

Evaluating the Efficacy of PCR in Detecting Canine Viral Infections

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Abstract This study evaluates the efficacy of polymerase chain reaction (PCR) techniques in detecting various canine viral infections, including canine parvovirus (CPV), canine distemper virus (CDV), canine coronavirus (CCV), and canine parainfluenza virus (CPIV5). The study demonstrated that PCR-based methods, including nested PCR, real-time quantitative RT-PCR, and multiplex PCR, offer high sensitivity and specificity in detecting canine viral pathogens. For instance, the nested PCR technique was found to be 100 times more sensitive than single PCR in detecting CPV DNA. A duplex real-time quantitative RT-PCR assay for CPIV5 showed superior diagnostic performance compared to previous assays. Additionally, a multiplex nanoparticle-assisted PCR assay was 100-fold more sensitive than conventional PCR in detecting CDV, CPV, and CCV in mixed infections. These methods were validated using clinical samples, showing higher detection rates compared to traditional diagnostic methods. The findings suggest that PCR-based diagnostic assays are highly effective for the rapid, sensitive, and specific detection of canine viral infections. These techniques can significantly improve the accuracy of clinical diagnoses and aid in the timely management of viral diseases in dogs.

Keywords PCR; Canine viral infections; Sensitivity, specificity; Diagnostic assay; CPV; CDV; CCV; CPIV5

1 Introduction

Canine viral infections are a significant concern in veterinary medicine due to their high prevalence and potential severity. These infections can affect various systems in dogs, including the respiratory and gastrointestinal tracts, leading to conditions such as canine parvovirus enteritis, canine distemper, and canine influenza (Piewbang et al., 2016; Gupta et al., 2017; Hao et al., 2019). The complexity of these infections is often compounded by the possibility of co-infections with multiple viral pathogens, which can exacerbate clinical symptoms and complicate treatment strategies (Deng et al., 2018; Hao et al., 2019).

Early and accurate detection of canine viral infections is crucial for effective disease management and control. Rapid diagnosis allows for timely intervention, which can significantly improve clinical outcomes and reduce the spread of infectious agents within canine populations (Decaro et al., 2005; Wang et al., 2017; Hao et al., 2019). Traditional diagnostic methods, such as hemagglutination and conventional PCR, have limitations in sensitivity and specificity, often leading to delayed or inaccurate diagnoses (Uwatoko et al., 1995; Decaro et al., 2005). Therefore, there is a pressing need for more advanced diagnostic tools that can provide reliable and swift detection of these viral pathogens.

Polymerase Chain Reaction (PCR) has emerged as a powerful diagnostic tool in the detection of viral infections due to its high sensitivity, specificity, and rapid turnaround time. Various PCR-based methods, including real-time PCR, multiplex PCR, and isothermal amplification techniques, have been developed to detect multiple canine viruses simultaneously (Deng et al., 2018; Hao et al., 2019; Jeon et al., 2023a). These methods have demonstrated superior performance in identifying viral pathogens at low titers, making them invaluable for early diagnosis and epidemiological surveillance (Piewbang et al., 2016; Gupta et al., 2017; Hao et al., 2019). For instance, multiplex PCR assays can detect several viruses in a single reaction, thereby streamlining the diagnostic process and providing comprehensive insights into the infection status of the patient (Deng et al., 2018; Hao et al., 2019). This research aims to compare the performance of different PCR techniques, including real-time PCR, multiplex PCR, and other novel amplification methods, in terms of sensitivity, specificity, and diagnostic accuracy. By assessing

these parameters, the study seeks to identify the most effective PCR-based diagnostic tools for clinical and epidemiological applications in veterinary practice. The scope of the study encompasses a range of common canine viral pathogens, including but not limited to canine parvovirus, canine distemper virus, and canine influenza virus, to provide a comprehensive evaluation of PCR's diagnostic capabilities.

2 Background on PCR Technology

2.1 Principles of PCR

Polymerase Chain Reaction (PCR) is a widely used technique in molecular biology that allows for the amplification of specific DNA sequences from small amounts of nucleic acid. The process involves repetitive cycles of denaturation, annealing, and extension, which are facilitated by a thermostable DNA polymerase, synthetic oligonucleotide primers, deoxynucleotide triphosphates (dNTPs), and a balanced buffer system. The target DNA can be isolated from various sources, and the reaction is automated to ensure precision and reproducibility. PCR's sensitivity and specificity make it a versatile tool in various clinical and research applications (Pelt-Verkuil and Witt, 2019).

2.2 Evolution of PCR techniques

Since its inception, PCR technology has undergone significant advancements. One notable development is the introduction of quantitative PCR (qPCR), which allows for the quantification of DNA in real-time by measuring the fluorescence emitted during the reaction. This method provides a single data point at the end of each thermal cycle, which can be limiting. However, continuous fluorescence monitoring has been developed to observe the reaction progress in real-time, offering over 100 data points per cycle. This advancement has led to better protocol optimization and enhanced PCR efficiency (Zhang et al., 2019). Another significant evolution is the droplet digital PCR (ddPCR), which encapsulates DNA/RNA in microdroplets, allowing for absolute quantification without a standard curve and high tolerance to PCR inhibitors (Kojabad et al., 2021).

2.3 Advantages of PCR in viral detection

PCR offers several advantages in the detection of viral infections. Its high sensitivity and specificity enable the detection of low levels of viral DNA or RNA, making it a powerful tool for early diagnosis. The method's rapid turnaround time allows for timely clinical decision-making. Additionally, PCR can be used to identify genetic polymorphisms and immune markers, providing insights into the mechanisms of viral infections and host responses (Shahi et al., 2018). The ability to perform absolute quantification with ddPCR further enhances its utility in virology, offering high reproducibility and efficacy (Kojabad et al., 2021).

2.4 Limitations and challenges of PCR

Despite its numerous advantages, PCR is not without limitations. One of the primary challenges is the need for high-purity DNA samples, as contaminants can inhibit the polymerization reaction. The technique's sensitivity also makes it prone to contamination, which can lead to false-positive results. Moreover, conventional qPCR methods provide limited data points per cycle, restricting a thorough understanding of the reaction kinetics. Continuous fluorescence monitoring and ddPCR have addressed some of these issues, but challenges remain in optimizing protocols for different applications (Pelt-Verkuil and Witt, 2019; Zhang et al., 2019). Additionally, the cost and complexity of advanced PCR techniques can be prohibitive for some laboratories, limiting their widespread adoption.

3 PCR in Canine Viral Infection Diagnosis

3.1 Common canine viral infections detected by PCR

3.1.1 Canine parvovirus

Canine parvovirus (CPV) is a highly contagious virus that causes severe gastroenteritis in dogs, particularly puppies. PCR methods, including quantitative real-time PCR (qRT-PCR) and multiplex PCR (mPCR), have been developed to detect CPV with high sensitivity and specificity. For instance, a study demonstrated that a multiplex PCR method could simultaneously detect CPV along with other viruses, providing a rapid and accurate diagnostic tool (Deng et al., 2018; Hao et al., 2019). Another study highlighted the development of a polymerase cross-linking spiral reaction (PCLSR) assay for early and rapid diagnosis of CPV-2, showing higher sensitivity and specificity compared to traditional methods (Xu et al., 2020).

3.1.2 Canine distemper virus

Canine distemper virus (CDV) is another significant pathogen affecting dogs, leading to respiratory, gastrointestinal, and neurological symptoms. Multiplex PCR assays have been designed to detect CDV alongside other viruses, enhancing the diagnostic process for mixed infections (Hao et al., 2019; Wang et al., 2021a). These assays offer a rapid and reliable method for identifying CDV in clinical samples.

3.1.3 Canine adenovirus

Canine adenovirus (CAV) includes two types: CAV-1, which causes infectious canine hepatitis, and CAV-2, associated with respiratory disease. PCR-based methods, including multiplex PCR, have been developed to detect CAV efficiently. These methods can simultaneously identify CAV along with other viral pathogens, streamlining the diagnostic process (Deng et al., 2018; Hao et al., 2019).

3.1.4 Canine coronavirus

Canine coronavirus (CCoV) is a common cause of enteric disease in dogs. PCR techniques, particularly multiplex PCR, have been employed to detect CCoV in conjunction with other viruses, providing a comprehensive diagnostic approach (Deng et al., 2018; Hao et al., 2019; Wang et al., 2021a). These methods are crucial for the rapid identification and management of CCoV infections.

3.1.5 Canine herpesvirus

Canine herpesvirus (CHV) is a significant pathogen in neonatal puppies, causing high mortality rates. While not as commonly included in multiplex PCR panels as other viruses, specific PCR assays have been developed to detect CHV, aiding in the diagnosis and control of this infection.

3.2 Sensitivity and specificity of PCR for different viruses

The sensitivity and specificity of PCR for detecting canine viral infections vary depending on the virus and the specific PCR method used. For example, a study comparing a point-of-care immunochromatographic test kit with PCR for CPV detection found that PCR had higher sensitivity and specificity, making it a more reliable diagnostic tool (Shima et al., 2021). Similarly, multiplex PCR assays have shown high sensitivity and specificity for detecting multiple viruses simultaneously, enhancing diagnostic accuracy (Deng et al., 2018; Hao et al., 2019; Wang et al., 2021a).

3.3 Comparison with other diagnostic methods

PCR is often compared with other diagnostic methods, such as immunochromatographic tests, enzyme-linked immunosorbent assays (ELISA), and lateral flow immunoassays, which are valued for their ability to deliver rapid results. However, these methods generally exhibit lower sensitivity and specificity compared to PCR, limiting their reliability, especially in detecting low-abundance targets. For instance, studies evaluating point-of-care tests for CPV detection consistently found PCR to outperform these methods in both sensitivity and specificity (Shima et al., 2021; Walter-Weingärtner et al., 2021). Furthermore, advancements in PCR technology have enhanced its diagnostic capabilities. Techniques like real-time PCR provide quantitative results, while the combination of PCR with fluorescent lateral flow immunoassay has been shown to produce fast, accurate outcomes, bridging the gap between speed and precision in diagnostics (Zhuang et al., 2019). These advantages underscore the pivotal role of PCR-based methods in modern diagnostic workflows, particularly in clinical and research settings where accuracy and reliability are paramount.

3.4 Factors influencing PCR accuracy in canine viral detection

Several factors can influence the accuracy of PCR in detecting canine viral infections. These include the quality and type of sample collected, the presence of inhibitors in the sample, and the specific PCR protocol used. For example, a study assessing the effect of sampling site on CPV diagnosis found that fecal samples had the highest sensitivity, while blood and pharyngeal samples were also suitable but less sensitive (Segev et al., 2022). Additionally, the use of advanced PCR techniques, such as nanoparticle-assisted PCR and polymerase cross-linking spiral reaction, can enhance the sensitivity and specificity of viral detection (Xu et al., 2020; Wang et al., 2021a).

4 Case Study

4.1 Case study 1: detection of canine parvovirus in a shelter outbreak

In a recent outbreak of canine parvovirus (CPV) at a local animal shelter, the efficacy of quantitative real-time PCR (qRT-PCR) was evaluated for diagnosing CPV infection. Samples were collected from various sites including the rectum, blood, and pharynx of the affected dogs. The study found that all dogs with clinical signs of CPV were positive by qRT-PCR in at least one sampling site, with 83% of the dogs testing positive across all sites. This highlights the sensitivity of qRT-PCR in detecting CPV, although specificity can be temporarily affected during the vaccination period (Segev et al., 2022). Additionally, an improved polymerase cross-linking spiral reaction (PCLSR) assay was utilized, which demonstrated rapid and highly sensitive detection of CPV-2, further confirming the presence of the virus in the shelter outbreak (Figure 1) (Xu et al., 2020).

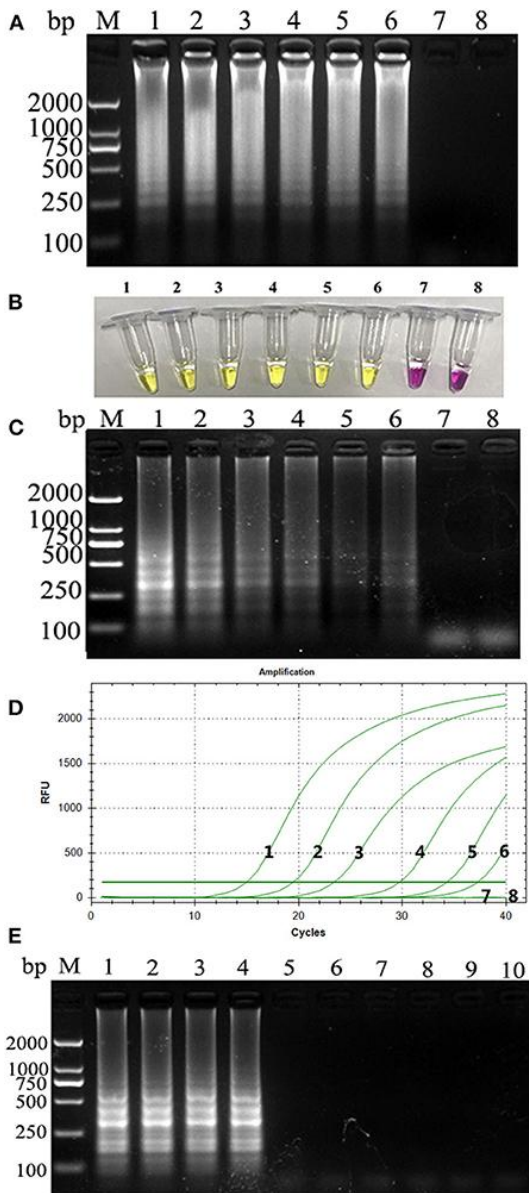


Figure 1 (A) Sensitivity of the CPV-PCLSR amplification; (B) results of indicator discoloration; (C) PSR amplification; (D) qPCR. M, 2 000 DNA ladder marker; 1-7, DNA template with 3.9×10^6 to 100 copies; 8, negative control; (E) specificity of the CPV-PCLSR amplification. M, 2 000 DNA ladder marker; 1, canine parvovirus 2 (CPV-2) from vaccine; 2, CPV-2a; 3, CPV-2b; 4, CPV-2c; 5, CDV; 6, CCov; 7, CCV; 8, Dogav-1; 9, Dogav-2; 10, negative control (Adopted from Xu et al., 2020)

4.2 Case study 2: PCR-based diagnosis of canine distemper virus in a clinical setting

In a clinical setting, a multiplex PCR/RT-PCR assay was employed to diagnose canine distemper virus (CDV) alongside other common canine viruses such as CPV and canine kobuvirus (CaKoV). This one-step multiplex

assay proved to be highly sensitive, with detection limits of 102.1 TCID₅₀ for CDV, 101.9 TCID₅₀ for CPV, and 103 copies for CaKoV. The assay did not amplify nonspecific DNA or RNA from other canine viruses, ensuring accurate diagnosis. This method facilitated rapid clinical detection and epidemiological surveillance of CDV, CPV, and CaKoV, proving to be a valuable tool in the clinical setting (Liu et al., 2018). Furthermore, a pan-genotypic RT-qPCR assay was validated for detecting various genotypes of CDV, which was particularly useful in regions with multiple circulating genotypes, ensuring comprehensive diagnostic coverage (Geiselhardt et al., 2022).

4.3 Lessons learned from case studies

The case studies underscore the importance of using advanced PCR techniques for the accurate and rapid diagnosis of canine viral infections. The qRT-PCR and PCLSR assays demonstrated high sensitivity in detecting CPV, even in complex outbreak scenarios, although specificity can be influenced by recent vaccinations (Xu et al., 2020; Segev et al., 2022). The multiplex PCR/RT-PCR assays provided a robust solution for simultaneous detection of multiple viruses, which is crucial in clinical settings where mixed infections are common (Liu et al., 2018; Hao et al., 2019). The pan-genotypic RT-qPCR assay for CDV highlighted the necessity of having diagnostic tools that can detect a wide range of viral genotypes, ensuring no cases are missed due to genetic variability (Geiselhardt et al., 2022). These findings emphasize the need for continuous improvement and validation of molecular diagnostic methods to enhance the management and control of canine viral infections.

5 Advances in PCR Technology for Canine Viral Detection

5.1 Real-time PCR and its applications

Real-time PCR (qPCR) has revolutionized the detection of canine viral infections by providing rapid, sensitive, and specific diagnostic capabilities. For instance, a duplex real-time quantitative RT-PCR assay was developed for the detection of canine parainfluenza virus 5 (CPIV5) and a canine endogenous internal positive control (EIPC). This assay demonstrated high sensitivity, specificity, and reliability, making it a promising tool for the rapid diagnosis and control of CPIV5 in dogs (Jeon et al., 2023). Additionally, a duplex SYBR Green I-based real-time PCR assay was established for the simultaneous detection of canine kobuvirus (CaKoV) and canine astrovirus (CaAstV), showcasing the ability to distinguish between viruses based on their melting temperatures (Wang et al., 2021b).

5.2 Multiplex PCR for simultaneous detection of multiple viruses

Multiplex PCR (mPCR) techniques have significantly advanced the field by enabling the simultaneous detection of multiple canine viruses in a single reaction. For example, a multiplex real-time RT-PCR assay was developed for the simultaneous detection of four avian respiratory viruses, demonstrating higher sensitivity and specificity compared to singleplex assays (Laamiri et al., 2018). Similarly, a study developed two new mPCRs for the simultaneous detection of canine respiratory and enteric viruses, allowing for rapid investigation and prompt evaluation of co-infections (Hao et al., 2019). Another mPCR method was designed to detect canine parvovirus (CPV-2), canine coronavirus (CCoV), and canine adenovirus (CAV) simultaneously, providing an efficient and accurate tool for clinical diagnosis and laboratory epidemiological investigations (Deng et al., 2018).

5.3 Digital PCR: enhancing sensitivity and quantification

Digital PCR (dPCR) has emerged as a powerful tool for enhancing the sensitivity and quantification of viral DNA. Unlike conventional qPCR, dPCR allows for absolute quantification without the need for a calibration curve. An IoT-integrated multiplexed dPCR (IM-dPCR) system was developed for the quantitative detection of infectious diseases, including canine viruses. This system demonstrated higher sensitivity and specificity, with the ability to analyze multiple RNA groups simultaneously (Choi et al., 2022). Additionally, a study comparing digital RNA quantification with real-time PCR for detecting respiratory syncytial virus (RSV) in children highlighted the high accuracy and robustness of dPCR, indicating its potential for large-scale epidemiological studies (Bouzas et al., 2018).

5.4 Future directions and innovations

The future of PCR technology for canine viral detection lies in the integration of advanced techniques and innovations. The development of IoT-integrated systems, such as the IM-dPCR, enables automatic processing,

data collection, and cloud storage, facilitating onsite detection of infectious diseases (Choi et al., 2022). Furthermore, the use of nanoparticle-assisted PCR (nanoPCR) combined with dual priming oligonucleotide (DPO) systems has shown to significantly enhance the sensitivity and specificity of multiplex assays, as demonstrated in the detection of canine distemper virus (CDV), canine parvovirus (CPV), and canine coronavirus (CCV) (Wang et al., 2021a). These advancements, along with continuous improvements in assay design and technology integration, will further enhance the diagnostic capabilities and efficiency of PCR-based methods for canine viral detection.

6 Clinical Implications and Practical Applications

6.1 Impact on veterinary clinical practice

The implementation of PCR-based diagnostic tools has significantly enhanced the accuracy and speed of detecting canine viral infections in veterinary clinical practice. For instance, the development of a duplex real-time quantitative RT-PCR assay for canine parainfluenza virus 5 (CPiV5) has shown higher diagnostic sensitivity compared to previous methods, thereby reducing the likelihood of false negatives and enabling more reliable diagnoses (Jeon et al., 2023a). Similarly, multiplex PCR methods have been established to detect multiple canine respiratory and enteric viruses simultaneously, which is crucial for timely and effective treatment of co-infections (Hao et al., 2019). These advancements in PCR technology ensure that veterinarians can make more informed decisions, leading to better patient outcomes and more efficient use of resources.

6.2 Role of PCR in outbreak management

PCR assays play a pivotal role in managing outbreaks of canine viral infections by providing rapid and accurate diagnostic capabilities. For example, the multiplex nanoparticle-assisted PCR assay developed for detecting canine distemper virus (CDV), canine parvovirus (CPV), and canine coronavirus (CCV) in mixed infections is 100-fold more sensitive than conventional PCR methods, allowing for early detection and intervention (Wang et al., 2021a). Additionally, the integration of PCR with fluorescent lateral flow immunoassay for CPV-2 detection facilitates quick and quantitative analysis, which is essential for outbreak investigations and timely response (Figure 2) (Zhuang et al., 2019). These tools enable veterinary professionals to swiftly identify and contain viral outbreaks, minimizing the spread of infections and protecting animal health.

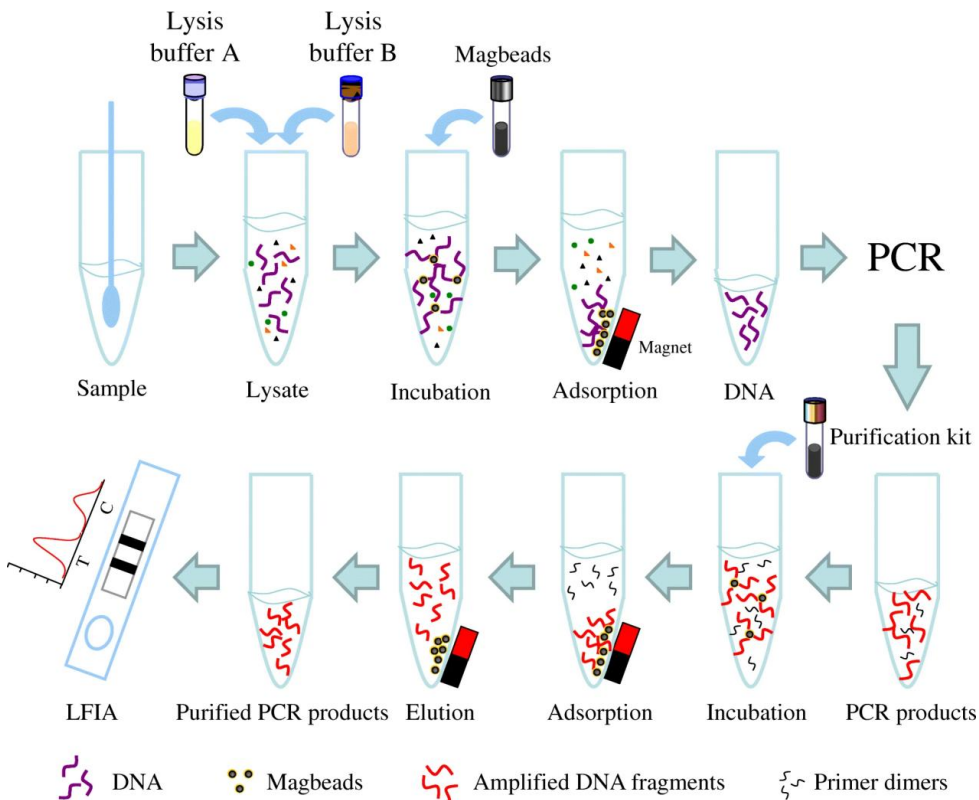


Figure 2 The illustration of PCR-LFIA for detection of CPV-2 (Adopted from Zhuang et al., 2019)

6.3 Integrating PCR with other diagnostic tools for comprehensive care

Integrating PCR with other diagnostic tools enhances the comprehensiveness of veterinary care. For instance, the use of species-specific internal positive controls in multiplex real-time RT-PCR assays for detecting SARS-CoV-2 in companion animals ensures high sensitivity and specificity, avoiding false-negative results and providing reliable diagnoses (Jeon et al., 2023b). Moreover, combining PCR with magnetic purification and fluorescent lateral flow immunoassay for CPV-2 detection offers a rapid and quantitative diagnostic approach, which is crucial for early and accurate disease management (Zhuang et al., 2019). By incorporating these advanced PCR techniques with traditional diagnostic methods, veterinarians can achieve a more holistic understanding of an animal's health status, leading to more effective treatment plans and improved overall care.

7 Challenges and Limitations

7.1 Technical challenges in pcr implementation

Implementing PCR for detecting canine viral infections presents several technical challenges. One significant issue is the sensitivity and specificity of the assays. For instance, while the duplex real-time quantitative RT-PCR assay developed for canine parainfluenza virus 5 (CPIV5) showed high sensitivity and specificity, it required careful optimization of primers and probes to avoid cross-reactivity with other canine pathogens. Additionally, the stability of the internal positive control, such as the canine 16S rRNA, is crucial to avoid false negatives, which can complicate the assay design and validation process (Jeon et al., 2021a). Another technical challenge is the detection of low viral loads, which can lead to false negatives. Digital PCR (ddPCR) has been shown to have higher sensitivity compared to traditional RT-qPCR, making it a more reliable method for detecting low viral loads, but it also requires more sophisticated equipment and expertise (Falzone et al., 2020; Suo et al., 2020; Poggio et al., 2021).

7.2 Cost and accessibility issues

The cost and accessibility of PCR-based diagnostic tools are significant limitations, especially in resource-limited settings. The development and implementation of advanced techniques like ddPCR, which offers higher sensitivity and specificity, are often cost-prohibitive (Falzone et al., 2020; Suo et al., 2020; Poggio et al., 2021). Additionally, the need for specialized equipment and trained personnel to perform these assays can limit their widespread adoption. Multiplex PCR methods, which can detect multiple pathogens simultaneously, offer a cost-effective solution by reducing the number of tests required, but they still require initial investment in technology and training. The high costs associated with these advanced diagnostic tools can be a barrier to their use in routine veterinary practice, particularly in low-income regions.

7.3 Interpretation of PCR results and false positives/negatives

Interpreting PCR results can be challenging due to the potential for false positives and negatives. False negatives can occur due to low viral loads, improper sample collection, or degradation of viral RNA (Falzone et al., 2020; Kucirka et al., 2020; Suo et al., 2020; Poggio et al., 2021). For example, the false-negative rate for SARS-CoV-2 RT-PCR tests varies significantly depending on the time since exposure and symptom onset, highlighting the importance of timing in sample collection (Kucirka et al., 2020). False positives, on the other hand, can result from contamination or non-specific amplification. The use of internal positive controls, such as the canine 16S rRNA, helps mitigate the risk of false negatives by ensuring the integrity of the PCR process, but it does not eliminate the possibility of false positives (Jeon et al., 2021a; Jeon et al., 2021b). Moreover, the diagnostic accuracy of PCR can be influenced by the choice of target genes and the design of primers and probes, which requires careful validation to ensure reliable results (Hao et al., 2019; Jeon et al., 2021a; Jeon et al., 2021b).

8 Future Prospects

8.1 Emerging technologies complementing PCR

The landscape of diagnostic technologies is rapidly evolving, with several emerging technologies showing promise in complementing and enhancing the capabilities of PCR. Next-generation sequencing (NGS) has revolutionized the field of pathogen detection by enabling comprehensive genomic analysis. NGS can detect a wide array of pathogens, including those that are unknown or unexpected, which traditional PCR methods might

miss due to their reliance on prior knowledge of the pathogen's genome (Anis et al., 2018; Wang et al., 2022; Gauthier et al., 2023). Additionally, CRISPR-based diagnostic methods, such as FLASH, have demonstrated significant potential in enriching low-abundance sequences, thereby improving the sensitivity and specificity of pathogen detection (Quan et al., 2018; Brown, 2022). These advancements suggest a future where PCR and NGS, along with CRISPR technologies, work synergistically to provide more accurate and comprehensive diagnostic solutions.

8.2 Potential for PCR in broader veterinary diagnostics

PCR has already established itself as a cornerstone in veterinary diagnostics, particularly for detecting viral infections in canines. However, its potential extends far beyond this application. The integration of targeted NGS with PCR can enhance the detection of a broader range of pathogens, including bacterial, fungal, and parasitic infections, which are often present in mixed infections (Anis et al., 2018; Kattoor et al., 2022). This comprehensive approach can streamline diagnostic workflows, reduce the need for multiple tests, and provide faster, more accurate results. Moreover, the development of multiplex PCR assays can further expand the utility of PCR in veterinary diagnostics by allowing simultaneous detection of multiple pathogens in a single test (Wright et al., 2021; Jeon et al., 2023a). This capability is particularly valuable in managing complex cases of fever of unknown origin (FUO) and other multifactorial diseases in animals.

8.3 Research needs and future directions

Despite the significant advancements in PCR and complementary technologies, several research needs and future directions must be addressed to fully realize their potential in veterinary diagnostics. First, there is a need for the development of more robust and user-friendly bioinformatics tools to handle the vast amount of data generated by NGS and CRISPR-based assays (Cantalupo and Pipas, 2019; Gauthier et al., 2023). These tools should be capable of accurately identifying and classifying pathogens while minimizing false positives and negatives. Second, further research is required to optimize the sensitivity and specificity of these diagnostic methods, particularly for low-abundance pathogens and in complex sample matrices (O'Flaherty et al., 2018; Quan et al., 2018). Additionally, studies focusing on the cost-effectiveness and scalability of these technologies are essential to facilitate their widespread adoption in clinical settings (Anis et al., 2018; Wright et al., 2021). Finally, ongoing surveillance and validation studies are crucial to ensure that these diagnostic tools remain effective against emerging and evolving pathogens, thereby safeguarding animal health and public safety (Brown, 2022; Wang et al., 2022).

9 Concluding Remarks

The research conducted on the efficacy of PCR in detecting canine viral infections has yielded several significant findings. Multiplex PCR (mPCR) methods have been developed to detect multiple canine respiratory and enteric viruses simultaneously, demonstrating high sensitivity and specificity. For instance, one study developed mPCR protocols capable of detecting seven different viruses in clinical samples, with a detection limit of 1×10^4 viral copies, thus enabling rapid and accurate diagnosis of co-infections in dogs. Another study introduced a nanoparticle-assisted PCR assay that was 100-fold more sensitive than conventional PCR methods, further enhancing the detection capabilities for mixed viral infections. Additionally, droplet digital PCR (ddPCR) has been highlighted for its superior sensitivity and accuracy in detecting low-abundance pathogens, making it a promising tool for clinical diagnostics. These advancements underscore the robustness and reliability of PCR-based methods in the veterinary field.

PCR-based methods, including mPCR, nanoPCR, and ddPCR, have proven to be highly effective in the detection of canine viral infections. The high sensitivity and specificity of these methods allow for the rapid and accurate identification of multiple pathogens, which is crucial for timely therapeutic interventions. The development of advanced PCR techniques, such as the duplex real-time quantitative RT-PCR assay and the IoT-integrated multiplexed digital PCR system, further enhances the diagnostic capabilities, providing reliable and comprehensive detection of viral infections in dogs. These methods not only improve the accuracy of diagnosis but also facilitate epidemiological surveillance and the management of viral outbreaks in canine populations.

The advancements in PCR technology have significant implications for veterinary medicine and animal health. The ability to rapidly and accurately diagnose multiple viral infections in dogs enables veterinarians to provide timely and effective treatments, thereby improving the health outcomes for affected animals. The use of PCR-based methods in routine diagnostics can also aid in the early detection and control of viral outbreaks, reducing the spread of infectious diseases within canine populations. Furthermore, the integration of PCR technology with IoT platforms and other advanced diagnostic tools can enhance the monitoring and management of animal health on a larger scale, contributing to better overall disease control and prevention strategies. As PCR technology continues to evolve, its application in veterinary medicine is likely to expand, offering new opportunities for improving animal health and welfare.

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Conflict of Interest Disclosure

The author affirms 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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