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Canine papillomavirus: status of diagnostic methods and vaccine innovations

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Abstract

Canine Papillomavirus (CPV) is a prevalent viral infection in dogs, characterized by the formation of benign warts or papillomas on the skin and mucous membranes. While most CPV types result in non-malignant growths, certain strains, particularly in immunocompromised dogs (e.g., sick or elderly animals), can lead to malignant transformations. This highlights the need for early, accurate diagnosis, alongside preventive vaccination, to manage the disease effectively. Diagnostic methods leverage CPV's unique characteristics, including histopathology with hematoxylin and eosin (H&E) staining for assessing neoplastic tissue growth and cytopathy, molecular techniques like polymerase chain reaction (PCR), rolling circle amplification (RCA), DNA in situ hybridization (ISH), and next-generation sequencing (NGS) for detecting CPV genomic DNA, immunohistochemistry (IHC) and enzyme-linked immunosorbent assay (ELISA) for identifying viral antigen such as the L1 protein, as well as transmission electron microscopy (TEM) for visualizing viral particles in lesions. These approaches display appreciable sensitivity and specificity and are often utilized in CPV research, though they also have certain intrinsic limitations, such as accessibility, technical complexity. Advancements in CPV vaccine development, including inactivated, live-attenuated, DNA-based, and recombinant protein-based formulations, show promise in achieving effective protection. However, a commercially available vaccine has yet to be developed. Furthermore, challenges persist in developing convenient, cost-effective diagnostics suitable for diverse clinical applications and in formulating affordable, cross-protective vaccines. This review emphasizes the importance of continued innovation in CPV diagnostics and vaccine development to mitigate both benign and malignant papillomatosis, enhance disease prevention, and safeguard canine health.

Keywords Canine papillomavirus (CPV), Diagnostic techniques, Vaccine development, Viral oncogenesis, Canine health management

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Introduction

Papillomaviruses (PVs) are a family of circular double-stranded DNA viruses that primarily infect epithelial cells, leading to benign or malignant tumors in the skin and mucous membranes of vertebrates [1]. While PVs have a broad host range, they exhibit strict species-specific tropism [2, 3]. Canine papillomavirus (CPV) infects dogs via contact with infected skin or mucosa and manifests in two clinical forms: low-risk types linked to benign warts and high-risk types associated with squamous cell carcinoma [4]. CPV infections typically present as oral papillomas, cutaneous papillomas, or viral pigmented plaques, with disease severity influenced by host immune status [5]. Immunocompromised dogs, such as those sick or elderly individuals, are at greater risk of malignant transformations [6], further underscoring the importance of early diagnosis and effective preventive measures to mitigate progression.

The prevalence of CPV infections varies by region and remains less systematically explored compared to human papillomavirus (HPV), yet it has a significant impact on canine health. For instance, a Swiss study detected CPV DNA in over 50% of oral and fingertip skin cell samples from healthy dogs [7], while a U.S. research identified CPV DNA in canine tissue samples, including nasal swabs, with a detection rate of 5.3%[(5)]. Research from Brazil, Switzerland and South Africa revealed diverse CPV genotypes and distinct infection rates across different populations [8, 9]. These findings highlight CPV's global prevalence and its significance in canine health.

Early detection of CPV is crucial, particularly for high-risk genotypes, as it facilitates timely intervention and reduces the risk of transmission. Diagnostic methods target various aspects of CPV, including cytopathic alterations, nucleic acids, antigens, and virions [10–12]. Key diagnostic tools including H&E staining, PCR, IHC, NGS, are pivotal in detecting infections, determining the genotypes involved, evaluating disease severity, and devising appropriate treatment strategies.

Vaccination remains the most effective strategy for preventing CPV infections. Numerous studies have explored several potential vaccine types, including inactivated autologous vaccines, DNA vaccines, and virus-like particle (VLP)-based vaccines, to provide immunity against CPV [13–17]. Among these, L1 protein-based VLPs have demonstrated robust humoral immune responses and promising efficacy in laboratory settings. However, the development of a comprehensive, commercially viable CPV vaccine remains a challenge and requires continuous efforts.

Thus, early and accurate diagnosis, combined with effective vaccination, is essential for controlling the spread of CPV and preventing potential malignant transformations. This review aims to examine current diagnostic methods and innovations in CPV vaccine development, emphasizing both progress and challenges in these areas to ultimately improve canine health.

Diagnostic methods for CPV

CPVs possess a small, circular, double-stranded DNA genome approximately 8 kb in size, comprising eight open reading frames (ORFs) that encode early proteins (E1, E2, E4, E5, E6, E7) and late proteins (L1, L2), along with a non-coding long control region (LCR) that regulates replication and transcription. These genes serve distinct functions in the viral lifecycle (as detailed in Fig. 1). CPVs are non-enveloped, icosahedral virions approximately 50–60 nm in diameter [5, 18]. These unique structural and genetic characteristics form the basis for CPV diagnostics and vaccine development, as discussed in subsequent sections.

Histopathology using hematoxylin and Eosin (H&E) staining

Histopathological examination using H&E staining remains a cornerstone in the diagnosis of CPV-associated lesions, providing a visual assessment of characteristic cytopathic alterations linked to papillomavirus infections. Key histological features include epithelial hyperplasia, orthokeratotic or parakeratotic hyperkeratosis, and granular keratinocytes with prominent keratohyalin granules. The presence of koilocytes-keratinocytes exhibiting perinuclear halos and nuclear pleomorphism, is a hallmark of papillomavirus infections. Additionally, intranuclear viral inclusions may occasionally be observed within keratinocytes, providing additional evidence for CPV diagnosis [19–22]. While H&E staining offers valuable morphological insights, it is inherently nonspecific and cannot distinguish between CPV types or confirm the presence of viral DNA or proteins without supplementary diagnostic techniques. Therefore, PCR and IHC are often employed in conjunction with H&E staining to achieve a comprehensive diagnosis [23, 24]. Furthermore, the histopathological changes associated with CPVs may overlap with those caused by other dermatological or neoplastic conditions, necessitating expert interpretation [5]and additional testing to ensure diagnostic accuracy. Despite of these limitations, H&E staining remains an essential preliminary tool in clinical practice, offering important contextual information that complements other molecular analyses like PCR and IHC for accurate identification of CPV infections and differentiation from other pathologies.

PCR

PCR is a highly sensitive and specific molecular technique for detecting CPV DNA. It is widely used for diagnosing infections, determining viral genotypes, and identifying novel variants. It amplifies conserved regions

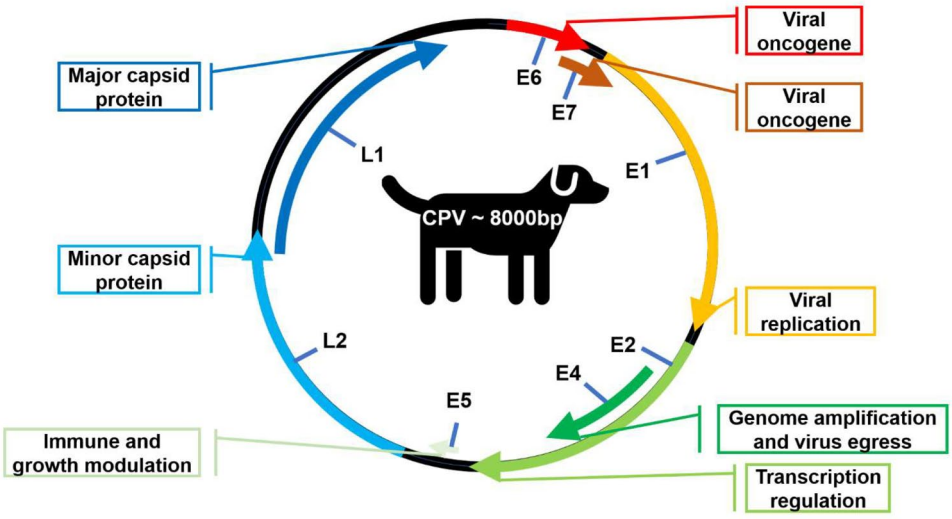

Fig. 1 Schematic representation of the CPV genome and its coding genes. CPV genomes range from 7.5 kb to 8.6 kb, reflecting variations in their genetic composition. Early genes (E1, E2, E4, E5, E6, E7) and late genes (L1, L2) perform essential functions in viral replication, immune evasion, and pathogenesis. E1 acts as a helicase, facilitating DNA unwinding and replication, while E2 regulates transcription, supports E1, and aids genome segregation. E4 contributes to genome amplification and viral release. E5 present only in several genotypes (e.g. CPV2, CPV11, CPV16, CPV19, and CPV20, is regarded to modulate growth factor receptors to enhance immune evasion and cell growth. E6 degrades the tumor suppressor protein p53, preventing apoptosis and enabling cell transformation, whereas E7 inactivates the Rb protein, driving the cell into S-phase for viral replication and oncogenesis. In the late phase, L1 forms the major capsid protein, assembling viral particles and eliciting immune responses, while L2, the minor capsid protein, assists in genome encapsidation and nucleus delivery. The coordination of these genes allows for the successful infection and propagation of CPVs

Table 1 Commonly used general or universal or degenerate primers and target genes of CPV

Primer Name	Target Gene	Primer Sequence (5′-3′)	Amplicon Size	References
MY09 MY11	L1	CGTCCATTYTAYCMACTGGT GCMCAGGGWCATAAYAATGG	~450 bp	[23]; [31]; [33]; [34]
FAP59 FAP64	L1	TAACWGTNGGNCAYCCWTATT CCWATATCWVHCATNTCNC-CATC	~480 bp	[31]
CanPVf FAP64	L1	CTTCCTGAWCCTAAYMAK-TTTGC CCWATATCWVHCATNTCNC-CATC	~480 bp	[30]
CP4 CP5	E1	ATGGTACARTGGGCATWTGA GAGGYTGCAACCAAAAMT-GRCT	~400 bp	[7]; [35]; [36]

Note: The use of degenerate bases in these universal primers

of the viral genome, such as the L1 and E1 genes, which are essential for viral replication and capsid formation [25–27]. Using primers tailored to these target regions, PCR amplifies specific CPV DNA sequences from clinical samples including biopsies, swabs, or formalin-fixed paraffin-embedded (FFPE) tissues. The resulting PCR products are typically visualized via gel electrophoresis or analyzed through sequencing [10, 19, 28]. To facilitate the detection of a broad-range of CPV genotypes, universal or general primers incorporating degenerate nucleotides have been developed, leveraging the relative conservation of these target genes. Several commonly used degenerate primers and their corresponding target genes are listed in Table 1. However, these primers have yet to be validated against all 26 currently identified CPV genotypes, highlighting the need for further optimization [7, 19, 21, 23, 29–32]. Despite of this, PCR remains a powerful diagnostic tool due to its exceptional sensitivity, enabling the detection of low viral DNA concentrations, and its high specificity, ensuring accurate differentiation of CPV types through precise primer design. Moreover, its versatility across various sample types enhances its utility in veterinary diagnostics. Frequently complemented by advanced sequencing methods, PCR provides critical insights into viral detection, diversity, and pathogenesis, making it indispensable for CPV research.

RCA

RCA is a molecular technique specifically designed to amplify circular DNA genomes, making it particularly suitable for detecting papillomavirus, including CPV. Using DNA polymerases with strand displacement activity, such as Phi29 DNA polymerase, RCA generates large quantities of DNA from minimal input material. The process begins with random hexamers or specific primers that anneal to circular DNA, initiating continuous amplification, and the resulting products are subsequently analyzed using restriction enzyme digestion, cloning, or sequencing to identify and characterize viral genome. For instance, this method has been successfully utilized to amplify and identify CPV genotypes such as

CPV-4 and CPV-9, enabling detailed genomic characterization and phylogenetic analysis [7, 37]. RCA-generated CPV genomes have revealed key genomic regions encoding early (E1, E2) and late (L1, L2) proteins, highlighting genetic diversity within the Papillomaviridae family [38–42]. The strength of this approach lies in its ability to produce large amounts of DNA from minimal input samples, facilitating the discovery of novel CPV genotypes and providing a deeper understanding of their genomic diversity and evolutionary relationships. However, this approach also has certain shortcomings, such as the potential for non-specific amplification of other circular DNAs present in the samples, including plasmids or mitochondrial DNA, necessitating stringent controls to avoid false positives [28]. Additionally, the method is less effective for detecting linear DNA or fragmented genomes. Despite its limitations, RCA is still a useful tool for CPV research.

DNA ISH

DNA ISH is a valuable technique for directly visualizing CPV genomes within tissue sections. By employing labeled probes complementary to specific viral DNA sequences, ISH enables the precise localization of CPV DNA within infected cellular compartments, often targeting conserved viral genes such as E6, E7, and L1. Hybridization signals predominantly appear in the nuclei of infected keratinocytes, particularly within the granular layer of the epidermis, where viral replication occurs, providing insights into viral activity and spatial distribution [19, 43]. ISH has proven effective in confirming CPV presence in both benign lesions, such as papillomas, and malignant cases, including squamous cell carcinoma. Studies have demonstrated its utility in identifying viral genomes in tissues exhibiting histopathological features like koilocytosis and hyperplasia [10, 28, 44–46]. For example, non-radioactive ISH methods employing digoxigenin-labeled probes have successfully demonstrated both safety and high diagnostic specificity in detecting CPV within epithelial samples [19]. However, ISH faces certain limitations, including reduced sensitivity due to formalin fixation and paraffin embedding, which may obscure hybridization signals, and lower sensitivity compared to PCR for detecting low-abundance viral genomes. As a result, it is often used alongside complementary techniques like PCR or IHC to enhance diagnostic accuracy. This combined approach offers critical spatial and molecular information, making ISH a valuable tool for understanding CPV pathogenesis, correlating viral distribution with lesion development, and informing diagnostics and therapeutic planning.

NGS

NGS and metagenomics are transformative methodologies that significantly enhance the detection and characterization of papillomaviruses, including CPV [12, 47–50]. These techniques enable high-through sequencing of viral genomes, providing comprehensive insights into both known and novel CPVs [12]. They are particularly effective in identifying diverse viral populations in complex biological samples, such as tissue biopsies, swabs, and feces. Bioinformatics tools such as BLASTx and de novo assembly, facilitate genome identification, classification, and annotation [12, 50]. Additionally, targeted sequence capture methods improve sensitivity by enriching viral DNA, allowing for the detection of CPVs even in low-abundance or co-infected scenarios [12]. These approaches have led to the discovery of novel CPV genotypes, including CPV9 and CPV21-23, shedding light on the genetic diversity and evolutionary dynamics of the Papillomaviridae family [12, 49]. Metagenomics further enhance CPV research by allowing unbiased detection of CPVs, offering valuable insights into multifactorial diseases such as respiratory or gastrointestinal syndromes [12]. Despite advantages in sensitivity, specificity, and the ability to sequence entire viral genomes, NGS and metagenomics are constrained by high costs, labor-intensive preparation, complex data analysis workflows, and the need for advanced computational infrastructure. Nonetheless, these methodologies remain pivotal in advancing the understanding of papillomavirus diversity, evolution, and their role in disease pathogenesis, offering transformative potential for CPV research and diagnostics [12, 50].

IHC

IHC is a powerful diagnostic tool for detecting CPV by identifying viral antigens, particularly the L1 capsid protein, within tissue samples. This approach utilizes specific antibodies, such as BPV-1/1H8 and CAMVIR-1, which recognize conserved epitopes across papillomavirus capsid proteins. By enabling precise localization of viral antigens IHC provides valuable insights into CPV infections [11, 21]. The procedure is typically performed on formalin-fixed, paraffin-embedded (FFPE) tissue sections, where antigen retrieval techniques enhance epitope accessibility. Positive immunostaining is generally observed in the nuclei or cytoplasm of keratinocytes, predominantly in the granular and keratinized layers of the epidermis, correlating with active CPV replication [22, 51]. This spatial localization provides critical insights into the distribution and activity of CPV infections within host tissues [52]. While IHC is effective, it has certain limitations, including potential cross-reactivity of antibodies originally developed for human or bovine papillomaviruses, which may reduce specificity. Additionally,

IHC has lower sensitivity compared to molecular methods like PCR, particularly in samples with low viral antigen levels. However, it has been instrumental in confirming CPV presence in papillomas and squamous cell carcinomas, with staining patterns reflecting the viral replication cycle [53]. When combined with molecular techniques such as PCR, IHC enhances diagnostic sensitivity and specificity, making it a useful tool for both clinical diagnostics and research applications.

ELISA

ELISA is a widely utilized technique for detecting CPV-specific IgG antibodies in canine sera, providing valuable insights into humoral immune responses. This assay involves coating microplates with antigens, such as authentic CPV particles or recombinant L1 proteins, which bind to antibodies present in serum samples. Horseradish peroxidase-conjugated goat anti-dog IgG serves as a secondary antibody to detect immune complexes, with the subsequent colorimetric reaction providing a quantitative measure of CPV-specific antibodies [54]. This technique has been instrumental in evaluating vaccine efficacy, immunogenicity, neutralizing antibody production, particularly in studies involving experimental CPV vaccines like VLP-based formulations. ELISA has also been employed to distinguish between infected and vaccinated animals by analyzing antibody titers against native and conformational epitopes of CPV capsid proteins [17, 55, 56]. Despite its versatility and ease of use, ELISA results are influenced by the quality of antigens and antibodies, with variations potentially affecting sensitivity and specificity. Nevertheless, its ability to handle batch and multiple-sample testing still makes it useful in CPV research, diagnostics, and vaccine evaluation [57, 58].

TEM

TEM is a high-resolution imaging technique useful for the detection and study of CPVs, with applications in both diagnostic and vaccine development contexts. TEM provides definitive visualization of CPV particles, which appear as non-enveloped, icosahedral structures approximately 50–60 nm in diameter. Viral particles are typically localized within the nuclei of infected keratinocytes, particularly in the granular and keratinized layers of the epidermis, correlating with active replication processes [20, 37, 41, 59–62]. This capability allows TEM to provide morphological evidence of infection and viral activity, offering insights into the virus's cellular effects [20, 41]. Beyond diagnostics, TEM plays a crucial role in validating the structural integrity of recombinant VLPs utilized in CPV vaccine development. Studies have shown that recombinant L1 VLPs produced in insect cell systems closely resemble native virions, as evidenced by TEM imaging, confirming their potential as effective immunogens [16, 54, 63, 64]. This validation ensures the structural and immunological fidelity of the VLPs, a critical step in the design and evaluation of CPV vaccines [41, 44, 59]. Moreover, TEM has been utilized to study CPV replication and assembly in experimental models, such as pigmented plaques, where it enables the concurrent detection of CPV-specific DNA, transcription activity, and virions, underscoring its role in comprehensive viral analysis [44, 59, 62, 65]. However, TEM is resource-intensive, requiring specialized equipment, technical expertise, and high viral loads for effective visualization, limiting its application primarily to research settings. Despite these challenges, its ability to provide direct evidence of viral presence and validate VLP-based vaccines ensures TEM's ongoing relevance in advancing CPV research and vaccine innovation [54, 63].

Evaluation and future directions for CPV diagnostics

Diagnostic methods for CPV detection vary widely in terms of sensitivity, specificity, and practical applications. Molecular techniques, such as PCR and NGS are considered the gold standard due to their high sensitivity and specificity. PCR efficiently detects low viral loads, while NGS provides comprehensive genomic insights and facilitates the discovery of novel CPV genotypes [12, 66]. Histological techniques like DNA ISH and IHC enhance diagnostics by enabling spatial localization of viral DNA and proteins within tissues [10]. TEM contributes by providing direct morphological evidence of CPV infection, visualizing intact viral particles within keratinocytes, and corroborating findings from molecular and histological analyses [20, 59].

However, these diagnostic methods also face practical challenges related to accessibility, cost, and resource requirements. Molecular approaches like PCR and NGS, demand sophisticated equipment, computational tools, and skilled personnel, limiting their feasibility in resource-constrained settings. Similarly, histological techniques such as IHC and DNA ISH, rely on high-quality antibodies or probes and involve meticulous sample preparation, making them labor-intensive and time-consuming. TEM is both expensive and technically demanding, requiring specialized training and advanced instrumentation. As a result, TEM is primarily confined to research laboratories and is less suited for routine veterinary diagnostics [19, 20, 28, 37]. A detailed comparison of various diagnostic methods for CPV detection is summarized in Table 2, while a classification of these methods based on their targets is illustrated in Fig. 2.

Advancing CPV diagnostics further requires the integration of molecular techniques with portable, user-friendly technologies. Innovations such as lab-on-a-chip systems [67], microfluidic assays [68], and

Table 2 Comparison of various diagnostic methods for CPV detection

Method	Sensitivity	Specificity	Strengths	Limitations
H&E	Low	Low	Basic morphological assessment	Cannot identify specific viral types
PCR	High	High	Rapid, highly sensitive, type-specific	Primer/probe design; not spatially resolved
RCA	Moderate	Moderate	Amplifies entire viral genomes	Prone to non-specific amplification
ISH	Moderate	High	Locates viral DNA in tissue samples	Time-consuming sample preparation
NGS	Very High	Very High	Comprehensive detection, identifies novel CPVs	High cost; computational expertise required
IHC	Moderate	High	Visualizes viral proteins in tissues	Antibody availability, lower sensitivity
ELISA	Moderate	Moderate-High	Simple, scalable, detects viral antigens	Requires high-quality antibodies
TEM	Low-Moderate	Very High	Definitive visualization of viral particles	Costly, technically demanding

smartphone-integrated diagnostic platforms [69] have the potential to revolutionize field applications by incorporating PCR, ELISA, and other techniques into compact, easy-to-use devices. These platforms enable rapid, real-time diagnosis even in remote areas or routine veterinary practices [70]. Future diagnostic tools should prioritize affordability, accessibility, and simplicity to ensure wide adoption in both specialized laboratories and general veterinary practices. Advancements in these aspects collectively enhance early detection, optimize interventions, and support CPV control initiatives.

Vaccine development for CPV

Inactivated and live-attenuated vaccines

Inactivated and live-attenuated vaccines are among the most traditional and widely studied approaches for viral immunization. Inactivated vaccines are produced by chemically or physically inactivating the virus, often using formalin or heat, while preserving the structural integrity of its immunogenic components. These vaccines are inherently safe, as they cannot replicate in the host, thereby reducing the risk of disease. In contrast, live-attenuated vaccines employ weakened viruses capable of replication with significantly reduced virulence, effectively inducing robust and long-lasting immune responses without causing severe illness. In the context of CPV and Canine Oral Papillomavirus (COPV), inactivated vaccines have shown promise by inducing high titers of neutralizing antibodies and preventing the development of mucosal papillomas following viral challenge [44, 60, 63]. Similarly, live-attenuated COPV vaccines have demonstrated their effectiveness in preventing mucosal infections in dogs when administered prior to exposure [71]. However, a study revealed potential risks associated with live vaccines, as the development of squamous cell carcinoma (SCC) at the injection site was observed in some dogs receiving an unattenuated COPV vaccine [62]. While inactivated vaccines offer a safer alternative, they often require adjuvants to boost their immunogenicity and repeated booster doses to maintain long-term protection. Additionally, the production process can be labor-intensive. Despite these challenges, both vaccine types remain valuable in veterinary medicine, offering protective immunity in dogs and serving as a foundation for further advancements in CPV vaccine development [72].

DNA-based vaccines

DNA-based vaccines have emerged as a promising approach for CPV immunization, leveraging genetic engineering to elicit robust immune responses. These vaccines utilize plasmids encoding key viral proteins, such as L1 or regulatory proteins like E1 or E2, under the control of strong promoters like the cytomegalovirus (CMV) promoter [15, 61, 73–75]. Once delivered into host cells, these plasmids drive the expression of viral proteins, thereby stimulating both humoral and cellular immunity. DNA vaccines offer advantages in safety, stability and the absence of live infectious agents, thereby eliminating the risk of reversion to virulence or severe immune reactions. Their cost-effective production and broad storage conditions further enhance their suitability for veterinary applications. Studies have demonstrated that DNA vaccines encoding the COPV L1 protein can elicit high titers of CPV-specific neutralizing antibodies, providing complete protection against experimental challenges [73, 74]. Advances such as codon optimization of E1 and E2 genes have further improved antigen expression and immune responses, resulting in reduced lesion size and shorter disease duration in challenged animals [15, 75]. Delivery methods also affect vaccine efficacy, with particle-mediated epidermal delivery systems, like gene guns, outperforming traditional intramuscular injections due to the

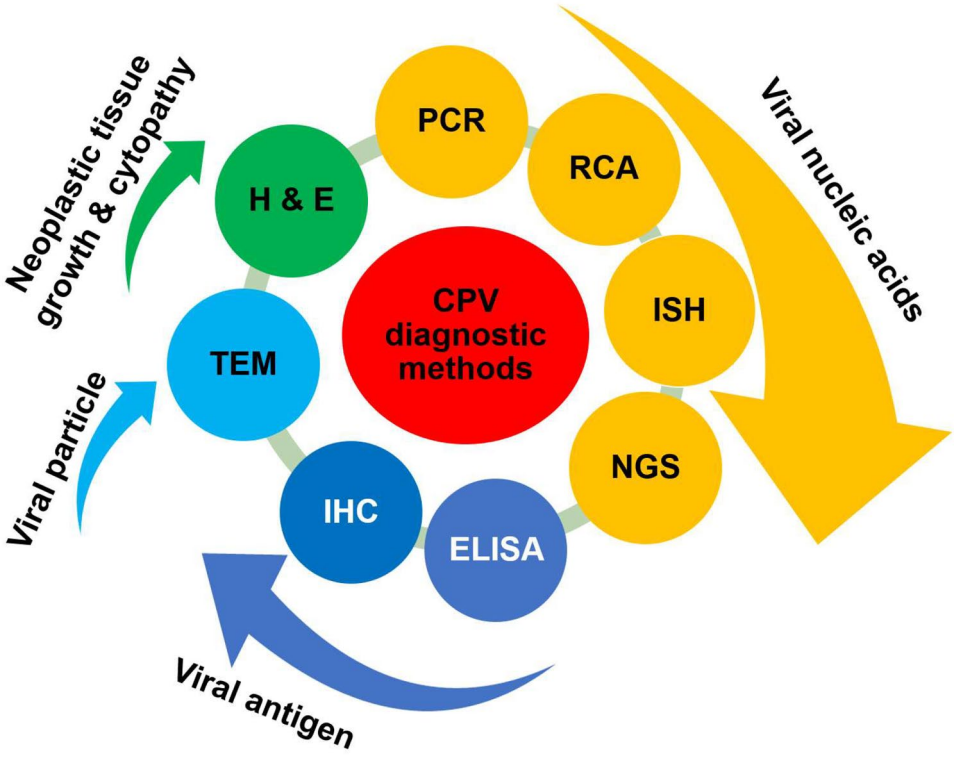

Fig. 2 The schematic illustrates how current diagnostic methods leverage the unique characteristics of CPV for detection. These approaches specifically target neoplastic tissue growth and cytopathy (H&E, Green), nucleic acids (PCR, RCA, ISH, NGS, Yellow), viral antigens (IHC, ELISA, Blue), and viral particles (TEM, light blue), respectively

higher density of antigen-presenting cells in the skin [73, 76]. However, DNA vaccines face challenges, including relatively low immunogenicity compared to protein-based counterparts. Current research is addressing these limitations through the development of advanced adjuvants and delivery technologies, such as electroporation and nanoparticle carriers, to enhance immune responses [77]. Furthermore, the adaptability of DNA vaccines allows for rapid modification to target emerging viral strains, underscoring their potential in combating evolving pathogens. As ongoing advancements refine delivery systems and formulations, DNA vaccines continue to represent a novel, versatile, and promising tool for CPV control in veterinary medicine.

Recombinant protein-based vaccines

Recombinant protein-based vaccines have demonstrated their potential in the prevention and management of CPV infection by harnessing the immunogenic properties of viral capsid proteins, particularly L1 and L2, as well as regulatory proteins like E1 and E2 [16, 17, 54, 57, 73]. The L1 protein, as the major capsid component, can self-assemble into virus-like particles (VLPs) that mimic native virion structures, presenting conformational epitopes critical for inducing robust, type-specific immune responses [16, 54]. Meanwhile, the L2 protein, a minor capsid component, provides conserved epitopes across various papillomavirus types, making it a promising candidate for cross-protective vaccines that address CPV's genetic diversity [17, 78]. Additionally, regulatory proteins such as E1 and E2, offer conserved epitopes that can enhance immune protection [57]. Incorporating these antigens into combination vaccines broadens immunogenicity and ensures both type-specific and cross-protective immunity [15, 57, 75, 79].

The production of recombinant protein-based vaccines leverages various expression systems, each with distinct advantages. Bacterial expression systems, such as Escherichia coli, are valued for their simplicity and cost-effectiveness, although challenges related to proper folding and post-translational modifications must be addressed [16, 17]. Baculovirus-infected insect cells are commonly employed due to their ability to produce correctly folded proteins and assemble them into VLPs [54, 64]. Adenoviral vectors have also been explored as platforms for delivering CPV antigens, providing high transgene expression and strong immunogenicity [57, 63]. Additionally, plant-based expression systems have emerged as innovative and scalable alternatives for producing recombinant L1 proteins, demonstrating retained immunogenicity and potential for large-scale vaccine production [80]. Novel platforms like the Tobacco Mosaic Virus

Table 3 Comparison of various CPV vaccines

Vaccine Type	Antigen(s)	Expression System	Adjuvant	Applied Effect	References
Inactivated and Live-attenuated vaccines	Whole CPV virus or COPV	Native virus	Alum, None	Provided protection against CPV; prevented oral papillomatosis but risked adverse outcomes like carcinoma	[13], [60], [44, 71]
DNA-Based Vaccines	L1, E1, E2 genes	Plasmid vectors	None specified	Induced humoral and cell-mediated immunity; protected against experimental challenge	[14]; [15]; [61]; [73]; [75]
Recombinant Protein Vaccines (e.g. Virus-Like Particles, VLPs)	L1 and L2 capsid proteins, as well as E1, E2	Baculovirus-infected insect cells (e.g., Sf9), adenovirus, tobacco plants, TMV-based platforms	Alum, QS21, or not specified	Induced robust humoral and cellular immune responses; prevented experimental papillomas; provided cross-protection and therapeutic efficacy in preclinical models	[54, 61, 63, 76, 80, 82]
Multimeric L2 Vaccines	L2 epitopes from multiple CPV types	E. coli, TMV-based platforms	Alum, RIBI	Elicited cross-neutralizing antibodies; effective against multiple papillomavirus types	[17]; [78]
Potential therapeutic Vaccines	E1, E2	Recombinant adenovirus	None specified	Reduced wart burden; prevented lesion development in therapeutic settings	[57]; [76]

(TMV) have been utilized to present L2 epitope in multimeric formats, significantly enhancing antibody titers and cross-neutralizing capabilities [78]. These advancements expand the protective efficacy of recombinant vaccines while addressing safety concerns, as recombinant vaccines lack viral DNA, eliminating risks of reversion to virulence seen with live-attenuated vaccines [81]. Moreover, multivalent formulations allow simultaneous targeting of multiple CPV genotypes.

Despite these advantages, recombinant protein-based vaccines face some challenges, including high production costs and complex manufacturing processes. Strategies to overcome these hurdles include codon optimization, more efficient expression systems, and innovative adjuvant formulations to enhance immunogenicity [17, 54, 57, 80]. For instance, combining L1 and L2 epitopes with adjuvants or incorporating them into adenoviral vectors could greatly enhance immune responses, further improving vaccine effectiveness [16, 17]. Additionally, continued refinement of bacterial and plant-based production platforms aims to achieve cost-efficient, large-scale manufacturing [16, 80]. As research progresses, recombinant protein-based vaccines are poised to deliver comprehensive, and cost-effective solutions for CPV prevention and management, providing robust protection and addressing the diverse needs of canine populations.

Advantages and disadvantages of different vaccines

CPV vaccines encompass a diverse array of approaches, each offering different advantages while facing distinct challenges. Traditional vaccines, such as inactivated and live-attenuated vaccines, have provided basic insights into CPV immunization. Chemically or physically inactivated vaccines have demonstrated their efficacy but often require the addition of adjuvants to boost immunogenicity, as well as repeated dosing to maintain long-term protection [61, 63]. Live-attenuated vaccines can induce robust and durable immunity, however, safety concerns such as the risk of reversion to virulence, and in rare cases, the development of squamous cell carcinoma, have limited their wide use [62]. Although both traditional approaches have shown effectiveness in preventing CPV infections and papillomatosis, their limitations underscore the need for further innovation in vaccine development.

Innovative vaccine platforms, such as DNA-based and recombinant protein vaccines, have emerged to address the limitations of traditional approaches. DNA-based vaccines offer a safe and stable alternative capable of stimulating both humoral and cellular immune responses. Their adaptability to target emerging viral strains makes them promising, though their efficacy depends on advanced delivery systems [74, 75]. Among the most advanced candidates, recombinant protein vaccines, particularly those based on virus-like particles (VLPs) mimic native virion structures without viral nucleic acids, inducing robust immune responses. Their large-scale production is feasible using platforms like baculovirus-infected insect cells, adenoviral vectors, and plant-based systems [16, 54, 63, 80]. Furthermore, multimeric L2 vaccines, through targeting conserved epitopes across CPV types, can elicit cross-neutralizing antibodies, thereby broadening the protective spectrum [17]. Additionally, therapeutic vaccines targeting E1 and E2 have shown potential in reducing lesion burden and preventing recurrence, leveraging recombinant adenoviral platforms for effective antigen delivery [57]. However, DNA-based and recombinant protein vaccines also face certain challenges, including production costs, the need for immunogenicity optimization, limited accessibility. Therefore, continuous advancements in codon optimization, adjuvant formulations, and delivery technologies are critical to addressing these issues and ensuring these vaccines meet the diverse needs of canine populations. A detailed comparison of these CPV vaccine types is summarized in Table 3.

Future vaccine innovations and challenges in CPV prevention

VLP-based vaccines have displayed great potential in the prevention of CPV due to their strong immunogenicity, effectively eliciting both neutralizing antibodies and cellular immune responses. Studies have shown that CPV L1 VLPs produced in insect cell systems confer robust, type-specific immunity and provide protection against experimental CPV challenges [54, 81]. Furthermore, efforts to develop multimeric L2 VLPs have contributed to addressing CPV's genetic diversity, allowing for cross-protection against multiple papillomavirus types [17]. These advancements position VLP-based vaccines as a cornerstone for CPV prophylaxis.

Emerging vaccine technologies, such as RNA-based vaccines, particularly mRNA platforms, represent a promising avenue for CPV immunization. These vaccines leverage synthetic RNA to encode viral antigens, offering scalability, rapid production, and the ability to elicit both humoral and cellular immune responses [83]. Although mRNA platforms have not yet been explored for CPV, their success in combatting human viral infections provides a compelling framework for future applications [84]. Innovations such as multivalent VLPs and chimeric constructs combining L1 and L2 antigens hold promise for eliciting broad-spectrum immunity. Coupled with advanced adjuvants like aluminum hydroxide and immunostimulatory molecules [85–87], these next-generation vaccines aim to overcome current limitations in CPV prevention by broadening protective efficacy and enhancing immunity.

Notably, a commercially available vaccine for CPV control has yet to reach the market, largely due to persistent challenges in the development and deployment. One of the major issues is the limited attention given to CPV infections in dogs, which has hindered research prioritization. Additionally, achieving cross-protective immunity remains a key hurdle. While L1-based VLP vaccines have indicated efficacy in type-specific protection, their ability to confer immunity against multiple CPV types has not been thoroughly investigated [54, 63]. Similarly, optimizing the incorporation of conserved L2 epitopes into multivalent or chimeric constructs is necessary to balance immunogenicity and broad-spectrum efficacy [17]. Another challenge is the accessibility and affordability of CPV vaccines. Therefore, scaling up cost-effective production methods, such as plant-based expression systems, could enhance vaccine availability. Additionally, integrating point-of-care diagnostic tools with vaccination programs may streamline preventive strategies and enhance disease control. Together, these innovations will shape the future of CPV prevention, thereby providing safer, more effective and accessible options for improving canine health.

Conclusions and perspectives

The advancements in CPV diagnostics and vaccine development signify substantial progress in addressing this prevalent canine disease. Diagnostic methodologies such as H&E, PCR, NGS, RCA, DNA ISH, IHC, and ELISA have significantly enhanced the detection, characterization and understanding of CPV infections. These approaches enable precise identification and genotyping of CPVs, facilitating detailed analyses of viral behavior, disease progression, and potential malignant transformations, as described above. However, challenges associated with high costs, limited accessibility, and the need for technical expertise remain barriers to broader application. Developing integrated and cost-effective diagnostic approaches is essential for achieving wide applicability in veterinary settings.

On the vaccine front, traditional approaches like inactivated and live-attenuated vaccines have laid the foundation for CPV immunization by effectively preventing papillomatosis. Nonetheless, safety concerns, including the risks of virulence reversion in live vaccines and the requirement for repeated boosters with inactivated vaccines, underscore the need for innovative solutions. Modern vaccine technologies such as recombinant protein-based vaccines, virus-like particles (VLPs), and DNA-based vaccines, in particular emerging RNA-platforms offer transformative potential. These advanced vaccines address safety concerns by excluding viral DNA, stimulate robust immunity, and utilize multivalent designs to target multiple CPV strains. Furthermore, innovations such as multimeric L2 constructs, and the application of novel adjuvants enhance immune breadth and efficacy, paving the way for broader protection against diverse CPV genotypes.

Future efforts should prioritize the integration of diagnostic tools with comprehensive vaccine strategies for sustainable CPV management. Portable diagnostics paired with advanced, cross-protective vaccines have the potential to revolutionize CPV control, particularly in resource-limited settings. Continuous advancements in scalable vaccine production, codon optimization, and novel adjuvants will play a critical role in overcoming current challenges. By addressing these aspects, researchers and veterinarians can significantly mitigate CPV's impact on canine health, ensuring broader protection, effective disease management, and an improved quality of life for affected animals.

Author contributions

Conceptualization, Y.F., K.W., D.D. and A.W.; data curation, Y.F., K. W.; funding acquisition, Y.F., and A.W.; resources, Y.F, K.W., D.Z., Y.Y., Y.C., J.W., H.S., X.H., X.P.; writing—original draft preparation, Y.F., K.W., D.Z., Y.Y., Y.C., J.W., H.S., X.H., X.P.; writing—review and editing, Y.F., K.W., D.Z., Y.Y., D.D. and A.W.; supervision, Y.Y., D.D. and A.W.; project administration, Y.Y., D.D., and A.W. All authors have read and agreed to the published version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Institutional review board
Not applicable.

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