REVIEW ARTICLE

CRISPR-Cas-Based Diagnostic Tools for Companion Birds: An Overview

Juana Darlene Virgit J.[1], Deepika Anitha[2], Raja Angamuthu[2]*

Abstract

Though pet bird ownership is increasing substantially, avian medicine faces a unique diagnostic challenge as birds often conceal signs of illness until the disease has progressed beyond treatment possibilities. Current diagnostic tools, such as qPCR and ELISA, are promising but rely heavily on expensive equipment and trained personnel. This results in long turn-around times, which makes them unsuitable for rapidly progressing avian diseases. This review evaluates the potential of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) systems to overcome these limitations. By utilizing the "collateral cleavage" activity of Cas12 and Cas13 nucleases, these platforms offer the sensitivity of qPCR with the speed and portability of point-of-care tools. The review also describes recently validated CRISPR-Cas-based models for poultry diseases such as Avian Leukosis Virus, Infectious Bronchitis Virus, and Duck Hepatitis A Virus and analyses their potential for translation into companion bird diseases. Further, the possibility of CRISPR-Cas to aid in detection of zoonotic threats like Psittacosis and Avian Influenza, alongside a few other critical avian pathogens is described. Despite challenges such as aerosol contamination and sample preparation, CRISPR-Cas systems combined with isothermal amplification represent a significant step toward accessible, lab-quality diagnostics for veterinarians, pet owners, and breeders.

Key words: Avian medicine, Companion birds, CRISPR-Cas, Point-of-care diagnostics, Zoonotic diseases.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.4.01

Introduction

Companion bird ownership has increased substantially in recent years, creating a growing need for effective diagnostic tools in avian medicine. A major unique challenge in diagnosing avian diseases is that birds, as prey species, often conceal clinical symptoms until the disease has progressed to advanced stages. This delay in detection reduces treatment success and increases the risk of disease transmission, particularly in the case of zoonotic infections such as psittacosis and avian influenza (Li *et al*., 2023). The transmission pathways and zoonotic risks of major avian pathogens to human populations are illustrated in Fig. 1.

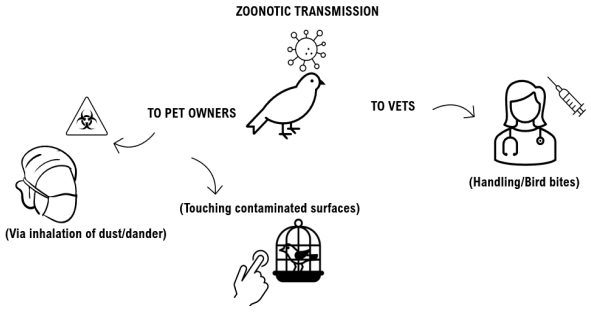

Fig. 1: Transmission pathways and zoonotic risk of avian pathogens to human populations

Current diagnostic methods, including quantitative PCR (qPCR) and enzyme-linked immunosorbent assay (ELISA), are highly sensitive and specific but rely on sophisticated laboratory infrastructure, trained personnel, and extended processing times (Zhang *et al*., 2020; Chen *et al*., 2024). These limitations make them unsuitable for rapid, point-of-care detection, especially in decentralized veterinary settings and among pet bird owners. The effectiveness of conventional diagnostics is further challenged by the rapid genetic evolution of avian pathogens. For instance, a study reported multiple genotypes of infectious bronchitis virus (IBV) in India, including unique emerging variants in chicken flocks, underscoring the need for sensitive and rapid detection tools capable of identifying diverse strains (Raja *et al.,* 2020). Similarly, a PPMV-1 outbreak in doves in Iran revealed a highly velogenic sub-genotype, highlighting viral evolution and cross boundary spread (Esmaeelzadeh-Dizaji *et al*., 2022).

[1]Department of Biotechnology, Anna University, Chennai-600025, Tamil Nadu, India

[2]Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600007, India

Corresponding Author: Dr. Raja A., Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600007, India. e-mail: raja.a@tanuvas.ac.in

How to cite this article: Juana Darlene, V. J., Deepika, A., & Raja, A. (2026). CRISPR-Cas-Based Diagnostic Tools for Companion Birds: An Overview. Ind J Vet Sci and Biotech, 22(4), 1-8.

Source of support: Nil

Conflict of interest: None

Submitted 02/05/2026 **Accepted** 17/05/2026 **Published** 10/07/2026

The occurrence of concurrent viral infections in poultry further complicates diagnosis, emphasizing the need for highly sensitive and specific detection methods (Fenton *et al*., 2005; Chitradevi *et al.,* 2018).

Recent advances in molecular diagnostics have introduced CRISPR-Cas systems as a promising alternative. By leveraging the sequence-specific targeting ability of guide RNAs and the collateral cleavage activity of Cas nucleases, these systems enable rapid, sensitive, and portable detection of nucleic acids. When combined with isothermal amplification techniques, CRISPR-based platforms eliminate the need for thermocycling equipment while maintaining diagnostic accuracy comparable to qPCR (Gootenberg *et al*., 2017; Chen *et al*., 2018). The development of several rapid diagnostic tools involving CRISPR-Cas for poultry diseases provides the perfect validated model for translation into the companion bird sector.

This review evaluates the performance of current established CRISPR-Cas-based diagnostic tools for a few poultry diseases. Further, we analyse the feasibility of using CRISPR for rapid point-of-care detection of companion bird diseases specifically. Finally, these findings are synthesized to identify crucial future targets for CRISPR adaptation, highlighting the potential of this technology to significantly enhance avian veterinary diagnostics.

Current Nucleic Acid-Based Disease Diagnosis

Molecular techniques like PCR and qPCR are the gold standards for avian diagnostics due to their high sensitivity and ability to detect latent infections (Madani and Peighambari, 2012). qPCR is widely used for precise viral load estimation and monitoring infection dynamics in avian diseases (Chitradevi *et al.,* 2024). While these methods provide definitive diagnosis by amplifying DNA or RNA directly from clinical samples, their reliance on specialized equipment and skilled personnel limits their utility in low-resource settings. As an alternative, isothermal amplification techniques such as Recombinase Polymerase Amplification (RPA) and Loop-mediated Isothermal Amplification (LAMP) operate at constant temperatures. RPA utilizes two primers for target amplification, while LAMP employs 4-6 primer pairs to recognize multiple sites within a sequence. LAMP enables rapid, highly sensitive nucleic acid detection under isothermal conditions, with studies demonstrating detection limits as low as 10 copies and high specificity without cross-reactivity to related avian viruses. Importantly, LAMP assays show performance comparable to PCR while eliminating the need for thermocyclers, making them well suited for field-level (Angamuthu *et al.,* 2012). Because these methods eliminate the need for temperature cycling and can provide simple colorimetric readouts, they are frequently integrated with CRISPR-Cas systems. This addresses the need for portable tools that deliver lab-quality diagnostics at the point-of-care without the requirement for sophisticated laboratory infrastructure.

Crispr-Cas for Molecular Diagnostics

Overview of the CRISPR-Cas System

The CRISPR-Cas system is derived from the adaptive immune mechanisms present in bacteria. Nucleases like Cas12a and Cas13a which are guided by RNA, can target single-stranded (ss) and double-stranded (ds) DNA and single-stranded (ss) RNA, respectively. Cas13 forms a complex with CRISPR RNA (crRNA) enabling it to specifically recognize and target ssRNA. This subsequently activates the HEPN domain of Cas13a and cleaves all surrounding ssRNA molecules (Gootenberg *et al*., 2017). This specific cleavage property has been exploited for the detection of diseases. Detection is based on the nonspecific nuclease activity of the Cas12a or Cas13a protein following target recognition mediated by the guide RNA (Chen *et al*., 2018).

Classification of CRISPR-Cas Systems

The CRISPR-Cas system can be broadly classified into two major classes and six distinct types based on the structure of their effector complexes (Makarova *et al*., 2019). Class 1 systems (Types I, III, IV) typically involve large multi-protein effect or complexes for nucleic acid detection and cleavage, making them less suitable for use in portable diagnostics (Hidalgo-Cantabrana and Barrangou, 2020). Unlike the class 1 systems, class 2 systems (Types II, V, VI) utilize one large multi-purpose protein (like Cas9, Cas12, or Cas13) to perform the detection (Makarova *et al*., 2019). Their relatively simple architecture and ability to cleave nearby ss nucleic acids non-specifically, makes them well suited for developing point-of-care diagnostic platforms. Approximately 95% of existing CRISPR-Cas based diagnostic platforms were developed using class II systems (Yang *et al*., 2023).

CRISPR-Cas based Disease Diagnosis

Recently, isothermal DNA amplification methods such as LAMP and RPA have been integrated with the CRISPR-Cas system to create point-of-care test kits with high-accuracy pathogen detection at constant temperatures, eliminating the need for sophisticated laboratory equipment (Mao *et al*., 2023). These systems utilize Cas effectors (Cas9, Cas12, Cas13, and Cas14) guided by crRNA to precisely target and cleave nucleic acids. Notably, Cas12 and Cas13 exhibit "collateral cleavage," where target recognition triggers the non-specific degradation of surrounding single-stranded nucleic acids, transforming molecular signals into quantifiable colorimetric or fluorescent readouts (Li *et al*., 2018, 2019). The mechanism and workflow of CRISPR-Cas-based diagnostic assays are illustrated in Fig. 2. Platforms that leverage this technology include SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking) (Khudeir *et al*., 2024), which pairs RPA with Cas13 for RNA detection, and

DNA-targeting tools like DETECTR and HOLMES) (Li *et al.,* 2018). These assays often incorporate lateral flow dipsticks for rapid visualization, as demonstrated in diagnostics for Nipah and Zika viruses (Gootenberg *et al*., 2017; Miao *et al*., 2023). Lateral flow dipsticks are widely used for rapid, equipment-free visualization in point-of-care diagnostics. For instance, immunochromatographic lateral flow assays have been successfully developed for on-farm detection of viral pathogens, demonstrating reliable sensitivity and specificity while enabling rapid preliminary diagnosis in resource-limited settings (Sambandam *et al.,* 2017). Compared to traditional gold standards like qPCR, CRISPR-based Point-of-Care Testing (POCT) offers comparable sensitivity and specificity while remaining cost-effective and easy to operate, making it ideal for rapid screening and outbreak containment. A comparison between conventional diagnostic methods and CRISPR-Cas systems is summarized in Table 1.

Recently, there has been a surge in the use of the CRISPR-Cas platform as a rapid point-of-care diagnostic tool in the poultry industry. They act as crucial field test models that have the potential for translation into the companion avian section. These platforms exploit isothermal amplification methods like Recombinase-Aided Amplification, Recombinase polymerase amplification, Reverse transcription-Recombinase polymerase amplification, Reverse transcription-enzymatic recombinase amplification, and combine them with Cas nucleases (e.g. Cas12a and Cas13a) to achieve the accuracy and sensitivity of qPCR while providing portability and rapid testing necessary for POCT. This makes CRISPR-Cas based diagnostics crucial for breeders, aviaries, and pet bird owners. Recently developed CRISPR-Cas-based diagnostic platforms for a few poultry diseases are discussed below and summarized in Table 2.

CRISPR-Cas12a based detection of Short beak and dwarfism syndrome virus in waterfowl

Short beak and dwarfism syndrome (SBDS) is a disease caused by the SBDS virus. As the name suggests, the disease is characterized by dwarfism and beak atrophy affecting primarily ducks and geese (Matczuk *et al*., 2020). Current diagnostic methods include virus isolation, Latex agglutination assay, and immunofluorescence assays (Chen *et al*., 2016) and ELISA (Zhang *et al*., 2020) which are cumbersome and time consuming. In a recent study, researchers have developed a diagnostic method to address the need for an accurate and rapid diagnosis of SBDSV. The study resulted in the development of a detection platform that has integrated recombinase-aided amplification with CRISPR-Cas12a. The results could be visualized via fluorescence on lateral flow readout strips (Chen *et al*., 2025). The assay's detection limit was found to be 10 copies/ µL and demonstrated high specificity in distinguishing SBDSV from other genetically related viruses namely, the Muscovy duck-origin goose parvovirus (GPV) and the classical GPV. Furthermore, this distinction could not be achieved by qPCR.

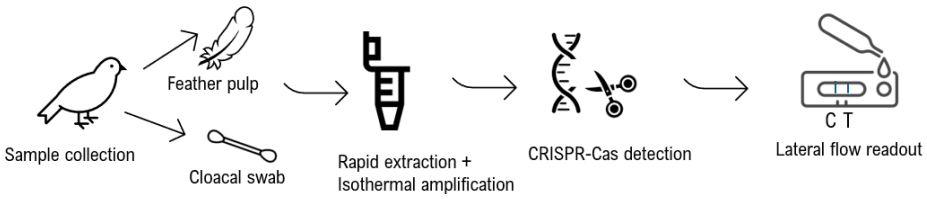

Fig. 2: Workflow for a point-of-Care (POC) CRISPR-Cas diagnostic assay with lateral flow readout.

Table 1: Comparison of traditional diagnostic methods with CRISPR-Cas

Feature	qPCR/ RT -PCR	ELISA	CRISPR-Cas systems
Time to result	2-4 h	4-6 h	30-60 min
Equipment	Thermocycler	Plate reader	Room temperature
Portability	Low	Low	High
Sensitivity	Extremely high	Moderate	High (equivalent to qPCR/ RT-PCR)
Visual readout	Digital	Colorimetric/Digital	Lateral flow (Dipstick)

Table 2: Summary of CRISPR-Cas based detection platforms for avian pathogens

Pathogen	Host	CRISPR type	Amplification method	Limit of detection (LoD)	Time	Reference
SBDSV	Waterfowl	Cas12a	RPA	10 copies/µL	~40 min	Chen *et al*., 2025
ALV	Chicken	Cas13a	RAA	100 copies/µL	40 min	Chen *et al*., 2024
C. psittaci	Psittacines	Cas12b	LAMP	aM	< 60 min	Wang *et al*., 2023
PPMV - 1	Pigeons	Cas12a	RAA	~10 copies/µL	50 min	Liang *et al*., 2024
AIV (H5N1)	General avians	Cas13a	RAA	copies/ µL	40 min	Li *et al*., 2023

CRISPR-Cas13a for Avian leukosis detection in chickens

One of the causative agents of cancer in chickens is the Avian leukaemia virus (ALV). Current methods of ALV detection include viral culture and isolation, ELISA and molecular techniques like PCR, qPCR, and LAMP. In a study by Chen *et al*. (2024), a rapid detection method for avian leukaemia virus was developed by integrating RAA with the CRISPR-Cas13 system. When the crRNA recognizes the target viral sequence, Cas13a cleaves reporting probes to produce a visible result on a lateral flow dipstick. To validate its efficacy, the RAA-CRISPR-Cas13a-LFD technique was benchmarked against the traditional PCR-agarose electrophoresis and qPCR using clinical samples. Further, the method showed good specificity and no cross-reactivity with other viruses like the Marek's disease virus (MDV), Fowl adenovirus (FAdV), New Castle disease virus (NDV), Infectious bronchitis virus (IBV), Infectious laryngotracheitis virus (ILTV) and Infectious bursal disease virus (IBDV). The platform also demonstrates superior performance by achieving a sensitivity threshold of 100 copies/ µL and a 10,000-fold increase over traditional PCR methods. Beyond its high sensitivity, the assay proved to be highly robust and reproducible. Results are produced within 40 min, and the crRNA-guided Cas13a activation ensures specificity to target.

CISPR-Cas13a based assay for Infectious bronchitis detection in poultry

Infectious bronchitis (IB) occurs in both vaccinated and unvaccinated chickens due to emergence of new variants. This highlights the need for accurate detection methods for the prevention of disease (Khudeir *et al*., 2024). Prior to CRISPR-based platforms, isothermal amplification methods such as reverse transcription loop-mediated isothermal amplification (RT-LAMP) were widely used for rapid detection of infectious bronchitis virus, demonstrating high sensitivity and specificity along with simple visual readouts suitable for field conditions (Chandrasekar *et al.,* 2015). Building on these principles, CRISPR-Cas13-based systems such as SHERLOCK integrate isothermal amplification with RNA-targeting nucleases to enable highly sensitive and rapid detection of IBV. A study developed an assay using CRISPR for IBV detection (Khudeir *et al*., 2024). According to the study, reverse transcription-Recombinase polymerase amplification (RT-RPA) coupled with CRISPR-Cas13 (SHERLOCK) was used for the first time to detect and visualize IBV. This novel detection platform was evaluated for its turn-around time, specificity, and sensitivity. The results revealed that the assay was highly selective and specific for IBV and showed no false positives or cross reactions. Another interesting aspect of the assay was that no instrument was used, and viral nucleic acid amplification was done at room temperature.

CRISPR-Cas12a for Muscovy duck parvovirus detection in *Cairina moschata*

The highly contagious Muscovy duck parvovirus is the causative agent of the Muscovy duck parvovirus disease, exclusively affecting *Cairina moschata* (Muscovy ducks) (Li *et al*., 2021). An equipment free CRISPR-based detecting platform for the Muscovy duck parvovirus was developed by Liang *et al*. (2025). It involved the coupling of RPA with CRISPR-Cas12a. The visualization was via a lateral flow readout strip. The platform achieved isothermal amplification of the viral nucleic acid at 37°C within 35 min while achieving high sensitivity, specificity, and no cross reactivity. This eliminated the need for a thermocycler or technical expertise. Validation of results was done using 98 clinical samples. This showed that the result obtained from this platform was 98.98% on par with that of qPCR. This highlights the reliability of this platform.

CRISPR-Cas12a for Hepatitis A virus detection in ducks

Another viral infection affecting ducks is the duck hepatitis A virus. Current detection methods include PCR, q-PCR and ELISA. In a study, researchers have engineered a diagnostic platform that integrates reverse transcription enzymatic recombinase amplification with Cas12a-for detection. This system utilizes both fluorescence-based assays and lateral flow strips for the detection and identification of DHAV-1 (Sun *et al*., 2025). The limit of detection for the platform was 10 copies/µL and could effectively differentiate DHAV-1 from other avian pathogens. The platform is user-friendly ideal for point-of-care detection. The results obtained were also consistent with that of RT-PCR.

These CRISPR-based platforms validated in poultry, which target pathogens like SBDSV, ALV, and IB provide technical blueprints for the companion bird sector. These models demonstrate that Cas12/13 collateral cleavage, combined with isothermal amplification, delivers lab-quality sensitivity without laboratory infrastructure. Specifically, the success of RPA-CRISPR-Cas12a in waterfowl offers a direct framework for detecting clinically similar pet bird infections, such as the Psittacine Beak and Feather Disease (PBFD). Furthermore, instrument-free assays like SHERLOCK enable rapid screening for backyard flocks and household aviaries, for proactive management of high-mortality viral threats.

Limitations of CRISPR-Cas Based Disease Diagnosis

Despite its advantages, several challenges hinder the widespread adoption of CRISPR-Cas-based diagnostic platforms. A primary drawback is the susceptibility of isothermal amplification (RPA/LAMP) to aerosol contamination, which can lead to false positives and reduced diagnostic reliability (Hu *et al*., 2022). Additionally, the requirement for a stable temperature for nucleic acid

extraction remains a hurdle, and the inability to directly process raw samples complicates point-of-care applications.

Standardization also poses a challenge due to variables such as sample type, sample preparation, reaction conditions, signal output interpretation, inhibitors, and contaminants (Rasool *et al*., 2024). Furthermore, CRISPR-Cas systems are often dependent on specific protospacer-adjacent motif (PAM) sequences, which can restrict targetable regions within a pathogen's genome and complicate assay design. Addressing these factors is essential for ensuring the consistency and the reliability of results.

Important Diseases of Companion Birds

1. Psittacosis

Psittacosis, alternatively known as avian chlamydiosis or ornithosis, is a zoonotic infection caused by the bacterial pathogen *C. psittaci* (Dembek *et al*., 2023). The disease primarily affects psittacines like budgerigars, parrots, cockatiels, lovebirds, and macaws. The disease is often underreported as infected birds usually mask the symptoms until the disease has progressed to its final stages. Infected birds can sometimes appear healthy but can shed the bacterium. The disease is highly contagious and is fatal if left untreated (Dembek *et al*., 2023). Current diagnostic methods include cell culture, qPCR, and ELISA. In recent times, data from metagenomic sequencing is use for diagnostic confirmation of the disease (Shi *et al*., 2021). Since the disease is also highly zoonotic, a CRISPR-Cas based diagnostic tool could provide rapid, and accurate results, allowing prompt decision-making which is an advantage for both the bird and the owner. The development of Cas12-based assays specifically targeting the ompA gene of *C. psittaci* represents a significant leap in managing this zoonotic threat (Wang *et al*., 2023).

2. Avian Influenza

Avian influenza (AI) also known as the "bird flu" is a contagious disease affecting the respiratory system of birds (Ayuti *et al*., 2024). Though avian influenza is often associated with poultry, psittacine species are also prone to different strains of the AI virus. The disease can be difficult to detect, as its symptoms resemble those of other avian diseases, such as infectious bronchitis, infectious laryngotracheitis, and Newcastle disease (Korteweg and Gu, 2008). Molecular characterization of local ILTV isolates has further highlighted the complexity of these respiratory outbreaks, emphasizing the necessity for high-resolution diagnostic tools to differentiate between clinically similar viral pathogens (Ponnusamy *et al.,* 2022). An advanced molecular diagnostic tool like CRISPR-Cas paves the way for accurate detection of the disease enabling timely intervention. This also proves to be useful for humans in close contact with birds, as avian influenza is zoonotic and contagious (Peiris *et al*., 2007). A CRISPR-Cas13 based diagnostic platform is particularly suited for AI because it directly targets the viral RNA without the need for complex cDNA synthesis. This allows bird owners and veterinarians to screen for the virus in minutes.

3. Bornavirus

Post-infection, avian bornavirus typically leads to high mortality. Some birds remain asymptomatic, acting as life-long carriers that facilitate persistent environmental shedding (Rubbenstroth, 2022). In a study, horizontal transmission of the disease was not seen after co-housing healthy cockatiels with those artificially inoculated with PaBV-2- or PaBV-4- for five months, thus implying that infected birds could be free of detectable virus for extended periods (Piepenbring *et al*., 2016). Infected birds are known for intermittent shedding of the virus via droppings or feather pulp (Rubbenstroth, 2022). Currently, RT-PCR assays are used for detection. However, due to the genetic diversity of the bornavirus, RT-qPCR based bornavirus detection can become unreliable (Sigrist *et al*., 2021) emphasizing the need for an advanced platform like CRISPR-Cas which can provide rapid, reliable results without the dependence of a thermocycler. Further, the robustness of CRISPR against inhibitors is a significant advantage for processing feather or faecal samples with the shed virus.

4. Psittacine Beak and Feather Disease (PBFD)

PBFD is caused by the highly contagious beak and feather disease virus (BFDV), affecting psittacine species which are often kept as pets. The virus is stable and known to survive in the surroundings of captive birds. This facilitates long term transmission of the disease either through direct contact or exposure to the contaminated environment (Kang *et al*., 2025). Beyond environmental stability, the pathogenesis of avian viral infections is often influenced by specific tissue tropism and host-virus interactions. For instance, chicken anaemia virus has been shown to preferentially target bone marrow and lymphoid tissues, highlighting the role of cellular tropism in disease progression (Suohu *et al.,* 2025). Similarly, BFDV primarily infects feather follicles and immune tissues, contributing to immunosuppression and persistent infection. An advanced molecular technique like CRISPR-Cas could overcome known limitations of conventional detection methods, and help with a faster, simpler, and cost-effective diagnosis. This could potentially help pet bird owners to test new birds before introducing them into aviaries to prevent mass infections.

5. Polyomavirus

Budgerigars, typically adult birds are often the carriers of the polyoma virus and shed the virus intermittently. This makes them responsible for the spread of the virus in nests, ultimately causing death of the offspring (Padzil *et al*., 2017). Currently, PCR is used as the confirmatory test for diagnosis. Though accurate, a CRISPR-Cas based tool could help breeders and pet store personnel to promptly check for the infection in birds before introducing them into aviaries to prevent infection from spreading to susceptible chicks.

Crispr-Cas Based Companion Bird Disease Diagnosis

1. CRISPR-Cas12b for detection of *Chlamydia psittaci*

In a study by Wang *et al*. (2023), researchers have developed two methods leveraging the LAMP-CRISPR-Cas12b assay for *C. psittaci* detection. Both methods yield results within 1 h and have a limit of detection of 102 aM. This was found to be 10-fold higher than that of LAMP. The performance of the assays was on par with qPCR and yielded a sensitivity of 93.8% and a perfect specificity of 100%.

2. RAA/CRISPR-Cas12a based assay for detection of Newcastle disease in Pigeons

A rapid point-of-care test kit involving the CRISPR-Cas system has been created particularly for PPMV-1 L (Liang *et al*., 2024). The kit involves combining RAA and CRISPR-Cas12a and the results can be obtained in just 50 min. The primers used for RAA are designed to amplify conserved regions within the L gene. This is followed by subsequent detection by the CRISPR-Cas12a system. Results are then visualized via a lateral flow dipstick. This diagnostic kit demonstrates high specificity and exhibits zero cross-reactivity with samples negative for PPMV-1. The results were also consistent with q PCR results.

3. *CRISPR*-Cas13 a for Avian Influenza detection

A recent study has developed a diagnostic tool for the H5 subtype of Avian Influenza which combines CRISPR-Cas13a with RAA (Li *et al*., 2023). The platform's detection limit was 0.1 copy/μL as well as no cross reactivity with other subtypes of Avian Influenza. The results were also comparable to qPCR. Furthermore, the results can be visualized in a lateral flow readout strip, and the entire process could be completed in 40 min at 37°C. The platform proves to be convenient for the accurate detection of the H5 subtype of avian influenza.

Currently, CRISPR-Cas based diagnostics have only been focussed on for high priority zoonotic diseases like Psittacosis and Avian Influenza. However, the use of CRISPR-Cas based diagnostic tools to ease the burden of other pet bird diseases like Avian Herpes virus, Polyoma virus, and Avian Borna virus have not been explored yet. Potential CRISPR-Cas diagnostic targets for high-priority companion bird diseases are outlined in Table 3.

Conclusions

In conclusion, this review supports the readiness of CRISPR-Cas systems to be used as rapid diagnostic tools for avian diseases. The successful development of several CRISPR-based platforms for poultry diseases, which exploits the collateral cleavage property of Cas12a and Cas13a nucleases combined with isothermal amplification processes, demonstrates a robust model for use in companion bird settings. These systems have shown to overcome limitations of traditional diagnostic methods, achieving the sensitivity and specificity of qPCR, while reducing time and dependence on a laboratory. Moreover, this platform makes it hassle free for use by pet owners at home due to the platform's portability and simple lateral flow read out systems. The sample requirement for CRISPR-Cas includes cloacal swabs, feather pulp, droppings, cage surfaces, and water, which are easy to collect. Importantly, these samples are well suited for CRISP/Cas systems as the platform can tolerate crude extracts, work with low amount of nucleic acid, and require minimal pre-processing. In addition to technological limitations, increasing evidence suggests that host-virus interactions are highly complex. Emerging studies indicate that host regulatory elements such as long non-coding RNAs can modulate viral infection and gene expression, adding further layers of complexity to disease progression and detection. Currently, CRISPR-Cas based diagnostics have only been focussed on for high priority zoonotic diseases like Psittacosis and Avian influenza. However, the use of CRISPR-Cas based diagnostic tools to ease the burden of other pet bird diseases have not been explored yet. Potential CRISPR-Cas diagnostic targets for high-priority companion bird diseases represent the next crucial area for the adaptation of CRISPR-based diagnostics. Developing point-of-care test kits for these high mortality pathogens is essential for early intervention and curbing outbreaks within aviaries and household companion birds. The use of CRISPR-Cas based diagnostics is promising to improve avian care by shifting from reactive disease management to proactive surveillance. Furthermore, future research could focus on multiplexing to detect multiple pathogens simultaneously. This potentially reduces cost and makes these tools accessible to veterinarians and pet bird owners alike.

Table 3: Potential CRISPR-Cas diagnostic targets for high-priority companion bird diseases

Target Pathogen	Disease	Need for CRISPR	Suggested approach
Avian Borna virus	PDD (Proventricular Dilatation Disease)	High mortality, intermittent shedding of virus requires high sensitivity.	RT-RPA + Cas13a (RNA target)
Psittacine Circo virus	PBFD (Beak and Feather Disease)	Highly contagious in aviaries, needs rapid onsite screening	RPA + Cas12a (DNA target)
Pacheco's Disease	Avian Herpesvirus	Rapid progression, birds often die before lab results return.	LAMP + Cas12a
Avian Polyoma virus	APV	Affects neonates; requires non-invasive field testing (feathers).	RPA + Cas12a

References

Angamuthu, R., Baskaran, S., Gopal, D.R., Devarajan, J., & Kathaperumal, K. (2012). Rapid detection of the marek's disease viral genome in chicken feathers by loop-mediated isothermal amplification. *Journal of Clinical Microbiology*, *50*(3), 961-965.

Ayuti, S.R., Khairullah, A.R., Lamid, M., Al-Arif, M.A., Warsito, S.H., Silaen, O.S.M., Moses, I.B., Hermawan, I.P., Yanestria, S.M., Delima, M., Ferasyi, T.R., & Aryaloka, S. (2024). Avian influenza in birds: Insights from a comprehensive review. *Veterinary World*, *17,* 2544-2555.

Chandrasekar, A., Raja, A., Dhinakar Raj, G., Thangavelu, A., & Kumanan, K. (2015). Rapid detection of avian infectious bronchitis virus by reverse transcriptase-loop mediated isothermal amplification. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*(3), 815-820.

Chen, J.S., Ma, E., Harrington, L.B., Da Costa, M., Tian, X., Palefsky, J.M., & Doudna, J.A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, *360*(6387), 436-439.

Chen, S., Li, Y., Liao, R., Liu, C., Zhou, X., Wang, H., Wang, Q., & Lan, X. (2024). Detection of avian leukosis virus subgroup J (ALV-J) using RAA and CRISPR-Cas13a combined with fluorescence and lateral flow assay. *International Journal of Molecular Sciences*, *25*(19), 10780.

Chen, S., Wang, S., Cheng, X., Xiao, S., Zhu, X., Lin, F., Wu, N., Wang, J., Huang, M., Zheng, M., Chen, S., & Yu, F. (2016). Isolation and characterization of a distinct duck-origin goose parvovirus causing an outbreak of duckling short beak and dwarfism syndrome in China. *Archives of Virology*, *161*(9), 2407-2416.

Chen, X., Zhang, S., Lin, S., Huang, M., Chen, S., & Wang, S. (2025). From lab to field: Innovative RPA–CRISPR-Cas12a platform for early short-beak and dwarfism syndrome virus nucleic acids detection. *Poultry Science*, *104*(7), 105191.

Chitradevi, S., Sukumar, K., Suresh, P., Balasubramaniam, G.A., Kannan, D., & Raja, A. (2024). Quantitative profiling of fowl adenoviral load in experimentally infected chicken using SYBR Green real time PCR. *Veterinarski Arhiv*, *95*(1), 39-50.

Chitradevi, S., Sukumar, K., Suresh, P., Balasubramaniam, G.A., Kannan, D., & Raja, A. (2018). Concurrent infections associated with fowl adenovirus in commercial broiler chicken. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, *39*(1), 20.

Dembek, Z.F., Mothershead, J.L., Owens, A.N., Chekol, T., & Wu, A. (2023). Psittacosis: An underappreciated and often undiagnosed disease. *Pathogens*, *12*(9), 1165.

Esmaeelzadeh-Dizaji, R., Molouki, A., Hosseini, H., Fallah-Mehrabadi, M.H., Ziafati-Kafi, Z., Takalou, A., Eram, N., Kumar, N., Ashuri, A., Sadri, N., & Ghalyanchi-Langeroudi, A. (2022). Molecular characterization of a pigeon paramyxovirus type 1 virus isolated from Eurasian collared doves in Iran, 2017. *Journal of Veterinary Science*, *23*(3), e29.

Fenton, S.P., Reddy, M.R., & Bagust, T.J. (2005). Single and concurrent avian leukosis virus infections with avian leukosis virus-J and avian leukosis virus A in Australian meat-type chickens. *Avian Pathology*, *34*(1), 48-54.

Gootenberg, J.S., Abudayyeh, O.O., Lee, J.W., Essletzbichler, P., Dy, A.J., *et al.* (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, *356*(6336), 438-442.

Hidalgo-Cantabrana, C., & Barrangou, R. (2020). Characterization and applications of Type I CRISPR-Cas systems. *Biochemical Society Transactions*, *48*(1), 15-23.

Hu, F., Liu, Y., Zhao, S., Zhang, Z., Li, X., Peng, N., & Jiang, Z. (2022). A one-pot CRISPR-Cas13a-based contamination-free biosensor for low-cost and rapid nucleic acid diagnostics. *Biosensors and Bioelectronics*, *202*, 113994.

Kang, E.G., Han, J.H., Shim, Y.J., Lee, D.N., Choi, K.S., & Yeon, S.C. (2025). Psittacine beak and feather disease: Global spread, international trade, and conservation challenges. *Animals*, *15*(20), 2947.

Khudeir, M.A., Alsultan, A.S., & Khudhair, Y. I. (2024). Novel CRISPR-Cas13- based assay for diagnosis of avian infectious bronchitis. *Iraqi Journal of Veterinary Sciences*, *38*(1), 63-69.

Korteweg, C., & Gu, J. (2008). Pathology, molecular biology, and pathogenesis of avian influenza A (H5N1) infection in humans. *The American Journal of Pathology*, *172*(5), 1155-1170.

Li, K.P., Hsu, Y.C., Lin, C.A., Chang, P.C., Shien, J.H., Liu, H.Y., Yen, H., & Ou, S.C. (2021). Molecular characterization and pathogenicity of the novel recombinant Muscovy duck parvovirus isolated from geese. *Animals*, *11*(11), 3211.

Li, S.Y., Cheng, Q.X., Liu, J.K., Nie, X.Q., Zhao, G.P., & Wang, J. (2018). CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. *Cell Research*, *28*(4), 491-493.

Li, Y., Li, S., Wang, J., & Liu, G. (2019). CRISPR-Cas systems towards next-generation biosensing. *Trends in Biotechnology*, *37*(7), 730-743.

Li, Y., Shang, J., Luo, J., Zhang, F., Meng, G., Feng, Y., Jiang, W., Yu, X., Deng, C., Liu, G., & Liu, H. (2023). Rapid detection of H5 subtype Avian influenza virus using CRISPR Cas13a based-lateral flow dipstick. *Frontiers in Microbiology*, *14,* 1283210.

Liang, L., Wang, D., Gao, Z., Tang, J., Li, X., Ren, P., Wang, Y., Gao, S., Wu, X., Guo, Y., Yang, B., & Li, J. (2024). RAA-CRISPR-Cas12a-mediated rapid, sensitive, and onsite detection of Newcastle disease in pigeons. *Veterinary Sciences*, *11*(10), 473.

Liang, Q., Chen, W., Wang, W., Liu, R., Fu, Q., Fu, G., Cheng, L., Jiang, N., Chen, H., & Huang, Y. (2025). Development of a rapid on-site nucleic acid detection method for new genotype Muscovy duck parvovirus based on RPA-CRISPR-Cas12a. *Frontiers in Veterinary Science*, *12*.

Madani, S.A., & Peighambari, S.M. (2012). PCR-based diagnosis, molecular characterization and detection of atypical strains of avian Chlamydia psittaci in companion and wild birds. *Avian Pathology*, *42*(1), 38-44.

Makarova, K.S., Wolf, Y.I., Iranzo, J., Shmakov, S.A., Alkhnbashi, O.S., *et al.* (2019). Evolutionary classification of CRISPR-Cas systems: A burst of class 2 and derived variants. *Nature Reviews Microbiology*, *18*(2), 67-83.

Mao, X., Xu, M., Luo, S., Yang, Y., Zhong, J., Zhou, J., Fan, H., Li, X., & Chen, Z. (2023). Advancements in the synergy of isothermal amplification and CRISPR-Cas technologies for pathogen detection. *Frontiers in Bioengineering and Biotechnology*, *11,* 1273988.

Matczuk, A.K., Chmielewska-Władyka, M., Siedlecka, M., Bednarek, K.J., & Wieliczko, A. (2020). Short beak and dwarfism syndrome in ducks in Poland caused by novel goose parvovirus. *Animals*, *10*(12), 2397.

Miao, J., Zuo, L., He, D., Fang, Z., Berthet, N., Yu, C., & Wong, G. (2023). Rapid detection of Nipah virus using the one-pot RPA-CRISPR-Cas13a assay. *Virus Research*, *332*, 199130.

Padzil, F., Mariatulqabtiah, A.R., & Abu, J. (2017). Avian Polyoma virus: A recent update. *Journal Veterinary Malaysia*, *None*, 9-13.

Peiris, J.S. M., de Jong, M.D., & Guan, Y. (2007). Avian influenza virus (H5N1): A threat to human health. *Clinical Microbiology Reviews*, *20*(2), 243-267.

Piepenbring, A. K., Enderlein, D., Herzog, S., Al-Ibadi, B., Heffels-Redmann, U., Heckmann, J., Lange-Herbst, H., Herden, C., & Lierz, M. (2016). Parrot Bornavirus (PaBV)-2 isolate causes different disease patterns in cockatiels than PaBV-4. *Avian Pathology*, *45*(2), 156-168.

Ponnusamy, P., Sukumar, K., Raja, A., Saravanan, S., Srinivasan, P., & Thangavelu, A. (2022). Characterization of infectious laryngotracheitis virus isolates from laying hens during 2019–2020 outbreaks in Tamil Nadu, India. *Archives of Virology*, *167*(9), 1819-1829.

Raja, A., Dhinakar Raj, G., & Kumanan, K. (2020). Emergence of variant avian infectious bronchitis virus in India. *Iranian Journal of Veterinary Research*, *21*(1), 33-39.

Rasool, H.M.H., Chen, Q., Gong, X., & Zhou, J. (2024). CRISPR /Cas system and its application in the diagnosis of animal infectious diseases. *The FASEB Journal*, *38*(24), e70252.

Rubbenstroth, D. (2022). Avian bornavirus research - A comprehensive review. *Viruses*, *14*(7), 1513.

Sambandam, R., Angamuthu, R., Kanagaraj, V., Kathaperumal, K., Chothe, S.K., Nissly, R.H., Barry, R.M., Jayarao, B.M., & Kuchipudi, S.V. (2017). An immuno-chromatographic lateral flow assay (LFA) for rapid on-the-farm detection of classical swine fever virus (CSFV). *Archives of Virology*, *162*(10), 3045-3050.

Shi, Y., Chen, J., Shi, X., Hu, J., Li, H., Li, X., Wang, Y., & Wu, B. (2021). A case of chlamydia psittaci caused severe pneumonia and meningitis diagnosed by metagenome next-generation sequencing and clinical analysis: A case report and literature review. *BMC Infectious Diseases*, *21*(1), 621.

Sigrist, B., Geers, J., Albini, S., Rubbenstroth, D., & Wolfrum, N. (2021). A new multiplex real-time RT-PCR for simultaneous detection and differentiation of avian bornaviruses. *Viruses*, *13*(7), 1358.

Sun, D., Zhu, Y., Wang, M., Wang, J., Cheng, W., Li, Z., Deng, Y., Ou, X., Jia, R., Chen, S., Zhu, D., Liu, M., Zhao, X., Yang, Q., Wu, Y., Zhang, S., Huang, J., He, Y., Wu, Z., & Cheng, A. (2025). A RT-ERA-CRISPR-Cas12a assay for rapid point-of-care duck hepatitis A virus detection. *Poultry Science*, *104*(8), 105316.

Suohu, S., Balasubramaniam, G.A., Arulmozhi, A., Gopalakrishnamurthy, T.R., & Raja, A. (2025). Determination of tissue tropism of chicken anaemia virus on chicken embryos: A molecular insight. *Journal of Veterinary Science*, *26*(3), e17.

Wang, R., Mao, X., Xu, J., Yao, P., Jiang, J., Li, Q., & Wang, F. (2023). Engineering of the LAMP-CRISPR-Cas12b platform for Chlamydia psittaci detection. *Journal of Medical Microbiology*, *72*(12), 1781.

Yang, H., Zhang, Y., Teng, X., Hou, H., Deng, R., & Li, J. (2023). CRISPR-based nucleic acid diagnostics for pathogens. *TrAC Trends in Analytical Chemistry*, *160*, 116980.

Zhang, S., Yang, J., Wang, Z., Chen, L., Diao, Y., & Tang, Y. (2020). Research Note: Development of an ELISA to distinguish between goose parvovirus infection and vaccine immunization antibodies. *Poultry Science*, *99*(3), 1332-1340.