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CURRENT PROBLEMS CAUSED BY THE AVIAN INFLUENZA VIRUS AND LABORATORY DIAGNOSTICS.

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Abstract. The scientific study of diseases in birds, known as avian pathology, focuses on the structural, functional, and molecular alterations in tissues and organs brought on by infections, poisons, malnutrition, or other factors. It is essential for preserving the health of chickens, guaranteeing food security, and fostering economic expansion. With a focus on developments in diagnostic techniques that improve avian illness identification and management, this review aims to highlight current trends, problems, and future directions in avian pathology. However, there is still little real-world use of cutting-edge technologies in avian pathology, especially in Ethiopia. Immunohistochemistry, molecular methods, and digital pathology are examples of recent diagnostic developments that have enhanced the identification, characterization, and treatment of poultry diseases. Future initiatives will focus on using machine learning and artificial intelligence (AI) for precise diagnosis, real-time illness monitoring, and outbreak prediction. Ethiopia has made great strides in the field of avian pathology, especially in the areas of histology and polymerase chain reaction. The poultry business still faces obstacles despite continuous progress, such as zoonotic risks, antibiotic resistance, emerging and reemerging infections, and restricted access to diagnostic infrastructure. Therefore, improving biosecurity procedures, encouraging the appropriate use of antibiotics, and increasing the use of molecular, digital pathology, and AI-supported diagnostic tools continue to be crucial tactics for safeguarding the public's health as well as the poultry population. Ethiopia should improve its diagnostic capabilities and professional training in avian pathology in order to further improve disease detection and control.
Keywords. digital pathology, avian pathology, diagnostics, artificial intelligence, poultry, diagnostic capabilities.

Introduction. Because it provides a plentiful supply of reasonably priced, high-quality protein in the form of meat and eggs, the poultry industry plays a crucial role in the world's food systems. Therefore, maintaining a steady supply of safe food, promoting economic growth, and improving societal wellness all depend on ensuring the health and welfare of chickens. Accordingly, by researching illnesses that impact both domestic poultry and wild bird populations, avian pathology—a specialized area of veterinary science—plays a critical role in preserving poultry health. Numerous factors, including geoclimatic conditions, population density, management practices, and immunization status, influence the occurrence of poultry diseases, which have significant economic and social ramifications. These factors include high morbidity and mortality rates in chickens, higher medication and veterinary costs, and significant losses in both production and market value. Furthermore, infectious agents, poisonous compounds, and nutritional imbalances further impair the health and production of chickens, with certain avian diseases, such avian influenza, having particularly severe consequences [1-6]. An influenza A virus (IAV) with a distinct environment, avian influenza virus (AIV) belongs to the Orthomyxoviridae family. Hemagglutinin (HA) and neuraminidase (NA) antigenicity has led to the identification of 16 H subtypes (H1–H16) and 9 N subtypes (N1–N9). Based on the amino acid sequence of the HA protein cleavage site, AIVs are further divided into two categories: high pathogenicity (HP) and low pathogenicity (LP). LPAIVs have a monobasic cleavage site that restricts replication to particular tissues, whereas HPAIVs, usually of the H5 or H7 subtypes, have a multi-basic cleavage site that permits global infection. Although AIV usually transcends species boundaries and infects a variety of mammals, including humans, waterfowl are the natural hosts. The identification of highly virulent strains frequently necessitates regulatory actions, such as trade restrictions on poultry, which may have a negative effect on the profitability of the sector. Diseases can enter poultry operations more easily when people and animals move about. In avian pathology, the diagnostic process has progressively shifted from the traditional veterinary approach, which focused on individual birds, to a comprehensive assessment of the health of entire flocks. On-farm, diagnostic activities comprise routine sampling and investigations in line with health control programmes [7-12]. In the field, diagnostic procedures are initiated as soon as flock health is compromised, with morbidity and/or mortality serving as primary indicators. Investigations begin with the compilation of a comprehensive case history encompassing relevant flock characteristics, management practices and infection or disease factors. Immunohistochemistry (IHC), digital pathology, and molecular approaches are examples of recent technology developments that are being used more and more to improve the identification, characterization, and tracking of avian diseases. These methods make it possible to thoroughly examine host-pathogen interactions and tissue-level alterations, which helps with early and precise illness diagnosis. It is clear that machine learning (ML) and artificial intelligence (AI) will probably be crucial to the future of illness diagnostics in both field and lab settings. As a result, avian pathology is acknowledged as an essential method for identifying a variety of poultry illnesses. Despite these developments, a number of obstacles still exist. There is little use of molecular, immunohistochemical, and digital pathology diagnostic methods. There are few comparisons between traditional and contemporary diagnostic techniques, and there is still a lack of research on the incorporation of AI/ML into standard avian diagnostics. By summarizing current technical developments, assessing their practical application, and outlining potential future approaches for enhancing the detection and treatment of avian diseases, this study fills in these gaps [13-21].

The main purpose of the presented manuscript is to provide a brief analysis of the diseases and laboratory diagnostics that cause the current problems of the avian influenza virus based on the results of prestigious scientific works.

Veterinary pathology is a broad area of veterinary medicine that focuses on diagnosing diseases in animals, including birds, which serves as the basis for avian pathology. It is the branch of pathology that investigates disease and disease processes in non-human species. The scientific field of avian pathology examines illnesses in populations of domestic poultry as well as wild birds. These authors contend that understanding and managing diseases in both domestic poultry and wild bird populations requires a grasp of avian pathology. Clinical observations, gross pathology, histopathology, microbiological culture, and serological methods have historically been used to diagnose avian diseases. Although these methods have yielded useful diagnostic data, their sensitivity, specificity, and efficiency are noticeably limited. For example, overlapping histological lesions and clinical symptoms among many avian diseases may lead to delayed or incorrect diagnosis, which could jeopardize prompt intervention. Further diagnostic difficulties in avian pathology are brought about by the advent of new bacterial and viral infections as well as illnesses with unknown causes. By improving specificity, sensitivity, and diagnostic speed, recent developments in IHC, digital pathology, and molecular diagnostics have revolutionized avian pathology. Together, these developments improve the precision and effectiveness of diagnosing and monitoring avian diseases [11-19].

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Avian pathology has always depended on morphological evaluation, which includes both macroscopic characteristics that are visible to the unaided eye and microscopic details that are discovered by histological analysis. Through the methodical assessment of tissues at the macroscopic level, gross pathology continues to be a crucial component of diagnostic practice, offering crucial information for disease diagnosis, prognosis, and treatment decision-making. These authors contend that in avian illness research, gross pathology still plays a crucial role in directing precise diagnosis, prognosis, and successful therapeutic decision-making. Tissue sample examination is considered the gold standard for diagnosing diseases and is essential for evaluating tissue pathology in all animal species, including birds. Using different tissue preparations to clarify structural and pathological changes, histopathological examination enables the assessment of host organ modifications at both the organ and cellular levels. To maintain architecture and stop autolysis, tissue specimens are usually first fixed in formalin [5-14]. To give structural support for thin sectioning, the samples are then dehydrated and embedded in paraffin. After that, sections are stained with hematoxylin and eosin (H&E), which makes it easier to see cellular and tissue morphology by highlighting nuclei and cytoplasmic components. Haematoxylin selectively stains the nuclei in H&E staining, while eosin counterstains the cytoplasm and other extracellular elements. In order to guarantee uniformity, precision, and repeatability of results, standardized methods are usually followed when doing histopathological analysis of avian tissues. Histological examination has been further improved by advances in digital pathology, especially with whole-slide imaging (WSI), which allows for extensive tissue evaluation employing methods for evaluating tumor margins, quantifying cell proliferation, and analyzing tissue architecture. However, variations in tissue preparation and staining procedures, which can impact image quality and analytical results, continue to limit the potential of digital histopathology. Innovative methods like multiplex staining techniques and three-dimensional histological reconstructions are being developed to solve these issues and offer more thorough insights into tissue morphology [21-27].

IHC Detection Techniques: Direct and Indirect. The primary antibody is directly attached to a reporter molecule, such as biotin, fluorochromes, or enzymes, in the one-step direct detection approach. This method is particularly helpful for highly expressed antigens or when secondary antibodies may result in undesired background staining because of the type of tissue or the species of the original antibody. For instance, it is better to employ labeled primary mouse antibodies when staining mouse lymph nodes since secondary antibodies against mouse immunoglobulins can also bind to the natural immunoglobulins found in the lymph nodes, resulting in high nonspecific staining. In these situations, direct detection using primary antibodies coupled to an enzyme or fluorophore is preferable. Among the many benefits of direct detection is the removal of the secondary antibody incubation step, which speeds up and simplifies the process. Nevertheless, this method has drawbacks, including decreased sensitivity for several antigens in frequently processed tissues and the requirement to conjugate each primary antibody separately with fluorophores or enzymes, which greatly raises the total cost [13-21]. Two-step, or indirect, detection techniques were developed in response to the demand for more sensitive detection systems for antigens with low expression levels. This method involves applying an unlabeled primary antibody first, then raising a labelled secondary antibody against the primary antibody and conjugating it to fluorophores or enzymes. Furthermore, because the primary antibodies in indirect methods are unlabeled, they maintain their full avidity, enabling numerous labelled secondary antibodies to bind to each primary antibody. This increases the intensity of the reaction and makes it possible to use less primary antibody to detect lower levels of antigen. Because multiple primary antibodies generated in the same species can be detected using the same labeled secondary antibody, indirect methods are also more practical than direct approaches. Indirect immunostaining techniques have some drawbacks despite the benefits mentioned above, such as the need for extra controls and blocking procedures when using secondary antibodies. As a result, when the secondary antibody attaches to unwanted tissue targets, nonspecific staining may happen. In these situations, the tissue slices must be treated with blocking reagents, which can be time-consuming and raise the IHC experiment's overall cost [22-27].

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Monitoring and simplified methods for controlling AIV. In order to stop additional adaptive development, host infections, and mortality, the main goal of an AIV incursion is to contain and "stamp out" the virus. In order to achieve this goal, we suggest using a thorough monitoring strategy that integrates environmental, human, and animal health to prevent zoonotic disease outbreaks and protect public health (One Health). The detection and monitoring of AIV during outbreaks and dissemination should be carried out using comprehensive and multifarious approaches by including both virological and serological surveillance, as indicated in the flow chart, due to the significant impact of AIV across numerous domains. It is possible to evaluate the intricate human-animal-environment relationship more precisely [11-19]. In order to detect AIV early, virological surveillance involves monitoring the environment (such as lakes and aquatic systems, agricultural areas, sewage systems, animal production facilities, and indoor air), various animal populations (such as wild birds, companion animals, stray animals, poultry, and livestock), the human population (including both at-risk groups and the general public), and food products. In line with the One Health framework, such an all-encompassing monitoring strategy enables the prompt implementation of public health interventions and the imposition of control measures by enabling the early detection of emergent strains, such as the presence of adaptive mutations and/or reassortment that may have pandemic potential. In conclusion, qPCR is still the best technique for detecting nucleic acids, but its use may be limited in settings with limited resources. The goal of the test (pure detection, serotyping, or whole genome sequencing), how quickly the results are required (on-site or laboratory detection), and the application (for farmed or wild animals) should all be taken into consideration when choosing an appropriate testing method [17-27].

Discussion. The creation and use of efficient vaccinations is a crucial component of more complete AIV preventative efforts under the "One Health" framework, in addition to thorough monitoring and the use of early control measures. Numerous vaccination platforms, including inactivated virus, live-attenuated virus, subunit or recombinant viral protein, nucleic acid (DNA and mRNA), and vector-based, have been the subject of much investigation. The WHO has already advised that human vaccine development should focus on the H5, H7, and H9 subtypes since these are the most common AIV viruses that cause zoonotic infections. Notably, as numerous official contingency plans demonstrate, pre-pandemic vaccination stockpiles are an essential part of pandemic preparedness. To vaccinate up to 20 million people, for example, the US keeps a stockpile of vaccines against influenza viruses with pandemic potential, including those that target particular AIV subtypes. It is widely known in the field of veterinary medicine that vaccination is a successful strategy for controlling avian influenza epidemics in poultry as well as for reducing and stopping the spread of the virus from avian hosts to humans [3-9]. More region-specific immunization tactics for both humans and animals are required because to the current AIV pandemic. More region-specific immunization tactics for both humans and animals are required because to the current AIV pandemic. In order to ensure that vaccines are distributed fairly for areas with limited resources, international institutions like the WHO and the WOAH should encourage and support inter-country collaboration and coordination. This will strengthen efforts to control AIV worldwide. In conclusion, developments in avian pathology—including histology, clinical pathology, IHC, molecular diagnostics, digital pathology, and artificial intelligence—have greatly enhanced the identification, monitoring, and treatment of poultry diseases. In chicken farming, these technical advancements improve epidemic prediction and early disease diagnosis. Ethiopia has made significant strides in the detection of avian diseases, especially in the use of PCR and histology. However, Ethiopia's system is still in its infancy and needs more funding, infrastructure, and training to fulfill similar international diagnostic standards in comparison to Western regions with sophisticated molecular platforms, digital pathology, and sequencing technology [13-19]. Poultry health and productivity are nevertheless threatened by enduring issues such disease introduction and reemergence, inadequate diagnostic infrastructure, biosecurity flaws, and antibiotic resistance. Strategic proposals to address these problems include improving diagnostic accessibility and field-level application through the development of quick, affordable, and farm-applicable instruments to enable early epidemic detection and containment. To stop the spread of disease and resistance, biosecurity must be strengthened and responsible use of antibiotics must be enforced. Investing in cutting-edge diagnostics, such as digital pathology, molecular methods, and AI-assisted technologies, will increase surveillance, efficiency, and accuracy. For veterinary diagnosticians, avian pathologists, and laboratory staff to be proficient in developing technologies, capacity building through focused training is essential. The development of high-sensitivity field diagnostics, the epidemiology of newly developing infections, and the incorporation of AI and digital pathology into routine surveillance should be the main areas of research. Lastly, early warning systems will be strengthened and illness management will be harmonized with a coordinated national approach that includes centralized reporting, laboratory networking, and data sharing [20-27].

Conclusions. In summary, developments in avian pathology, including histopathology, clinical pathology, IHC, molecular diagnostics, digital pathology, and artificial intelligence, have greatly enhanced the identification, monitoring, and treatment of poultry diseases. In chicken production, these technical advancements assist epidemic prediction and enable early disease diagnosis. Ethiopia has made significant strides in the diagnoses of avian pathology, especially in the use of PCR and histology. However, Ethiopia's system is still in its infancy and needs more funding, infrastructure, and training to fulfill similar international diagnostic standards when compared to Western regions with sophisticated molecular platforms, digital pathology, and sequencing technology. Poultry health and productivity are nevertheless threatened by enduring issues such disease introduction and reemergence, inadequate diagnostic infrastructure, gaps in biosecurity, and antibiotic resistance.

The zoonotic and pandemic threats posed by AIVs are increased by the recent incursion of H5N1 into a new host species (bovine) with associated human illnesses. The One Health framework is becoming more and more necessary to reduce the impact of AIV transmission by fostering agency collaboration and creating cutting-edge vaccinations and diagnostics. Additionally, there are a number of unresolved problems that need to be addressed. In order for more regions to take part in the monitoring and management of AIVs, it is crucial to reduce the gap in diagnosis, surveillance, and preventative actions between resource-rich and resource-poor regions. Second, vaccines put selective pressure on viral evolution even though they are an economical way to prevent AIV.

This problem is made worse by raising animals that are not all fully vaccinated together, which creates a vulnerable population where mutant viruses can proliferate. Third, virus surveillance is necessary along these inaccessible migration pathways because migrating birds can fly great distances across isolated areas. It's interesting to note that a well-established infrastructure48 contributed to the discovery of H5N1 in Antarctica. As a result, funding is required to create a larger network for AIV surveillance. In order to use serology data for infection status, vaccine efficacy, and general prevalence—all vital information for a thorough risk assessment—it is imperative to create new or additional reagents and procedures.

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