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Molecular Diagnostics of Water Buffalo Diseases: A Comparative Analysis

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Abstract This study provides a comprehensive analysis of molecular diagnostic tools and their applications in the detection of key diseases affecting water buffaloes, including bovine tuberculosis, brucellosis, and foot-and-mouth disease. Through a detailed review of PCR-based techniques, high-throughput sequencing, and immunological diagnostics, the study compares the efficacy of these methods in diagnosing specific water buffalo diseases. A case study of a disease outbreak highlights the practical application of molecular diagnostics in controlling outbreaks and improving disease management strategies. The study also explores future directions, emphasizing emerging technologies, integration with veterinary surveillance systems, and overcoming implementation barriers in developing regions. The findings underscore the potential of molecular diagnostics to revolutionize disease detection and management in water buffalo populations, with broader implications for global livestock health.

Keywords Water buffalo; Molecular diagnostics; PCR; Disease outbreaks; Veterinary surveillance

1 Introduction

Water buffaloes (*Bubalus bubalis*) play a crucial role in agriculture and the economy, particularly in regions like the Philippines, Brazil, and Thailand. They are integral to the livestock industry, contributing significantly to dairy production and serving as a sustainable alternative to cattle in areas like the Amazon River Delta (Galon et al., 2019; Guedes et al., 2020; Nguyen et al., 2020). Despite their economic importance, water buffaloes face challenges related to disease diagnosis and management. They can act as reservoirs for various pathogens, including tick-borne pathogens (TBPs) and hemoprotozoan parasites, which can affect both buffaloes and cattle (Sivakumar et al., 2014; Silveira et al., 2016).

Diagnosing diseases in livestock, including water buffaloes, presents several challenges. Traditional diagnostic methods, such as microscopic examination, often lack sensitivity and specificity, making it difficult to detect infections accurately (Silveira et al., 2016). Moreover, diseases like anaplasmosis and babesiosis, caused by rickettsial bacteria and protozoan parasites, respectively, are prevalent in buffaloes and can lead to significant economic losses (Sivakumar et al., 2014; Nguyen et al., 2020). The presence of pathogens such as *Anaplasma marginale*, *Babesia bovis*, and *Theileria spp.* in buffaloes highlights the need for effective diagnostic tools to manage and control these infections (Silveira et al., 2016; Galon et al., 2019; An et al., 2021).

Molecular diagnostics have emerged as a powerful tool in veterinary science, offering enhanced sensitivity and specificity for detecting pathogens in livestock. Techniques such as PCR and recombinase polymerase amplification (RPA) have been employed to identify and characterize pathogens at the molecular level, providing insights into their prevalence and genetic diversity (Galon et al., 2019; An et al., 2021). These methods have been instrumental in detecting infections in water buffaloes, including those caused by *Leptospira spp.*, *Babesia spp.*, and *Theileria spp.*, thereby facilitating better disease management and prevention strategies (Mingala et al., 2009; Silveira et al., 2016; Guedes et al., 2020).

This study attempts to explore the comparative analysis of molecular diagnostics in detecting and characterizing diseases in water buffaloes, discuss the effectiveness of various molecular techniques, and provide an overview of their role in enhancing our understanding of pathogen prevalence and diversity in buffalo populations. By

evaluating these methods, the research aims to contribute to improved disease management and control in the livestock industry.

2 Overview of Common Diseases in Water Buffaloes

Water buffaloes, an essential component of the livestock industry, are susceptible to a variety of diseases that can significantly impact their health and productivity. These diseases can be broadly categorized into infectious and non-infectious types, each with distinct implications for animal health, economic stability, and public health.

2.1 Infectious diseases affecting water buffaloes

Infectious diseases are a major concern for water buffaloes, with several pathogens posing significant threats. Anaplasmosis, caused by rickettsial bacteria in the genus *Anaplasma*, is prevalent in water buffaloes and has been identified in regions such as Thailand and the Philippines. This disease is transmitted by ticks and can lead to severe health issues in infected animals (Galon et al., 2019; Nguyen et al., 2020). Babesiosis, another tick-borne disease caused by *Babesia* species, is also a significant concern, particularly in China, where it leads to substantial economic losses (An et al., 2021). Brucellosis, caused by *Brucella abortus*, is a zoonotic disease that affects water buffaloes and can be transmitted to humans, highlighting its public health importance (Lecchi et al., 2019; Grandoni et al., 2023). Other notable infectious diseases include bovine tuberculosis, caused by *Mycobacterium bovis*, and Johne's disease, caused by *Mycobacterium avium* subspecies paratuberculosis, both of which have been reported in water buffalo populations (Figure 1) (Uy et al., 2018; Cadavid et al., 2024).

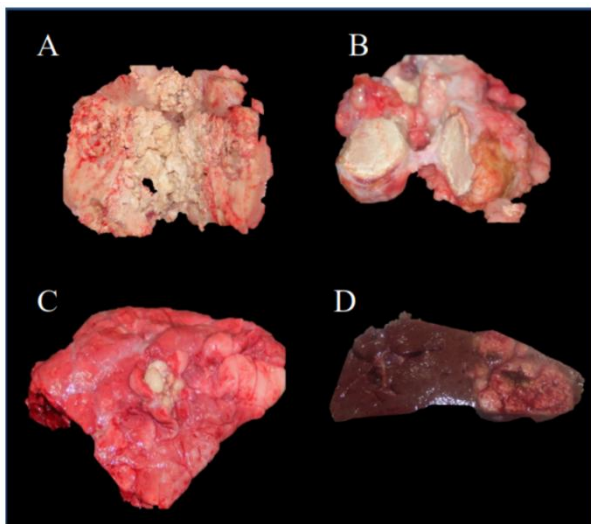


Figure 1 Macroscopic lesions in tuberculin-positive water buffaloes (Adopted from Cadavid et al., 2024)

Image caption: A. Granuloma lesion in retropharyngeal lymph node. B. Granuloma lesion in a mediastinal lymph node. C. Granulomatous lesion in lung tissue. D. Granulomatous lesion in the liver (Adopted from Cadavid et al., 2024)

2.2 Non-infectious diseases and their impact

While infectious diseases are more commonly discussed, non-infectious diseases also affect water buffaloes, though they are less frequently documented in the literature. These conditions can include nutritional deficiencies, metabolic disorders, and environmental stressors that impact the overall health and productivity of the animals. The management of these non-infectious diseases is crucial for maintaining the welfare and productivity of water buffalo herds.

2.3 Economic and public health consequences of disease outbreaks

Disease outbreaks in water buffaloes have significant economic and public health consequences. Economically, diseases like anaplasmosis and babesiosis lead to reduced productivity, increased veterinary costs, and potential trade restrictions (Nguyen et al., 2020; An et al., 2021). The impact of brucellosis extends beyond economic losses, as it poses a zoonotic risk, potentially affecting human health through direct contact with infected animals or consumption of contaminated products (Lecchi et al., 2019). The presence of diseases such as foot and mouth

disease (FMD) can lead to high morbidity and mortality rates, further exacerbating economic losses and necessitating stringent control measures (Damaty et al., 2021). Effective disease management and prevention strategies are essential to mitigate these impacts and ensure the sustainability of water buffalo farming.

In summary, both infectious and non-infectious diseases pose significant challenges to water buffalo health, with far-reaching economic and public health implications. Addressing these challenges requires comprehensive disease surveillance, effective management practices, and robust prevention strategies.

3 Molecular Diagnostic Tools

3.1 PCR-based techniques in disease diagnosis

Polymerase chain reaction (PCR) is a cornerstone in the molecular diagnosis of diseases in water buffaloes. It is widely used for detecting various pathogens, including *Anaplasma marginale* and *Babesia* species. For instance, PCR assays have been employed to identify tick-borne pathogens in water buffaloes in the Philippines, revealing a significant prevalence of *Anaplasma marginale* and *Babesia bovis* (Galon et al., 2019). Similarly, nested PCR techniques have been used to detect *Cryptosporidium spp.* in buffaloes, showing a higher sensitivity compared to traditional microscopic methods (Al-Amery et al., 2022). PCR-based methods are also crucial in diagnosing *Mycobacterium avium* subspecies paratuberculosis, the causative agent of Johne's disease, with qPCR assays providing a higher detection rate than serological tests (Uy et al., 2018).

3.2 High-throughput sequencing and its applications

High-throughput sequencing technologies, such as Next Generation Sequencing (NGS), have advanced the molecular diagnostics of infectious diseases in water buffaloes. These technologies allow for comprehensive profiling of pathogen genomes and host responses. For example, NGS has been utilized to characterize miRNA signatures in water buffaloes infected with *Brucella abortus*, identifying potential biomarkers for non-invasive diagnosis (Lecchi et al., 2019). This approach provides insights into the host-pathogen interactions and can aid in the development of targeted diagnostic and therapeutic strategies.

3.3 Serological and immunological diagnostics in molecular testing

Serological and immunological diagnostics complement molecular techniques by providing additional layers of information about disease status and immune response. Enzyme-linked immunosorbent assays (ELISA) are commonly used to detect antibodies against pathogens such as *Mycobacterium avium* subspecies paratuberculosis, although they may have lower sensitivity compared to molecular methods like qPCR (Uy et al., 2018). In the context of schistosomiasis, multi-epitope recombinant antigens have been developed to improve the sensitivity and specificity of serological tests, demonstrating potential for enhanced diagnostic accuracy (Lv et al., 2018). Additionally, immunophenotyping using flow cytometry has been applied to assess lymphocyte alterations in buffaloes with brucellosis, providing valuable data for disease diagnosis and management (Grandoni et al., 2023).

In summary, the integration of PCR-based techniques, high-throughput sequencing, and serological diagnostics offers a comprehensive toolkit for the molecular diagnosis of diseases in water buffaloes. These methods enhance the detection, characterization, and understanding of various pathogens, contributing to improved disease management and control strategies.

4 Comparative Analysis of Molecular Diagnostics for Specific Diseases

4.1 Diagnostic approaches for bovine tuberculosis

Bovine tuberculosis (bTB) in water buffalo is primarily caused by *Mycobacterium bovis*. Traditional diagnostic methods, such as the intradermal tuberculin test, have limitations in sensitivity and specificity. Recent studies have explored alternative diagnostic methods, including histopathological analysis, microbiological culture, and molecular techniques like PCR-HRM (High-Resolution Melting) analysis. In a study conducted in Colombia, histopathological tests revealed that 52% of tuberculin-positive buffaloes had lesions compatible with bTB, while PCR-HRM confirmed *Mycobacterium bovis* in 34% of cases, even in the absence of histological lesions (Jaiswal

et al., 2021; Cadavid et al., 2024). These findings suggest that molecular diagnostics can enhance the detection accuracy of bTB in water buffaloes, providing a more reliable alternative to traditional methods.

4.2 Molecular tools in brucellosis detection

Brucellosis, caused by *Brucella abortus*, poses significant health risks to water buffaloes and can be transmitted to humans. Molecular diagnostics, particularly the use of circulating microRNAs (miRNAs), have emerged as promising tools for non-invasive detection. A study identified specific miRNA signatures in *Brucella*-infected buffaloes, which were validated using RT-qPCR. These miRNAs showed potential as biomarkers for brucellosis, with ROC curve analyses supporting their diagnostic value (Craig et al., 2015; Lecchi et al., 2019). Additionally, immunophenotyping has revealed significant alterations in lymphocyte subsets in brucellosis-positive buffaloes, further aiding in the disease's diagnosis (Grandoni et al., 2023). These molecular approaches offer a novel and effective means of diagnosing brucellosis in water buffaloes.

4.3 Foot-and-mouth disease diagnostics: pcr and beyond

Foot-and-mouth disease (FMD) in water buffaloes is a highly contagious viral disease, with the SAT-2 serotype being particularly prevalent. Molecular diagnostics, such as reverse transcriptase PCR (RT-PCR), have been instrumental in identifying and characterizing FMDV strains. In Egypt, RT-PCR was used to confirm the presence of the SAT-2 serotype in water buffaloes, revealing a new lineage with high virulence (Figure 2) (Damaty et al., 2021). This molecular characterization is crucial for understanding the epidemiology of FMD and for developing effective control strategies. The study highlights the importance of continuous molecular surveillance to detect new FMDV topotypes and lineages, which can inform vaccination and outbreak management efforts (Elhaig and Elsheery, 2014; Zeedan et al., 2020; Wong et al., 2020).

In summary, molecular diagnostics offer significant advantages in the detection and characterization of diseases in water buffaloes. These methods provide higher sensitivity and specificity compared to traditional diagnostic approaches, enabling more accurate disease management and control.

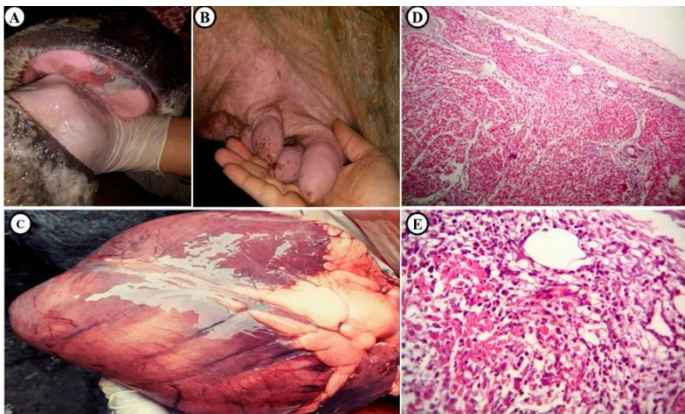


Figure 2 Clinical and histopathological signs in FMD-infected buffaloes (Adopted from Damaty et al., 2021)

Image caption: (A) Blisters and ulcers in the dental pad. (B) Blisters in teats. (C) Heart of buffalo showing grayish-white stripping, particularly in the left ventricle near the septum (Tiger heart). (D) Heart of buffaloes showing pericarditis and myocarditis $\times 120$ H&E; and (E) Heart of buffaloes showing non-suppurative myocarditis characterized by replacement of necrotic cardiac myocytes by edema, lymphocytes, and some plasma cells $\times 400$ H&E (Adopted from Damaty et al., 2021)

5 Case Study: Application of Molecular Diagnostics in a Specific Outbreak

5.1 Outbreak background and initial diagnosis approach

In a recent outbreak of foot and mouth disease (FMD) in Egyptian water buffaloes, the disease was characterized by high morbidity and mortality rates, particularly affecting smallholder production systems. The initial diagnosis was based on clinical symptoms such as oral lesions and lameness, which are typical of FMD. However, due to the high mutation rate of the FMD virus, traditional diagnostic methods faced challenges in accurately identifying the specific serotype responsible for the outbreak (Damaty et al., 2021).

5.2 Diagnostic techniques employed in the case study

To address the limitations of conventional diagnostic methods, molecular diagnostics were employed. Reverse transcriptase PCR (RT-PCR) was used to confirm the presence of the FMD virus in samples collected from affected buffaloes. This technique allowed for the precise identification of the SAT-2 serotype, which was closely related to the lineage of lib12, topotype VII. The molecular approach provided a rapid and accurate diagnosis, which was crucial for implementing timely control measures (Damaty et al., 2021). Additionally, phylogenetic analysis was conducted to understand the genetic relationship of the circulating virus with other known strains, further aiding in the outbreak management (Ayebazibwe et al., 2010).

5.3 Impact of molecular diagnostics on control measures

The application of molecular diagnostics had a significant impact on controlling the FMD outbreak. By accurately identifying the SAT-2 serotype, authorities were able to tailor vaccination strategies to include vaccines effective against this specific strain, thereby enhancing the efficacy of the vaccination program. Moreover, the rapid diagnosis facilitated by molecular techniques allowed for quicker implementation of quarantine and movement restrictions, reducing the spread of the virus among buffalo populations. This case study highlights the critical role of molecular diagnostics in managing infectious disease outbreaks in livestock, providing a model for future interventions (Clavijo et al., 2004; Wong et al., 2020; El-Ansary et al., 2023).

In summary, the use of molecular diagnostics in this outbreak of FMD in Egyptian water buffaloes demonstrated the advantages of precise and rapid pathogen identification, which is essential for effective disease control and prevention strategies.

6 Future Directions in Molecular Diagnostics for Water Buffalo Diseases

6.1 Emerging technologies in molecular diagnostics

Emerging technologies in molecular diagnostics are paving the way for more accurate and rapid detection of diseases in water buffalo. Techniques such as recombinase polymerase amplification-lateral flow dipstick (RPA-LF) have shown promise in detecting *Babesia orientalis* with high sensitivity and specificity, outperforming conventional PCR methods (An et al., 2021). Additionally, the use of multi-epitope recombinant antigens has been explored to enhance the sensitivity of serological tests for diseases like schistosomiasis, indicating a potential for improved diagnostic accuracy (Lv et al., 2018). The development of SYBR Green-based real-time PCR systems for the detection of *Babesia* species further exemplifies advancements in molecular quantification, offering a more precise tool for epidemiological studies (Rehman et al., 2020).

6.2 Integrating molecular diagnostics with veterinary surveillance

Integrating molecular diagnostics with veterinary surveillance systems can significantly enhance disease monitoring and control efforts. The molecular detection and characterization of tick-borne pathogens in water buffaloes, such as *Anaplasma marginale* and *Babesia* species, provide critical data for crafting effective surveillance and prevention programs (Uy et al., 2018; Galon et al., 2019). The use of molecular tools like PCR assays allows for the identification of pathogens at a genetic level, facilitating targeted interventions and improving the overall health management of water buffalo populations (Martucciello et al., 2024).

6.3 Overcoming barriers to widespread implementation in developing regions

Despite the advancements in molecular diagnostics, several barriers hinder their widespread implementation in developing regions. Challenges include the high cost of advanced diagnostic equipment, the need for technical expertise, and limited access to necessary resources. Efforts to overcome these barriers could involve the development of cost-effective diagnostic methods, such as the RPA-LF assay, which is simple to operate and suitable for field conditions (An et al., 2021). Additionally, enhancing local capacity through training and infrastructure development is crucial for the successful adoption of these technologies. Collaborative efforts between research institutions and local governments can facilitate the integration of molecular diagnostics into routine veterinary practices, ultimately improving disease management in water buffalo (Obregón et al., 2016).

In summary, the future of molecular diagnostics for water buffalo diseases lies in the continued development of innovative technologies, their integration with existing surveillance systems, and addressing the challenges of implementation in resource-limited settings. These efforts will contribute to more effective disease control and improved health outcomes for water buffalo populations.

7 Concluding Remarks

The comparative analysis of molecular diagnostics in water buffalo diseases has highlighted several critical insights. Studies have demonstrated the prevalence and genetic characterization of various pathogens, such as *Anaplasma marginale* and *Babesia* species, in water buffalo populations across different regions, including Thailand and the Philippines. The development of advanced diagnostic methods, such as recombinase polymerase amplification for *Babesia orientalis*, has shown increased sensitivity and specificity compared to traditional PCR methods. Additionally, the use of multi-epitope recombinant antigens has improved the sensitivity of serological tests for diseases like schistosomiasis. The identification of miRNA signatures as potential biomarkers for *Brucella abortus* infection further underscores the potential of molecular diagnostics in non-invasive disease detection.

The findings from these studies have significant implications for the management and control of water buffalo diseases. The high prevalence of tick-borne pathogens and the identification of water buffalo as reservoirs for diseases like anaplasmosis and babesiosis suggest a need for targeted surveillance and control measures. The development of rapid and sensitive diagnostic tools, such as the RPA-LF assay for *Babesia orientalis*, offers promising applications for field diagnostics, enabling timely interventions. Furthermore, the use of miRNA biomarkers for *Brucella abortus* could revolutionize non-invasive diagnostic practices, facilitating early detection and management of brucellosis.

To advance molecular diagnostic practices in water buffalo disease management, several recommendations can be made. First, there is a need to standardize and validate novel diagnostic assays, such as multi-epitope antigens and miRNA biomarkers, across different geographical regions and buffalo breeds to ensure their broad applicability and reliability. Second, integrating molecular diagnostics with traditional surveillance methods could enhance the accuracy and efficiency of disease monitoring and control programs. Lastly, fostering collaborations between researchers, veterinarians, and policymakers will be crucial in translating these diagnostic advancements into practical disease management strategies, ultimately improving the health and productivity of water buffalo populations globally.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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