Sokoto Journal of Veterinary Sciences, Volume 23 (Number 1). March, 2025

REVIEW ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v23i1.1

Raji *et al.*/*Sokoto Journal of Veterinary Sciences*, **23**(1): 1 - 13.

Revolutionising avian pathology: The impact of molecular diagnostics

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Publication History:
Received: 18-10-2024
Revised: 10-02-2025
Accepted: 27-02-2025

The field of avian pathology has experienced substantial advancements over the past few decades, primarily due to the integration of molecular diagnostics. This paper explores the transformative role of molecular diagnostic techniques in the detection, characterization, and management of avian diseases. The historical context of diagnostics in avian pathology was studied, tracing its evolution from conventional methods to the advent of molecular diagnostics. The paper highlights the technologies that have been central to this shift, including polymerase chain reaction (PCR), quantitative PCR (qPCR), next-generation sequencing (NGS), and CRISPR-based diagnostics. By comparing these methodologies with traditional diagnostic practices, we illuminate their advantages in terms of sensitivity, specificity, and speed. The discussion extends to the implications of molecular diagnostics on avian disease management, epidemiology, and conservation efforts, showcasing several case studies where these techniques have led to significant breakthroughs in disease control and prevention. Additionally, we address the challenges posed by rapid technological advancements, such as technical complexities and the need for standardisation. The paper concludes with a prospective view, examining emerging trends and future directions in the molecular diagnostics of avian diseases, emphasising the potential for personalised medicine and the integration of artificial intelligence. Through this comprehensive analysis, the review underscores the pivotal role of molecular diagnostics in revolutionising avian pathology, offering new horizons for research, clinical practice, and policy-making in avian health.

Keywords: Avian pathology, Disease identification, Molecular diagnostics, Next-generation sequencing, Sensitivity, Specificity

Introduction

Avian pathology studies diseases affecting avian species, including domestic poultry and wild bird populations. These diseases have significant implications for food production, wildlife conservation, and public health (Ishtiaq, 2021). Avian influenza (AI) and Newcastle disease (ND), for example, have had devastating impacts on poultry farms, resulting in economic losses and affecting the availability and affordability of poultry products. Avian malaria and avian botulism have also caused declines in wild bird populations and disrupted ecosystems (Ishtiaq, 2021). Some avian diseases, like AI, can be transmitted to humans and potentially lead to outbreaks and pandemics. By understanding and managing these diseases through avian pathology, we can mitigate their negative impacts and safeguard food security, biodiversity, and public health (Evans, 2011; Kozdruń *et al.,* 2015).

Traditional diagnostic methods used in avian pathology, such as clinical sign observation, gross and histopathology, microbiology, and serological techniques, have been valuable tools for disease diagnosis (Liebhart *et al.,* 2023). However, these methods have limitations in terms of sensitivity, specificity, and time consumption. Relying solely on clinicopathology, for instance, can result in misdiagnosis or delayed diagnosis due to similarities in signs and lesions exhibited in many avian diseases (Liebhart *et al.,* 2023; Raji & Omar, 2022). Histopathology also presents challenges, as it requires time-consuming tissue processing and may not always provide definitive diagnoses (Ahmed *et al.,* 2018). Avian pathology also faces difficulties with emerging bacterial and viral infections and diseases with unknown causes (Tyson-Pello & Olsen, 2020). Therefore, the ability to diagnose avian diseases rapidly and accurately is crucial for effective management, prevention, and control (Liebhart *et al.,* 2023).

The limitations of traditional diagnostic methods have underscored the critical need for the emergence of molecular diagnostics as a revolutionary force in avian pathology. Molecular diagnostics, encompassing techniques such as polymerase chain reaction (PCR), gene sequencing, metagenomics, and microarrays, have gained significant popularity in recent years (Liu *et al.,* 2023; Raji *et al.,* 2022b; Schädler *et al.,* 2019). This is particularly vital for identifying and differentiating between various avian diseases, as well as detecting emerging pathogens. Moreover, molecular diagnostics offer faster results compared to traditional approaches, facilitating swift disease management and control. Furthermore, these techniques are relatively cost-effective, user-friendly, and easily accessible to individuals without extensive technical expertise (Liebhart *et al.,* 2023).

The multitude of benefits linked to molecular diagnostics have driven their increasing popularity and integration into avian pathology (Liebhart *et al.,* 2023). PCR is used to amplify specific DNA sequences, facilitating the detection and identification of pathogens (Valones *et al.,* 2009). Next-generation sequencing (NGS) enables the rapid sequencing of large amounts of DNA or RNA, providing accurate identification and characterization of large-sized avian pathogens (Satam *et al.,* 2023; Vashisht *et al.,* 2023). Quantitative polymerase chain reaction (qPCR) is a technique that detects and quantifies specific nucleic acid sequences in real-time, yielding rapid and precise results (Valones *et al.,* 2009). The CRISPR-Cas system is a revolutionary gene editing tool that can selectively target and modify specific genes in avian pathogens (Ebrahimi *et al.,* 2023; Vashisht *et al*., 2023). Microarrays detect and measure gene expression levels, offering a comprehensive analysis of gene expression patterns (Zimdahl & Hübner, 2008). These techniques have revolutionised disease diagnosis in avian pathology by delivering more accurate and rapid results compared to traditional methods. They have been successfully applied to diseases such as AI, ND, and other threats to poultry and wild bird populations (Kubacki *et al.,* 2022).

Applications of molecular diagnostics in avian pathology

The incorporation of molecular diagnostics in avian pathology has resulted in the creation of convenient and user-friendly platforms for on-site testing (Liebhart *et al.,* 2023). These advancements have the potential to transform disease diagnosis and surveillance in avian populations, particularly in resource-limited settings and during disease outbreaks. Similarly, the continuing trajectory of advancements in molecular diagnostics of avian diseases has not only enhanced disease identification but has also fundamentally reshaped the landscape of epidemiological studies of avian diseases (Bustin & Jellinger, 2023). This integration of molecular diagnostics into epidemiological studies extends beyond domestic flocks to wildlife conservation (Gebreyes *et al.,* 2020; Horvat *et al.,* 2022; Hou *et al.,* 2020). The developmental outfit in avian pathology diagnosis is schematically presented in Figure 1, while, the molecular advancement is shown in Figure 2.

Loop-Mediated Isothermal Amplification (LAMP)

The loop-mediated isothermal amplification (LAMP) assay is a prime example of how molecular diagnostics have contributed to this revolution (Notomi *et al.,* 2015). This technique, which does not require specialized equipment or highly skilled personnel, has shown great promise in providing rapid and accurate detection of various avian pathogens, facilitating early intervention and control measures (Han *et al*., 2019). Its successful

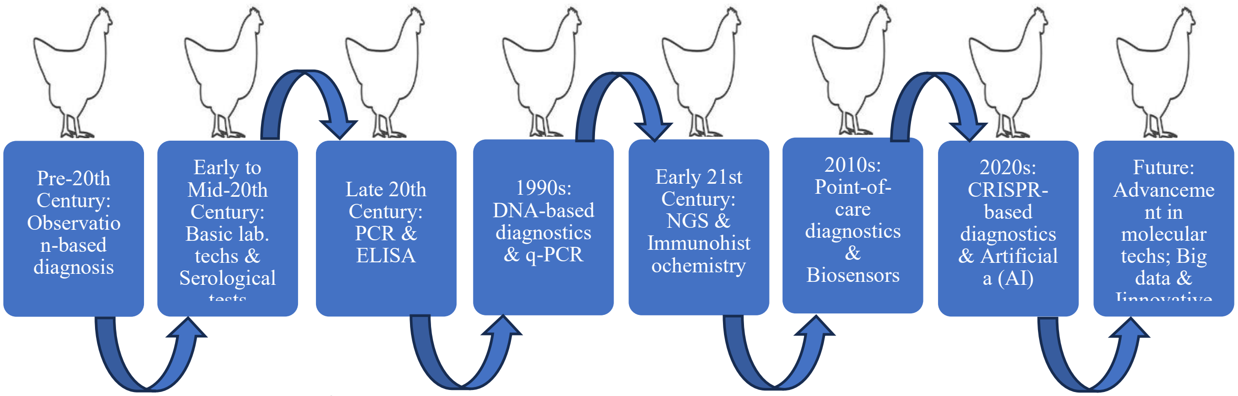
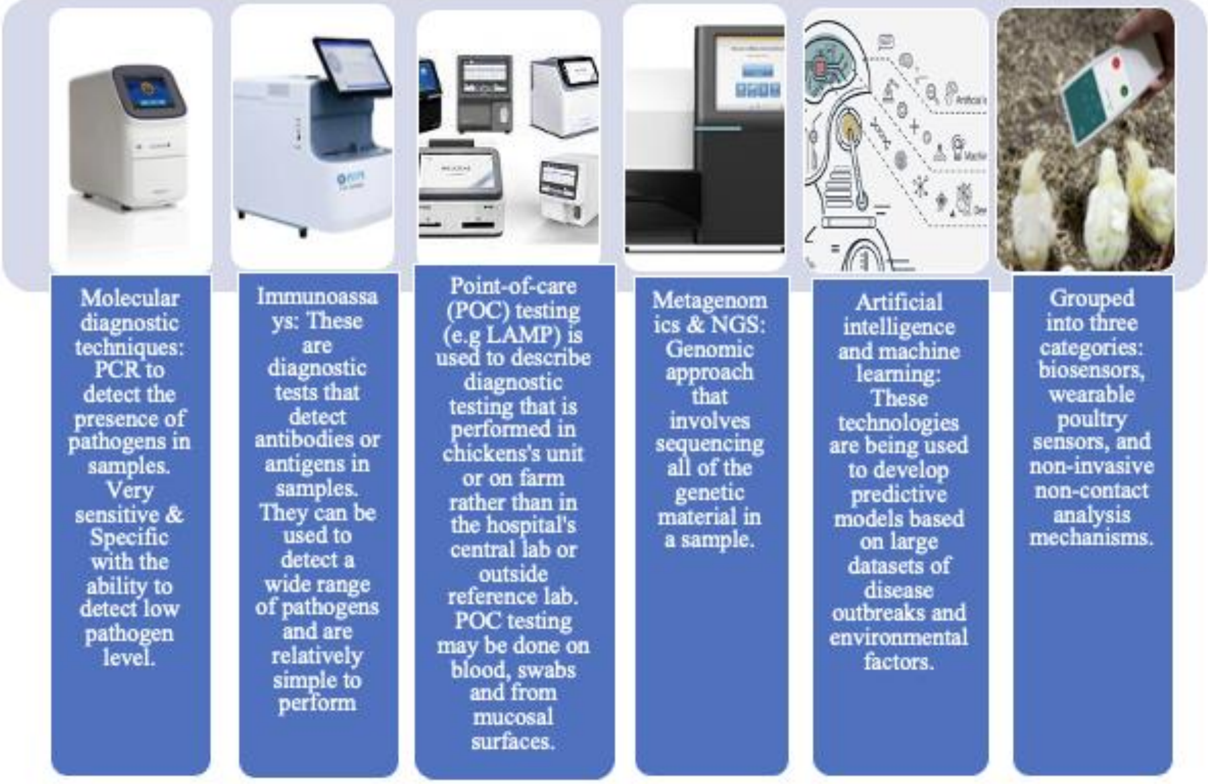


Figure 1: Timeline evolution of avian disease diagnosis



Molecular diagnostic techniques: PCR to detect the presence of pathogens in samples. Very sensitive & Specific with the ability to detect low pathogen level.

Immunoassays: These are diagnostic tests that detect antibodies or antigens in samples. They can be used to detect a wide range of pathogens and are relatively simple to perform

Point-of-care (POC) testing (e.g LAMP) is used to describe diagnostic testing that is performed in chickens's unit or on farm rather than in the hospital's central lab or outside reference lab. POC testing may be done on blood, swabs and from mucosal surfaces.

Metagenomics & NGS: Genomic approach that involves sequencing all of the genetic material in a sample.

Artificial intelligence and machine learning: These technologies are being used to develop predictive models based on large datasets of disease outbreaks and environmental factors.

Grouped into three categories: biosensors, wearable poultry sensors, and non-invasive non-contact analysis mechanisms.

Figure 2: Devices and gadgets used in molecular diagnostics of avian diseases

implementation in field settings and resource-limited regions has made it a valuable tool for enhancing avian disease detection and surveillance in developing countries, where access to sophisticated diagnostic tools is limited (Han *et al.,* 2019; Padzil *et al.,* 2022). The isothermal nucleic acid-based method can amplify RNA or DNA targets a billion-fold in less than an hour (Oliveira *et al.,* 2021). The LAMP utilises four primers that target six specific nucleotide regions in a given sequence with a Bst polymerase, which does not require DNA denaturation, facilitating the process (Notomi *et al.,* 2015). The reaction can be performed at a constant temperature, enabling rapid and cost-effective detection without the need for expensive equipment. Positive results can be obtained within 20 to 30 minutes (Notomi *et al.,* 2000).

In addition to the LAMP assay, other emerging techniques in molecular pathology have further advanced the field of avian pathology (Padzil *et al.,* 2022). One such technique is the application of nanomaterial-based sensors, which with their small size and rapid detection capabilities, offer significant advancements in revolutionizing avian pathology diagnostics by providing sensitive and specific detection of avian pathogens (Wei-Wen Hsiao *et al.,* 2023). Nanomaterial-based sensors, with their compact size and rapid detection capabilities, are ideal for deployment in first responder scenarios and point-of-care applications, ensuring quick and accurate pathogen identification (Wei-Wen Hsiao *et al.,* 2023). These sensors are a big step forward in quickly and accurately identifying avian pathogens in a wide range of settings, such as in the field and in hospitals (Vidic *et al.,* 2017; Wei-Wen Hsiao *et al.,* 2023). Some other well-known isothermal amplification methods are helicase-dependent amplification (HDA), which utilizes helicase enzymes to separate DNA strands; rolling circle amplification (RCA), known for its isothermal amplification of circular DNA templates; nucleic acid sequence-based amplification (NASBA), a technique that amplifies RNA sequences specifically; transcription-mediated amplification (TMA), a method for amplifying RNA targets; and recombinant polymerase amplification (RPA), which uses recombinant polymerases for nucleic acid amplification (Padzil *et al.,* 2022).

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based

Clustered regularly interspaced short palindromic repeats (CRISPR)-based molecular diagnostics is an emerging technique for detecting avian pathogens (Khudeir *et al.,* 2024; Srivastava *et al.,* 2020). This technology has the potential to revolutionise diagnostics by offering a highly specific and sensitive method for identifying the nucleic acids of avian pathogens, such as viruses and bacteria (Khudeir *et al.,* 2024; Zhao *et al.,* 2022). Its precision and versatility make it a valuable tool for quickly and accurately identifying various avian pathogens, promising advancements in disease management and control strategies in avian medicine. Also, CRISPR-based molecular diagnostics can identify any genetic changes or mutations in avian pathogens. This helps us understand how these pathogens have evolved and spread (Khudeir *et al.,* 2024) and is very useful. This technique can be easily adapted for high-throughput screening, allowing for rapid and cost-effective testing of large populations of birds (Khudeir *et al.,* 2024; Srivastava *et al.,* 2020). With ongoing advancements in CRISPR technology, it is anticipated that this diagnostic technique will continue to improve and play a crucial role in avian disease surveillance and prevention.

Artificial intelligence

Integrating artificial intelligence and machine learning algorithms into molecular diagnostics for avian pathogens has opened up new possibilities for rapid and automated data analysis (Sadeghi *et al.,* 2023). AI-powered diagnostic tools have the potential to enhance the sensitivity and specificity of avian pathogen detection, as well as streamline the interpretation of complex molecular data (Walsh *et al.,* 2019). Gradient boosted trees, a specific technique in machine learning, can be used to accurately assess the likelihood of isolating AI Virus (AIV) from samples obtained from wild birds. The model, which considers variables such as age, gender, bird species, and rRT-PCR data, exhibits a strong predictive capability (Walsh *et al.,* 2019). The model has the ability to prioritise samples for isolation and efficiently assess surveillance schemes, hence maximising the chance of viral isolation (Machuve *et al.,* 2022; Walsh *et al.,* 2019). Harnessing the power of AI enables researchers and clinicians to make more informed decisions regarding disease management and treatment strategies in avian species. A global-scale predictive map of AIV in wild birds was developed using the random forests algorithm (RFA), which identified northern areas of the globe as having the highest relative predicted risk of outbreak. Important predictor variables included low temperatures and low annual precipitation (Herrick *et al.,* 2013). A study that looked at the autoregressive integrated moving average (ARIMA) and RFA time series models, found that the RFA model was better at predicting when highly pathogenic (HP) AI (H5N1) would spread in Egypt than the ARIMA model (Kane *et al.,* 2014). The RFA model provides enhanced predictive ability for the prediction of infectious disease outbreaks, including AI (Kane *et al.,* 2014).

Furthermore, machine learning algorithms can be employed to analyse poultry surveillance data and detect when disease behaviours or activities arise (Astill *et al.,* 2018). Techniques such as vocalisation and imaging analysis, as well as robotic surveillance, have potential for detecting avian pathogens. Additionally, machine learning models can be developed to classify chicken health based on colour data extracted from digital images of chicken combs (Bakar *et al.,* 2023).

Pulsed-Field Gel Electrophoresis (PFGE) and Multi-Locus Sequence Typing (MLST)

Genetic fingerprinting techniques, such as pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST), have emerged as invaluable tools for tracing the origin of avian pathogens (Cao *et* *al.,* 2018; Ghanem & El-Gazzar, 2019). Pulsed-Field Gel Electrophoresis (PFGE) has been instrumental in identifying the source of a Salmonella outbreak on a poultry farm (Peighambari *et al.,* 2018). PFGE is a rapid laboratory technique used to determine genetic relatedness among Salmonella species, allowing researchers to compare the DNA fingerprints of different strains to trace back their origin. It is widely considered the most dependable method for categorizing different types of Salmonella at the molecular level (Jain *et al.,* 2018). A research note from 2021 said that PFGE analysis showed a lot of genetic similarity between Salmonella strains taken from chicken samples. This suggests that the infection spread through clones on the farm (Ha *et al.,* 2021). Additionally, PFGE has been used extensively in outbreak investigations to link strains based on their unique DNA fingerprints, helping to pinpoint the source of contamination and prevent further spread of the disease (Chen *et al.,* 2011). In contrast, MLST is a method that relies on the sequence of nucleotides in DNA to examine variations in specific genes. It provides rapid results with high reproducibility, and exchangeability between laboratories (Tang *et al.,* 2019). MLST is a valuable tool for refining strain identification, aiding in understanding the epidemiology of infections, and facilitating disease prevention and control efforts. It also has the potential for higher throughput. It is complementary to whole genome sequencing (WGS) (Tang *et al.,* 2019). In the context of poultry disease diagnosis, MLST has been used to identify genetic variations within pathogens like *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale*, *Clostridium* perfringens, and *Mycoplasma gallisepticum* (Ghanem & El-Gazzar, 2019; Matucci *et al.,* 2020), enabling a more precise diagnosis and enhancing disease management strategies.

Metagenomics and Next-Generation Sequencing

Metagenomics and next-generation sequencing (NGS) techniques have significantly enhanced the understanding of avian microbiomes by allowing the examination of the genetic material of all microorganisms in avian samples simultaneously (Afonso & Afonso, 2023; Shah *et al.,* 2016). Random NGS is a powerful tool that obtains the full genome of viruses that are difficult to cultivate or distinguish by other techniques. Even with a small number of NGS reads, metagenomic analysis becomes achievable and offers sufficient data to utilize more sensitive tools for detailed examination. These approaches have provided valuable insights into the diversity, dynamics, and functional potential of avian microbial communities, shedding light on the mechanisms of disease transmission and host-pathogen interactions in avian species (Chua & Rasmussen, 2022). Metagenomic studies have uncovered a wide variety of viruses in both healthy and clinical samples from chickens. In healthy chickens, the virome is dominated by viruses, including those from the *Adenoviridae, Caliciviridae, Circoviridae, Parvoviridae, Picobirnaviridae, Picornaviridae, Astroviridae,* and *Reoviridae* families (Day *et al.,* 2010; Shah *et al.,* 2016). Clinical samples have a wider range of RNA and DNA viruses than healthy samples. These consist of viruses from families like *Astroviridae, Caliciviridae, Picornaviridae, Parvoviridae, Coronaviridae, Siphoviridae*, and *Myoviridae* (Devaney *et al.,* 2016). These findings highlight the potential of metagenomics in identifying and characterising the viromes of healthy and clinical chicken samples. Additionally, a study based on metagenomics characterised the development of the RNA viral community in the small intestines of healthy broiler chickens from hatch through 6 weeks of age (Shah *et al.,* 2016). While the breeder source only affected the RNA viral community at hatch, bird age had a more pronounced impact (Shah *et al.,* 2016). The most abundant RNA viral families detected relative to age were *Astroviridae* and *Picornaviridae*. In contrast, the abundance of Astroviridae decreased with age, whereas the abundance of Picornaviridae increased with age (Shah *et al.,* 2016). These shifts in the RNA viral community reflect the changing environment and immune system maturation in young birds, suggesting that the gut microenvironment plays a role in shaping the viral community composition (Shah *et al.,* 2016). Having complete genome sequencing information for avian pathogens has made it easier to find genetic variations, factors affecting disease severity, and drug resistance mechanisms, leading to the development of more precise and efficient treatment approaches for avian diseases.

Polymerase Chain Reaction (PCR) and Quantitative Polymerase Chain Reaction (qPCR)

Over the years, PCR and qPCR have been valuable tools in the diagnosis of avian diseases, providing high sensitivity and specificity. Researchers widely use these techniques to detect and characterise various avian pathogens, including viruses and bacteria. Recent research has demonstrated the utility of PCR and qPCR in diagnosing various avian diseases such as avian pox, AI, and avian haemosporidians (Baek *et al.,* 2020; Galvin *et al.,* 2022).

In 2020, researchers focused on using qPCR to detect avipoxvirus DNA in various sample types from hummingbirds. The research emphasized the high sensitivity of qPCR in detecting avian pox infection in hummingbirds, underscoring its value for diagnosis, particularly in field sampling. The study also emphasised the quantitative analysis capability of qPCR, which allows for the estimation of viral loads without the need for additional diagnostic tests (Baek *et al.,* 2020). The "Riems Influenza A Typing Array," a qPCR-based low-density array created in another study, was made to help with better subtyping of avian influenza viruses (Hoffmann *et al.,* 2016). This diagnostic tool offers enhanced sensitivity and specificity for the differential diagnosis of AI viruses, particularly in complex viral co-infections (Hoffmann *et al.,* 2016). The study emphasised how important it is to keep technical tools up-to-date and use RT-qPCR to accurately diagnose and classify avian flu viruses (Hassan *et al.,* 2022).

Furthermore, a qPCR-based protocol has been developed for the detection of avian haemosporidians, demonstrating the effectiveness of qPCR as a large-scale screening tool for avian blood samples. The study emphasized that qPCR allows for faster processing, reduced screening time, and cost-effectiveness, making it a valuable tool for studying the diversity of avian haemosporidians. A more recent study developed a one-step qPCR for the detection of a novel variant of IBDV (genotype A2dB1) currently circulating in the poultry industry in Asia (Wang *et al.,* 2023). The work explored the potential and reliability of the real-time TaqMan analysis for detecting and distinguishing the novel variant IBDV from other variant strains. The technique provides a simple, rapid, sensitive, and specific diagnostic method for novel variant identification, and it is a useful tool in clinical diagnosis.

Pros and cons

In the field of avian molecular pathology research, both challenges and opportunities shape the future of avian disease management. However, one of the key challenges is the need to expand our understanding of the intricate interactions between avian hosts and pathogens at the molecular level. Understanding how host-pathogen interactions work and how birds are more likely to be infected, even with progress in molecular diagnostics, is still a difficult and multifaceted task (Videvall, 2019). To tackle this challenge effectively, it is essential to foster interdisciplinary collaborations and make concerted efforts to elucidate the molecular determinants that influence avian immune responses and disease outcomes. Several factors implicated in altering vector performance and avian disease susceptibility are still understudied (Näpflin *et al.,* 2019; Videvall, 2019). Furthermore, the development and validation of molecular diagnostic assays specific to avian pathogens are crucial to improving diagnostic accuracy and reliability. Making progress in molecular diagnostic assays that are specific to avian pathogens will be key to solving this problem. These assays will help find infectious agents more accurately and give us more information about how disease is spread in bird populations (Videvall, 2019).

In addition, avian molecular pathology research is faced with the necessity of unravelling the complexities of pathogen evolution and adaptation (Fu *et al.,* 2023). Avian pathogens exhibit remarkable genetic plasticity, enabling them to evolve rapidly and overcome host immunity and management strategies (Lu *et al.,* 2023). Understanding the genomic diversity and evolutionary dynamics of avian pathogens is essential for designing effective control measures and developing robust intervention strategies (Altizer *et al.,* 2003). Leveraging advanced genomic sequencing and bioinformatic tools is crucial for understanding the evolutionary changes in avian pathogens over time. This will give us important information for making targeted and adaptive control strategies (Videvall, 2019).

Despite these challenges, the field of avian molecular pathology research presents exciting opportunities for the discovery of novel therapeutic targets and the development of innovative intervention strategies (Näpflin *et al.,* 2019; Videvall, 2019). The integration of multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, offers a comprehensive view of host-pathogen interactions and the molecular signatures of disease progression (Jax *et al.,* 2018; Dehau *et al.,* 2022). By leveraging these technologies, researchers can identify potential biomarkers of disease resistance and susceptibility, paving the way for the development of precision medicine approaches tailored to individual avian species or populations (Lee *et al.,* 2017). This knowledge can inform the development of novel diagnostic tools and therapeutic interventions tailored to the unique genetic traits of avian pathogens, ultimately enhancing disease control and prevention strategies.

Additionally, the development of advanced genetic editing tools such as CRISPR-based genome editing and gene drive technologies has made it possible to change the genes of avian hosts in a way that makes them resistant to certain avian pathogens. This makes it easier to use targeted disease management strategies (Islam *et al.,* 2020; Khudeir *et al.,* 2024).

Conversely, a significant challenge in avian molecular pathology research is the need to address the impact of environmental and ecological factors on avian disease dynamics. Putting together ecological and environmental data with molecular pathogen surveillance can give us a full picture of how avian diseases spread, which in turn allows us to create complete disease control strategies that work in different ecological settings (Struelens *et al.,* 2024). For example, the prevalence of avian malaria is influenced by factors such as climate change and alterations in land use patterns (Ishtiaq, 2021). Understanding the complex interplay between environmental factors and avian health can provide valuable insights into disease emergence, persistence, and transmission dynamics. The integration of ecological and environmental data with molecular pathogen surveillance not only provides a holistic view of avian disease ecology but also facilitates the tailored development of comprehensive disease control measures suited to specific ecological contexts. Avian malaria, for example, is influenced by land use changes and climate change (Ishtiaq, 2021), while human movements and climate change may be driving factors in the spread of avian pathogens (Fuller *et al.,* 2012). For example, studies have revealed the impact of deforestation on pathogen spread and the role of migratory patterns in disease transmission dynamics (Ishtiaq *et al*., 2021).

Furthermore, the application of advanced bioinformatics and computational modelling approaches presents an opportunity to harness big data analytics for predictive modelling of avian disease dynamics (Astill *et al.,* 2018). Through the integration of genomic and epidemiological data, researchers can develop predictive models that enable the anticipation of disease outbreaks, assessment of transmission risks, and optimization of surveillance strategies (Cavanagh *et al.,* 1997). These data-driven approaches can empower proactive decision-making and intervention, enabling early detection and rapid response to emerging threats in avian populations (Shittu *et al.,* 2014).

Additionally, the advancement of vaccine development and antigen discovery through molecular pathology research offers promising avenues for enhancing avian health. Using information from molecular factors that affect how a host and a pathogen interact, researchers can find new vaccine targets and improve vaccine formulations to protect against a wide range of avian pathogens (Shrestha *et al.,* 2018). This proactive approach to vaccine development aligns with the principles of 'one health,' emphasizing the interconnectedness of human, animal, and environmental health. It contributes significantly to global efforts in mitigating the spread of zoonotic diseases and safeguarding public health (Cunningham *et al.,* 2017).

The challenges and opportunities in avian molecular pathology research underscore the critical need for interdisciplinary collaboration, technological innovation, and data-driven approaches to advance avian health management. By addressing the complexities of host-pathogen interactions, pathogen evolution, environmental influences, and leveraging cutting-edge technologies, the veterinary community can enhance its capacity to mitigate avian diseases and safeguard the well-being of avian species, emphasizing the importance of interdisciplinary collaboration and technological innovation in avian health management (Cunningham *et al.,* 2017). As the field continues to evolve, ongoing research and collaboration will be paramount in harnessing the full potential of molecular pathology for proactive disease surveillance, precise diagnostics, targeted interventions, and sustainable avian health management.

Compelling case studies

Numerous case studies demonstrate how molecular diagnostics have revolutionized avian pathology. An important example is the application of molecular diagnostics in monitoring and managing avian influenza outbreaks (Okamatsu *et al.,* 2016; Wei *et al.,* 2020). Avian influenza is a highly contagious viral disease that affects birds, poultry, and wild bird populations. Through the employment of advanced molecular diagnostic techniques, including qPCR and sequencing methods, authorities were able to swiftly identify the strains of the virus responsible for outbreaks (Fu *et al.,* 2023; Vilela *et al.,* 2020). This swift identification of the virus strain enabled authorities to take targeted actions, including conducting vaccination campaigns, culling infected and at-risk birds, and setting up quarantine zones to contain the disease spread (de Vries *et al.,* 2022; Hill *et al.,* 2018). Additionally, molecular diagnostics allowed tracking the evolution of the virus, essential for updating vaccines and anticipating future outbreaks (Quer *et al.,* 2022).

Additionally, in a case study, Han *et al.* (2019) successfully used loop-mediated isothermal amplification (LAMP) for the detection of chicken anaemia virus (CAV) in chickens. The findings highlighted the cost-effectiveness and speed of LAMP in detecting specific avian pathogens. On-site testing facilitated early infection detection, reduced economic losses, and improved treatment strategies on commercial poultry farms, as supported by Padzil *et al.* (2022).

Another case highlights the fight against Newcastle Diseases (ND), a devastating viral disease affecting poultry worldwide (Moustapha *et al.,* 2023). By leveraging next-generation sequencing technologies, researchers have identified and characterised various strains of the Newcastle disease virus (Dey *et al.,* 2023; Pereira *et al.,* 2022). This enhanced understanding of the virus' genetic diversity and transmission dynamics has played a crucial role in developing more effective vaccines and strengthening biosecurity measures (Harder *et al.,* 2023). As a result, the incidence of ND has seen significant reductions in areas where these modern molecular diagnostic techniques have been applied (Chen *et al.,* 2020).

In addressing bacterial pathogens, molecular diagnostics have demonstrated significant success in identification and management (Gerace *et al.,* 2022). For example, the identification of *Mycoplasma gallisepticum* infections, which cause respiratory distress in poultry, has been greatly improved (Fujisawa *et al.,* 2019). Traditional diagnostic methods frequently necessitated prolonged bacterial culturing, leading to delays in timely interventions (Jafar & Noomi, 2019). CRISPR-based diagnostics and qPCR now enable direct detection of bacterial DNA from clinical samples. This capability allows for prompt confirmation of infections and immediate measures to prevent disease spread within flocks (Vilela *et al.,* 2020).

One other area where molecular diagnostics has made a significant stride is in the detection and management of chicken astrovirus, a pathogen causing enteritis, runting-stunting syndrome, white chick syndrome, and gout in chickens (Baxendale & Mebatsion, 2004; Raji *et al.,* 2022a; Smyth, 2017). These diseases lead to considerable economic losses in the poultry industry due to decreased flock performance (Long *et al.,* 2017; Smyth *et al.,* 2013; Smyth, 2017). The application of molecular diagnostics, particularly polymerase chain reaction and next-generation sequencing, has opened new avenues for the rapid and precise identification of this virus (Adebiyi *et al.,* 2021; Raji *et al.,* 2022a). For instance, researchers have used these techniques to detect and characterise different strains of chicken astrovirus, providing valuable insights into the transmission dynamics and geographic distribution of the virus (Patel *et al.,* 2017).

An additional area where molecular diagnostics have made a remarkable difference is in the battle against Psittacine Beak and Feather Disease (PBFD), which affects parrots and related species (Haddadmarandi *et al.,* 2018). Utilising molecular techniques like PCR, researchers can now detect the circovirus responsible for PBFD with great accuracy, long before clinical signs appear in affected birds (Dolatyabi *et al.,* 2022). Early detection is critical for preventing the spread of this devastating disease, as it allows for the immediate isolation of infected individuals and helps protect other birds from exposure. Molecularly characterising the virus also makes it easier to make specific vaccines and treatment plans, which gives us hope for managing and maybe even getting rid of PBFD in wild and captive parrot populations (Haddadmarandi *et al.,* 2018; Dolatyabi *et al.,* 2022).

Through the lens of these case studies, it is clear that molecular diagnostics have not only transformed the approach to managing avian diseases but have also established a foundation for innovative research and discovery. By allowing for the rapid, accurate, and sensitive detection of pathogens, these techniques are catalysing a paradigm shift in avian pathology and medicine from reactive to proactive and preventive strategies.

The expansion of molecular diagnostics into more accessible and cost-effective formats continues to make even greater strides in avian disease management. Point-of-care diagnostics and portable sequencing devices are among the developments rendering molecular diagnostics more readily available in remote and resource-limited settings. This accessibility is vital for timely surveillance and control of avian diseases, particularly in regions with limited access to traditional laboratory facilities.

Future directions

Research in avian health is progressing quickly due to advancements in DNA sequencing and computer analysis tools. These advancements enable large-scale studies, such as population-wide pathogen surveillance projects and comparative genomics analyses, to better understand the complex mechanisms behind immune responses and pathogen evasion strategies. The fields of functional genomics and evolutionary biology play a crucial role in uncovering the adaptive strategies employed by avian pathogens, such as identifying genetic variations that confer pathogen resistance and tracing the evolutionary history of virulence factors. By integrating environmental and ecological data, such as climate patterns and habitat changes, with molecular pathogen surveillance, researchers can gain a comprehensive understanding of avian disease ecology.

Funding

No funding was received.

Conflict of Interest

The authors declare that there is no conflict of interest.

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