombus (embolus) can detach from it and flow downstream (embolism) to distant sites, a process referred to as thromboembolism. Thrombi can also propagate (become larger) or resolve via fibrinolysis and thrombolysis. The clinical significance of a thrombus is dependent mainly on its location and size, as well as on the susceptibility of the involved tissues to ischemia. Thromboembolism can often lead to tissue ischemia and subsequent infarcts (Fig.50-55).

INFLAMMATION

In inflammation, several cell types, as well as fluid proteins from the blood are added to the tissues, creating the hallmarks of the inflammatory process (redness, swelling, heat, pain, and loss of function). There is a reasonable correlation between the type of exudate and certain types of causal agents (Table 2). Look for these correlations when describing an inflammatory lesion. Inflammation is classified according to the exudate (suppurative, fibrinous, hemorrhagic, necrotizing, and granulomatous, although these can overlap), intensity (mild, moderate, and severe, which are subjective and typically linked with the viewer's experience), and age (acute, subacute, and chronic).

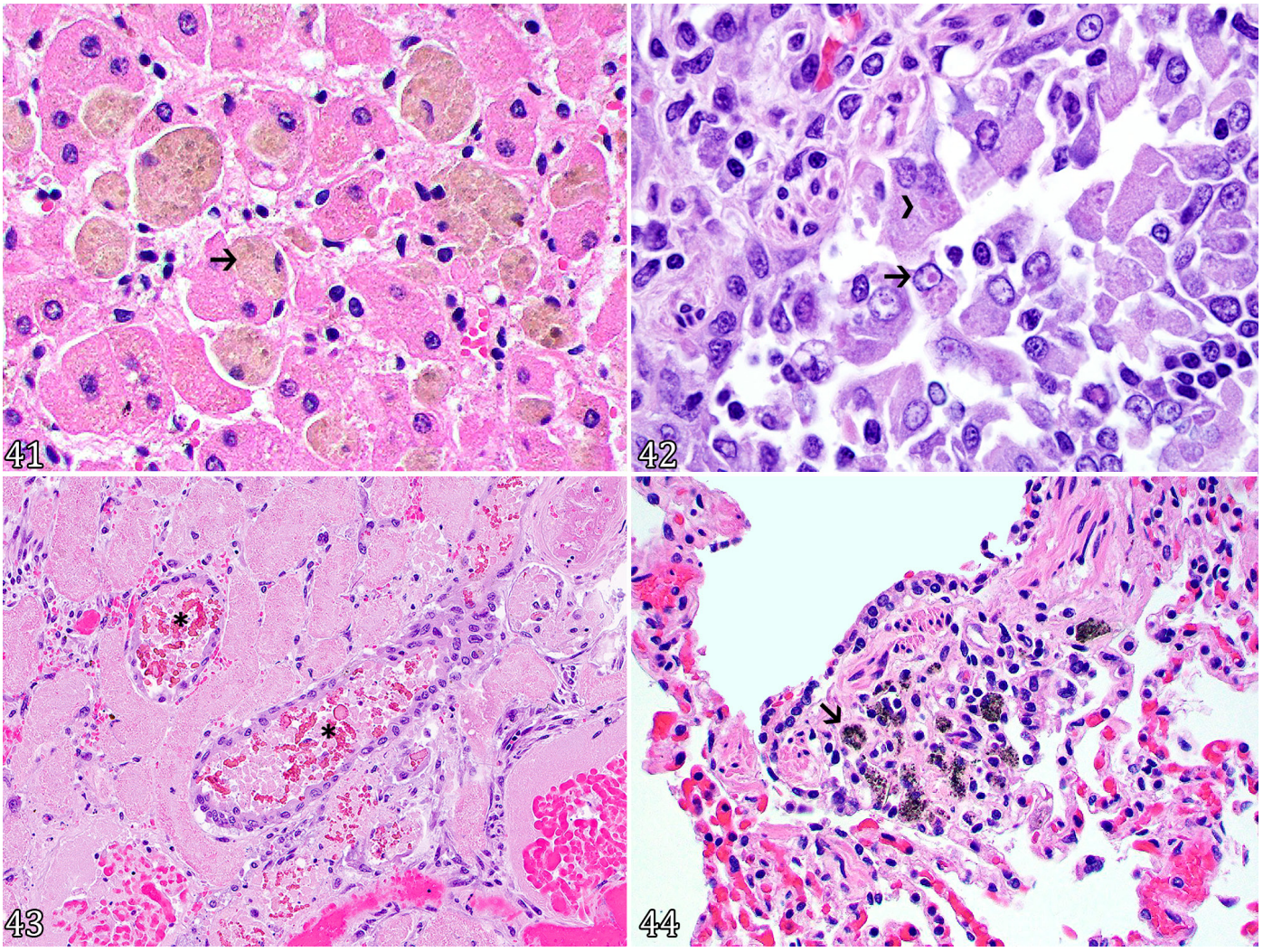


Fig.41-44. (**41**) Lipofuscinosis, liver, sheep. Hepatocytes contain abundant light brown pigment in the cytoplasm (arrow). HE, obj.40x. (**42**) Canine distemper, lung, dog. Bronchiolar epithelial cells exhibit intranuclear (arrow) and intracytoplasmic (arrowhead) eosinophilic viral inclusions. HE, obj.40x. (**43**) Renal tubular necrosis, kidney, sheep. The epithelial tubular cells have undergone coagulative necrosis associated with luminal clusters of red granular hemoglobin pigment (asterisks). HE, obj.10x. (**44**). Anthracosis, lung, dog. Clusters of macrophages (arrow) with black cytoplasmic pigment (carbon) surround a bronchiole. HE, obj.20x.

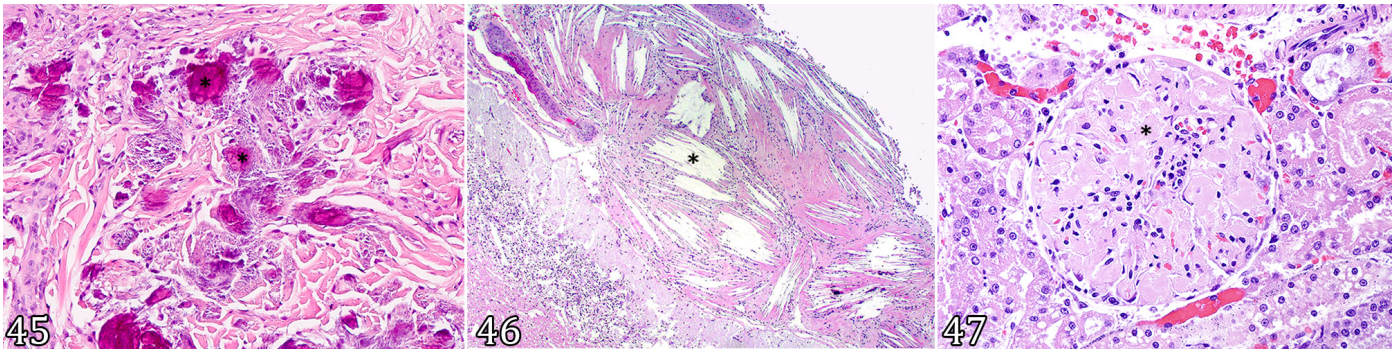


Fig.45-47. (**45**) Mineralization, skin, dog. Collagen bundles have undergone mineralization, characterized by deposition of granular, basophilic to eosinophilic extracellular material (asterisks). HE, obj.20x. (**46**). Cholesterol granuloma, fourth ventricle, horse. The granuloma contains numerous acicular (needle-shaped) cholesterol clefts (asterisk) surrounded by inflammatory cells. HE, obj.4x (**47**). Amyloidosis, kidney, dog. The glomerulus is hypocellular and partially effaced and compressed by eosinophilic, fibrillar, acellular amyloid (asterisk). HE, obj.40x.

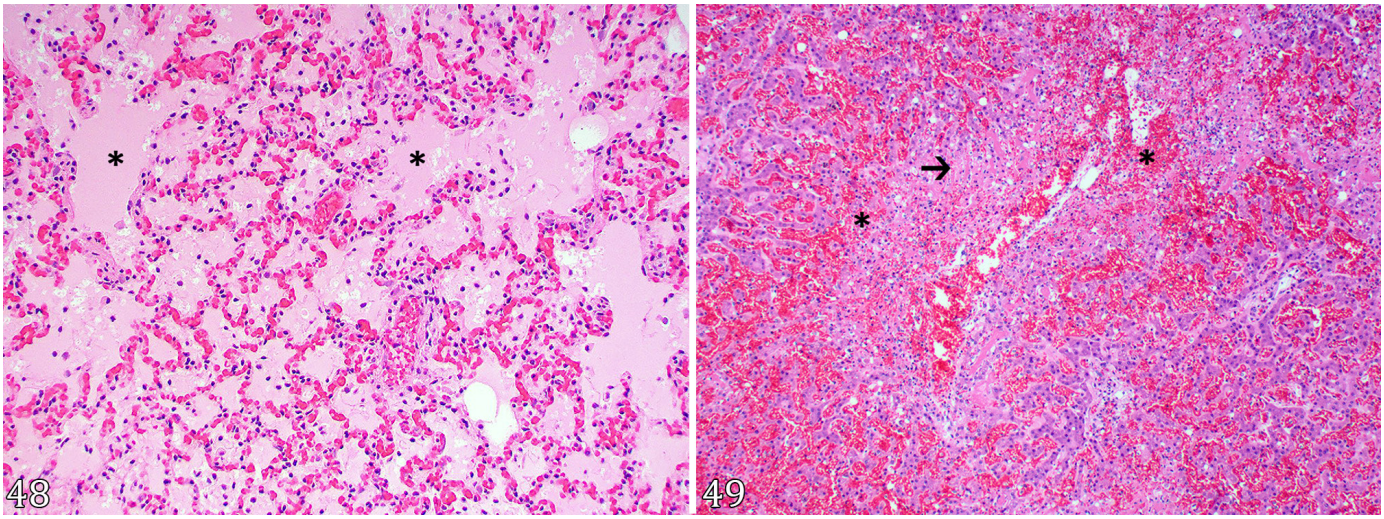


Fig.48-49. (**48**) Alveolar edema, lung, dog. Alveolar spaces are filled and expanded with eosinophilic edema fluid (asterisks). Compare it with the normal alveolar spaces in Figure 4. HE, obj.20x. (**49**) Chronic passive congestion, liver, dog. Centrilobular sinusoids (asterisks) are expanded by red blood cells that compress adjacent hepatocytes. Chronic congestion will also cause hypoxia and eventual hepatocellular necrosis (arrow). This is a hallmark of congestive heart failure. HE, obj.10x.

Table 2. Inflammatory patterns and commonly associated causes and diseases in veterinary diagnostic pathology

Exudate	Most common etiology	Specific diseases
Fibrinous	Bacteria	Blackleg (fibrinous pericarditis)
Suppurative/fibrinosupurative	Bacteria	Bovine pleuropneumonia
Granulomatous/pyogranulomatous	Bacteria/fungi/algae/protists/parasites	Tuberculosis Actinobacillosis Aspergillosis Candidiasis Mucormycosis Blastomycosis Pythiosis Protothecosis *Halicephalobus gingivalis* infection
Hemorrhagic/necrotizing	Virus	Bovine herpesvirus-1 and -5 meningoencephalitis
	Bacteria	Blackleg
Lymphocytic/plasmacytic	Virus	Rabies
Fibrinoid change	Virus	Feline infectious peritonitis
		Malignant catarrhal fever
		Classic swine fever
		Equine herpesvirus-1 myeloencephalopathy
	Bacteria	Thrombotic meningoencephalitis Bacterial septicemia
	Non-infectious	Steroid responsive meningitis-arteritis

In acute inflammation, neutrophils predominate, but this by no means equals suppurative inflammation. Suppurative inflammation requires pus (extensive collections of neutrophils and cell debris with or without bacteria) (Fig.58). As the inflammation progresses, macrophages, lymphocytes, and plasma cells take over (Fig.59). Macrophages originate from monocytes that leave the circulation and enter sites of inflammation or enter migrate to specific tissues during fetal life, the so-called resident macrophages (Sompayrac 2012). Granulomatous inflammation (Fig.60) (Thomson 1978b, Ackermann 2017) consists of aggregates of epithelioid macrophages, lymphocytes, plasma cells, and, occasionally, multinucleated giant cells or neutrophils (pyogranulomatous inflammation). Epithelioid macrophages differ from the usual macrophages in that they have a more abundant and eosinophilic cytoplasm. Its cellular limits are closely connected so that the entire structure has an aspect that resembles the epithelial layer of the epidermis. Granulomas form in response to bacteria and fungi that resist elimination by the immune system (e.g., *Mycobacterium bovis*, *Mycobacterium avium* subsp. *paratuberculosis*, or *Histoplasma capsulatum*) or substances that cause a strong cell-mediated rather than a humoral type of immune response (Sompayrac 2012). Under the influence of suitable mediators, epithelioid cells fuse to form multinucleated giant cells (Fig. 61). Granulomas or granulomatous inflammation not associated with an

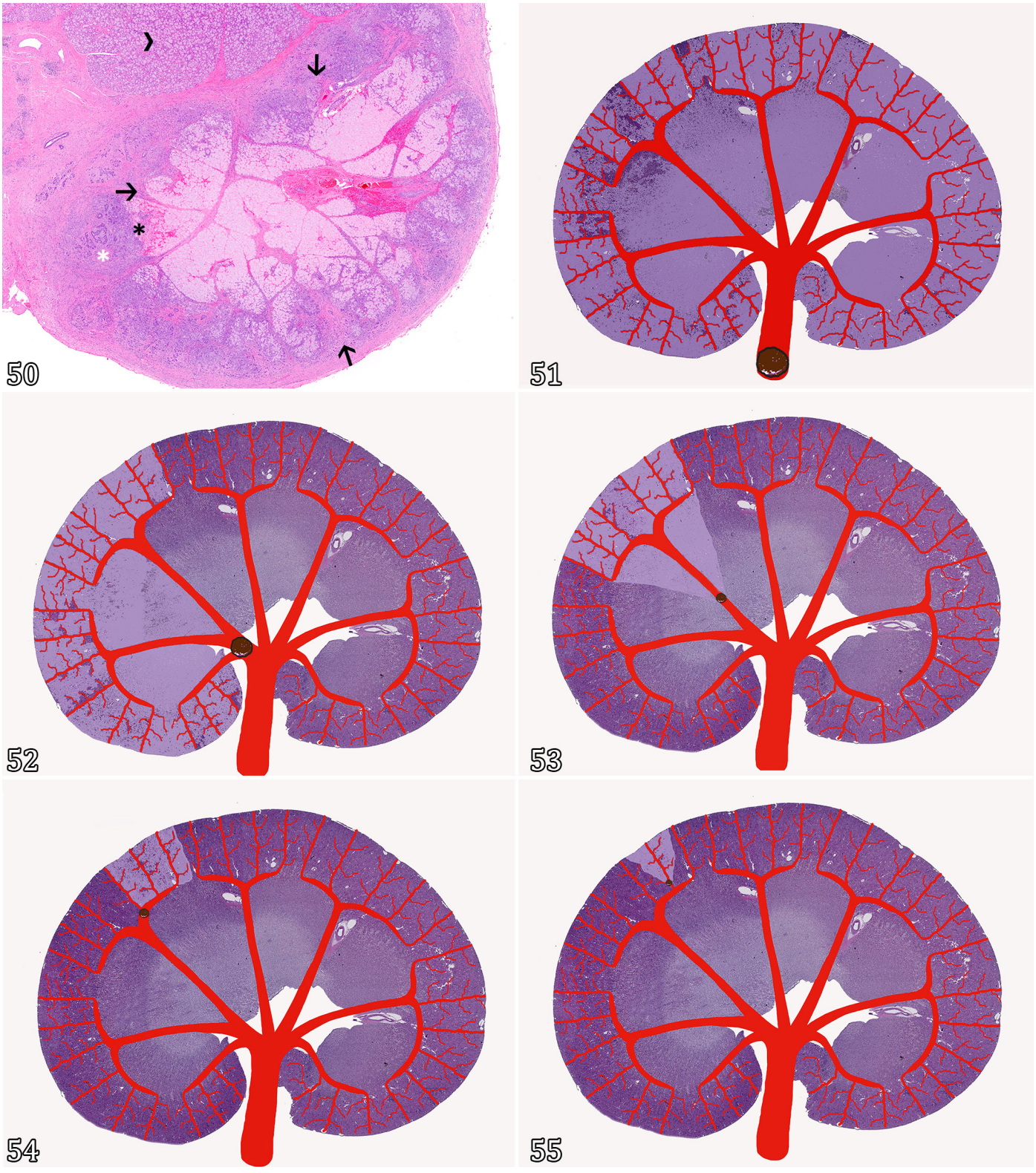


Fig. 50-55. (**50**) Necrotizing sialometaplasia, salivary gland, dog. A well-demarcated area of coagulative necrosis (infarct) effaces part of the glandular tissue (arrows). The necrotic area is separated from the normal salivary gland tissue (arrowhead) by areas of hemorrhage and inflammation (black asterisk) and fibrosis (white asterisk). HE, obj.2x. (**51**). Renal infarct, kidney, dog. Diffuse or total renal infarct due to vascular thrombosis and occlusion of the renal artery. (**52**). Renal infarct, kidney, dog. Focally extensive or subtotal renal infarct due to vascular thrombosis and occlusion of an extra-renal branch of the renal artery. (**53**). Renal infarct, kidney, dog. Corticomedullary (triangular) renal infarct due to vascular thrombosis and occlusion of an interlobular artery. (**54**). Renal infarct, kidney, dog. Cortical (trapezoid) renal infarct due to vascular thrombosis and occlusion of an arcuate artery. (**55**). Renal infarct, kidney, dog. Cortical (wedge-shaped) renal infarct due to vascular thrombosis and occlusion of a radiate artery.

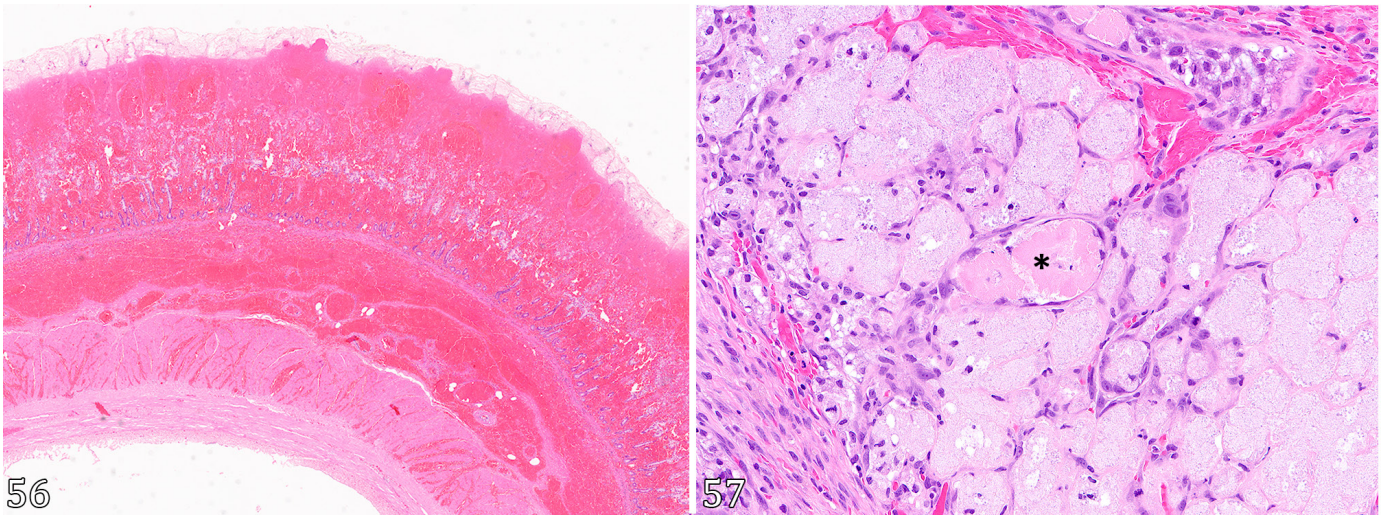


Fig.56-57. (**56**) Intestinal volvulus with hemorrhage, intestine, horse. The volvulus has caused impaired blood outflow and widespread congestion and hemorrhage throughout the affected intestinal segment. Such dramatic vascular changes will usually lead to hypoxia and ischemic necrosis of the affected area. HE, obj.2x. Image: Department of Pathology, University of Georgia College of Veterinary Medicine. (**57**) Vascular thrombosis, salivary gland, dog. A blood vessel is occluded by a solid cluster of eosinophilic fibrin strands (asterisk). The epithelial cells forming adjacent acini have undergone coagulative necrosis due to tissue ischemia caused by vascular compromise. HE, obj.20x.

infectious origin include those caused by the ingestion of toxic plants (Fighera & Barros 2004) (Fig.62) or tissue reactions to exogenous particles such as surgical suture or vaccine adjuvant (foreign body-type granuloma) (Panziera et al. 2016). Eosinophils (Fig.63) can participate in both acute and chronic inflammatory processes. They typically reach the site of inflammation after neutrophils and are associated mainly with allergic and parasitic reactions (Thomson 1978b).

One of the main characteristics of chronic inflammation is the excessive deposition of fibroblasts and collagen (fibrovascular tissue or granulation tissue). One of the main examples of granulation tissue is the so-called "proud flesh" (Fig.11) that usually follows cutaneous wound repair in horses (Theoret et al. 2013).

GROWTH DISORDERS

A tumor is a growth or an increase in size and volume of a particular tissue. It can be inflammatory, hyperplastic, cystic, or neoplastic (Reese 1981). A neoplasm is a type of tumor that consists of a proliferation of pre-existing cells indigenous to the site that grow autonomously. Common exceptions to that rule are the canine transmissible venereal tumor (Rogers et al. 1998) and the devil facial tumor disease of Tasmanian devils (Pye et al. 2016), which grow from naturally implanted grafts rather than indigenous cells. While the term neoplasm designates the tumor itself, the term neoplasia should be used to refer to the mechanism(s) generating a neoplasm. As an analogy, the term "thrombus" is to "neoplasm" what "thrombosis" is to "neoplasia". In a simplistic view, neoplasms are classified by the cells they originate from (histogenetic classification) or by their clinical behavior (benign or malignant). A relationship usually exists between histogenetic classification and behavior.

The main characteristics of neoplasia are summarized in Table 3, indicating the main differences between benign and malignant neoplasms. The differentiation of a neoplasm is based on how similar neoplastic cells are to the tissue that gave rise to the tumor. Benign tumors tend to be well to moderately differentiated (Fig.64), whereas malignant tumors tend to be moderately to poorly differentiated (Fig.65). The lack of differentiation is the same as anaplasia (Kemp et al. 2008). There is an argument among pathologists that differentiation is a somewhat subjective topic (Kemp et al. 2008), but we see a reasonably accurate parameter at practice.

In general terms, benign tumors are expansile but non-invasive, slow growing, and many times encapsulated. The mitotic activity is usually low, and mitoses are typically normal. Exceptions for the rule include follicular tumors, which tend to be less demarcated, and canine cutaneous histiocytomas, which tend to have a high mitotic activity. In contrast, malignant tumors are expansile and invasive, fast growing (Fig.66), and tend to have a higher mitotic count (not always) with atypical mitoses (Donovan et al. 2020). Therefore, although histopathologic features do not always suffice to distinguish between benign and malignant neoplasms, tumor invasion into surrounding tissues, cell and nuclear atypia, and increased mitotic activity are typically seen in malignant tumors (Fig.67). In addition, metastasis (Fig.68) is an exclusive feature of malignant neoplasms (Kemp et al. 2008). In oncology, metastasis refers to the transfer of neoplastic cells from one site (primary neoplasm) to a distant site (metastasis or secondary neoplastic site). For a neoplasm to be considered a metastasis, there must be no anatomical connection between the primary and the secondary tumor (Slauson & Cooper 2002, Kemp et al. 2008).

The primary and most commonly utilized tumor diagnosis method is the routine HE staining on formalin-fixed, paraffin-embedded tissues typically sectioned at 5µm (Abrahamsohn 2017). Tumor description and nomenclature should follow adequate and recent classification and grading systems, such as the World Health Organization classification guidelines for human or preferably veterinary medicine (Slayter et al.

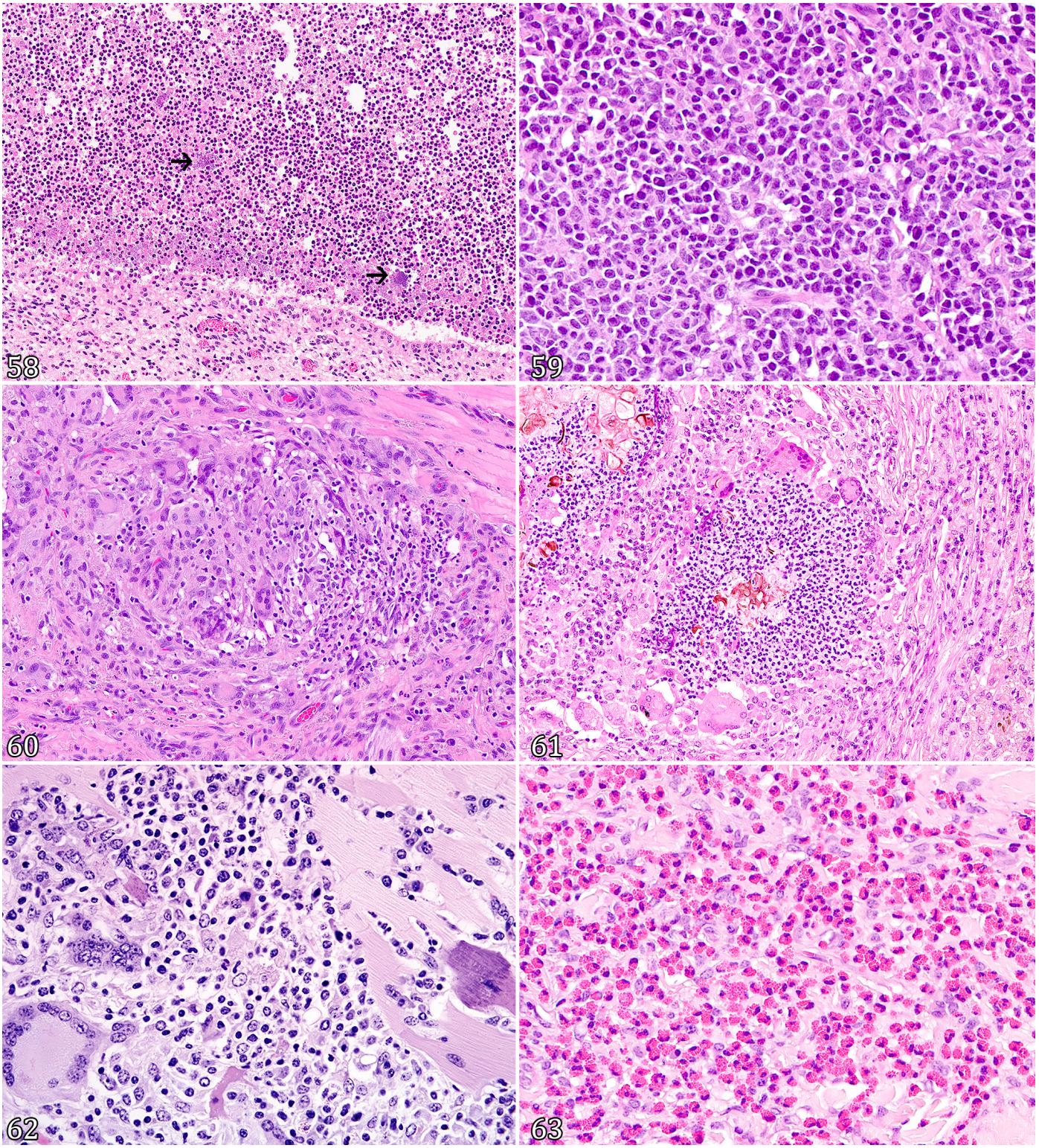


Fig.58-63. (**58**) Suppurative ependymitis, brain, goat. Suppurative inflammation characterized by collections of neutrophils and cell debris admixed with bacteria (arrows) within the lateral ventricle of a goat with septicemia. HE, obj.10x. (**59**). Lymphoplasmacytic inflammation, skin, dog. Inflammatory cells consist of lymphocytes, plasma cells, and fewer macrophages. HE, obj.40x. (**60**) Pythiosis, intestine, dog. Granulomatous inflammation is characterized by the presence of epithelioid macrophages and/or multinucleated giant cells, with a variable number of lymphocytes, plasma cells, and, occasionally, neutrophils (pyogranulomatous inflammation). HE, obj.20x. (**61**) Mycotic dermatitis (pseudomycetoma), skin, cat. Multinucleated giant cells with abundant cytoplasm and multiple nuclei surround clusters of fungal hyphae. HE, obj.20x. (**62**) Granulomatous myocarditis, heart, cow. Granulomatous inflammation can also occur due to noninfectious causes such as *Vicia villosa* (hairy vetch) poisoning. HE, obj.40x. (**63**). Eosinophilic dermatitis, skin, horse. Numerous eosinophils efface the dermis in a horse with allergic dermatitis. HE, obj.40x.

1994, Goldschmidt et al. 1998, 2018, Hendrick et al. 1998, Kennedy et al. 1998, Dungworth et al. 1999, Koestner et al. 1999, Misdorp et al. 1999, Head et al. 2002, Valli et al. 2002, Wilcock et al. 2002, Meuten et al. 2004, Roccabianca et al. 2019, Zappulli et al. 2019), as well as textbooks and scientific articles (Meuten et al. 2016, Meuten 2017). Depending on the circumstances, IHC (Fig.69) will be necessary to guide or confirm the diagnosis and add information relevant to the prognosis of certain neoplasms. IHC uses labeled antibodies of known specificity to detect antigen epitopes in tissues (Table 4). Enzymatic reactions with red or brown chromogens (Lindberg & Lamps 2018) are incorporated into the tissue around the antibody binding site, which can then be visualized (Klopfleisch & Bauer 2016). This technique can be used in formalin-fixed, paraffin-embedded tissues. However, fixation may impair antigenicity and require the use of antigen retrieval methods (Lindberg & Lamps 2018).

HOW TO FORMULATE A DIAGNOSIS

We will wrap things up by giving clues on how to formulate a morphologic and etiologic diagnosis, how to determine

Table 3. Morphologic features of non-neoplastic cell disorders, benign neoplasms, and malignant neoplasms

Morphology	Hyperplasia	Metaplasia	Dysplasia	Benign neoplasm	Malignant neoplasm
Cellular organization	Maintained	Maintained	Partially or completely lost	Lost	Lost
Cell differentiation	Normal	Normal	Low atypia	Typically low atypia	Moderate or marked atypia
Is cell type typical to the location?	Yes	No	Yes	Yes	Yes, in the case of primary neoplasm
Mitotic count	Low or moderate	Low	Low or moderate	Low or moderate	Low, moderate, or high
Atypical mitoses	Absent	Absent	Absent	Absent	Frequent
Pleomorphism (cellular and nuclear variation)	Absent or low	Absent or low	Low or moderate	Low or moderate	Moderate or marked
Increase of the nucleus and nucleolus	Low	Minimal	Low or moderate	Low or moderate	Moderate or marked
Grotesque-looking cells	Absent	Absent	Absent	Absent	May be present
Necrosis	Absent	Absent	Absent	Absent or mild	Mild to severe
Local invasion	Absent	Absent	Absent	Typically absent	Typically present
Vascular invasion	Absent	Absent	Absent	Absent	May be present

Modified from: Rowland (2004).

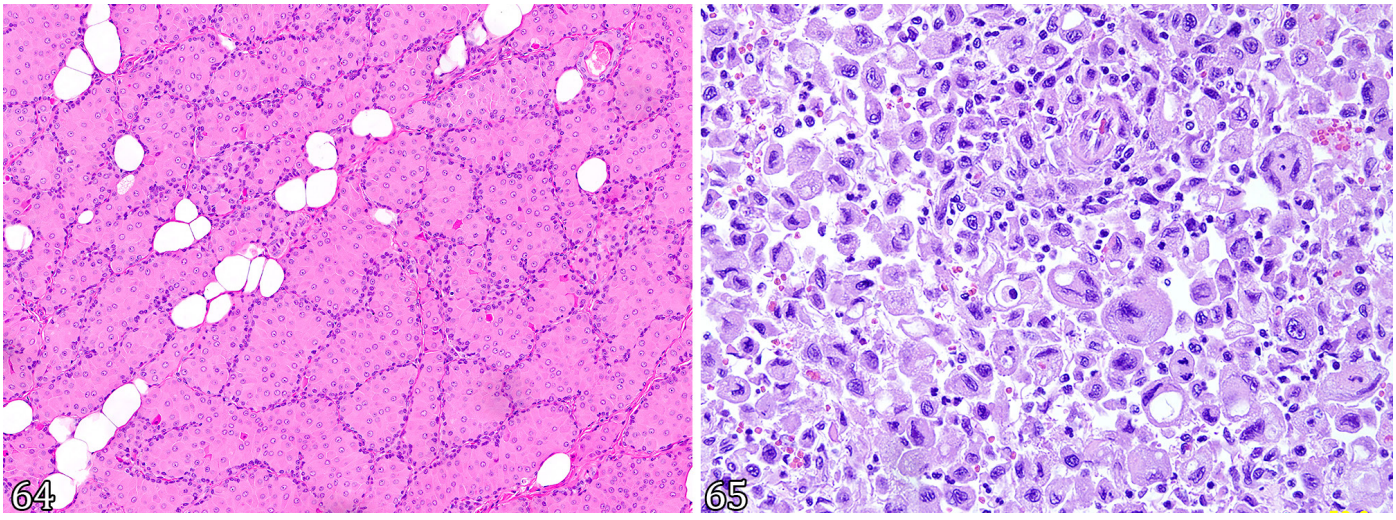
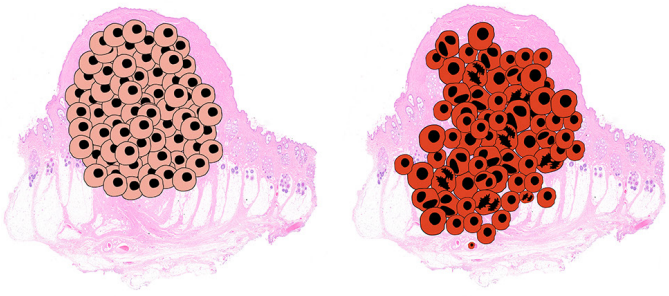
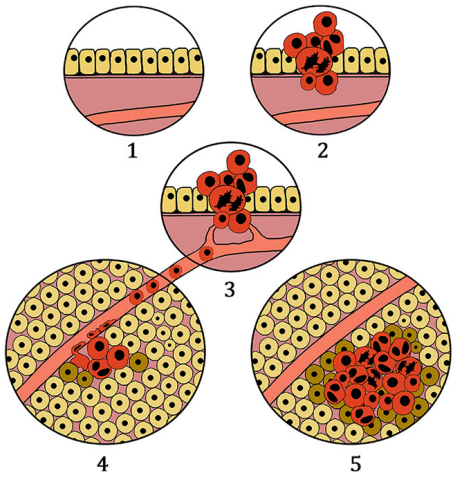


Fig.64-65. (**64**) Perianal gland adenoma, skin, dog. A well differentiated benign neoplasm in which neoplastic cells are morphologically similar to the normal cells that form the perianal gland. HE, obj.10x. (**65**) Histiocytic sarcoma, brain, cat. A poorly differentiated malignant neoplasm in which neoplastic cells display marked pleomorphism, with clear variations in size and shape of the cytoplasm and nucleus. The diagnosis of such poorly differentiated neoplasms often requires immunohistochemistry for confirmation. HE, obj.40x.

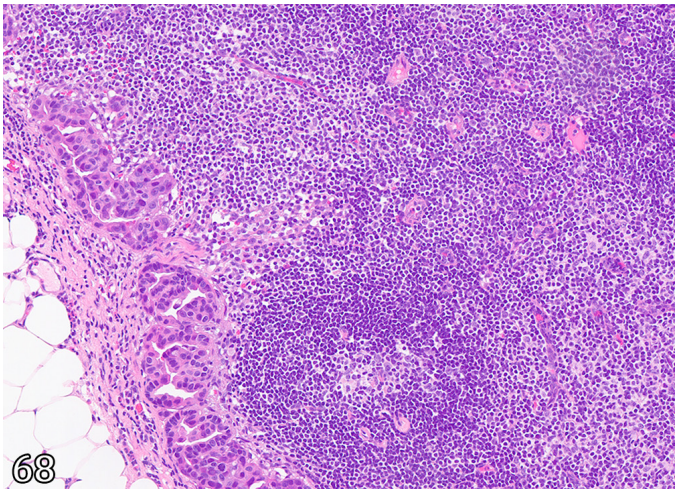


66



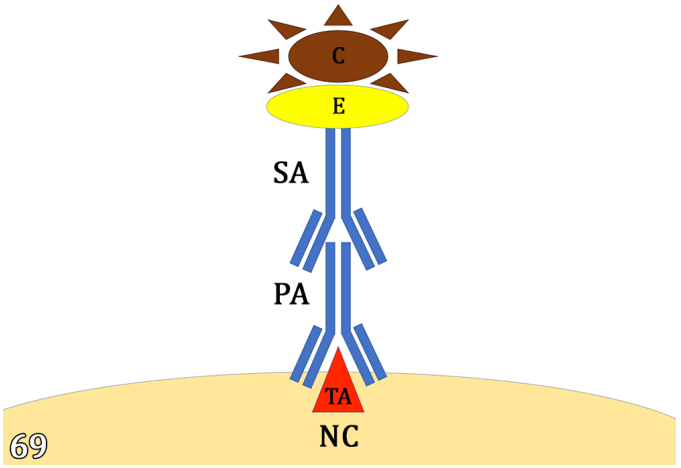
67

Fig.66-67. (**66**) Morphologic differences usually observed between benign versus malignant neoplasms. Benign neoplasms (left) tend to be expansile but non-invasive, slow growing, and typically have low mitotic activity. In contrast, malignant neoplasms (right) tend to be expansile and invasive, fast growing, and have a higher mitotic activity. **(67)** Metastatic spread in malignant neoplasms. Metastasis is an exclusive feature of malignant neoplasms, and refers to the transfer of neoplastic cells from one site (primary neoplasm) to a distant site (metastasis or secondary neoplastic site). In this example, a normal epithelium (1) undergoes malignant transformation (2), with subsequent vascular invasion by neoplastic cells (3). Intravascular neoplastic cells adhere to the endothelial lining at a distant site and invade the adjacent tissues (4), in which they form a secondary (metastatic) neoplasm (5). There is no anatomic connection between the primary and the secondary tumor.



68

Fig.68. Metastatic mammary carcinoma, lymph node, dog. Neoplastic epithelial cells (left) originating in the mammary gland tissue infiltrate the subcapsular sinus of a regional lymph node. HE, obj.10x.



69

Fig.69. Immunohistochemistry in a nutshell. A primary antibody of known specificity is utilized to detect antigens on the surface of neoplastic cells. Secondary antibodies attach to the primary antibody and enzymatic reactions using chromogens are incorporated and then visualized under the microscope. Chromogen (C), enzyme (E), secondary antibody (SA), primary antibody (PA), tissue antigen (TA), neoplastic cell (NC).

the etiology (cause), and how to name the condition (the disease itself), while warning the reader that it is not always possible to attain the entire task. The morphologic diagnosis summarizes the pathologic process or processes observed in the tissue section. The organ (anatomic location) and the type and distribution of the lesions should always be listed in the morphologic diagnosis. It is also desirable that, whenever possible, a morphologic diagnosis contains information on the approximate age (acute, subacute, chronic) and severity of the lesion (mild, moderate, severe). A rule of thumb when assessing severity is to consider a lesion mild when it affects less than 20% of the tissue, moderate when it affects between 20 and 60% of the tissue, and severe when it affects more than 60% of the tissue. An adequate morphologic diagnosis in the following example (Fig.70) of a canine heart would be "Myocardium: Multifocal to coalescing, subacute, severe lymphoplasmacytic myocarditis with intracytoplasmic protozoan amastigotes".

The etiologic diagnosis is a sentence indicating the process and the general category of the causative agent causing the lesion. In the current case, the etiologic diagnosis is "protozoal myocarditis". If you know the cause you can be more specific and include that information in your etiologic diagnosis ("trypanosomal myocarditis").

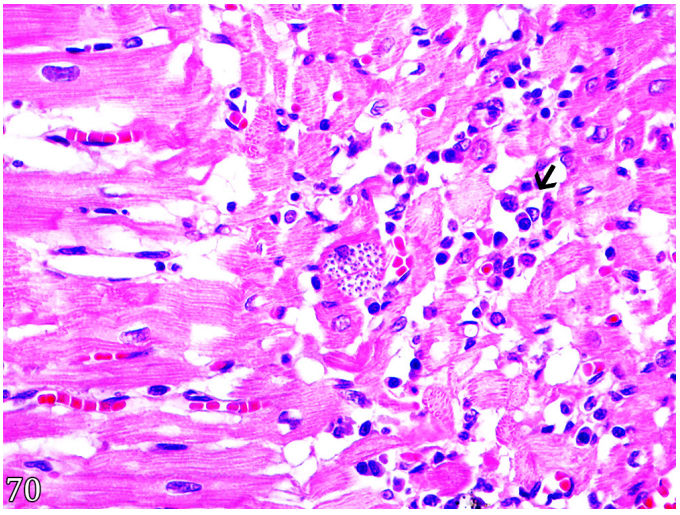


Fig.70. Cardiac trypanosomiasis, heart, dog. A moderate number of lymphocytes and plasma cells disrupt groups of cardiomyocytes (arrow). An affected myofiber (center) is expanded by a sarcoplasmic protozoal cyst measuring approximately 40µm in diameter and containing 3µm round amastigotes with a central basophilic nucleus and an adjacent rod-shaped kinetoplast (not evident). The morphologic diagnosis in this case would be "Myocardium: Multifocal to coalescing, subacute, moderate lymphoplasmacytic myocarditis with intracytoplasmic protozoan cysts and amastigotes". The etiologic diagnosis would be "protozoal myocarditis" or "trypanosomal myocarditis", the etiology would be *Trypanosoma cruzi*, and the name of the disease would be "canine American trypanosomiasis". HE, obj.40x.

In this case, the etiology is *Trypanosoma cruzi*, and the name of the condition or disease is "canine American trypanosomiasis". Remember, do not capitalize the name of diseases, unless grammatically necessary (eponymous diseases such as Johne's disease, Aujeszky's disease, etc.; or diseases named after geographic locations such as West Nile fever, Ebola hemorrhagic fever, Borna disease, etc.).

FINAL CONSIDERATION

None of the concepts we have introduced here are new or original. Variations of these methods and ideas have been around since the first morphologic descriptions of lesions and diseases by Hippocrates (around 2400 years ago) and have grown following the systematic dissection of humans and other animals by Alexandrian scientists around 300 BCE (Van den Tweel & Taylor 2010). Observations became more detailed and precise over the next centuries and culminated in the examination of the microscopic features of normal and diseased tissues after the advent of the microscope in the mid-nineteenth century (Van den Tweel & Taylor 2010). We have now examined the big and the small and we have had the chance to explore the intricacies of everything in the middle. Molecular biology took us further and into the subcellular world. And new technologies will surface. Digital pathology is here, and as it becomes more accepted by our peers, it will be more widely utilized for diagnostic routine, research, and teaching (Bertran & Klopfleisch 2017). Whether we use a microscope or a computer screen to examine our pathology specimens, we still rely on our power of observation and pattern

Table 4. Common tumor immunomarkers utilized in veterinary diagnostic pathology

Immunomarker	Target cell	Immunolabeling
CD117 (KIT)	Mast cells, intertitial cells of the gastrointestinal tract	Membranous and cytoplasmic
CD20	B lymphocytes	Membranous
CD3	T lymphocytes	Membranous
CD31	Endothelial cells	Membranous
CD45R	B lymphocytes	Membranous
CD79a	B lymphocytes	Membranous
Chromogranin A	Neuroendocrine cells	Cytoplasmic
Cytokeratin	Epithelial cells	Cytoplasmic
Desmin	Skeletal and smooth muscle	Cytoplasmic
E-cadherin	Meningothelial cells	Membranous and cytoplasmic
Factor 8-related antigen	Endothelial cells and megakaryocytes	Cytoplasmic
Glial fibrillary acidid protein	Astrocytes, ependymal cells	Cytoplasmic
IBA1	Monocytes/macrophages	Cytoplasmic
Melan A	Melanocytes, steroid-producing cells	Cytoplasmic
Myoglobin	Skeletal muscle	Cytoplasmic
MUM1	Plasma cells, subsest of lymphocytes, and histiocyties	Nuclear
Olig2	Oligodendrocytes and astrocytes	Nuclear
Pax-5	B lymphocytes	Nuclear
PNL2	Melanocytes	Cytoplasmic
S-100	Neuroectodermal cells	Cytoplasmic
Smooth muscle actin	Smooth muscle cells	Cytoplasmic
Synatophysin	Neuroendocrine cells	Cytoplasmic
Vimentin	Mesenchymal cells	Cytoplasmic

recognition to achieve a diagnosis. Artificial intelligence may one day take over our capacity to perform these tasks, but until then we judge that a basic knowledge of morphologic description and interpretation of tissues and tissue changes is a fundamental part of veterinary pathology training.

Conflict of interest statement.- The authors have no competing interests.

REFERENCES

Abrahamsohn P. 2017. Preparação de espécimes para exame microscópico, p.2. In: Ibid (Ed.), Histologia Básica, Texto e Atlas. Guanabara Koogan, Rio de Janeiro.

Ackermann M.R. 2017. Inflammation and healing, p.73-131. In: Zachary J.F. (Ed.), Pathologic Basis of Veterinary Disease. 6th ed. Elsevier, St. Louis.

Agnew D.W. & MacLachlan N.J. 2017. Tumors of the genital systems: tumors and tumor-like lesions of the prostate, p.715. In: Meuten D.J. (Ed.), Tumors in Domestic Animals. 5th ed. Wiley Blackwell, Ames.

Bertran C.A. & Klopfleisch R. 2017. The pathologist 2.0: an update on digital pathology in veterinary medicine. Vet. Pathol. 54(5):756-766. <https://dx.doi.org/10.1177/0300985817709888> <PMid:28578626>

Bracegirdle B.A. 1986. History of Microtechnique. 2nd ed. Science Heritage, Lincolnwood, p.69-72.

Brooks J.S.J. & Perosio P.M. 2007. Adipose tissue: metaplasia, p.177. In: Mills S.E. (Ed.), Histology for Pathologists. 3rd ed. Lippincott Williams and Wilkins, Philadelphia.

Cook H. 2000. Evolution of histology. Biomed. Sci. 9:825-827.

Donovan T.A., Moore F.M., Bertram C.A., Luong R., Bolfa P., Klopfleisch R., Tvedten H., Salas E.N., Whitley D.B., Aubreville M. & Meuten D.J. 2020. Mitotic figures-normal, atypical, and imposters: a guide to identification. Vet. Pathol. 58(2):243-257. <https://dx.doi.org/10.1177/0300985820980049> <PMid:33371818>

Dungworth D.L., Hauser B., Hahn F.F., Wilson D.W., Haenichen T. & Harkema J.R. 1999. World Health Organization Histological Classification of Tumors of the Respiratory System of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 71p.

Fighera R.A. & Barros C.S.L. 2004. Systemic granulomatous disease in Brazilian cattle grazing pasture containing vetch (*Vicia* spp.). Vet. Hum. Toxicol. 46(2):62-66. <PMid:15080204>

Gerber M.A. & Thung S.N. 1988. Obituary: Hans Popper, M.D. Ph-D. November 24, 1903-May 6 1988. Am. J. Pathol. 133:13-14.

Goldschmidt M.H., Dunstan R.W., Stannard A.A., Tscharner C.V., Walder E.J. & Yager J.A. 1998. World Health Organization Histological Classification of Epithelial and Melanocytic Tumors of the Skin of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 106p.

Goldschmidt M.H., Munday J.S., Scruggs J.L., Klopfleisch R. & Kiupel M. 2018. Surgical Pathology of Tumors of Domestic Animals. Volume 1: Epithelial Tumors of the Skin. Davis-Thompson Foundation, Gurnee, IL. 241p.

Hadlow W.J. 1994. Pathologic lesions: they're the worst kind. Vet. Pathol. 31(2):290-291. <https://dx.doi.org/10.1177/030098589403100229> <PMid:8203105>

Ham A.W. & Cormack D.H. 1979. Histology. Lippincott Philadelphia, Pennsylvania, p.26.

Head K.W., Cullen J.M., Dubielzig R.R., Else R.W., Misdorp W., Patnaik A.K., Tateyama S. & Van der Gaag I. 2002. World Health Organization Histological Classification of Tumors of the Alimentary System of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 257p.

Hendrick M.J., Mahaffey E.A., Moore F.M., Vos J.V. & Walder E.J. 1998. World Health Organization Histological Classification of Mesenchymal Tumors of Skin and Soft Tissues of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 64p.

Henrich M. 2016. Hematopoietic tumors, p.238. In: Klopfleisch R. (Ed.), Veterinary Oncology: a short textbook. Springer International Publishing, Cham, Switzerland.

Kemp W.L., Burns D.K. & Brown T. 2008. Pathology: the big picture. McGraw-Hill, New York, p.30-37.

Kennedy P.C., Cullen J.M., Edwards J.F., Goldschmidt M.H., Larsen S., Munson L. & Nielsen S. 1998. World Health Organization Histological Classification of Tumors of the Genital System of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 79p.

Kiernan J.A. 2008. Histological and Histochemical Methods. 4th ed. Scion, Publishing Bloxham, United Kingdom, p.276-281.

Klopfleisch R. & Bauer N. 2016. Basic principles of cancer diagnostics, p.141. In: Klopfleisch R. (Ed.), Veterinary Oncology: a short textbook. Springer International Publishing, Cham, Switzerland.

Koestner A., Bilzer T., Fatzer R., Schulman F.Y., Summers B.A. & Van Winkle T.J. 1999. World Health Organization Histological Classification of Tumors of the Nervous System of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 71p.

Kumar V., Abbas A.K. & Aster J.C. 2015. Dysplasia, p.171. In: Ibid. (Eds), Robbins and Cotran Pathologic Basis of Disease. 9th ed. Elsevier Saunders, Philadelphia.

Lindberg M.R. & Lamps L.W. 2018. Immunohistochemistry of normal tissues, p.28-29. In: Lindberb M.R. & Lamps L.W. (Eds), Normal Histology. 2nd ed. Elsevier, Philadelphia.

Meuten D.J. 2017. Tumors in Domestic Animals. 5th ed. John Wiley and Sons, Ames. 989p.

Meuten D.J., Everitt J. & Inskeep W. 2004. World Health Organization Histological Classification of Tumors of the Urinary System of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 70p.

Meuten D.J., Moore F.M. & George J.W. 2016. Mitotic count and the field of view area: time to standardize. Vet. Pathol. 53(1):7-9. <https://dx.doi.org/10.1177/0300985815593349> <PMid:26712813>

Miller M.A. & Zachary J.F. 2017. Mechanisms and morphology of cellular injury, adaptation, and death, p.2-43. In: Zachary J.F. (Ed.), Pathologic Basis of Veterinary Disease. 6th ed. Elsevier, St. Louis, Missouri.

Misdorp W., Else R.W., Hellmen E. & Lipscomb T.P. 1999. World Health Organization Histological Classification of the Mammary Tumors of the Dog and Cat Histological Classification of Endocrine Tumors of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 57p.

Mosier D.A. 2017. Vascular disorders and thrombosis, p.44-72. In: Zachary J.F. (Ed.), Pathologic Basis of Veterinary Disease. 6th ed. Elsevier, St. Louis.

Panziera W., Rissi D.R., Galiza G.J.N., Giaretta P.R., Bianchi R.M., Bazzi T. & Barros C.S.L. 2016. Pathology in Practice: Spinal cord compression associated with vaccine granulomas in 2 calves. J. Am. Vet. Med. Assoc. 249(5):483-485. <https://dx.doi.org/10.2460/javma.249.5.483>

Pye R.J., Woods G.M. & Kreiss A. 2016. Devil facial tumor disease. Vet. Pathol. 53(4):726-736. <https://dx.doi.org/10.1177/0300985815616444>

Reese A.J.M. 1981.The principles of pathology, p.126. 2nd ed. Wright PSG, Bristol, United Kindom.

Reisner H.M. 2015. Lesão, morte e envelhecimento da célula, p.21-43. In: Ibid. (Ed.), Patologia: uma abordagem por estudo de casos. McGraw-Hill, New York.

Robinson W.F., Huxtable C.R. & Pass D.A. 1980. Canine parvoviral myocarditis: a morphologic description of the natural disease. Vet. Pathol. 17(3):282-293. <https://dx.doi.org/10.1177/030098588001700302> <PMid:7368523>

Roccabianca P., Schulman F.Y., Avallone G., Foster R.A., Scruggs J.L., Dittmer K. & Kiupel M. 2019. Surgical Pathology of Tumores of Domestic Animals. Volume 3: Tumors of Soft Tissue. Davis-Thompson Foundation, Gurnee, IL. 307p.

Rogers K.S., Walker M.A. & Dillon H.B. 1998. Transmissible venereal tumor: a retrospective study of 29 cases. J. Am. Anim. Hosp. Assoc. 34(6):463-470. <https://dx.doi.org/10.5326/15473317-34-6-463> <PMid:9826280>

Rowland P.H. 2004. O papel do patologista na oncologia veterinária, p.53-67. In: Rosenthal R.C. (Ed.), Segredos em Oncologia Veterinária. ArtMed, Porto Alegre.

Sipe J.D. & Cohen A.S. 2000. Review: History of the amyloid fibril. J. Struct. Biol. 130(2/3):88-98. <https://dx.doi.org/10.1006/jsbi.2000.4221> <PMid:10940217>

Slauson D.O. & Cooper BJ. 2002. Mechanisms of Disease: a textbook of comparative general pathology. Mosby, St. Louis, p.341.

Slayter M.V., Boosinger T.R., Pool R.R., Dammrich K., Misdorp W. & Larsen S. 1994. World Health Organization Histological Classification of Bone and Joint Tumors of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 50p.

Sompayrac L. 2012. How the Immune System Works. Wiley-Blackwell, West Sussex, UK, p.1-23.

Theoret C.L., Olutoye O.O., Parnell L.K. & Hicks J. 2013. Equine exuberant granulation tissue and human keloids: a comparative histopathologic study. Vet. Surg. 42(7):783-789. <https://dx.doi.org/10.1111/j.1532-950X.2013.12055.x> <PMid:24015864>

Thomson R.G. 1978a. General Veterinary Pathology. W.B. Saunders, Philadelphia, p.262-298.

Thomson R.G. 1978b. General Veterinary Pathology. W.B. Saunders, Philadelphia, p.152-261.

Titford M. 2006. A short history of histopathology technique. J. Histotechnol. 29(2):99-110. <https://dx.doi.org/10.1179/his.2006.29.2.99>

Titford M.l. 2009. Progress in the development of microscopical techniques for diagnostic pathology. J. Histotechnol. 32(1):9-19. <https://dx.doi.org/10.1179/his.2009.32.1.9>

Valli V.E., Jacobs R.M., Parodi A.L. & Vernau W. 2002. World Health Organization Histological Classification of Hematopoietic Tumors of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 190p.

Van den Tweel J.G. & Taylor C.R. 2010. A brief history of pathology: Preface to a forthcoming series that highlights milestones in the evolution of pathology as a discipline. Virchows Archiv 457:3-10. <https://dx.doi.org/10.1007/s00428-010-0934-4> <PMid:20499087>

Waugh E.M., Gallagher A., Hayley H., Johnston P.E.J., Marchesi F., Jarrett R.F. & Morris J.S. 2016. Optimisation and validation of a PCR for antigen receptor rearrangement (PARR) assay to detect clonality in canine lymphoid malignancies. Vet. Immunol. Immunopathol. 182:115-124. <https://dx.doi.org/10.1016/j.vetimm.2016.10.008> <PMid:27863542>

Wilcock B., Dubielzig R.R. & Render J.A. 2002. World Health Organization Histological Classification of Ocular and Otic Tumors of Domestic Animals. Armed Forces Institute of Pathology, Washington, D.C. 72p.

Yam P. 2003. The Pathologic Protein: mad cow, chronic wasting, and other deadly prion diseases. Spring Verlag, New York, p.94.

Zappulli V., Pena L., Rasotto R., Goldschmidt M.H., Gama A., Scruggs J.L. & Kiupel M. 2019. Surgical Pathology of Tumores of Domestic Animals. Volume 2: Mammary Tumors. Davis-Thompson Foundation, Gurnee, IL. 270p.