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Article Review: Advancements in Veterinary Histopathology: Linking Tissue Pathology to Clinical Outcomes

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ABSTRACT: stressors. Histopathologists in veterinary pathology are therefore engaged in examining tissue alterations resulting from the direct or indirect influence of diseases on cells, tissues, organs, and/or body systems. Such microscopic alterations are collectively considered as 'lesions,' and the terminology used to describe these changes is unique to histopathology. Although mainly involved in Histopathology comprises the study of morphological changes occurring in tissues and organs during various pathological conditions or following exposure to chemicals, drugs, and other environmental diagnostic work, the veterinary histopathologist often plays a substantial role in understanding the pathogenesis of an individual case or collection of cases and may contribute to fundamental studies of the etiology and pathogenesis of a wide range of diseases affecting different species of animals. In review, where the aim is to understand why and how a disease develops, the broad term "anatomical pathology" is used.

Keywords: Clinical, Histopathology, Veterinary, Lesions, Anatomical Pathology

1. INTRODUCTION

1. Introduction to Veterinary Histopathology

Veterinary histopathology, therefore, benefits from the knowledge and information obtained in all other specialist areas of preclinical veterinary studies to draw conclusions and make inferences pertinent to the health and welfare of domestic, farmed, or wild animals [1].

Histopathological changes (lesions) can occur during many physiological, toxicological, and pathological compromises. Collectively, the array of histopathological data can indicate, for example, how a diseased animal became affected, which tissue or cell is primarily involved, the nature and extent of the inflammation generated, and ultimately support practices for clinical, therapeutic, and/or preventive interventions. The early use of histopathology focused on natural diseases seen in traditional domestic and production animals and was generally descriptive. The evolution and advancements in technologies now provide, increasingly so, unlimited information on various disease processes and improved understanding of lesions that arise during normal, naturally occurring diseases and their subsequent translation across to humans. In a similar strategic approach, toxicologically and toxicodynamic characterization of active substances in the process of drug development is accelerated using mechanistic-based reasoning. In some cases, this evidential value of

histopathology has shown to be capable of accurate predictive capabilities between animals and humans, which is pertinent in medicines' regulatory assessment during policy initiation [2].

1.2. Importance in Veterinary Medicine

Histopathology is an interface between pathology, histology, and biochemistry, essential in diagnostics and treatment of various diseases. As tissue examination is regarded as the "gold standard" for disease diagnosis and is vital for tissue disease examination in all types of potential animal patients. Reviewing histopathology in the final year curriculum, this section will provide students with an appreciation of the essence of linking basic histology to disease pathogenesis and is often the "clincher" for making a diagnosis. Many scientists and students in different veterinary courses believe that accurate histopathology of the animal tissues helps in making responsible decisions, leading to effective treatment and successful recovery in the animal. Irrespective of the animal species and/or pet, latent histopathological examination is essential in making the decision for diagnostic procedures, pathology investigations, conservation, and curatorship, and if/when to start breeding and stem cell research studies [5].

Hence, it is paramount to:

• Accurately recognize the tissue pathology and decide whether treatment/procedure is to be started.

• Determine the type/nature of disease.

• Assess the long-term effects of the treatment.

• Correlate the tissue pathology with the clinical signs. One good example is in the overuse of diuretics, often indicated by unregistered or laypersons, where gastrointestinal lesions are not identified and treated, often incurring extra costs for owners. The veterinarian has a responsibility to establish the efficacy of treatment on the disease through routine or special pathology investigations. Furthermore, skin tissue biopsies are often essential to provide a tissue diagnosis through histopathology and also gauge treatment levels for improvement of the patient’s condition. Histopathology, through structural pathogenesis, can indicate the approximate duration of an animal’s condition [6].

2. BASIC PRINCIPLES OF HISTOPATHOLOGY

Histopathology is the study of tissue in disease and is central to veterinary diagnostic pathology. The aim is to generate a diagnosis and discuss the possible differential diagnoses, which typically requires analyzing both gross and histopathological findings. This text provides an introduction to veterinary histopathology and covers a few main topics of interest in veterinary medicine [7].

Histopathology begins with handling the tissue: it should be promptly and thoroughly fixed in formalin for at least 24 hours, followed by being trimmed to procedural blocks for accurate tissue processing, where capillaries, surface areas of mucosa, and the center of thick tissues are removed and replaced with tightly packed formalin-fixed tissue. Trimming columella requires rendering the tissue into several thinner sections. Specific anatomic landmarks and any gross lesions can then be chiseled into the block and must be amongst the paraffin for accurate cutting. Routinely processing a tissue can produce a thin paraffin-embedded and sectionable tissue block. These thin sections are the basis for all veterinary histopathology. Once generated, they undergo a process called staining, which involves putting increasing numbers of dyes on the section and then removing them. The stains provide necessary contrast to examine cellular detail and give unique colors for specific structures or pathological alterations. The most widely used stains in veterinary histopathology are the hematoxylin and eosin stain, which stains nuclei blue-black and cytoplasm pink-red, and the periodic acid-Schiff stain, which stains glycogen-rich cytoplasm bright magenta. Tissues can also be special stained to demonstrate specific structures or abnormalities, using various stains [8].

Sectioning the histological block requires glass knives and a microtome or a freezing microtomy system, and mounted sections can be viewed using a light microscope. An oil immersion lens can be used, if required, to visualize the finest of cell details. Histopathology evaluation takes time and patience; however, it is a technique that is only mastered after meticulous and repetitive training. The interpretation rests on familiarity with normal structure and function, which allows the pathologist to have an eye for abnormalities. Indeed, as with all sciences, the use of histopathology for diagnostic work and research in veterinary medicine requires sound technical expertise to produce good scientific results [9].

2.1. Tissue Fixation and Processing

2.1. Types of tissues

The process can use different types of biopsy tissues, such as muscles, glands, bone marrow, etc. These can be processed using the following steps to benefit the clinical diagnosis [10].

2.1.2. Fixation

To obtain optimal results during histopathological tissue evaluation, strict adherence to standard protocols in the pre-analytic phase is mandatory. Most specifically, the process of tissue fixation is absolutely essential in order to limit ex vivo post-collection changes as much as possible and to preserve cellular and subcellular components capable of providing clear diagnostic support. The main purpose of tissue fixation is to coagulate and denature proteins, particularly enzymatic proteins, which can cause autolysis to proceed and also prevent the evolution of fixation to coagulation and further to complete necrosis. The process of fixation must occur as quickly as possible in order to prevent any or all of these processes from proceeding while the tissue is still being perfused with blood, ideally at the time of collection [10].

2.1.3. Dehydration of tissues

Once tissue has been effectively fixed, the next important step is its dehydration to extract water from fixed cells. Water-filled cells can cause evaporation at a later date, and this accelerated dehydration using absolute alcohol can minimize further changes in tissue architecture.

2.1.4. Paraffin embedding

Following dehydration, the tissue must then be either infiltrated as well as cleared in a medium of low boiling point and low surface tension that fills the interstices between the tissue and the embedding medium subsequently used. The final process required for successful embedding is spinning the tissues to expel clearing media and replace it with molten paraffin wax in a mold to form the tissue block. These initial procedures are not representative of 'visible' microscopic disease processes and instead are very basic and detailed technical steps. However, performing these steps in the correct way is essential for the diagnostic success of any histopathology investigation, as suboptimal handling of the tissue may result in unsatisfactory application for microscopic evaluation or incorrect results from subsequent fixation and processing artifacts [11].

2.2. Microscopic Examination Techniques

Reviewing tissue histopathology is an essential diagnostic step and an important method used to correlate clinical findings with outcomes. Tissues have been examined under the microscope since the origins of medicine. Today, technological advancements have changed the microscopes we use from basic bright field microscopes to confocal, two-photon, and scanning electron microscopes, which can produce 3D representations of tissue. It is important for the histopathology practitioner to understand the basics of all these microscopes and what their use might bring to the patient to provide longer, healthier lives. This is a rather lengthy section, and so relevant chapters from recent texts will be discussed. Fixation and processing of tissue are necessary to allow microscopy to be possible, and these techniques, along with staining methods, will be discussed in detail. Very briefly: examining current practices of fixed, processed, and stained

tissues under a microscope can allow the examiner to delineate tumor tissue from normal, dedifferentiated from well-differentiated, diagnose tedious cases, or generally assess the tissue for treatment and determine prognosis. To conduct a fair and reliable microscopic examination, the techniques one uses, time, and care in examining the tissue must be above reproach or question. Artifacts can easily be produced in fixed and processed tissue and high-speed rendered images. Nurses, veterinary technicians, and histotechnicians must take this into account at every step. Ultimately, the microscopic examination, while still using our long-learned techniques and light expressed by a mirror and focused optical view of the tissue under a physical lens at 100x oil immersion, shows that with improvements in photographic and computer technology, our examination of the tissues is exponentially supported and increased. More than ever, in our practice of all types of histopathology, microscope viewing can be seen on a screen by multiple people at the same time, and digitized images can be sent to pathologists and veterinary colleges for consultation [12].

3. TECHNOLOGICAL INNOVATIONS IN VETERINARY HISTOPATHOLOGY

Digital pathology (or virtual microscopy) is the practice of using whole slide imaging to convert glass microscope slides into high-resolution digital images. Whole Slide Imaging (WSI) can then be stored, viewed on the computer monitor, and shared with others remotely. The stained tissue sections can also be viewed in different image layers to view the light from individual layers of the specimen, capturing tumor margins or complicated tissue layers. WSI also replaces the need to file and retrieve glass slides and to have wet ink printouts. Studies have shown that WSI image quality and slide swapping accuracy are similar to traditional light microscopy. WSI digital pathology technology has been adopted in human oncology settings by several hospitals and facilities around the world. Veterinary and human pathology service providers are also incorporating this technology [13, 14].

Routine access to relatively high-resolution digital histopathology images is a major milestone in the digitization of veterinary pathology as a service. The resolution of modern slide scanning equipment also lends itself to a wide range of digital tools, in particular for image analysis with artificial intelligence. Whole slide images have enabled several improvements in the diagnostic workflow, particularly in terms of access to cases for review or collaborative reporting. Additionally, AI algorithms for image analysis have shown potential for pattern recognition of specific visual features, assisting in the diagnosis of disease states. It is hoped that these advances could lead to improvements in the efficiency, standardization, and objectivity of the diagnostic process, particularly when the provision of a diagnosis based exclusively on WSIs meets the clinical requirements of the case. Many steps will be required to meet these ambitions, and some significant technical and ethical considerations must be resolved before pathologists' use of AI algorithms becomes a clinical reality [15].

3.1. Digital Pathology and Whole Slide Imaging

Digital pathology is a rapidly developing area that is becoming more accessible to veterinary pathologists. The main concept related to digital pathology is whole slide imaging or scanning a histopathological slide in order to digitize it. After scanning, a digitized histopathological slide is a collection of digitized images, gathered into one image. A digitized histopathological slide can be viewed on a screen using a software viewer, and the entire slide can be reviewed, both at low powers and under high magnification. Veterinary histopathological diagnostic services across the world have adopted this technology, mainly for the review of cases and digitization of histopathological education resources. Image analysis is an expected 'game changer' in the pathology field, as it can provide harvestable data from the microscopic examination of tissue [16].

Over and above facilitation of collaboration, whole slide imaging allows new ways to visualize tissues, and with this, new ways to access, report on, and visualize tissue morbidities, and so potentially shift or improve our diagnostic abilities and outcomes. At the same time, scanning takes a pathologist off the microscopy for around 20 minutes and is not instant. The pathologist who will find these methods most relevant to their clinical practice is the one who interacts with clients directly when making a diagnosis and assists with case monitoring with regular consults [17].

3.2. Artificial Intelligence Applications

Leveraging capabilities in computer imaging, the field of AI is emerging as a vertical that can potentially disrupt veterinary histopathology as it continues to intersect with medical imaging, similar to digital pathology. The rapidly changing and complex nature of histological data makes pathology a unique specialty within the medical profession. However, pathologists are currently using AI algorithms that leverage computer capabilities to assess patterns in the histological findings, although the technology is still not an autonomous diagnostician. The theory upon which machine learning is built is an algorithm that learns from data [3].

Principles of machine learning can be used to scrutinize a pattern, or in pathology's case a digitized histological image, to discern subsets of the data that might not be readily visible. It is particularly good at identifying patterns. Currently, AI in the veterinary academic literature is in the developing stages when applied to real cases. A pilot study on its use in oncology concluded that AI improved diagnostic turnaround time, disease detection, and resident confidence. Coronary artery disease has also been determined in cattle, with AI showing near-perfect concordance to the gold standard. Such accuracies, particularly in the under-reported diseases for which there are no antibodies, show promise for AI in veterinary pathology in the future. Furthermore, the commercial and medical applications of AI in combination diagnostics for histopathology are increasing. Despite the application of AI in animal models being limited by the lack of access to large libraries of digitized histological images required to effect machine learning, AI offers a glimpse into the future of how technology might be used to assist veterinarians in this field. It is likely that the future of histopathology will involve a hybrid of the traditional and more recent technology. To a certain extent, AI is already changing the workload of veterinary pathologists by standardization of diagnoses and reduction of elements of a subjective assessment, such as interobserver agreement and institutional variability. However, the practical application of AI is challenging in terms of algorithm currency, transparency when algorithms are proprietary, repetition of learning, verification, and data privacy [18,19,20].

4. INTEGRATION OF HISTOPATHOLOGICAL FINDINGS WITH CLINICAL DATA

Veterinary practice has increasingly looked into integrating histopathological findings with animal clinical data such as clinical signs, laboratory results, and imaging findings to make a more accurate diagnosis or help consider a better, tailored treatment for each individual case. Nevertheless, there is still a large pool of cases in which histopathological findings are regarded as purely descriptive entities, and their communicability and reproducibility are often challenged. Each histopathological case can be regarded as unique in itself, with very little advantage of direct comparability among them. By providing clinical examples of individual animals, the advantages of directly correlating histopathological findings with original clinical data may be better elucidated [7].

Case studies and clinical examples are very useful in elucidating the integration of tissue pathology with clinical signs. There exist various anecdotal publications in the veterinary literature, which are able to improve our work in pathological diagnosis by complementing the pathologist's perspective with a clinician's insight of the clinical picture and physical findings as well as suggesting further disease diagnostics and animal management to establish an evidence-based

diagnosis. The information gathered from the successful examples suggests the need for a more collaborative collaboration between clinicians and pathologists, whose interactions are, however, currently jeopardized by the diminished availability of shared working spaces and increasing working time constraints. The latest information technology advances have allowed us to overcome the above obstacles by sharing vital patient data and exchanging views. Misinterpretations of histopathological findings sometimes stem from isolated observations and do not always coincide with the development of animal patient management. Critically addressing those misleading comments might be the first mandatory step towards the progress of our field. Data-sharing environments, having different collaborative approaches at the basis of producing clinical reports from a single case, are likely to promote a multidisciplinary approach. Data from well-characterized and clinically validated cases might ascertain the need for pathological and clinical integration and serve as a tool for the extensive propagation of this concept among colleagues. Integrating pathology advances protocols and improvements in common practice disagreements could, thus, be a trigger to promoting increased diagnostic accuracy in veterinary practice. Integrating the information provided by pathology would not only benefit patient management but might also directly result in advantages for the veterinary pathologist's work [17,21].

4.1. Case Studies and Examples

The case submissions were selected for their diversity in species, disease, and patient outcome, and the represented species include marine mammals, model research species, and treatment-refractory patients who ultimately succumbed to disease [17]. Practically integrating histopathological findings with an understanding of the patients' medical history and clinical course can be extremely challenging in a delayed communication setting. Multiple independent data sources may be inaccurate or incomplete, and human error in both directions may exaggerate apparent inconsistencies. Timely arrival at a verifiable and diagnostically helpful interpretation may be thwarted by barriers between professional communities, including confidentiality of sensitive data [22]. These submissions are selected to help document the array of cases served by veterinary histopathologists, and in doing so describe for the reader some as-yet unnoticed reasons in support of that important role [23].

5. QUALITY ASSURANCE AND STANDARDIZATION IN VETERINARY HISTOPATHOLOGY

The frequent artifacts that occur in the veterinary histopathology are some alterations in the main features of collected tissues and happen during different steps of tissue processing, such as swelling, shrinkage, and autolysis, etc.

The primary aim of the veterinary pathologist is to perform a histopathological tissue evaluation and transmit the information obtained into a diagnosis and a research conclusion. Numerous studies have shown that histopathological examination is one of the main methods to achieve the accuracy and reliability of pathology diagnostics in veterinary medicine. Because of the wealth of diagnostic information that can be gained from evaluating tissues pathologically, the use of biopsy material in veterinary clinical settings has increased dramatically over the last several decades. Indeed, this shift has led to a growing demand for the integration of histological results with clinical parameters and patient outcomes. Moreover, veterinarians and researchers alike have turned their interest to standardizing the pathological description and context for even further diagnostic accuracy, progress in medical practice, comparative medicine, and research [11, 24].

To address these concerns, a series of global initiatives have been undertaken. The goals of these initiatives are to promote good practice in histology across laboratories and to improve the provision and quality of histopathology data to researchers and regulatory authorities. The quality of testing presented on a laboratory report from the histopathologists depends on a multistep process: the appropriate collection and preparation of the tissue by the submitter; the correct process of tissue processing and slide preparation by the laboratory technician; the accurate generation of the histology

slides by the laboratory pathologist; the accurate evaluation of the slide by the pathologist; and the preparation of the report. Substantial variability exists due to the variations in techniques, technology, tissue handling, and interpretations used to generate and test data among independent laboratories in veterinary pathology. Variations in diagnosis correlate with standardized procedures. The adoption of the best possible practices at each of these levels of testing by the laboratory and pathologist, from more detailed grossing and trimming to the management of the parallel analysis, has a positive effect on overall outcomes. It can impact how well the veterinary clinical pathologist is able to use the results in the diagnostic work-up [25, 26]

Accreditation programs are available for laboratories involved in veterinary diagnostics and toxicologic pathology. These programs require submission of appropriate documentation as well as an onsite inspection followed by a report. Subsequent surveillance audits and participation in certified external quality assessment programs are required to maintain accreditation. Participation in an accredited quality assessment program is also an important aid to assure the quality of laboratory test results and to demonstrate due diligence in the provision of test data, which is typically required for submission for regulatory and publication programs. Ongoing training and regular participation in education and external quality assurance schemes are recommended by regulatory bodies for the role of clinical pathologist in order to guarantee the quality of the histopathology report. Due to the general pathologist's low visitation level, a great program for ongoing training and education is essential to guarantee the quality of the histopathology report. It is suggested that a new pathologist should be trained for 3–5 years before he is able to be a commercial gold standard [27].

6. CHALLENGES AND FUTURE DIRECTIONS IN THE FIELD

The continuous evolution in diagnostic methodologies poses a formidable challenge. The way cases are diagnosed and annotated is highly variable, and as diagnostic approaches advance, the systems tend to fragment even further into more specialized practices. Frequently, deviations from normal tissue or cellular architecture can be attributed to alterations in one or more domains; the challenge of the future lies in integrating many levels of evidence from a variety of new techniques into a comprehensive narrative that explains tissue pathology. The need for lifelong learning cannot be overemphasized. Training in pathology is multidisciplinary, integrative, and all-encompassing, and a quest towards continuous education will be mandatory to keep abreast of the cutting-edge science and technology that can affect the field [28].

The area related to data management is the bottleneck of any scientific research, and this is accentuated as the volume and complexity of data collected increases. Additionally, there is an increased amount of practical and ethical concern, such as what is considered "normal." In the case of hyper-specialized metrics development, there is a need for ethical guidelines that permit deviation from tumor cell pathology in the name of increasing animal welfare—yet normative values for such advanced metrics currently do not exist [29]. Standardization for this type of measurement would be beneficial, and there is potential for significant veterinary science and human health progress if and when it ever happens. The challenges outlined in veterinary histopathology underscore a complex area that will require ongoing method development and research, from single-cell evaluations using emerging microscopy techniques that make observations more finely actionable to the development of rapidly sequencing RNA in fixed tissue [30]. Further, interdisciplinary efforts that include functional clinical trials are probably required to link the ever-advancing molecular biology to clinical outcomes and veterinary healthcare. Trends such as AI and telepathology open up new areas of interest and research, for example, the development of algorithms capable of mapping functional relationships between tissue structure, function, and health states [31].

7. CONCLUSION

The field of veterinary histopathology has undergone significant advancements, enhancing our understanding of tissue pathology and its direct correlation to clinical outcomes in various animal species. These advancements have been primarily driven by the integration of advanced imaging techniques, molecular diagnostics, and a deeper understanding of disease mechanisms. As a result, veterinarians are now better equipped to diagnose and manage various conditions, ultimately improving patient care. This advancement has not only enhanced diagnostic accuracy but also facilitated the development of targeted therapies, thereby fostering a more personalized approach to veterinary medicine. Moreover, these advancements have enabled veterinarians to better correlate histopathological findings with clinical signs, leading to improved prognostic assessments and treatment strategies. This integration of histopathology with clinical outcomes not only enhances the accuracy of diagnoses but also fosters a more personalized approach to veterinary medicine.

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