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REVIEW ARTICLE

The future of Veterinary Pathologists in current scenario of artificial intelligence

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We are in an era of rapid change and Veterinary Pathology can and will remain one of the leading biomedical discipline, provided recognize forces around and maintain our currency[1].

Veterinary Pathology is the branch of pathology concerned with the investigation of disease and disease processes in non-human species. Veterinary pathology is a multifaceted field within veterinary medicine that focuses on diagnosing diseases in animals, a foundational discipline for maintaining animal health and, by extension, human health. Veterinary pathology encompasses diagnostic, research, and preclinical facets, each serving a critical role in understanding and managing animal diseases. Diagnostic veterinary pathology is concerned with the examination of tissues, body fluids, and organs to diagnose diseases in animals. Veterinary pathologists play a crucial role in identifying and characterizing diseases, aiding in treatment decisions and disease management. Research veterinary pathology relates to investigating the mechanisms, causes, and treatments of diseases in animals contributing to advancing veterinary medicine, developing new therapies, and understanding disease processes. Preclinical veterinary pathology plays crucial role on assessing the safety and efficacy of pharmaceuticals, chemicals, and other interventions in animals before they are applied clinically minimizing risks to animal and human health. It underpins all aspects of clinical disease management in veterinary medicine and is essential to biomedical research, human and animal drug development and animal health surveillance, which protects human food supplies.

Veterinary Pathologists are veterinary surgeons who usually have post-graduate training in clinical or anatomic pathology. Veterinary anatomic pathology is concerned with the investigation of pathological changes in tissues, and veterinary clinical pathology is concerned with the investigation of changes in body fluid or cellular samples. Veterinary pathologists tend to specialize in particular species groups, including laboratory animals, small domestic animals, large domestic animals, fish, exotic species and birds, also veterolegal cases. We are a group with tremendously diverse interests and expertise that includes basic and clinically applied research, drug discovery and preclinical toxicologic testing, diagnostic testing and quality assurance, public health and governmental policy, public outreach, and education.

Our diversity of interests is both a strength and a challenge[2]. The veterinary pathology discipline is experiencing dramatic changes. The diagnostic techniques used by veterinary pathologists are incorporating new methodologies with a focus on molecular detection, digitalisation and the incorporation of digital analysis and artificial intelligence. New technologies are also being used in research pathology, to understand the pathogenesis of disease and to be in line with the 3Rs[3].

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As per FAO, animal diseases can have a devastating impact on animal production, livestock and its products trade, food security, livelihoods and consequently, on the overall process of economic and social development, emphasizing veterinary pathologists' role in disease diagnosis and control.

Diagnosis

Future research in veterinary pathology should focus on the development of low-cost, high-accuracy diagnostic tools and the integration of AI to aid in rapid and precise diagnoses[4].

The bases for diagnosis of diseases are history, clinical signs, physical examination and using appropriate laboratory tests. Role of Veterinary Pathologists changing rapidly, and they play vital role in finding anatomical changes *viz.* i. necropsy (Gk: *Nekros*-Dead; *opsis*-Sight) diagnosis and ii. microscopical findings in spontaneous diseases and reading experimentally induced lesions in laboratory animals. Pathologists should develop the art of differential diagnosis and help clinicians for prognosis. There is a call for

international guidelines for veterinary tumour pathology. The authors felt that this initiative (a continuation of efforts published in veterinary pathology journal in 2011) will facilitate collaboration and reproducibility between pathologists and institutions, increase case numbers, and strengthen clinical research findings, thus ensuring continued progress in veterinary oncologic pathology and improving patient care. Synoptic reporting as opposed to narrative reporting will facilitate reporting specific pieces of prognostically relevant data in a discrete[5].

Third dimension being impact of molecular biology, veterinary pathologist felt it e.g. neoplasms, altered genes, rearrangements of genes, surface receptors like identifying lymphoid tumours, T or B cell lineages, intracellular markers, neoplasms producing polypeptide hormones or histologically relevant receptors cannot be identified morphologically. Further developments in future will reflect on advances in tissue handling, technology, application of molecular biology in pathology and greater use of telepathology in teaching, quality assurance and continuing veterinary education for professional development. Hence, veterinary pathology is increasingly integrated and interdependent. Making final diagnosis by veterinary pathologists involve microbiologists, parasitologists, toxicologists, radiologists, immunologists and clinicians. We should be aware of local settings like small holdings or farms-Dairy, sheep/goat or large highly commercialized poultry/dairy farms. Hence, diagnosis starts from local investigation to the involvement of District/State/University laboratories, national referral laboratories. Identification of markers and developing relevant diagnostics, treatment protocols and vaccines are important in veterinary medicine. Poultry disease diagnosis is complicated since most diseases are multifactorial. In poultry, the market adopts various strategies for preventing the diseases rather than treatment.

Electron microscopy

Ultrastructural studies also provide information on presence of viruses and their structure which will help in identifying the virus. Morphology of other organisms are discernible in detail[6].

Histochemistry

Special stains can bring about the features of causative organisms such as Gram's staining for bacteria acid fast-tubercle bacilli, silver stain for leptospires, GG and PAS for fungi[7] and so on.

Immunohistochemistry (IHC)

Under the action of invisible ultra violet light (350-400 nm wavelength) acting as an exciting light, the different cellular components re-emit variable lengths of visible light waves according to their molecular density. Primary Ab to specific cell components (Ag) are exposed to secondary Ab directed against the primary Ab. The secondary Ab is linked to peroxidase or avidin biotin peroxidase complexes. The peroxidase catalyzes a reaction in presence of dye which precipitates on the complex. Ag-Ab binding is demonstrated with a coloured histochemical reaction visible by light microscopy or flurochromes with UV light. The use of immunoperoxidase staining is rapidly expanding with the increasing number of new commercially and non-commercially available specific Ab. The choice of Ab depends on the nature and amount of the available specimen and the intended differential diagnosis. Molecular markers, antibody against organism can identify the pathogens *in situ* and location can also be identified-intra- and/or extracellular[8].

IHC: Molecular tumour markers

Markers against intermediate filaments identify type of cells (Cytokeratin-Epithelium; Vimentin-Fibroblasts; Desmin-Muscle; Glial fibrillary acidic protein-GFAP-Glial cells; Neurofilaments-Neural origin-These just identify the cells not the tumour), malignancy (Caveolin-Adenocarcinoma of mammary gland[9], Ki67, hormone receptor markers ER, PR, recurrence/metastasis-CSC[10], PCNA[11], Caspase 3-Apoptotic marker, TWIST 1-Metastatic marker (Upregulates N-cadherin and downregulates E-cadherin expression Induce metastasis, angiogenesis, EMT and chromosomal instability, Negatively associated with p53 protein); Cathepsin D-Metastatic marker-E-cadherin, proteolytic action by degrading the cysteine cathepsin inhibitor cystatin C-Attacks basement membrane, digest extracellular matrix, liberates growth factors, increase angiogenesis and results in metastasis, proapoptotic-Bax, antiapoptotic-BCL2, arginase-1-Hepatic tumour etc.[12,13].

IHC markers in different types of the tumours: Mammary tumor: Ki-67, CK 7, 18, 5, 8, ER, HER 2, Bcl-2, p63; Pancreatic cancer: CK19, Colo-rectal cancer: CK 7, 20; Stomach cancer: SMA, CD 34; Oral cancer: EGP-40; Bladder cancer: CK 7, 20, 14, GATA-3; Liver tumor: GP-73, TAG-72; Kidney tumor: PAX-2, 8, CD-10.

Epithelial-mesenchymal transition is the loss of epithelial characteristics and the acquisition of a mesenchymal phenotype in epithelial cells. Apart from reversible change during embryogenesis and wound healing, it also occurs in malignancy for the acquisition of invasive properties. Cells lose expression of epithelial markers like cytokeratins and E-cadherin and acquire a mesenchymal phenotype, expressing vimentin, N-cadherin[14].

Cancer stem cells

Cancer stem cell (CSC) theory: In this model, cancers can be considered an abnormal organ in which the bulk of tumour growth is provided by a small population of

cells, CSCs, that divide asymmetrically to produce more CSCs and a non-tumorigenic population of cancer cells. This asymmetric division is thought to contribute to the heterogeneity of solid tumours. CSCs can be considered to be cells that have the ability to self-renew and are capable of asymmetric cell division. Osteosarcoma: Embryonic stem cells (ESCs)-Oct4, Nanog and STAT3 and the mesenchymal stem cell (MSC) marker Stro-1[11,15].

Solid cancer consists of heterogeneous cells that contain a subpopulation of tumour cells with stem cell properties, including self-renewal capacity, differentiation potential, tumorigenicity in immunodeficient mice, and resistance to chemotherapy and radiation. Such tumour cells, termed CSCs or tumour-initiating cells (TICs), are generated either from mutational events in normal tissue stem cells or from the acquisition of stem cell properties by differentiated cells; these tumour cells exist at the apex of a hierarchy of cancer tissues. In various cancers such as skin, liver, and glioblastoma, CSCs are organized as tree-like hierarchies. CSCs have been shown to drive tumour initiation, recurrence, and metastasis. Morphologically, canine mammary mixed tumours comprise epithelial components, including luminal and/or myoepithelial cells and mesenchymal cells such as osteoblasts and chondrocytes. However, the cellular origin of mammary mixed tumours remains unclear. Based on comparisons of DNA alterations, the cellular components in most mixed tumours are thought to share a common origin, such that the various cell types observed in mixed tumours may originate from common CSCs[16]. A triple immunohistochemistry was done using $CD44^+$ $CD24^-$ and ESA in canine mammary tumour to identify CSC[10]. Use of sphere-forming assay, surface markers, CD24, 44 expression and Aldeflour assay (ALDHA) in CSC in canine mammary tumour was reported[17]. Each molecular mammary tumor subtype corresponds with a different histological type, grade, tumor aggressiveness, and prognosis. This helps to standardize treatment options and to estimate prognosis, but it is still not very precise and further studies are needed[18].

Experimental pathology/Toxicological pathology/Biomedical research

Animal models have been fundamental in preclinical and biomedical research for revealing key biochemical and physiologic processes, clarifying disease mechanisms, and translating biomedical discoveries into effective clinical treatments for human disease. Animal models have been critical in the development of history's most seminal breakthroughs in medicine. Although the use of animals in research is widespread and has advanced the understanding of human disease, concerns about the limitations of preclinical animal research and its ability (or often inability) to reliably predict clinical trial success have received considerable attention. Too frequently, historical precedent, availability and funding drive selection of animal models, rather than critical analysis of the model system, and how it can answer the research question[19].

Gene editing technology

Gene editing process involves the deletion, insertion, or modification of specific DNA sequences in the genome, allowing the host machinery to repair or modify this defect. Knockouts, knockins, and other manipulations can be generated with this technology. Base editing introduces precise, single nucleotide substitutions in a DNA or RNA strand. Prime editing, a recent technique, unlike base editing, not limited to transition substitutions and allows a broader range of mutations, including insertions and deletions. Prime editors utilize an engineered reverse transcriptase fused to a Cas9 nickase (nicks the DNA strand at precise locations) and a prime editing guide RNA (pegRNA) to introduce edits.

CRISPR-based diagnostics (nucleic acid detection)

A common gene editing tool called clustered regularly interspaced short palindromic repeat (CRISPR) is based on the adaptive immune system of bacteria. CRISPR and CRISPR-associated (CRISPR-Cas) adaptive immune systems contain programmable endonucleases that can be leveraged for CRISPR-based diagnostics (CRISPRDx). CRISPR-cas (Caspase) is a low cost, less complex and easy for multiple edits system. It comprises two components i. a guide RNA (gRNA) and ii. Cas9 nuclease, which together form a ribonucleoprotein (RNP) complex. The presence of a specific protospacer adjacent motif (PAM) in the genomic DNA is required for the gRNA to bind to the target sequence. The Cas9 nuclease then makes a double strand break in the DNA (denoted by the scissors). Endogenous repair mechanisms triggered by the double strand break may result in gene knockout via a frameshift mutation or knock-in of a desired sequence if a DNA template is present. The Cas protein is a pair of molecular scissors and gRNA is the GPS that guides it to the appropriate site. In prokaryotes, the gRNA guides the nuclease to viral DNA, but as a biotechnological tool, the design specifications of the gRNA can be altered to target any organism's genome at virtually any location.

Currently, CRISPR-Cas systems can be divided, according to evolutionary relationships, into two classes, six types and several subtypes. The classes of CRISPR-Cas system are defined by the nature of the ribonucleoprotein effector complex: class 1 systems are characterized by a complex of multiple effector proteins, and class 2 systems encompass a single crRNA-binding protein. The design of crRNAs for the different effector proteins used in CRISPR diagnostics follow the same principles as those of other CRISPR applications. Among the diverse CRISPR systems, class 2 systems have primarily been applied for diagnostics, as these systems are simpler to reconstitute.

They include enzymes with collateral activity, which serves as the backbone of many CRISPR-based diagnostic assays. Class 1 systems (such as the type III effector nuclease Csm6 or Cas10) have also been engineered for diagnostics, either in combination with components of the class 2 system or with the native type III complex[20,21,22].

While some Cas enzymes target DNA, single effector RNA-guided RNases, such as Cas13a, can be reprogrammed with CRISPR RNAs (crRNAs) to provide a platform for specific RNA sensing. Upon recognizing its RNA target, activated Cas13a engages in "collateral" cleavage of nearby non-targeted RNAs. This crRNA-programmed collateral cleavage activity allows Cas13a to detect the presence of a specific RNA *in vitro* by nonspecific degradation of labeled RNA.

Uses: CRISPR and similar gene editing tools have been suggested as potential treatments and cures for a variety of conditions including monogenic diseases (cystic fibrosis), metabolic disease (type 2 diabetes), cancer (melanoma), infectious diseases, septic shock, and neurodegenerative diseases (Alzheimer's disease). Challenges: unexpected challenges that sometimes arise that require new approaches and technologies in animal model development and utilization, as illustrated by COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). Institutional research enterprises were largely postponed, supply chains were disrupted, and personal protective equipment was scarce. Opportunities: New types of animal models were developed and new research techniques (single cell transcript analysis) were applied to advance studies on SARS-CoV2 cellular predilection, pathogenesis and therapies[23]. Neurodegeneration Initiative (iNDI) for Alzheimer's Disease and related dementia; potential cell therapies - triple-knockout, hypoimmunogenic 'off-the-shelf' T cells-immunotherapy in multiple disorders while evading any immune response; Development of precise transgenic mouse models for disease progression and therapeutic research in a relatively short span of time[22].

CRISPR gene editing is useful in humanization of mouse models (by replacing specific mouse genome sequences with the human equivalent) which are more physiologically relevant systems than their conventional transgenic counterparts, and key to understanding and treating human diseases and can solve the most difficult of life sciences problems and enable explore dimensions of the genome that have not been studied hitherto. Thus, CRISPR technology is promising in human therapeutics, agricultural biology, biofuels, and basic scientific research.

Modified version of CRISPR is now used for exploring epigenomics as CRISPR complex that is capable of acetylating histone proteins at precise locations dictated by the complex's gRNA has been developed and can correlate relationship between epigenetic markers and gene expression. CRISPR-edited cells are effective tools to generate disease models. CRISPR-based editing of induced pluripotent stem cells (iPSCs) provides a versatile bandwidth to generate isogenic disease models with genetically matched controls allowing high throughput modeling of complex diseases involving multiple genes and mutations[22]. Experimental models on veterinary diseases using cell cultures or molecular technologies e.g. organoid technology, infancy stage. The progress from 2D to 3D culture systems and the incorporation of stem cells into organoids. Insights on experimental and diagnostic pathology given[3].

Advantages: The CRISPR-based diagnostic (CRISPR-Dx), providing rapid DNA or RNA detection with attomolar sensitivity and single-base mismatch specificity. Cas13a-based molecular detection platform has been used to detect specific strains of viruses, distinguish pathogenic bacteria, and genotype human DNA. The reaction reagents can be lyophilized for cold-chain independence and long-term storage, and readily reconstituted on paper for field applications. CRISPR-Dx open new avenues for rapid, robust and sensitive detection of biological molecules. Disadvantages: The sample preparation requires a separate step, and incubation temperatures higher than room temperature necessitate heating devices.

Zoonotic disease: Rudolf Virchow coined term zoonosis who is father of cellular pathology

A zoonotic disease is a disease or infection that can be transmitted naturally from vertebrate animals to humans or from humans to vertebrate animals. More than 60% of human pathogens are zoonotic in origin. This includes a wide variety of bacteria, viruses, fungi, protozoa, parasites, and other pathogens. Factors such as climate change, urbanization, animal migration and trade, travel and tourism, vector biology, anthropogenic factors, and natural factors have greatly influenced the emergence, re-emergence, distribution, and patterns of zoonoses. Most humans are in contact with animals in a way or another. As time goes on, there are more emerging and re-emerging zoonotic diseases.

One health/medicine/pathology-Public health-Comparative pathology

One Health concerns with human, animal, plant and environment. The American Medical Association and the American Veterinary Medical Association have recently approved resolutions supporting 'One Medicine' or 'One Health' that bridge the two professions. The concept is far from novel. Rudolf Virchow, the Father of Modern Pathology, and Sir William Osler, the Father of Modern Medicine, were outspoken advocates of the concept. The concept in its modern iteration was re-articulated

in the 1984 edition of Calvin Schwabe's Veterinary Medicine and Human Health. The veterinary and medical pathology professions are steeped in a rich history of 'One Medicine,' but they have paradoxically parted ways. The time has come for not only scientists but also all pathologists to recognize the value in comparative pathology, the consequences of ignoring the opportunity and, most importantly, the necessity of preparing future generations to meet the challenge inherent in the renewed momentum for 'One Medicine.' The impending glut of new genetically engineered mice creates an urgent need for prepared investigators and pathologists[24-26].

Epigenetics and genetics

The genomes of several animal species have been sequenced and annotated, including dogs, cats and horses. These resources have allowed us to employ genome-wide association mapping to identify genetic abnormalities in inbred animals with simple disease traits that have helped inform similar diseases and normal development in humans. These breakthroughs have in turn resulted in the development of diagnostic tests for the genetic traits, which has potential for reducing disease prevalence. Genetic screening has also revealed associations with specific loci for complex diseases. Further investigation of these loci may lead us to discover causative genes and develop relevant diagnostic tests. Despite the rapid advances in genomic mapping, many challenges remain. For example, we are just beginning to explore epigenetic patterns in animals but lack important tools, such as promoter arrays. We also need more information on cancer genomes in animals.

Nano-engineering/Nanotechnology

Nanoparticles defined as particles which measure 1-100 nm, allow unique interaction with biological systems at the molecular level. The burgeoning field of biomedical engineering has rapidly morphed from basic research into translational medicine, with the application of engineering principles to cancer diagnostics, drug delivery, imaging, and infectious disease detection, to name a few application. This field, together with genomic analysis, has ushered in personalized medicine in humans. Application of nanoengineering tools has just begun in veterinary medicine, but the goal of personalized medicine for pet animals is likely to come sooner rather than later. As pathologists, we hold a unique position where we can be both leaders and an integral member of multidisciplinary teams in the charge toward personalized medicine.

There is a critical need for novel immunopotentiators and delivery vehicles capable of eliciting humoral, cellular and mucosal immunity. Various vaccine adjuvants and delivery vehicles are being developed that are approximately nanoscale in size. PLGA and CaP coupled NDV inactivated vaccines elicited stronger and prolonged immune responses in comparison to commercial live vaccine. Nanotechnology and anticancer effects: Effect of aqueous nano-neem leaf extract against mammary tumour was impressive[27] and that of solid nano-lipid curcumin was encouraging against hepatocarcinogenesis[28] in rats.

Nanotechnology-based strategies for rapid detection

The advancements in nanotechnology offer innovative solutions to improve current diagnostic strategies for managing infectious diseases. The nanomaterials (1 and 100 nm) have tunable optical, magnetic, electrical, thermal, and biological properties and can be engineered with different shapes, sizes, chemical compositions and surface functionalities. These properties enable them to be exploited for improving the detection of biological molecules or whole pathogens. Nanomaterials, namely Quantum dots (QDs), Gold nanoparticles (GNPs), and Magnetic nanoparticles (MNPs), have been extensively used in developing various *in vitro* diagnostics due to their unique optical, magnetic, electrical and thermal properties. Nanodiagnostics have various detection modalities like fluorescence, surface-enhanced Raman, magnetic, electrochemical, colorimetric, and thermal. Advantages: The nanodiagnostics is rapid, precise, and economical. Disadvantages: Limited use for the clinical samples.

Regenerative medicine

Regenerative medicine is the replacement or regeneration of human/animal cells, tissue or organs to restore or establish normal function which is an emerging multidisciplinary field that aims to restore, maintain or enhance tissues and hence organ functions. Regenerative medicine is considered to have the potential for developing new treatments for previously untreatable, or difficult to treat diseases. Regeneration of tissues can be achieved by the combination of living cells, which will provide biological functionality and materials, which act as scaffolds to support cell proliferation. Nanotechnology is not only an excellent tool to produce material structures that mimic the biological ones but also holds the promise of providing efficient delivery systems.

Clinical application of regenerative medicine e.g. replacement of skin for burns patients, wounds, pressure sores or diabetic foot ulcers. Also bone and cartilage regeneration, bladder repair, vascular tissue engineering, the use of stem cells in tissue regeneration, repair of damaged heart muscle following heart attack, restoration of peripheral nerve or spinal cord following injury, regeneration of pancreatic tissue to produce insulin for people with diabetes, to replace lost organ function the stabilization and maintenance of the viability of tissue prior to regeneration, control of environmental contaminants, interfaces between tissues and devices, such as the artificial retina the fate of biomaterials and

implant materials within the environment of the body. Check for biocompatibility and stability.

Diagnostic Imaging

The introduction of powerful advanced techniques, such as imaging flow cytometry and histology-directed imaging mass spectrophotometry, is yielding new insights into disease pathogenesis. As dedicated instruments become more commonplace and cost-effective, it will only be a matter of time before these technologies enter the diagnostic realm, the opportunity that these modalities represent. Pinpoint accuracy for infectious disease diagnosis, novel insights into biochemical pathways that lead to pathology - the possibilities are endless.

Human-wildlife boundaries/conflict

Human activity continues to encroach upon uninhabited areas perturbing ecosystems and wildlife populations. A consequence of bringing human populations into close contact with previously isolated wild species is the interspecies transmission of infectious disease. Recently seen outbreaks of Ebola and Zika viruses in humans, viruses that have previously been largely restricted to wildlife. Conversely, viruses that are typically considered pathogens of domestic animals, such as canine distemper, have spread into native wildlife, with devastating effects. Technologic advances, such as hydrofracking, have allowed for the increased utilization of previously inaccessible natural resources. Yet, these come at a health cost to animals and humans. Wildlife species are sentinels of environmental changes, the proverbial "canary in the coal mine." The emergence and spread of fungal diseases, such as *Pseudogymnoascus destructans* and chytridiomycosis *(Batrachochytrium dendrobatidis)*, emphasizes our need to be continually and actively vigilant at screening wildlife for environmental changes that may affect human and animal health. Veterinary pathologists will continue to play a vital and prominent role in identification and investigation of these diseases.

Diagnostics

Diagnostics can be used in various contexts - testing of symptomatic individuals, at-risk presymptomatic individuals, confirmatory testing, differential diagnosis, testing of patients with previous exposure, surveillance at sites of outbreaks and environmental monitoring (Foundation for Innovative New Diagnostics (FIND). The use case determines the way in which diagnostic tests are used optimally. Different Platforms for Veterinary Diagnostic Kits: Serological diagnostic assays, Nucleic acid-based diagnostic assays a. Hybridization methods, b. Amplification methods, Novel and high-throughput assays, a. Microarray, b. Peptide nucleic acid and aptamers, c. Biosensors, d. Next-generation sequencing-based methods, e. POC diagnostics[29-31] and f. Patented diagnostic technologies.

Lateral Flow Assays

Lateral flow assays (LFAs) are point-of-care (POC) devices currently used for qualitative and semi-quantitative diagnosis in non-laboratory settings. The parts of a LFA include sample application pad, conjugate pad, nitrocellulose membrane and adsorption pad. Nitrocellulose membrane is imprinted with test and control lines[32]. Pre-immobilized reagents on the LFA become active upon flow of liquid sample and buffer inducing immune complex formation. Since one of the reagents is coupled with a reporter dye such as colored latex or colloidal gold, concentration of this tagged reagent in a narrow zone exhibits itself as a colored line. Advantages: Single step assay, rapid, user friendly, no instrumentation required and long term stability. Disadvantages: Low sensitivity, qualitative and difficult to optimize.

Enzyme Linked Immunosorbent Assay (ELISA)

ELISA is one of the most commonly used protein-based assays to detect the presence of an antibody or an antigen in a sample. Different ELISA platforms are available for detection of antigen or antibody. The immune capture or sandwich ELISA uses capture and detecting antibodies (either specific MAbs or polyclonal antibodies) and is used for antigen detection. The Competitive ELISA (cELISA) is used to detect or quantify antibody/antigen using a competitive method[33]. The cELISA for detection of specific antibodies has largely replaced the Indirect (iELISA) for large-scale screening and sero-surveillance. Advantages include that it is a rapid, scalable and specific assay, very useful for mass screening, qualitative or quantitative means and objective result interpretation. Limitations are that these, highly variable, needs skilled personnel, stability of reagents and needs specific equipment such as ELISA reader.

Polymerase chain reaction (PCR)

PCR is an enzymatic amplification method that permits amplification of an exact DNA fragment from a complex pool of DNA[29,34]. PCR can be performed using DNA from various sources including tissues, microbes, fluid samples, swabs, semen etc. Only trace amounts of DNA is required for PCR. Advantages: Quick, reliable, sensitive, relatively easy and specific. Limitations: Need for equipment, aerosol contamination leading to false positive results, possibility of cross reactivity and non-specific amplification.

Quantitative real time PCR

Real time PCR (qRT-PCR) has become a handy tool for disease diagnosis, identification of species, quantifying gene expression and monitoring viral loads during therapy. A DNA binding dye for real-time detection allows PCR amplification to be monitored. However, the DNA binding dyes does not distinguish between signals generated by either specific or non-

specific products. Thus, any mispriming events that lead to spurious bands observed on electrophoretic gels will generate false positive signal when a generic DNA binding dye is used for real-time detection[35,36].

Real-time systems for PCR were improved by probe-based, rather than intercalator-based, PCR product detection. The fluorogenic probe is an oligonucleotide with both a reporter fluorescent dye and a quencher dye attached. While the probe is intact, the proximity of the quencher greatly reduces the fluorescence emitted by the reporter dye. If the target sequence is present in the template, the probe anneals downstream from one of the primer sites and is cleaved by the 5' nuclease activity of *Taq* DNA polymerase as this primer is extended. This cleavage of the probe separates the reporter dye from quencher dye, increasing the reporter dye signal. Cleavage removes the probe from the target strand, allowing primer extension to continue to the end of the template strand. Thus, inclusion of the probe does not inhibit the overall PCR process. Additional reporter dye molecules are cleaved from their respective probes with each cycle, affecting an increase in fluorescence intensity proportional to the amount of amplicon produced. The advantage of fluorogenic probes over DNA binding dyes is that specific hybridization between probe and target is required to generate fluorescent signal. Thus, with fluorogenic probes, non-specific amplification due to mis-priming or primer-dimer artifact does not generate signal. Another advantage of fluorogenic probes is that they can be labeled with different, distinguishable reporter dyes. By using probes labeled with different reporters, amplification of two distinct sequences can be detected in a single PCR reaction. The disadvantage of fluorogenic probes is that different probes must be synthesized to detect different sequences. Advantages: Quantitative, no post PCR processing, TaqMan probe eliminated nonspecific amplification, UDG eliminates carry over contamination, real time, large range of quantitation and more sensitive than PCR[37,38]. Limitations: Costly equipment needed, and presence of PCR inhibitors. Use of qRT-PCR methods for diagnosis of some of the animal and poultry viruses. In situ PCR was also used in rabies diagnosis[39].

Isothermal Amplification assays

Isothermal amplification-based tests are generally being used in the detection of pathogen genomes from clinical samples. DNA polymerases with strand displacement activity or DNA polymerases combined with strand displacement enzymes and proteins are being used in the isothermal amplification of DNA/RNA. Isothermal amplification assays with higher temperature requires thermostable reverse transcriptase[40]. There are seven types of isothermal assays for the detection of animal and poultry viruses, of which LAMP is most used.

Loop Mediated Isothermal Amplification (LAMP)

It is a widely applied modification of PCR which avoids the need for the expensive thermal cycler and allows the technique to be carried at field level. This methodology uses an auto cycling strand displacement DNA synthesis approach that is done in isothermal condition within a short period of time. In addition, the results of the LAMP can be detected visually and does not require post PCR methodologies. Advantages: Does not require a thermal cycler and can be carried out in a water bath, does not require post amplification procedures for result visualization, results are available in an hour, sensitivity is higher than the regular PCR and tolerance to inhibitory substances. Limitations: Size of the target sequence should not be more than 300 bp, more chances of carry over contamination and need for high strand displacement enzyme[41].

Imaging flow cytometry (IFC)

This platform combines the workflow of flow cytometry and fluorescent microscopy. IFC can be used for analyzing the morphology and fluorescence information of not only a single cell but also a population of cells. Morphological and spatial information at single-cell resolution can be obtained using IFC[42]. IFC has been used to study apoptosis in relation to alterations of nuclear morphology and structure, cell cycle progression based on chromatin condensation, protein and molecule translocation and/or colocalization in different cellular compartments, and cytoskeleton structures. Thus, IFC can be a better tool for oncology. Due to the high throughput nature of IFA, analysis of rare cell types like leukemia circulating in blood can be identified. Advantages: Thousands of morphological and spatial properties can be measured from each individual cell. IFC has the capacity to image non-adherent or dissociated cells, hence, bodily fluids like blood, whose structures can be distorted by placement onto a slide. Limitations: Currently the technology is in preliminary stages and requires costly equipment; User-friendly, robust, and standardized workflows that can facilitate machine learning is essential.

Microarray

Microarray technology is a general laboratory approach that involves binding an array of thousands to millions of known nucleic acid fragments to a solid surface referred to as a "chip". The chip is then bated with DNA or RNA isolated from a study sample (cells or tissue, organisms). The steps involve isolation/ preparation, hybridization, washing and image analysis. Main types are spotted arrays, in-situ arrays and self-assembled arrays. It may be gene expression microarray and tissue microarray (TMA) and also cDNA based microarray and oligonucleotide based microarray[35,43,44]. Microarray is useful in infectious organisms/toxin and

also lymphoma cell identification and in ALL. Normal or apparently healthy samples are used along with affected tissue to compare the results.

Next generation sequencing (NGS)

NGS also referred to High-thorough put sequencing encompasses all modern sequencing technologies that can sequence large number of genes or whole genome in short time, simultaneously at an affordable cost. The generations of sequencing include: First Generation - Sanger sequencing; Second generation - Pyrosequencing; Third generation - Single molecule fluorescent sequencing, Nanopore; Fourth generation - genomic analysis directly in the cell. While whole genome sequencing of individual microorganisms/isolates was once the primary target for microbial NGS technologies, the substantial increase in throughput has led to the adoption of metagenomic sequencing approaches for disease diagnosis. This possibility to rapidly identify the entire microbial content of a target sample provides a unique and novel strategy for pathogen detection and identification. Compared to the targeted approaches such as PCR or qPCR, metagenomic approaches are less biased and require no prior knowledge of the pathogen involved. In addition, metagenomic data also allows for the detection and identification of antibiotic resistance genes and virulence factors that can be used to guide treatment options and improve antibiotic stewardship. As NGS technology matures, it has the potential to be used in routine diagnosis clinically[45].

Proteomics

Protein expression represents the accumulation or end product of genetic information. DNA is transcribed into RNA which is then translated into proteins often phosphorylated the global understanding can be both qualitative and quantitative and can western blotting, immunohistochemistry and various novel non-candidate proteomic approaches. Proteomics, the main tool for proteome research, is a relatively new and extremely dynamically evolving branch of science, focused on the evaluation of gene expression at proteome level. Proteome is a set of proteins in a given time and space, as its composition may vary from tissue to tissue or even from cell to cell. A protein, the basic unit of a proteome, is a molecule composed of single amino acids, further forming secondary, tertiary, and quaternary three-dimensional structures. The rapid development of proteomics was made possible by progress in analytical instrumentation, especially in mass spectrometry (MS) with the introduction of new, cutting-edge types of mass spectrometers and improvements of soft ionization techniques. Although the amino acid sequence is defined by the appropriate gene, the genetic information itself cannot provide the complete information about a protein. In contrast to the stable, rigid, single-dimensional genomic information based on a combination of four nucleotides, the information encoded in proteins is not exclusively limited to the amino acid sequence. Thus, protein expression in a two-dimensional polyacrylamide gel electrophoresis (2D) is a technique used to separate proteins by both their isoelectric point (pl; in the first dimension) and by their mass (second dimension)[46].

Biomarker

A biomarker is a characteristic one that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes or pharmacological responses to a therapeutic intervention. It may be also defined as an *in vivo* derived molecule present at levels deviating significantly from the average in association with specific conditions of health. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes or pharmacological responses to a therapeutic intervention (FDA). It may be also defined as an *in vivo* derived molecule present at levels deviating significantly from the average in association with specific conditions of health[47].

Biosensor

Biosensor recognizes a target biomarker, characteristic for particular pathogen, via an immobilized sensing element called bioreceptor (monoclonal antibody, RNA, DNA, glycan, lectin, enzyme, tissue, and whole cell). The bioreceptor is a crucial component as its biochemical properties assure high sensitivity and selectivity of the biomarker detection and permit to avoid interferences from other microorganisms or molecules present in the tested sample. The specific biochemical interaction between the biomarker and the bioreceptor is converted into a measurable signal by the transductor signal recording and display should, then, allow qualitative and quantitative pathogen identification. There are two principal challenges to develop a biosensor for pathogen detection: (i) elaboration of a bioassay for biomarker detection, and (ii) improving the robustness of the bioassay to adapt it for applications in field and/or on complex biological samples. Indeed, many bioassays that work well on the bench with purified biomarker molecules fail to detect them in complex media like blood or serum. Different sensing strategies based on DNA receptors, glycan, aptamers and antibodies are presented. Besides devices still at development level some are validated according to standards of the World Organization for Animal Health and are commercially available. Especially, paper-based platforms proposed as an affordable, rapid and easy to perform sensing systems for implementation in field condition are included. In addition, diagnostics of infection disease require high sensitivity since pathogens might spread rapidly before that any clinical sign appears in animals. e.g. *E. coli*, avian

influenza, mycoplasma and other pathogen mastitis, foot and mouth disease, blue tongue, *Clostridium perfringens* toxins[48].

Emerging diseases

An emerging disease is a new infection or infestation resulting from the evolution or change of an existing pathogenic agent, a known infection or infestation spreading to a new geographic area or population, or a previously unrecognized pathogenic agent or disease diagnosed for the first time and which has a significant impact on animal or public health (OIE, WOAH). A known or endemic disease is considered to be re-emerging, if it shifts its geographical setting, expands its host range, or significantly increases its prevalence. Otherwise, emerging diseases are diseases that appear in a population for the first time e.g. Avian influenza, african swine fever, lumpy skin disease in cattle or that may have existed previously but are rapidly increasing in incidence or geographic range and re-emergence is the reappearance of a known disease after a significant decline in incidence e.g. glanders.

Most (75%) emerging infectious diseases (EID) are caused by zoonotic pathogens. Factors contributing to EID include population growth, spread in health care facilities, aging population, global travel, and changing vector habitats related to climate change. Environmental changes, human and animal demography, pathogen changes and changes in farming practice are among the factors that lead to emerging diseases. Social and cultural factors such as food habits and religious beliefs play a role too. Re-emergence may happen because of a breakdown in public health measures for diseases that were once under control and can also happen when new strains of known disease-causing organisms appear. Emerging diseases have economic repercussions well beyond their immediate health costs. They may impede trade and travel or cause disproportionate alarm. The answer to the international threat from these diseases is through well-coordinated global surveillance and response.

Digital pathology - Rudolf Virchow - Father of modern cellular pathology (1858)

Digital pathology is a transformative approach to pathology that involves the digitization of pathology information, including slides and data. It encompasses the acquisition, management, sharing, and interpretation of pathology data in a digital environment. Slide scanners consist of 4 components i. light source, ii. robotics to move the slide holder, iii. 1 or several objectives, and iv. an associated camera to capture the images (Digitized by high resolution charge-coupled device-CCD camera). Magnifications (Objective lens 2x to 100x). 40x for haematopoietic tumours and subtle cellular changes and 100x for cytological diagnosis. Use of 20x to 1000x objectives and file size doubling mag. 400 MB to 1.6 GB. Scanning time: 5 to 20 minutes. One of the important advantages of digital pathology is that pathological AI-based models can be easily used during the diagnostic process and collection and management of pathologic big data. Pathological big data can be used for learning various AI diagnostic models needed for pathological diagnosis and can be used as various educational materials. Histopathological processing, paraffin embedding, sectioning and staining need to be standardized[49].

Digital pathology helps in quantitative analysis of the WSI, can identify and quantify specific cell types quickly and accurately and can quantitatively evaluate histological features, morphological patterns, and biologically relevant regions of interest (e.g. tumoral or peritumoral areas, relationships between different immune cell populations, areas of expression, presence of metastasis). The challenges of digital pathology include expensive equipment, data security, etc., Real-time reporting demands a fast transfer of data. Strong connections between the internet, laboratory information systems and electronic medical records are necessary for clinical implementation and require new systems of storage due to large file sizes.

Benefits of digital pathology include improved quality, productivity, and innovation. Pathologists are faced with a workforce shortage, and digital technology adoption offers solutions to enhance analysis and collaboration[49-51]. Digital microscopy (DM) can be further separated into robotic microscopy, region of interest (ROI) digital microscopy, or whole-slide imaging (WSI). Visualization of the sample on a computer is the common thread that links these modalities, but they differ in how much of the slide is available for viewing (individual fields, ROIs, or the entire slide) and whether the image is stored (static telepathology, WSI, or ROI scans) or viewed in "real-time" (robotic microscopy)[52].

Digital pathology benefits patient by rapid reference for expert advice on diagnoses, improves laboratory workflow and connectivity and increases flexibility and efficiency of the workforce, helping creation of digital training resources for specialists, improving pathology profession by slide sharing and can combine AI for advancement of pathology services[53].

Telepathology

Telepathology, since 2015, as a subset of teleconsulting, is pathology interpretation performed at a distance. Teleconsulting gained prominence during the COVID-19 pandemic as veterinary clinics looked for alternatives to in-person consultation. Telepathology following significant advances in information technology and telecommunications coupled with the pandemic led to unprecedented sophistication, accessibility, and use

of telepathology in human and veterinary medicine. Furthermore, telepathology can connect veterinary practices to distant laboratories and provide support for underserved animals and communities. Despite the widespread use of digital microscopy in large veterinary diagnostic laboratories. But, there is a significant gap in validation of WSI for primary diagnosis and underutilization of telepathology to support postmortem examinations conducted in the field indicating a potential area for service development. Telepathology involves the acquisition of cytologic, hematologic, histologic, or macroscopic images for transmission along telecommunication pathways for diagnosis, consultation, education, and research. This process can include *static* telepathology, also known as offline or store and-forward, and *dynamic* or "real-time" pathology[49].

Bioinformatics

Bioinformatics is application of techniques from computer science to problems from biology. Bioinformatics is a key discipline that combines computer science, mathematics, statistics, engineering, and biology to help answer biological issues. To designate the study of informatics processes in biotic systems, Hogeweg and Hesper introduced the word "bioinformatics" in 1970. A field of science that uses computers, databases, math, and statistics to collect, store, organize, and analyze large amounts of biological, medical, and health information. Information may come from many sources, including genetic and molecular research studies, patient statistics, tissue specimens, clinical trials, and scientific journals. It is also called as computational biology. Bioinformatics is conceptualizing biology in terms of macromolecules (in the sense of physical-chemistry) and then applying "informatics" techniques (derived from disciplines such as applied maths, computer science, and statistics) to understand and organize the information associated with these molecules, on a large-scale. Since the publication of the *Haemophilus influenzae* genome, complete sequences for nearly 300 organisms have been released, ranging from 450 genes to over 100,000. As a result of this surge in data, computers have become indispensable to biological research. Such an approach is ideal because of the ease with which computers can handle large quantities of data and probe the complex dynamics observed in nature. Bioinformatics, the subject of the current review, is often defined as the application of computational techniques to understand and organise the information associated with biological macromolecules[54]. Bioinformatics involves structural bioinformatics, drug designing, phylogenetics, computational biology, and gene prediction[55].

Aims of bioinformatics

1. Organize biological data in an easy-to-use format that allows biologists and researchers to save and access current data.
2. Create software tools to aid in data analysis and management.
3. To analyze and interpret the results in a biologically meaningful manner using these biological data.
4. To aid pharmaceutical industry researchers in better understanding protein structure those contribute to the development of medicines.
5. In order to enable and assist physicians in understanding the gene architecture that will aid in the recognition and diagnosis of diseases like cancer. The execution of BLAST is fast and reliable, whose search from the query sequence (Query) is compared to the database to be used[56].

***In silico* analysis**

This is also known as computational therapeutics, computational pharmacology. Of scientific experiments or research, conducted or produced by means of computer modelling or computer simulation "in silico analysis of the human genome".

Drug discovery: Pharmacology over the past 100 years has had a rich tradition of scientists with the ability to form qualitative or semiquantitative relations between molecular structure and activity *in cerebro*. To test these hypotheses they have consistently used traditional pharmacology tools such as *in vivo* and *in vitro* models. Computational (in silico) methods have been developed and applied to pharmacology hypothesis development and testing. These in silico methods include databases, quantitative structure-activity relationships, pharmacophores, homology models and other molecular modeling approaches, machine learning, data mining, network analysis tools and data analysis tools that use a computer. In silico methods are primarily used alongside the generation of *in vitro* data both to create the model and to test it. Such models have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization[57].

Docking

Docking is a molecular modeling technique designed to find the proper fit between a ligand and its binding site (receptor). Dock pose: A ligand molecule can bind with a receptor in a multiple positions, conformations, and orientations.

Artificial intelligence

Artificial intelligence (AI) is intelligence manifested by machines, as opposed to the natural intelligence demonstrated by humans and animals. Machines mimic cognitive function associated with the human mind *viz*., learning and problem assessment. AI was invented as an academic discipline in 1959. AI is intelligence manifested by machines and has developed into subfields five; i.

Machine learning (ML): ML is the process of utilizing mathematical models of data to make computer learn without direct instruction given. ii. DL (Deep learning) is a promising subfield of machine learning, composed of multiple layers, uses raw data as input, and improves the representations of data. Deep learning algorithms are classified into categories; Convolutional neural network (CNN), Restricted Boltzmann Machines, Auto encoder and Sparse Codingta. iii. Natural language processing (NLP): A subfield of AI concerned with enabling computers to process, understand, and generate human language, iv. Natural language generation (NLG): A subfield of NLP that involves generating humanlike text from structured data. NLG is commonly used for tasks such as chatbots, automated writing, and content generation. v. Computer Vision: The field of AI that deals with enabling computers to interpret and understand visual information from the world, such as images and videos[58-62].

Bioinspired ML in bioinformatics and applications

The exponential growth in the size and rate of capture of biomedical data during the "big data" age is posing a challenge to traditional analysis methods. DL, a subset of machine learning methods with biological origins, promises to use massive data sets to find hidden patterns and make precise predictions. ML has a lot of potential for the analysis of biological data sets, as is known. Building complex models that reveal their underlying structure theoretically enabling greater exploitation of the accessibility of increasingly large and high-dimensional data sets. The learned models include advanced properties, enhanced interpretability, and a better knowledge of the structure of biological data[63].

Livestock farming is utilizing AI-based technology to improve cattle welfare and productivity where sensors, cameras and data analytics, are being used by farmers to monitor their livestock's feeding habits, behavior, fertility and the spread of illnesses. Disease diagnosis, predictive analytics, therapy optimization, remote monitoring, behavior analysis and data-driven research are just a few examples of how AI is being employed in animal healthcare[64].

The incorporation of AI-supported surgical robots in veterinary diagnostics has shown promising results in enhancing precision and reducing human error. Exploring the use of machine learning algorithms to predict disease outbreaks and developing standardized protocols for diagnostic procedures are also crucial areas for future study[4]. Integration of AI and ML in veterinary pathology holds great promise. AI algorithms can assist in analyzing necropsy data, identifying patterns, and detecting anomalies that may be overlooked by human observers. This can lead to more accurate diagnoses and a more efficient necropsy process[65].

AI accelerates the pace of veterinary research and drug development. ML algorithms can analyze complex veterinary data and identify potential drug candidates. This expedites the discovery process and contributes to developing novel therapies for various veterinary conditions. The prospects of AI in veterinary science are undeniably transformative, redefining animal healthcare. AI offers a spectrum of benefits from rapid and precise diagnostics to personalized treatment plans and proactive disease management. However, embracing this future requires careful navigation of ethical considerations and a collaborative synergy between human expertise and AI capabilities.

We can tap the transformative potential of AI and ensure a future of improved animal health and well-being. As we unleash AI in veterinary science, a new era of compassionate, data-driven and efficient animal care emerges, promising a healthier future for our animal companions[66].

In recent years, the emergence of AI has led to a new direction in biomedical research, especially in translational research with great potential, promising to revolutionize science. AI is applicable in antimicrobial resistance (AMR) research, cancer research, drug design, vaccine development, epidemiology, disease surveillance, and genomics. The potential impact of various aspects of AI in veterinary clinical practice and biomedical research, as a key tool for addressing pressing global health challenges across various domains were discussed[67].

AI applied on veterinary education in two different cases showed need for further study, improvement and caution. One on suturing skills of veterinary students[68] and another on examination on evaluation of the knowledge level and consistency[69]. In a swine production and machine learning study, a balanced accuracy of 85.3% on any disease in the first system and balanced accuracies (average prediction accuracy on positive and negative samples) of 58.5%, 58.7%, 72.8% and 74.8% on porcine reproductive and respiratory syndrome, porcine epidemic diarrhea virus, influenza A virus, and *Mycoplasma hyopneumoniae* in the second system, respectively, using the six most important predictors in all cases. These models provide daily infection probabilities that can be used by veterinarians and other stakeholders to more timely support preventive and control strategies on farms[70].

Implementation framework that can support veterinary practices in adopting AI[71]

1. Establish an AI implementation team consisting of stakeholders across the organization.
2. Ensure appropriate training of team members to understand AI basics.

3. Define the use case or purpose of the AI system.
4. Determine data needs, availability, and quality.
5. Develop or procure the appropriate model.
6. Consider ethical and legal obligations.
7. Create an implementation plan (a) Training and engagement; (b) Ongoing monitoring.
8. Manage change.
9. Stay relevant.

AI applications in veterinary medicine

Epidemiology and population health: To collect and analyze massive amounts of data, perform disease surveillance tasks, and predict disease outbreaks. Livestock production: Disease detection, behavior recognition, environmental management, and growth evaluation, to score teat ends and lameness in dairy cows, predict calving based on behavior and assess meat quality in production animals. Image analysis in tissue and cytological samples-Storage and archiving. Clinically to predict the need for surgery and survivability in colicking horses by using history and clinical information. NN: Predict the onset of chronic kidney disease within 12 months in cats with 88% accuracy. NNs: Classification of the severity of canine ulcerative keratitis based on corneal photographs. To perform the time-intensive task of converting written medical records to digital documents or searching digital documents for keywords or phrases. AI and ML are also used in biomedical and translational research settings and 3D printing of biological materials[72].

AI applications in veterinary pathology

Image analysis: AI analyzes images, aiding pathologists in identifying abnormalities and diagnosing diseases, including tumor detection, cell classification, and quantification of features. Identify subtle patterns and abnormalities that may not be apparent to the human eye; reduce interpretation variability by standardizing analysis methods and criteria; reduce workload and improve turnaround times; able to reduce fatigue by reducing the need for laborious or repetitive tasking e.g. mitotic figures or cell counts, measuring nuclear or cell diameters), or searching for fine details like binucleated or multinucleated cells amongst neoplastic mononuclear cell populations. Pattern recognition: AI recognizes patterns, aiding pathologists in identifying disease trends, correlations, and biomarkers, particularly useful for research uncovering disease mechanisms. Diagnostic assistance: AI systems can serve as diagnostic aids, providing pathologists with second opinions and assisting in decision-making. Predictive modeling: AI can help predict disease using patient history, lab results, and imaging, helping pathologists anticipate progression and tailor treatments for better outcomes. AI-based telepathology systems can overcome geographical barriers by enabling remote consultation and diagnosis[58].

AI offers five crucial benefits to veterinary pathologists

1. *Enhanced accuracy:* AI algorithms can analyze large volumes of data with high precision, leading to more accurate diagnoses and prognoses.
2. *Time savings:* AI automates repetitive tasks, allowing pathologists to process samples and interpret results more quickly.
3. *Improved productivity:* With AI handling routine tasks, veterinary pathologists can devote more time to complex cases, research endeavors, and professional development.
4. *Consistency:* AI ensures consistency in diagnostic interpretation by standardizing analysis methods and criteria. This consistency is particularly valuable in multicenter studies and longitudinal research projects, where uniformity is essential for accurate comparisons and conclusions.
5. *Expanded knowledge base:* AI facilitates the aggregation and analysis of vast amounts of data from diverse sources, leading to the discovery of new disease markers, therapeutic targets, and treatment strategies[58].

Ethics in AI

Ethical issues for veterinary AI are accuracy and reliability, over diagnosis, transparency, data security, trust and distrust, autonomy of clients, information overload and skill erosion, responsibility of AI-influenced outcomes, environmental effects. Ethical guidance is in the interest of ethicists, veterinarians, clinic owners, veterinary bodies and regulators, clients, technology developers and AI researchers[73].

Future prospects, perspectives, contributions of Veterinary Pathology/gists

Work as a veterinary pathologist in interdisciplinary biomedical research teams offers exciting opportunities to impact human and animal health. Projects and discoveries may be directly translatable to the clinic in terms of improved diagnostic strategies or novel therapeutics, truly making a difference in many lives. Future comparative veterinary pathologists will continue to play important roles in identifying and researching emerging diseases and pandemics, critically assessing and developing models to meet these and other challenges. The recent SARS-CoV2 pandemic illustrates the need for new models and approaches for emerging diseases, as well as roles for comparative pathologists in developing and interpreting the models to develop and qualify vaccines and other interventions with rigor and efficiency. When global events impact society, as in 2020, pathology provides mission critical insights into feasible solutions. With these challenges come significant opportunities for pathologists to advance our roles and opportunities in biomedical research and pharmaceutical drug development, and beyond[23].

Virtual microscopy is increasingly applied in all fields of pathology and poses a unique chance to improve the performance of pathologists as diagnosticians, researchers, and teachers, despite all current minor weaknesses. Major advantages of DP and WSI include remote and off-site access to digitalized slides, easy handling, improved ergonomics, and quantitative measurements. Although digital veterinary pathology is in its fledgling stage, automated image analysis will improve in the next decade and will certainly facilitate and broaden the pathologist's work by providing higher reproducibility and reliability of qualitative and quantitative diagnoses. These innovations will inevitably influence the pathologist's routine workflow regardless of whether we are skeptics or promotors of digital pathology. Current and future pathologists should therefore learn and be taught information technologies and image editing to keep up with the inevitable digitization of their profession[49].

In the field of veterinary and toxicologic pathology (few publications, acceptance of regulatory authorities, GLP), evidence for acceptable diagnostic concordance of DM is largely lacking and further validation study publications are needed, especially for specific applications in our fields[50]. There are gaps in the literature on the use and validation of telepathology in veterinary medicine despite the widespread use of DM by private veterinary diagnostic laboratories[52].

Consensus statements and standardization of testing (One Pathology)

New guidelines for our vast array of assays, such as cytologic/histopathologic tumor grading, clonality assays and reporting, and flow cytometric methods. These guidelines should be grounded in evidence-based medicine and have a built-in plan for re-evaluation. American Society of Veterinary Clinical Pathology has given guidelines and consensus statements for several diagnostic activities, including reference interval establishment, prognostic markers in cancer, flow cytometric reporting in canine hematopoietic neoplasia, immunocytochemical staining, and viscoelastic-based hemostasis testing etc. Similarly, cytologic and histologic grading schemes have been proposed for various tumors. Importantly, these guidelines are the culmination of efforts of multiple investigators and truly represent a global approach ("One Pathology"). Their frequency of citation and routine application during diagnostic testing attests to the usefulness of these guidelines.

Recommendations to Veterinary Pathologists

1. Training on statistical analysis and experimental design that helps in data analysis.
2. Developing skill in the use of micro-computational resources designed to allow them to collect and interpret large bodies of data.
3. Develop and standardize a) a discipline-wise nomenclature for all lesions, and b) terms for semiquantitative expression of lesion severity.
4. Using quantitative methods in morphology to provide as secure a quantitative base as possible for lesion occurrence, size, degree of organ involvement, severity of lesion etc[1].

To meet increased pressures councils like ICVP, ACVP, ECVP, JCVP, RCVP etc. to update knowledge by

1. Conducting annual education programme-Continuing education on one topic.
2. Training programmes for speciality groups.
3. Short courses including laboratory techniques and methods to complement diagnostic process.
4. To share spontaneously occurring lesions including experimentally induced.

Scope of employment for veterinary pathologists is wide, Dept Animal Husbandry - Veterinary Assistant Surgeons (Specialist post - IAVP propose to Governments), Teaching profession Universities - Assistant Professor - Professor), Lab animal facilities: Institutes (Central), CROs, pharmaceuticals - Veterinary Officers/Researchers/ preclinical toxicology/drug discovery National-ICAR/ ICMR - Scientists, Fisheries, Self-employment, private organizations - Lab and abroad etc.

Considering various opportunities available as discussed veterinary pathologists need to develop day one skills, necropsy, gross pathology, histopathology, clinical pathology, veterolegal cases, wild animal pathology, one health, zoonotic diseases, aquatic animal pathology, molecular methods to imbibe methods in currency like digital pathology, telepathology, AI, drug discovery and in future. These skills will immensely help veterinary pathologists in getting employed.

Machine Learning and Veterinary Pathology: Be Not Afraid! [58].

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