

REVIEW ARTICLE

Self-assembling fowl adenovirus virus-like particles: Mechanistic insights and emerging strategies for vaccine development



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ABSTRACT

Fowl adenoviruses (FAdVs) are important pathogens of poultry and are associated with economically significant diseases, including inclusion body hepatitis, hepatitis-hydropericardium syndrome, and gizzard erosion. The substantial genetic and antigenic diversity among FAdV species and serotypes can limit cross-protection provided by conventional vaccines, emphasizing the need for safer and more adaptable vaccination strategies. Virus-like particles (VLPs) offer a promising approach because they mimic the structural organization of native virions while lacking viral genetic material. This review summarizes current knowledge of FAdV capsid architecture and the molecular mechanisms governing self-assembly and examines their relevance to VLP-based vaccine development. Particular attention is given to the structural and immunological functions of hexon, fiber, and penton base proteins, including their roles in capsid formation, host-cell interactions, antigenicity, and protective immunity. The review also evaluates current strategies for recombinant expression and VLP production, including bacterial, insect cell–baculovirus, and mammalian expression systems, together with approaches for improving protein folding, assembly efficiency, yield, purification, and stability. Available experimental evidence indicates that FAdV capsid proteins can induce humoral and cellular immune responses and that recombinant, chimeric, and multivalent antigen designs may broaden protection against antigenically diverse serotypes. However, evidence specifically demonstrating the performance of fully assembled FAdV-derived VLP vaccines remains limited, with many studies focusing on individual recombinant capsid proteins or early experimental vaccine candidates. Major barriers to practical application include efficient particle assembly, scalable manufacturing, structural stability, preservation of immunogenicity, production cost, and validation of cross-protective efficacy under field conditions. Advances in structural analysis, computational modeling, antigen engineering, expression platform optimization, and formulation strategies may facilitate the rational development of more stable and broadly protective VLP vaccines. Overall, FAdV VLP technology represents a promising platform for next-generation poultry vaccines, but further mechanistic studies, standardized characterization, controlled challenge experiments, and large-scale field validation are required before widespread commercial application.

Keywords: capsid assembly, fowl adenovirus, hexon protein, poultry vaccines, recombinant vaccines, self-assembly, vaccine development, virus-like particles.

INTRODUCTION

Fowl adenoviruses (FAdVs) are non-enveloped, double-stranded DNA viruses classified within the genus *Aviadenovirus* of the family *Adenoviridae* [1]. They are associated with several diseases of major economic importance in poultry, including inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS), and gizzard erosion [1, 2]. These diseases can cause increased mortality, impaired growth and production performance, and substantial economic losses to the poultry industry [2].

The FAdV genome encodes multiple proteins involved in viral replication, modulation of host-cell functions, and evasion of host immune responses. FAdVs exhibit considerable genetic and antigenic diversity, with multiple

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species and serotypes recognized on the basis of phylogenetic and antigenic characteristics [3]. This diversity reflects viral adaptation and has important consequences for pathogenicity, host interactions, immune recognition, and disease control.

Structural proteins, particularly hexon and fiber, contribute to virulence, tissue tropism, and recognition by the host immune system [4]. Consequently, antigenic differences among serotypes may limit the protection provided by vaccines against heterologous strains, thereby complicating effective vaccination under field conditions [4]. Clinical outcomes also vary considerably according to viral strain, host species, age, and immune status, ranging from subclinical infection to severe disease [5–7].

Virus-like particles (VLPs) have emerged as promising vaccine platforms because they can reproduce important structural and antigenic features of native viruses without containing the viral genome [8, 9]. Their repetitive and organized antigenic surfaces can facilitate efficient antigen presentation and stimulate both humoral and cell-mediated immune responses while eliminating the risk of replication or reversion to virulence associated with replication-competent vaccine platforms [8]. Although VLP-based vaccines have been investigated for numerous viral diseases, their application to FAdV remains comparatively underexplored [9].

The absence of viral genetic material is an important safety characteristic of VLP-based vaccines. Unlike live-attenuated vaccines, VLPs cannot replicate or revert to virulence, while their structural resemblance to native virions can preserve relevant antigenic characteristics and promote strong immune responses [10, 11]. The repetitive presentation of antigens on VLP surfaces can enhance immune recognition and stimulate both humoral and cellular responses. These properties have supported the investigation of VLPs as adaptable vaccine platforms for routine immunization and situations requiring rapid vaccine development [10–12].

Conventional inactivated and live-attenuated FAdV vaccines may provide predominantly serotype-specific protection, potentially limiting cross-protection against genetically and antigenically diverse FAdV serotypes circulating in commercial poultry populations [13]. This limitation is particularly relevant where pathogenic serotypes such as FAdV-4 and FAdV-8b co-circulate or occur sequentially. Antigenic variation in major capsid proteins, particularly hexon and fiber, can influence recognition by neutralizing antibodies and thereby restrict cross-reactivity among serotypes. Consequently, vaccinated flocks may remain susceptible to heterologous FAdV infections or coinfections, with potential consequences for mortality, production performance, and economic losses [3, 10, 13].

The development of scalable vaccines capable of providing broader protection against diverse FAdVs therefore remains an important objective in poultry health. Disease-related mortality, impaired feed conversion, reduced productivity, and veterinary interventions contribute to the economic burden associated with FAdV infections [3, 14]. VLP technology provides a potential platform for addressing some limitations of conventional vaccines because native-like structural organization can promote antigen presentation, whereas modular engineering may permit the incorporation or modification of antigenic determinants from different serotypes [11]. These characteristics have encouraged investigation of VLPs and related recombinant technologies as next-generation approaches for controlling adenoviral diseases in poultry [15, 16].

Recent advances in molecular biology, structural virology, and vaccinology have facilitated the development and evaluation of several vaccine strategies against FAdVs. These include inactivated vaccines, live-attenuated vaccines, including host-adapted and gene-deletion approaches, viral-vector vaccines, and recombinant subunit strategies incorporating capsid proteins, VLP-related platforms, and chimeric proteins [17]. Understanding the advantages and limitations of these approaches is important for identifying strategies capable of providing safe, effective, and broader protection against FAdV infections.

Evidence relevant to FAdV vaccine development varies considerably in experimental design and level of validation. Studies include *in vivo* poultry challenge experiments, experimental vaccine evaluations, structural and immunological investigations, and *in vitro* analyses of capsid proteins and assembly mechanisms [18–21]. Findings obtained exclusively from *in vitro* experiments or theoretical models require cautious interpretation when extrapolated to protective efficacy in poultry. Conversely, controlled *in vivo* challenge experiments and studies with clearly described experimental procedures can provide more direct evidence regarding vaccine immunogenicity and protective potential [20, 21].

Despite increasing interest in VLP-based vaccines (Figure 1), direct evidence concerning fully assembled FAdV-derived VLPs remains limited. Much of the available research has focused on recombinant individual capsid proteins or subunit vaccine candidates rather than complete, structurally characterized VLPs [22]. Furthermore, although multivalent inactivated vaccines and chimeric fiber-based strategies have shown encouraging

protection against different FAdV serotypes in experimental poultry models [17], these approaches do not fully resolve mechanistic questions about FAdV capsid self-assembly or establish how to translate this knowledge into reproducible VLP production. Important uncertainties remain about the contributions and interactions of hexon, fiber, penton base, and associated structural components during particle assembly; how expression systems affect protein folding and assembly; whether antigenic epitopes are preserved; and whether engineered particles can provide cross-protection against antigenically diverse serotypes. In addition, production efficiency, particle stability, scalability, manufacturing consistency, and validation under commercial poultry conditions remain important barriers to practical application [22, 23]. A synthesis connecting the molecular basis of FAdV capsid assembly with VLP engineering and vaccine development is therefore needed to identify current evidence gaps and priorities for future research.

This narrative review aims to critically synthesize current knowledge of FAdV capsid architecture and the molecular mechanisms governing the self-assembly of its principal structural proteins, with particular emphasis on hexon, fiber, and penton base proteins. It further examines how these structural and mechanistic insights can inform the rational design of FAdV-derived VLP vaccines. The review evaluates current evidence on recombinant expression and particle-production strategies, immunogenicity and protective responses, and emerging approaches involving engineered, chimeric, and multivalent antigens. It also examines the principal challenges affecting VLP assembly efficiency, structural stability, scalability, manufacturing, and cross-serotype protection and identifies research priorities required to advance FAdV VLPs from experimental vaccine candidates toward practical application in poultry production [8, 17, 22–24].

Because this article adopts a narrative evidence-based approach rather than a formal systematic review design [18, 19], the evidence is interpreted according to its experimental context and level of validation. Particular emphasis is placed on peer-reviewed *in vivo* poultry challenge studies, experimentally evaluated vaccine candidates, and investigations providing structural or immunological characterization of FAdV capsid proteins and vaccine platforms [20, 21]. Where direct evidence for fully assembled FAdV-derived VLPs is limited, findings from relevant recombinant FAdV platforms and carefully selected comparable avian viral systems are considered to provide mechanistic context while maintaining a distinction between direct and indirect evidence [8, 9, 24].

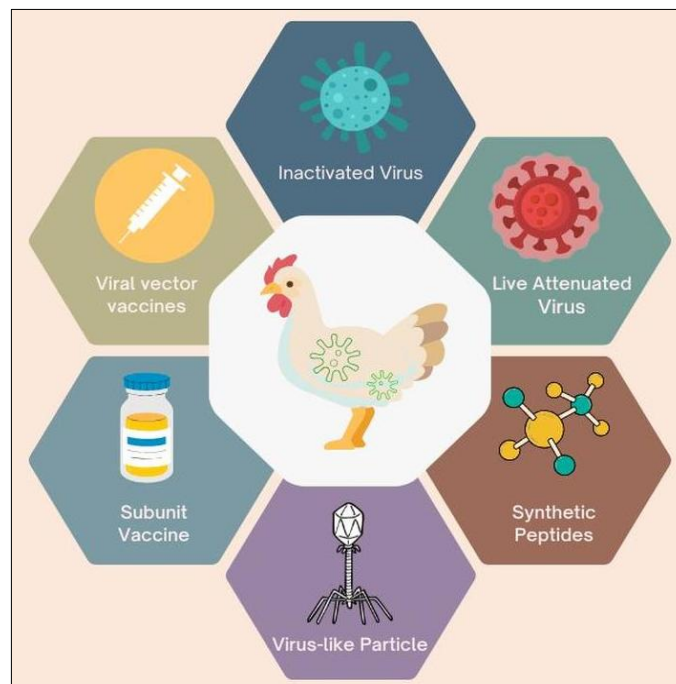


Figure 1: Fowl adenovirus vaccine development approaches [The figure was prepared by the authors using Canva (Canva Pty Ltd, Sydney, Australia)].

CURRENT STATUS OF FAdV VLP DEVELOPMENT

Compared with other viral systems, research on FAdV VLPs remains limited. Nevertheless, several studies have demonstrated the potential of FAdV structural proteins as components of recombinant vaccine platforms. Early investigations primarily evaluated individual capsid proteins, particularly hexon and fiber, for their immunogenicity and ability to induce protective immune responses.

Subsequent research has increasingly explored recombinant expression and combinations of structural proteins intended to reproduce antigenic features of the native virion. Gupta *et al.* [25] investigated VLPs and recombinant fiber proteins as vaccine candidates against FAdV-8b, providing evidence supporting the potential application of FAdV structural proteins in vaccine development. However, the available literature should distinguish carefully between fully assembled VLPs and individual recombinant capsid proteins or VLP-like structures because these vaccine platforms are not directly equivalent.

Although progress has been made, several challenges continue to limit the development of FAdV VLP-based vaccines. These include optimization of recombinant protein expression, appropriate folding and interaction of capsid components, efficient and reproducible particle assembly, structural stability, preservation of relevant antigenic determinants, and the development of protection against antigenically diverse FAdV serotypes. Further mechanistic studies are therefore required to characterize the structural and molecular determinants governing FAdV VLP formation and to determine how these properties influence immunogenicity, protective efficacy, and the feasibility of scalable vaccine production.

LITERATURE SEARCH STRATEGY AND REVIEW APPROACH

This review adopted a narrative, evidence-based approach to synthesize current knowledge of FAdV capsid structure, VLP formation, and emerging vaccine development strategies. Relevant peer-reviewed publications were identified through searches of PubMed, Scopus, Web of Science, and Google Scholar. Literature searches used combinations of terms related to FAdV biology and vaccine development, including “FAdV,” “virus-like particles,” “hexon,” “fiber,” “penton base,” “adenovirus assembly,” and “avian adenovirus vaccines. The flow diagram for selection of the study is presented in Figure 2.

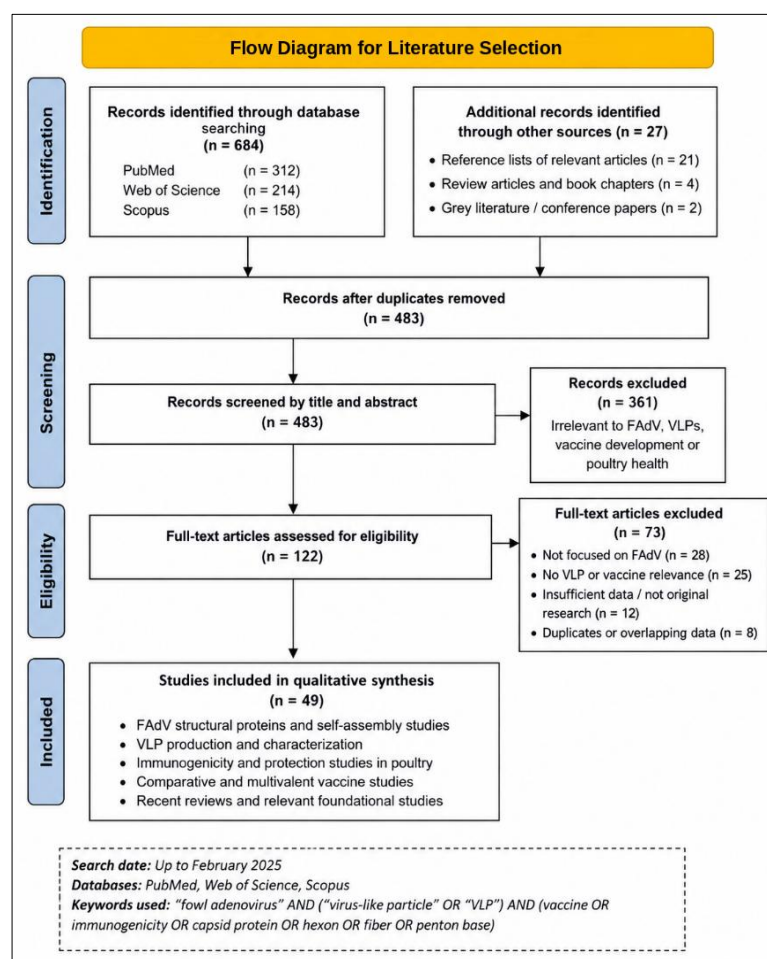


Figure 2: Flow diagram of the literature search and study-selection process.

Greater emphasis was placed on studies published after 2015 to capture recent advances in structural virology, recombinant vaccine engineering, and multivalent or chimeric vaccine platforms. Earlier landmark studies were included when they provided essential background information or fundamental mechanistic insights into FAdV structure, assembly, antigenicity, or immunogenicity.

Because this article was designed as a narrative review rather than a formal systematic review, complete PRISMA procedures were not applied. Nevertheless, best-evidence review principles were incorporated to improve scientific balance and transparency. Evidence from different vaccine platforms was compared, conflicting or emerging findings were considered, and studies with limited experimental or field validation were interpreted cautiously. Particular attention was given to distinguishing evidence obtained from fully assembled VLPs from that derived from recombinant subunit proteins, inactivated vaccines, and viral-vector approaches [12, 15, 17, 26–28].

STRUCTURAL ORGANIZATION OF FAdV CAPSID PROTEINS

The FAdV capsid has a complex icosahedral organization in which 240 hexon trimers form the principal capsid shell and 12 penton bases occupy the vertices, where they are associated with one or two serotype-dependent fiber proteins [3, 29]. Minor capsid and core proteins contribute to capsid stabilization, genome organization, and virion integrity. Among the major structural components, hexon and fiber proteins have particular importance for viral antigenicity and host interactions. The hypervariable regions (HVRs) of hexon contain major antigenic determinants involved in serotype-specific immune recognition and neutralizing antibody responses, whereas fiber proteins contribute to receptor interactions and host-cell tropism [3, 6, 29]. These structural and antigenic properties make the major capsid proteins important targets for molecular characterization and rational vaccine development.

The conserved pedestal domains of hexon, including P1 and P2, contribute to trimer formation and maintenance of the icosahedral capsid architecture [29–31]. High-resolution structural analysis of FAdV-4 has provided further insight into the organization and stability of the virion. FAdV-4 virions were reported to exhibit substantial thermal stability despite structural differences from members of *Mastadenovirus* and *Atadenovirus*. The FAdV-4 hexon contains distinctive features, including an insertion in the trimer equatorial region and an extended N-terminal tail. The half-transition temperature associated with the maximum rate of DNA release was reported as $T_{0.5} = 57.3 \pm 0.01^\circ\text{C}$ for FAdV-4 compared with $T_{0.5} = 47.5 \pm 0.06^\circ\text{C}$ for HAdV-C5 [32].

Three-dimensional structural characterization further demonstrated that the FAdV-4 capsid follows a pseudo- $T = 25$ icosahedral organization, with hexons forming the principal capsid facets and penton bases occupying the vertices. Internal cementing proteins IIIa and VIII are located on the inner capsid surface. The overall architecture of the 937-amino-acid FAdV-4 hexon monomer is highly conserved relative to other adenoviruses, including the 952-amino-acid HAdV-C5 hexon. The structure contains two jelly-roll motifs, P1 and P2, which together form a pseudohexagonal base, as well as flexible loop regions containing hypervariable regions [32]. These conserved and variable structural elements are relevant to capsid stability, antigenic diversity, and the selection of candidate regions for vaccine engineering.

The penton base represents another functionally important component of adenovirus capsid architecture. In adenoviruses possessing the relevant receptor-binding determinants, interactions involving integrins contribute to viral internalization and subsequent entry processes [33, 34]. Structural conservation of functionally important capsid domains may therefore provide opportunities for identifying vaccine targets or antiviral strategies. However, the extent to which receptor-binding mechanisms characterized in other adenoviruses can be directly extrapolated to individual FAdV species and serotypes requires careful consideration.

Although FAdVs and other adenoviruses share a fundamental icosahedral capsid architecture, substantial variation occurs in surface-exposed antigenic determinants, fiber configurations, and capsid-stabilizing interactions [35, 36]. Hypervariable loops of hexon and receptor-interacting regions of fiber exhibit considerable sequence diversity, contributing to differences in antigenicity, cellular tropism, and host interactions. Such diversity can also restrict cross-reactive immunity and complicate the development of vaccines capable of protecting against multiple serotypes [29, 35–37].

Fiber diversity is particularly relevant to FAdV biology and vaccine development. Depending on the serotype, differences occur in fiber number, sequence, length, and receptor-interacting regions. FAdV-4 possesses a dual-fiber architecture, emphasizing the functional diversity of these surface proteins [29, 37]. Because fiber proteins are exposed on the virion surface and participate in host-cell interactions while also containing antigenically relevant regions, they represent important candidates for recombinant and chimeric vaccine development.

Chimeric fiber-based approaches have consequently been investigated as a strategy for broadening protection against pathogenic FAdV serotypes. De Luca *et al.* [38] evaluated a recombinant chimeric fiber construct incorporating antigenic components associated with FAdV-4 and FAdV-11 and reported protection against HHS and IBH in chickens. Vaccinated birds reportedly exhibited reduced clinical manifestations, decreased

indicators of hepatic injury and viral loads, and improved preservation of lymphoid organs after challenge. Protection was associated with antibody responses before challenge, whereas neutralizing antibody responses were limited or absent, suggesting that mechanisms other than classical virus neutralization may have contributed to the observed protection [38]. These findings indicate that chimeric fiber constructs warrant further investigation as potential components of vaccines intended to broaden protection against antigenically distinct FAdVs.

SELF-ASSEMBLY MECHANISMS OF FAdV CAPSID PROTEINS

The assembly of native adenovirus involves coordinated interactions among structural proteins, genome packaging, and proteolytic maturation. These processes should be distinguished from the assembly of genome-free FAdV VLPs, which are designed to reproduce selected structural and antigenic features of the viral capsid without incorporating the viral genome. In native virions, genome packaging, DNA condensation, and protease-mediated maturation contribute to the formation of infectious particles; however, these processes should not be considered prerequisites for FAdV VLP assembly. For VLP production, particular attention is instead placed on the expression, folding, oligomerization, and assembly of the selected capsid proteins, as well as the structural integrity and antigenic properties of the resulting particles. [12, 28, 29, 39].

Native adenovirus capsid formation has been described in terms of nucleation, particle growth, and maturation; however, the extent to which this framework applies specifically to FAdV VLP assembly remains to be established. Hexon monomers must fold correctly and assemble into stable trimers before incorporation into the developing capsid. Molecular chaperones contribute to this process by facilitating protein folding and preventing inappropriate aggregation. The viral L4-100K protein has an important role in hexon folding and trimerization and facilitates the formation of assembly-competent hexon structures.

Host-cell chaperones may also contribute to adenovirus protein folding and replication. T-complex polypeptide 1 subunit eta (TCP1 η), also known as chaperonin-containing TCP1 subunit 7 (CCT7), is a component of the eukaryotic chaperonin TRiC (TCP1-ring complex). In FAdV-4-infected cells, CCT7 has been reported to interact with the viral hexon protein and to contribute to hexon stability and viral replication [40, 41]. However, direct involvement of CCT7/TRiC in FAdV capsid self-assembly has not been experimentally established. This approximately 900-kDa complex consists of two stacked rings, each containing eight distinct subunits (CCT1-CCT8), and assists in the folding of several cellular proteins [40]. Evidence cited in the manuscript indicates an interaction between adenovirus hexon and CCT7, suggesting that host chaperone machinery may contribute to processes required for viral replication [3]. However, the extent to which this mechanism has been demonstrated specifically for FAdV capsid self-assembly should be distinguished from evidence obtained from other adenovirus systems.

Following the formation of assembly-competent structural units, capsid development proceeds through interactions among hexon trimers, penton-associated components, and minor capsid proteins. Protein concentration, folding efficiency, and the intracellular environment can influence these interactions and consequently affect assembly efficiency. Differences in minor capsid composition among adenovirus genera are also important when extrapolating assembly mechanisms to FAdV. Members of *Mastadenovirus* contain proteins V and IX, whereas the corresponding genes are absent from members of *Aviadenovirus*, including FAdV [41, 42]. These differences indicate that assembly and capsid stabilization mechanisms characterized in mammalian adenoviruses cannot necessarily be transferred directly to FAdV.

The final maturation of native adenovirus particles involves proteolytic processing of precursor capsid and core proteins by adenovirus protease (AVP). This processing induces structural rearrangements required for maturation and infectivity, including activation of functions associated with protein VI [28]. Maturation is closely coordinated with other stages of virion formation and contributes to the transition from immature particles to infectious virions.

The relationship between native adenovirus maturation and FAdV VLP production requires particular consideration. VLPs lack the viral genome and therefore do not reproduce all events involved in native virion assembly, genome packaging, and maturation. Nevertheless, understanding the interactions among structural proteins and the conformational changes associated with native capsid formation can inform strategies for producing particles with appropriate architecture and antigen presentation. The requirement for AVP-mediated processing in FAdV VLP production, however, depends on the specific VLP design and should not be assumed from native adenovirus maturation alone.

Environmental and intracellular conditions can also influence protein folding, capsid assembly, and particle stability. Factors such as pH, ionic strength, temperature, protein concentration, redox conditions, and the availability of molecular chaperones may alter protein-protein interactions and assembly dynamics [28]. Host chaperones, including HSP70 and HSP90, together with virus-specific factors such as L4-100K, may facilitate appropriate folding, limit aggregation, and promote productive interactions among structural components. These factors are relevant to recombinant VLP production because inappropriate folding or subunit interactions can reduce assembly efficiency and compromise particle stability.

PRODUCTION STRATEGIES FOR FAdV VLPs

Selection of an appropriate expression system is a critical consideration in FAdV VLP production because the platform can influence protein yield, folding, assembly, product quality, scalability, and manufacturing cost. Bacterial, insect-cell, and mammalian-cell expression systems offer different advantages and limitations, and their suitability depends on the structural requirements of the target capsid proteins and the intended vaccine product [43–49].

Escherichia coli provides a comparatively rapid, economical, and scalable platform for recombinant protein production and can be useful for producing individual capsid proteins and conducting proof-of-concept studies [44]. However, bacterial expression can be limited by protein misfolding, formation of inclusion bodies, and the absence of eukaryotic post-translational processing. These limitations may be particularly important when correct folding and multimeric assembly are required to reproduce conformational epitopes or native-like particle architecture.

Insect-cell/baculovirus systems provide a eukaryotic environment that supports protein folding and post-translational processing and have been widely used for recombinant protein and VLP production [45, 46]. However, differences between insect- and vertebrate-cell post-translational processing may influence the properties of some recombinant proteins. Stable insect-cell expression systems represent another potential production approach, although their optimization requires careful control of cell density, culture conditions, retention strategies, and process parameters. High cell density cultures and continuous production may improve overall productivity but can increase process complexity and manufacturing requirements. The insect cell–baculovirus expression vector system (IC-BEVS) can achieve high recombinant protein yields over relatively short production periods; however, lytic infection, proteolytic degradation, and limitations in continuous processing may affect product quality and process efficiency [43].

Mammalian-cell expression systems can support complex eukaryotic protein folding and post-translational processing and may therefore be useful when native-like structural characteristics are required. Nevertheless, their comparatively high production costs, longer development timelines, and greater process complexity may limit their suitability for large-scale veterinary vaccine production. Consequently, selection of an expression platform should consider the target antigen, structural requirements, production scale, product quality, regulatory requirements, and economic feasibility [2, 47–49].

Optimization of VLP yield requires coordinated control of molecular design, expression conditions, and downstream processing [48, 50]. Strategies described across VLP production systems include codon optimization, fusion-tag design, optimization of culture conditions, and improved purification procedures. Codon optimization can enhance heterologous expression by adapting coding sequences to the translational characteristics of the selected host, whereas fusion tags may improve protein solubility, stability, or purification. However, fusion sequences can potentially influence protein conformation or antigenicity and may require removal during downstream processing.

Culture conditions, including induction timing, temperature, pH, nutrient availability, and cell density, can substantially influence recombinant protein expression and particle assembly. Optimization must therefore be performed for the individual expression system and target protein rather than applying conditions established for unrelated VLP platforms directly to FAdV. Studies involving other viral systems have demonstrated that optimization of coding sequences, protein engineering, and expression conditions can substantially improve VLP production [51, 52]. These findings provide useful technological context but do not by themselves establish equivalent improvements in FAdV VLP production.

Evidence from recombinant FAdV vaccine studies further illustrates the potential of capsid proteins as vaccine antigens. A study evaluating hepatitis B virus core protein-fused hexon epitopes reported protection in broiler chickens following vaccination [53]. Other recombinant FAdV vaccine approaches have produced variable

levels of protection under experimental conditions [53]. Such studies provide evidence for the immunogenic potential of FAdV-derived antigens but should be distinguished from studies evaluating fully assembled FAdV VLPs.

Overall, optimization of FAdV VLP production requires balancing expression yield with appropriate protein folding, structural integrity, particle assembly, antigenicity, and manufacturing feasibility. Potential strategies include selection of suitable expression hosts, optimization of culture conditions, control of proteolytic degradation and aggregation, and development of scalable upstream and downstream processes [47]. Importantly, production approaches established for VLPs derived from unrelated viruses should be considered supporting technological evidence rather than direct evidence of their effectiveness for FAdV VLP manufacture.

FADV VLP-BASED VACCINE DEVELOPMENT

Humoral immunity and neutralizing antibody responses

Protective immunity induced by FAdV vaccination involves humoral responses directed against viral structural antigens. Following vaccination, antigen-specific B-cell activation and antibody production can contribute to protection by limiting viral attachment, entry, and subsequent replication. Studies of conventional FAdV vaccines have demonstrated measurable antibody responses after immunization. For example, inactivated FAdV-4 vaccines have provided protection against homologous challenge, whereas oil-emulsion FAdV-8a vaccines have induced substantial antibody responses and protective immunity [54–56]. These findings provide important information regarding FAdV vaccine immunology, although they should not be interpreted as direct evidence of immune responses induced by VLPs.

Hexon and fiber are important targets of the humoral response. Hypervariable regions of hexon contribute to serotype-specific antigenicity and contain epitopes recognized by neutralizing antibodies. Fiber also contains antigenically relevant regions and participates in host-cell attachment. Consequently, antibodies directed against these surface-exposed capsid components may interfere with different stages of infection. However, antigenic variation among FAdV serotypes can restrict heterologous protection, and strong antibody responses against one serotype do not necessarily indicate equivalent protection against antigenically distinct FAdVs [15, 55, 56].

Evidence from recombinant FAdV vaccine candidates supports the immunogenic potential of structurally presented capsid antigens. Gupta *et al.* [25] evaluated VLPs and recombinant fiber proteins against FAdV-8b, demonstrating the potential of these antigenic platforms for poultry vaccination. Nevertheless, the available evidence remains insufficient to conclude that FAdV VLPs consistently provide broader or stronger protection than established vaccine platforms.

Reported protection levels should therefore be interpreted on a study-specific basis and should not be generalized across FAdV VLP candidates, serotypes, vaccine formulations, or challenge models.

Cellular immune responses

Cell-mediated immunity may complement antibody-mediated protection against FAdV infection. Antigen presentation by professional antigen-presenting cells can activate CD4⁺ helper and CD8⁺ cytotoxic T-cell responses, thereby contributing to cytokine production, immune regulation, and elimination of infected cells. VLPs can promote efficient antigen uptake and presentation because their repetitive particulate architecture resembles that of native virions while lacking the complete infectious viral genome [11].

A proposed mechanism involves of Toll-like receptor 2 (TLR2) signaling, dendritic cell activation, increased expression of costimulatory molecules such as CD40 and CD86, major histocompatibility complex class I and II antigen presentation, and subsequent CD4⁺ and CD8⁺ T-cell responses. These mechanisms are biologically relevant to VLP immunology [57–59]; however, their specific demonstration for FAdV-derived VLPs requires direct experimental evidence. Therefore, they should not be presented as established mechanisms of FAdV VLP vaccination unless the cited studies specifically demonstrate these responses.

Comparison with conventional FAdV vaccine platforms

Conventional live-attenuated and inactivated vaccines remain important approaches for FAdV control. Experimental studies have demonstrated substantial protection with some of these platforms. For example, cell-culture-derived inactivated FAdV-4 vaccine has been reported to provide protection against homologous challenge [56], and inactivated FAdV-8a oil-emulsion vaccine has also been evaluated for immunogenicity and protective efficacy [55].

VLP-based strategies offer a different approach because they can display viral structural antigens without

containing a complete infectious viral genome. This property avoids the risk of reversion to virulence associated with replication-competent live-attenuated vaccines and provides opportunities for antigen engineering [25]. However, current evidence does not support the conclusion that FAdV VLP vaccines consistently outperform conventional vaccines in humoral responses, cellular responses, or protective efficacy. Direct comparisons are further complicated by differences in vaccine composition, antigen dose, adjuvants, administration routes, bird age, challenge strains, and experimental endpoints.

Accordingly, the reported protection achieved by conventional FAdV vaccines, recombinant capsid proteins, chimeric constructs, and VLP candidates should be interpreted according to the specific vaccine platform evaluated. Evidence generated with an inactivated or live-attenuated FAdV vaccine cannot be used directly to establish the efficacy of a FAdV VLP vaccine.

Potential for multivalent and cross-protective vaccine development

The modular nature of VLP platforms provides a potential basis for incorporating or displaying selected antigens from multiple FAdV serotypes. Such approaches could be particularly relevant to infections involving FAdV-4, FAdV-8b, and FAdV-11, which are associated with economically important disease manifestations in poultry. However, broad cross-protection requires identification and appropriate presentation of antigenic determinants capable of inducing protective responses against heterologous serotypes.

Related VLP and recombinant FAdV approaches provide evidence that multivalent protection is experimentally achievable, although these approaches should not be classified as VLP vaccines. The manuscript describes recombinant and chimeric vaccine candidates targeting combinations of FAdV-4, FAdV-8b, and FAdV-11 and reports protection against multiple challenge serotypes [38, 60–65]. These findings support continued investigation of multivalent antigen design but do not establish that equivalent protection will necessarily be obtained with FAdV VLP formulations. The manuscript itself also recognizes that differences in vaccine formulations, antigen doses, immunization protocols, challenge models, and endpoints limit direct comparisons among studies.

Adjuvant and immunological engineering strategies

Adjuvant selection represents another potential strategy for improving the immunogenicity of recombinant and VLP-based vaccines. TLR agonists can stimulate innate immune signaling and influence antigen-presenting cell activation and subsequent adaptive responses. In avian species, differences in innate immune receptor biology must be considered when translating adjuvant strategies developed in mammalian systems. For example, nucleic acid-based immunostimulatory approaches involving avian TLR pathways have been investigated as potential means of enhancing vaccine-induced immunity [10, 12].

Combinations of antigens and immunostimulatory molecules may offer opportunities to enhance humoral and cellular responses, but their safety, dose, delivery system, and species-specific effects require experimental validation. Nanoparticle-based delivery and antigen-adjuvant co-formulation may further improve antigen presentation and immune stimulation. However, these concepts should currently be considered potential engineering strategies rather than established components of FAdV VLP vaccines.

Multivalent vaccination strategies targeting FAdV together with other economically important avian pathogens may also be of interest. Nevertheless, the proposed combinations involving Newcastle disease virus, infectious bronchitis virus, and infectious bursal disease virus require direct experimental evidence before claims regarding simultaneous protection or improved field control can be made. Further studies should therefore determine whether engineered FAdV VLP platforms can accommodate heterologous antigens while retaining appropriate particle assembly, antigenicity, stability, and protective efficacy.

CHALLENGES AND FUTURE DIRECTIONS

Challenges in large-scale vaccine production

Large-scale production remains an important challenge for the translation of VLP-based vaccines from laboratory development to routine vaccination programs. Expansion from laboratory-scale optimization to commercial manufacturing requires substantial investment in production infrastructure, biosafety systems, bioreactor capacity, containment facilities, cleanrooms, downstream processing, and quality control procedures. The economic requirements of these systems can substantially influence the feasibility of large-scale vaccine production [12, 28].

Biological variability in raw materials, genetic changes in production cell lines, environmental fluctuations,

and microbial contamination can contribute to batch-to-batch variation and increase production costs. Such variability may also affect product quality, consistency, and potentially vaccine performance. Maintaining uniformity becomes increasingly challenging during scale-up because changes in raw-material characteristics, mixing efficiency, culture conditions, and downstream processing can become more consequential as production volumes increase [12, 28].

Several approaches could potentially improve the resilience and flexibility of vaccine-manufacturing systems. These include repurposing appropriate veterinary vaccine production infrastructure, establishing regional manufacturing hubs, using flexible single-use technologies, and developing modular manufacturing facilities that can be reconfigured for different production requirements. Technologies requiring lower antigen doses or simplified production processes may also offer economic advantages. However, manufacturing strategies developed for other vaccine platforms, including RNA-based technologies, require platform-specific evaluation before their relevance to FAdV VLP production can be established [12, 28].

Stability and storage of VLPs

VLP stability represents another major challenge because structural integrity must be preserved during production, purification, formulation, transport, and storage. Thermal stress can weaken protein-protein interactions and promote particle disassembly or aggregation. Studies involving other viral VLP systems have demonstrated substantial differences in thermal sensitivity according to capsid composition and particle architecture. Foot-and-mouth disease virus-derived particles, for example, have been investigated extensively as models for capsid destabilization and stabilization. Similarly, adenovirus-derived particles can undergo structural alterations and aggregation under unfavorable physicochemical conditions [38, 56].

Enveloped and non-enveloped VLPs also differ in their physicochemical properties. Enveloped particles contain a lipid membrane that introduces additional sensitivity to temperature and other environmental stresses, whereas non-enveloped particles depend predominantly on interactions among structural proteins for particle integrity [15, 57]. Consequently, stability requirements should be determined individually for each VLP platform rather than extrapolated directly among unrelated viral systems.

Storage conditions may further influence VLP integrity. Temperature fluctuations and repeated freeze-thaw cycles can promote aggregation, dissociation, or conformational changes in some VLP systems. Studies of unrelated VLP platforms have reported substantial losses of structural integrity after repeated freeze-thaw exposure, emphasizing the importance of appropriate formulation and storage conditions [15, 57]. These observations provide relevant technological context for FAdV VLP development, although corresponding stability limits and shelf life characteristics must be determined experimentally for FAdV-derived particles.

Several approaches have been investigated to improve VLP stability. Protein engineering can introduce stabilizing interactions, including strategically positioned disulfide bonds or amino acid substitutions designed to reduce unfavorable electrostatic interactions. Formulation approaches using nonionic surfactants may reduce aggregation under mechanical stress, whereas chemical cross-linking can potentially increase particle rigidity and thermotolerance [15, 56, 57]. However, excessive structural stabilization could alter antigenic epitopes or interfere with the conformational flexibility required for optimal immune recognition. Therefore, improvements in physicochemical stability must be evaluated together with antigenicity and immunogenicity.

Lyophilization represents another potential approach for reducing dependence on continuous cold-chain storage. Cryoprotectants such as trehalose and sucrose can help preserve protein and particle structure during freezing and dehydration. Optimization of lyophilization conditions may therefore improve the storage stability of VLP formulations. Analytical techniques, including nanoparticle tracking analysis, can additionally facilitate monitoring of particle size distributions and aggregation during formulation development [15, 56]. Nevertheless, the optimal formulation, storage temperature, and shelf life of FAdV VLP vaccines remain to be established experimentally.

Advanced structural characterization of FAdV particles

Advanced structural methods have considerable potential to improve understanding of FAdV capsid organization and assembly. High-speed atomic force microscopy (HS-AFM), cryogenic electron microscopy, and related high-resolution techniques can provide complementary information regarding viral particle architecture and, in suitable experimental systems, dynamic protein interactions. Combining experimental structural methods with computational modeling could therefore facilitate investigation of capsid-protein interactions, assembly intermediates, and environmental factors influencing particle formation [26, 58, 59].

HS-AFM has been used in viral and protein-assembly research because it can monitor molecular processes dynamically rather than providing only static structural snapshots. Such approaches could potentially be applied to investigate the association of hexon trimers with penton- and fiber-containing complexes and to examine how parameters such as pH, ionic strength, temperature, and protein concentration influence FAdV VLP assembly. At present, however, findings obtained from other viral assembly systems should be regarded as a methodological basis for future FAdV studies rather than direct experimental evidence of FAdV assembly kinetics.

High-resolution structural studies have provided important information on FAdV-4 capsid organization and stability. The FAdV-4 capsid exhibits a pseudo-T = 25 icosahedral architecture, with hexons forming the principal capsid shell and penton bases occupying the vertices. Structural features of the FAdV-4 hexon, including the P1 and P2 domains and surface-exposed hypervariable regions, are relevant to capsid stability, antigenic diversity, and potential vaccine engineering [32]. These findings provide a structural framework for understanding FAdV capsid organization and for guiding future studies of VLP assembly and stability.

Epitope mapping and rational antigen design

Detailed mapping of antigenic regions can complement structural studies and guide rational vaccine design. Chai *et al.* [60] used peptide-array scanning, truncation analysis, and alanine-scanning mutagenesis to identify three linear B-cell epitopes within the fiber-1 knob domain: 319SDVGYLGLPPH329, 328PHTRDNWYV336, and 407VTTGPIPFYSYQ417. Structural analysis indicated that the two contiguous epitopes, 319SDVGYLGLPPH329 and 328PHTRDNWYV336, were prominently exposed on the exterior of the fiber-1 knob trimer, whereas 407VTTGPIPFYSYQ417 occupied a different position within the three-dimensional structure [60].

Identification of such epitopes provides opportunities for designing recombinant antigens, chimeric proteins, and potentially engineered VLPs displaying selected immunologically relevant regions. However, recognition of a linear B-cell epitope does not itself establish protective efficacy. Candidate epitopes require further evaluation for accessibility on assembled particles, immunogenicity, neutralizing capacity, conservation among circulating serotypes, and protective efficacy in controlled challenge studies.

Computational modeling of capsid assembly

Multiscale computational modeling provides another potential approach for investigating VLP assembly. Atomistic molecular dynamics, coarse-grained modeling, and related computational approaches can be used to examine protein-protein interactions, estimate energetic barriers, and identify structural regions that may influence particle assembly or stability. When integrated with experimental structural data, these approaches could help prioritize candidate modifications for subsequent laboratory testing [26, 59].

Models of capsid self-assembly developed for other viruses, including MS2 bacteriophage, demonstrate how computational approaches can describe nucleation, particle growth, and alternative assembly pathways [26]. Such frameworks could potentially inform the development of FAdV-specific models. However, predicted stoichiometric requirements, energetic interaction hotspots, assembly intermediates, or stabilizing mutations should not be assigned to FAdV unless they have been derived from and experimentally validated in FAdV systems.

Machine-learning approaches may eventually complement these methods by identifying relationships among sequence, structure, environmental conditions, and assembly outcomes. A future closed-loop strategy could integrate computational prediction with experimental particle characterization, allowing proposed modifications to be tested iteratively. This approach may accelerate identification of constructs with improved assembly efficiency and stability, although its application to FAdV VLP development remains an emerging research direction.

Broader applications of engineered VLPs

Beyond vaccination, VLP technology is increasingly being investigated as a platform for therapeutic delivery and molecular engineering. Engineered VLPs (eVLPs) can potentially exploit viral capsid architecture and cellular uptake mechanisms while avoiding the complete infectious genome of the parental virus. Their modular structure allows encapsulation or display of different biological cargos and surface ligands, creating opportunities for applications extending beyond conventional vaccination [2].

In broader biomedical research, eVLP platforms have been investigated for delivery of gene editing components, nucleic acids, and therapeutic proteins. Experimental systems have explored delivery of Clustered regularly interspaced short palindromic repeats (CRISPR)-associated components, base-editing or related molecular machinery, antisense molecules, mRNA, and functional proteins. These applications illustrate the

engineering versatility of VLP platforms, although they are distinct from FAdV VLP vaccine development and should not be interpreted as evidence of equivalent capabilities for FAdV-derived particles.

VLP-based approaches have also been investigated in cancer immunotherapy and targeted antigen delivery. Preclinical studies using non-FAdV systems have demonstrated that engineered particles can enhance antigen presentation and stimulate cellular immune responses [2, 61]. The modular architecture of VLPs can facilitate antigen incorporation and surface functionalization with targeting ligands, potentially supporting tissue-specific delivery or customized antigen presentation [10, 62]. These developments provide a broader technological perspective on VLP engineering but remain outside the direct evidence base for FAdV vaccines.

STRATEGIES TO OVERCOME CURRENT LIMITATIONS OF FAdV VLP DEVELOPMENT

Enhancing vaccine production capacity

Flexible modular manufacturing: Modular and reconfigurable manufacturing facilities could allow production capacity to be adapted to different vaccine platforms and production volumes. For FAdV VLPs, such systems could facilitate scale-up if compatible expression and purification processes are established.

Regionalized production: Establishment of regional manufacturing hubs could reduce dependence on extended supply chains and potentially improve access to veterinary vaccines in regions with substantial poultry production.

Reuse of existing infrastructure: Existing veterinary vaccine or biotechnology facilities could potentially be adapted for VLP manufacture where production systems, biosafety requirements, and quality control standards are compatible. The extent of regulatory revalidation required would depend on the production platform and jurisdiction.

Alternative high-productivity platforms: Emerging technologies that reduce antigen requirements, shorten production cycles, or improve expression efficiency could provide useful manufacturing concepts. However, technologies such as self-amplifying RNA represent fundamentally different vaccine platforms and should not be described as direct solutions to FAdV VLP production without comparative evaluation.

Reducing production costs and improving process efficiency

Procurement and supply-chain optimization: Coordinated procurement and reliable regional supply networks could reduce vulnerability to shortages of essential raw materials and consumables.

Component and process innovation: Development of lower-cost culture media, purification materials, and production reagents could improve the economic feasibility of veterinary VLP manufacture.

Flexible consumables and automation: Appropriate single-use or reusable process components, combined with automation of monitoring, documentation, and quality control procedures, could potentially improve manufacturing efficiency while maintaining sterility and product quality.

Process analytical technologies: Real-time monitoring of critical process parameters could help reduce batch-to-batch variability and facilitate control of particle quality during scale-up.

Cell-line and environmental control: Stable production cell lines and tightly controlled culture parameters, including pH, temperature, nutrient availability, and ionic conditions, may improve reproducibility of capsid-protein expression and particle assembly.

Closed-loop optimization: Integration of computational predictions with real-time or high-resolution experimental measurements could eventually enable iterative optimization of VLP assembly and production conditions.

Raw-material standardization: Consistent sourcing and rigorous quality assessment of raw materials are essential for reducing process variability and maintaining reproducible vaccine quality.

Improving VLP stability and storage

Protein engineering: Rational amino acid substitutions or engineered intermolecular interactions could potentially improve particle stability. Any structural modification must be evaluated for effects on assembly, antigenicity, and immunogenicity.

Stabilizing excipients: Surfactants, stabilizers, or carefully selected cross-linking approaches may reduce aggregation and improve resistance to mechanical or thermal stress, although their compatibility with FAdV VLPs requires experimental validation.

Optimized lyophilization: Development of lyophilized formulations containing suitable cryoprotectants, including trehalose or sucrose, may improve storage stability and potentially reduce cold-chain dependence.

Particle monitoring: Nanoparticle tracking analysis and complementary physicochemical methods could be incorporated during formulation development to monitor particle size, aggregation, and stability.

Integrating advanced structural and computational tools

Advanced imaging: HS-AFM and other high-resolution structural approaches could be combined with computational modeling to investigate FAdV capsid-protein interactions and assembly mechanisms.

Machine-learning approaches: Once sufficiently large and experimentally validated FAdV datasets become available, machine-learning models could potentially assist in predicting assembly behavior and prioritizing candidate modifications.

In silico simulations: Atomistic and coarse-grained molecular simulations could support hypothesis generation concerning capsid interactions, stability, and antigen engineering, followed by experimental validation.

Expanding the engineering potential of VLP platforms

Modular antigen design: The modular nature of VLPs could be exploited to optimize antigen presentation or incorporate selected determinants from multiple FAdV serotypes, provided that modifications preserve particle assembly and immunogenicity.

Engineered delivery platforms: Broader eVLP research demonstrates the feasibility of using engineered particles to deliver biological cargos. Although these applications extend beyond FAdV vaccination, advances in particle engineering may eventually provide technologies applicable to veterinary vaccine design.

Integrated development strategy: Progress in FAdV VLP development will require coordinated improvements in expression and assembly, scalable manufacturing, quality control, particle stability, antigen engineering, and experimental validation. Integration of structural biology, computational modeling, advanced analytical technologies, and controlled *in vivo* evaluation may facilitate development of reproducible and economically feasible FAdV VLP vaccines.

KEY FEATURES OF FAdV CAPSID PROTEINS RELEVANT TO VLP VACCINE STRATEGIES

Table 1 [22, 25, 26, 29, 34] summarizes the principal structural and immunological characteristics of FAdV capsid proteins and their potential relevance to VLP-based vaccine design. Fiber is an important surface-exposed capsid component involved in host-cell attachment and contributes to viral tropism and antigenicity. The receptor-interacting knob domain is particularly relevant because it contains antigenic determinants that can be targeted by vaccine-induced antibodies. These characteristics have supported the investigation of fiber-based vaccine candidates, including approaches targeting FAdV-8a and FAdV-8b associated with IBH. However, the antigenic diversity of fiber among serotypes may restrict heterologous protection and represents an important consideration when designing vaccines intended to provide broader coverage.

Table 1: Key structural and functional features of Fowl adenovirus capsid proteins and their potential roles in virus like particle-based vaccine development, including current evidence levels and cross-protection potential.

Protein	Function	Role in VLP	Evidence Level	Cross-protection potential	Reference
Hexon	Major capsid protein	Structural stability, immunogenicity	Experimental	Moderate–High	[22, 26, 29]
Fiber-1	Cell attachment (secondary role)	May enhance immune response	Experimental (emerging)	Moderate	[25, 26, 29]
Fiber-2	Primary attachment, virulence-associated	Key antigenic determinant	Strong experimental	High (but variable)	[25, 26, 29]
Penton base	Virus internalization	Supports particle assembly	Experimental	Low–Moderate	[26, 34]
Combined proteins	Full capsid mimicry	Essential for VLP formation	Experimental	High	[22, 26]

Hexon constitutes the major structural component of the capsid and contains both conserved structural domains and surface-exposed HVRs. The latter contribute substantially to serotype-specific antigenicity and immune recognition. This combination of structural conservation and antigenic variability makes hexon an important target for serotyping, molecular characterization, and vaccine development. However, the ability of hexon-based vaccines to provide protection against multiple serotypes depends on the conservation, accessibility, and protective relevance of the incorporated antigenic determinants.

The penton base occupies the capsid vertices and interacts structurally with fiber while contributing to processes associated with viral entry. Its position within the capsid architecture makes it relevant to the formation of native-like particles. Consequently, the coordinated incorporation of hexon, fiber, and penton base may provide a structural basis for producing particles that more closely reproduce important antigenic features of the native FAdV capsid. Nevertheless, the assumption that combining these three proteins necessarily results in broader cross-protection requires experimental confirmation because structural similarity alone does not establish protective efficacy.

Evidence from FAdV vaccine studies demonstrates the potential value of incorporating antigens from multiple serotypes. Recombinant and inactivated multivalent approaches targeting FAdV-4, FAdV-8b, and FAdV-11 have generated substantial immune responses and protection under experimental challenge conditions [63–65]. These findings are relevant to multivalent antigen design but should be distinguished from evidence obtained specifically with fully assembled VLP vaccines.

For example, an inactivated recombinant construct incorporating antigens associated with FAdV-4, FAdV-8b, and FAdV-11 reportedly induced immune responses against all three serotypes and provided clinical protection following challenge [64]. In another approach, recombinant FAdV-4 expressing FAdV-8b fiber (FA4-F8b), generated using CRISPR-Cas9 and homologous recombination, was evaluated as an inactivated vaccine and induced neutralizing antibody responses together with protection against FAdV-4 and FAdV-8b challenge [64]. A recombinant inactivated vaccine targeting FAdV-4 and FAdV-8b was also reported to provide protection against both serotypes in 3-day-old specific-pathogen-free (SPF) chickens [65].

A chimeric recombinant subunit vaccine incorporating antigenic components from FAdV-4 and FAdV-11 has likewise been reported to protect 1-day-old SPF chickens against challenge [38]. In addition, experimental coinfection studies involving infectious bursal disease virus (IBDV) and FAdV-4 have investigated protection against simultaneous or combined disease challenges [66]. Collectively, these studies demonstrate the feasibility of multivalent and chimeric FAdV vaccine strategies, although they represent different vaccine technologies and should not be interpreted collectively as direct evidence of FAdV VLP efficacy.

Despite encouraging experimental findings, comparisons among FAdV vaccine studies remain difficult because of substantial variation in vaccine formulation, antigen dose, adjuvant use, vaccination schedule, route of administration, age of birds, challenge strain, and experimental endpoints [67]; some studies also provide limited characterization of cellular immune responses, restricting interpretation of the mechanisms underlying protection [68, 69]. In addition, the absence of standardized challenge models and consistent efficacy endpoints complicates direct comparison of protection among vaccine platforms [69]. Future studies should therefore combine standardized experimental designs with detailed humoral, cellular, virological, pathological, and clinical assessments to determine whether multicomponent VLP formulations provide reproducible advantages over existing FAdV vaccine approaches.

VLP-based vaccine strategies are increasingly being investigated as alternatives or complements to conventional FAdV vaccines because they can reproduce important structural and antigenic characteristics of viral particles without containing a complete infectious viral genome [8]. Consequently, VLPs avoid the risk of reversion to virulence associated with replication-competent live-attenuated vaccines while retaining repetitive antigen presentation that may promote effective immune recognition [8, 9].

Despite these advantages, development of FAdV VLP vaccines remains at a relatively early stage [13, 70]. Important limitations include the small number of comprehensive experimental studies, difficulties in achieving efficient and reproducible capsid-protein expression and particle assembly, challenges in large-scale manufacturing, and inconsistent cross-protection among antigenically distinct serotypes [22, 71]. Future development should therefore focus on improving capsid-protein expression, optimizing particle assembly, and identifying conserved protective antigenic regions that could support broader protection [4, 72]. Integration of structural biology, computational modeling, and protein engineering may facilitate rational design and optimization of next-generation FAdV VLP candidates [9, 73].

STRUCTURAL PROTEINS AS TARGETS FOR VLP DEVELOPMENT

This review focuses primarily on the three major FAdV capsid proteins, hexon, fiber, and penton base, because of their central roles in capsid architecture, host-cell interactions, immune recognition, and potential VLP formation. These proteins have been extensively investigated and represent important targets for understanding FAdV biology and developing recombinant vaccine strategies.

Other minor capsid and core-associated proteins may also contribute to virion stability, maturation, and assembly. However, available evidence defining their specific contributions to FAdV VLP generation remains limited; consequently, these proteins are addressed more briefly in this review.

Studies involving adenoviruses outside the FAdV group, including human adenoviruses, when they provided relevant structural, mechanistic, or immunological information to help interpret FAdV assembly pathways, viral biology, or VLP engineering. Earlier landmark studies were also included where necessary to establish fundamental concepts, although this review primarily emphasizes literature published from 2015 onward to capture recent advances in structural virology, recombinant vaccine platforms, and VLP-based strategies relevant to avian adenoviruses [3, 14, 29].

ADVANTAGES AND LIMITATIONS OF VLP-BASED STRATEGIES

Inactivated and live-attenuated vaccines have played important roles in FAdV control. Nevertheless, conventional platforms can have limitations related to manufacturing, safety, antigenic diversity, and incomplete cross-protection against heterologous serotypes [13, 69]. VLPs provide a distinct approach because their particulate organization can mimic important features of the native viral surface without containing a complete infectious genome. This characteristic eliminates the possibility of reversion to virulence arising from replication of the vaccine particle itself and provides opportunities for controlled antigen engineering.

The repetitive presentation of antigens on VLP surfaces can promote B-cell recognition and antibody responses, while uptake and processing by antigen-presenting cells may also support cellular immune responses [16]. However, the magnitude and quality of these responses depend on VLP composition, antigen organization, dose, adjuvant, administration route, and host factors. Therefore, strong humoral and cellular immunity should not be assumed for every FAdV VLP formulation.

The modular characteristics of VLP platforms also provide opportunities to display selected epitopes or heterologous antigens. In principle, this flexibility could support chimeric or multivalent formulations incorporating antigenic determinants from several FAdV serotypes or, potentially, unrelated avian pathogens [16, 25]. Such strategies could be valuable where multiple FAdV serotypes co-circulate. Nevertheless, successful incorporation of multiple antigens does not necessarily establish broad protection, and candidate formulations require experimental evaluation of particle assembly, antigen accessibility, immunogenicity, and protective efficacy.

EXPERIMENTAL EVIDENCE AND CURRENT LIMITATIONS

Available studies provide encouraging evidence for FAdV-derived recombinant antigens and selected VLP candidates, but the evidence base remains heterogeneous. Fully assembled FAdV VLPs should be distinguished from recombinant hexon or fiber proteins, epitope-display systems, recombinant viral constructs, inactivated vaccines, and other particulate vaccine platforms. This distinction is particularly important when interpreting protection, reduction in viral replication, pathological lesions, or virus shedding.

Claims that FAdV VLP vaccines have already been extensively validated under laboratory and field conditions are premature. Current evidence supports continued investigation of these platforms, but it is insufficient to conclude that FAdV VLP vaccination consistently interrupts transmission or establishes population-level herd immunity. Likewise, direct evidence demonstrating reduced environmental shedding and indirect protection of unvaccinated birds remains limited.

The potential incorporation of marker antigens could support differentiation of infected from vaccinated animals (DIVA) strategies. However, DIVA capability is not an intrinsic characteristic of all VLP vaccines. It requires deliberate antigenic design together with a compatible diagnostic assay capable of distinguishing vaccine-induced responses from those generated by natural infection. Accordingly, FAdV VLPs should be considered potentially compatible with DIVA strategies rather than inherently DIVA-capable.

The modularity of VLPs may also facilitate modification of antigen composition in response to emerging strains. Nevertheless, the claim that antigenic updates can routinely be completed and implemented within weeks is not yet established for FAdV VLP vaccines because redesign must be followed by characterization of particle assembly, stability, antigenicity, immunogenicity, protective efficacy, manufacturing reproducibility, and regulatory assessment. Current research should therefore focus on developing adaptable platforms while maintaining rigorous validation of modified vaccine candidates [16].

MATERNAL ANTIBODIES AND FIELD-RELATED FACTORS

Maternal immunity and environmental conditions can substantially influence vaccination outcomes in commercial poultry production, yet these factors have not been comprehensively investigated for FAdV VLP vaccines [74]. Maternal antibodies may alter the timing and magnitude of vaccine-induced responses, making vaccination schedules an important consideration when translating experimental candidates into field use.

A study cited in the manuscript reported differences in Enzyme-linked immunosorbent assay (ELISA)-measured maternal antibody levels against FAdV-8a in progeny derived from grandparent flocks of different ages. A geometric mean titer (GMT) of 927 was reported for progeny associated with 30-week-old grandparents compared with 3393 for those associated with 50-week-old grandparents after vaccination of the grandparent flocks at 10 and 17 weeks of age [75]. Antibodies were no longer detectable by ELISA at 3 weeks of age in the evaluated groups. These observations indicate that breeder vaccination history, flock age, maternal antibody transfer, and possible field exposure should be considered when determining vaccination schedules. However, the suggested contribution of field infection to the higher antibody level in older flocks remains an interpretation rather than a demonstrated causal mechanism.

RECOMBINANT EPITOPE-DISPLAY APPROACHES

Epitope-based recombinant approaches provide additional information relevant to rational FAdV vaccine development. A study cited in the manuscript evaluated three predicted FAdV-4 hexon epitope regions displayed using hepatitis B virus core protein (HBc)-based particles. Following two immunizations administered 2 weeks apart, the constructs exhibited immunogenicity but differed in protective efficacy. The most promising candidate, incorporating the Asp348-Phe369 region, reportedly achieved 90% survival in 7-day-old broiler chickens following administration of 100 µg of recombinant antigen [52].

These findings support the potential of particulate epitope-display strategies but should not be interpreted as evidence for a fully assembled FAdV capsid-derived VLP. HBc-based particles represent a heterologous display platform carrying selected FAdV epitopes. Such approaches may offer advantages for antigen engineering and recombinant production, but their immunological and manufacturing characteristics differ from those of VLPs assembled from FAdV structural proteins.

Multi-epitope vaccine strategies incorporating B- and T-cell epitopes may also facilitate rational antigen selection and recombinant production. However, claims of faster or less expensive production than conventional vaccines require economic and manufacturing comparisons specific to the selected expression system and production scale. At present, commercialization of FAdV VLP vaccines remains limited, with most candidates remaining experimental, whereas inactivated and live vaccines continue to represent established approaches [17, 74, 76].

STABILITY, DELIVERY, AND ADJUVANT DEVELOPMENT

Improving storage stability represents an important objective for future FAdV VLP development, particularly where continuous refrigeration is difficult to maintain. Formulation and protein engineering strategies that improve resistance to thermal stress could facilitate vaccine distribution in resource-limited settings. However, increased physicochemical stability must be achieved without compromising antigen conformation or immunogenicity.

Improved delivery systems and adjuvants represent another important research direction. Dendritic cells (DCs) are potent antigen-presenting cells and therefore represent relevant targets for strategies intended to enhance antigen uptake and subsequent adaptive immune responses [77]. Fusion of antigens with immunostimulatory molecules has also been explored as a means of enhancing vaccine responses.

Salmonella flagellin acts as an agonist of Toll-like receptor 5 and has been investigated as an immunostimulatory component in vaccine formulations. Fusion of flagellin with selected antigens can combine antigen delivery with innate immune stimulation, although the effects depend on antigen structure, route of administration, dose, and host species [78]. Such approaches may inform future FAdV vaccine design but should be distinguished from direct evidence involving FAdV-derived VLPs.

MUCOSAL DELIVERY USING RECOMBINANT PROBIOTICS

Mucosal vaccination represents another potential strategy for improving the practicality of FAdV

immunization in poultry. One study evaluated recombinant probiotic bacteria displaying FAdV-4 Fiber-2 on their surface. *Enterococcus faecalis* strain MDXEF-1 and *Lactococcus lactis* NZ9000 were investigated as delivery hosts for surface-anchored Fiber-2. Engineered recombinant bacteria were administered orally to chickens using three vaccinations at 2-week intervals [79].

The *E. faecalis* pTX8048-DCpep-Fiber2-CWA construct reportedly provided 100% protection against homologous FAdV-4 challenge in SPF chickens following oral administration at 1.0×10^{10} colony-forming units (CFU) [79]. This finding demonstrates the potential of mucosal delivery of recombinant FAdV antigens. However, this platform is a recombinant bacterial surface-display vaccine rather than a FAdV VLP vaccine and should therefore be discussed as a complementary vaccine development strategy.

IMMUNOGLOBULIN (IgY)-ANTIGEN IMMUNIZATION STRATEGIES

An additional approach has combined yolk IgY with inactivated FAdV-4 antigen as co-immunogens [80]. In SPF chickens, the IgY-antigen formulation reportedly induced stronger humoral responses than a conventional inactivated vaccine, as assessed using serum neutralization and ELISA assays. A single administration at 14 days of age was reported to provide protection against subsequent lethal FAdV-4 challenge [80].

This strategy is relevant to the broader development of improved FAdV vaccines, particularly with respect to antigen formulation and enhancement of humoral immunity. However, the IgY-antigen complex is not a VLP vaccine and should not be presented as evidence of FAdV VLP efficacy. The unusually broad reported protection period and challenge schedule should also be verified directly against reference [80] before the precise duration is retained.

NANOPARTICLE-ASSISTED VACCINE DELIVERY

Traditional FAdV vaccine strategies include inactivated, live-attenuated, recombinant subunit, and viral-vector approaches. Depending on the platform, vaccination schedule, and target serotype, booster immunizations or adjuvants may be required to maintain protective immunity, while heterologous cross-protection can remain variable [81]. Nanotechnology-based delivery systems provide another approach for enhancing antigen stability, delivery, and immunogenicity.

Nanoparticle-based adjuvants may protect antigens from degradation, facilitate uptake by antigen-presenting cells, and modulate innate and adaptive immune responses [82]. Their effects nevertheless depend strongly on nanoparticle composition, size, surface properties, dose, and route of administration, and their safety must be evaluated alongside immunogenicity.

A recent study cited in the manuscript evaluated an inactivated FAdV vaccine formulated with silver nanoparticles (AgNPs) in broiler chickens [83]. The AgNPs were synthesized using *Pongamia pinnata* leaf extract and characterized using ultraviolet-visible spectroscopy. Forty-five broiler chickens were allocated among an AgNP-adjuvanted vaccine group, a commercial alum-adjuvanted vaccine group, and an unvaccinated control group. Vaccination was performed at 7 and 21 days of age, followed by oral FAdV challenge on day 28.

The AgNP-adjuvanted group reportedly developed significantly higher antibody responses and GMTs than the alum-adjuvanted and control groups ($p < 0.05$). Following challenge, survival was reported as 80% in the AgNP-adjuvanted group, 73% in the commercial vaccine group, and 13% in the unvaccinated control group [83]. These findings suggest that nanoparticle-based adjuvant systems warrant further investigation for FAdV vaccines. Nevertheless, this study evaluated an inactivated FAdV vaccine with a nanoparticle adjuvant, rather than a VLP vaccine, and therefore provides supporting evidence for formulation development rather than direct evidence for FAdV VLP efficacy.

FUTURE TRANSLATIONAL PERSPECTIVES

FAdV VLPs may eventually provide opportunities extending beyond conventional antigen delivery. The modular architecture of VLPs permits incorporation or surface presentation of additional biological components, raising the possibility of dual-function platforms that combine vaccination with delivery of immunomodulatory molecules or other bioactive agents. Such applications remain largely conceptual in the context of FAdV and require experimental validation before their relevance to poultry health can be established [13, 16, 25, 69].

Integration of vaccination programs with digital flock-health monitoring could also improve evaluation of vaccine performance under commercial conditions. Production records, serological surveillance, disease occurrence, environmental variables, and other flock-level data could potentially support more responsive

vaccination programs. However, individualized real-time vaccination based on electronic health records has not been established for FAdV VLP vaccines and should be considered a prospective application rather than a current capability.

More broadly, successful development of FAdV VLP vaccines could contribute to improved prevention of FAdV-associated disease and potentially support animal health and production efficiency. Claims that these vaccines will directly reduce antimicrobial use, transform global poultry production, or produce population-level improvements in biosecurity and animal welfare require appropriate field evidence and should not be assumed from experimental vaccine efficacy alone.

Evidence gaps and requirements for field translation

Research specifically addressing FAdV VLP vaccines remains limited compared with the broader literature on recombinant, subunit, inactivated, and multivalent FAdV vaccines [1, 48]. Much of the available evidence derives from laboratory studies conducted under controlled experimental conditions, and relatively few investigations have evaluated fully assembled FAdV VLPs in poultry challenge models [25]. Consequently, findings from recombinant capsid proteins, chimeric constructs, viral vectors, or conventional vaccines should not be considered direct evidence of the efficacy of fully assembled FAdV VLPs.

This limitation is particularly important when considering translation to commercial poultry production. Experimental challenge studies cannot completely reproduce the effects of maternal antibodies, environmental stress, concurrent infections, management conditions, nutritional status, or exposure to multiple circulating FAdV serotypes. Large-scale field trials and direct comparative studies will therefore be required to establish the effectiveness, duration of immunity, cross-protective capacity, safety, and practical advantages of FAdV VLP vaccines under commercial conditions [69].

Manufacturing and interdisciplinary development

Development and commercialization of FAdV VLP vaccines involve interconnected structural, immunological, manufacturing, and regulatory challenges. Scalable production systems must support efficient expression and assembly of the required structural proteins while maintaining product quality and economic feasibility. Improvements in physicochemical stability and shelf life will also be important, particularly where dependence on cold-chain infrastructure limits vaccine distribution. Downstream processes must provide adequate purification while preserving particle integrity and controlling process-related impurities [15, 23].

Standardized methods for evaluating particle identity, structural integrity, antigen content, potency, safety, and protective efficacy will be required to support reproducible manufacturing and comparison among vaccine candidates. Regulatory requirements for veterinary biological products can also vary among jurisdictions, making early consideration of quality control and regulatory requirements important during product development [23].

Addressing these challenges will require collaboration among structural biologists, virologists, immunologists, veterinarians, protein engineers, bioprocess specialists, computational scientists, vaccine manufacturers, and regulatory authorities. Advances in cryogenic electron microscopy, high-resolution structural analysis, computational modeling, synthetic biology, and data-driven optimization could contribute to the rational design of particles with improved assembly, stability, and antigen presentation. However, modifications predicted computationally must be confirmed experimentally and evaluated for their effects on antigenicity, immunogenicity, safety, and manufacturability.

Collaboration among academic institutions, veterinary vaccine manufacturers, regulatory agencies, animal health organizations, and poultry-industry stakeholders may facilitate translation of promising experimental candidates into scalable vaccines. Such collaboration may be particularly important in regions where poultry production contributes substantially to food supply and economic livelihoods and where vaccine affordability, storage requirements, and distribution infrastructure influence practical implementation [15, 16, 23].

Emerging multivalent and chimeric approaches

Although VLPs offer several potential advantages, including the absence of a complete infectious genome, native-like antigen presentation, and compatibility with deliberately designed DIVA strategies, evidence demonstrating their effectiveness specifically against FAdV remains comparatively limited [8, 24]. Reported protection from experimental FAdV vaccine studies must be interpreted according to the platform evaluated because protection achieved using recombinant proteins, inactivated vaccines, viral vectors, or chimeric constructs cannot automatically be attributed to FAdV VLPs.

Recent multivalent and subunit approaches provide useful benchmarks for future VLP development [84, 85].

The modular characteristics of VLPs could potentially permit incorporation of antigenic determinants from multiple FAdV serotypes within a single particle or formulation [24, 73]. This feature is relevant because substantial antigenic diversity among FAdVs complicates development of broadly protective vaccines [71, 84].

Chimeric fiber constructs incorporating components from different serotypes, including constructs targeting FAdV-4 and FAdV-11, have demonstrated the feasibility of broadening the antigenic composition of experimental FAdV vaccines [85]. Such studies support the underlying concept of multivalent antigen engineering. However, chimeric recombinant proteins and recombinant viral vaccines are distinct from fully assembled multivalent VLPs. Their protective efficacy therefore provides a rationale for investigating multivalent VLPs rather than direct evidence that such VLP formulations will provide equivalent cross-protection.

Regulatory, production, and field validation requirements

Several challenges must be addressed before FAdV VLP vaccines can be considered for widespread commercial application. Veterinary vaccine development requires evaluation of product identity, quality, safety, potency, consistency, and protective efficacy. These requirements may be particularly demanding for multicomponent particles because alterations in expression or assembly can affect particle structure and antigen presentation [24].

Batch-to-batch consistency and particle stability during manufacture, storage, transportation, and administration are therefore critical development considerations. Appropriate analytical methods will be needed to monitor capsid composition, particle size and integrity, aggregation, antigenicity, and biological potency.

Large-scale field evaluation is equally important. Environmental stress, maternal immunity, coinfections, flock management, vaccination route, bird age, and circulating viral diversity may influence vaccine performance under commercial conditions [69, 71]. Controlled laboratory efficacy should therefore be followed by appropriately designed field studies before conclusions regarding commercial effectiveness are made.

Future development could additionally investigate advanced adjuvant systems, improved stabilization and delivery technologies, and complementary combinations with other vaccine approaches [24, 73]. Whether VLPs can be combined effectively with subunit or vector-based platforms to improve cross-protective immunity should be determined experimentally rather than assumed from the theoretical compatibility of these technologies [17, 69].

LIMITATIONS AND EMERGING EVIDENCE

Despite growing interest in VLP-based vaccines as a next-generation approach for controlling FAdV infections, several limitations of the available evidence must be recognized. A substantial proportion of the reported protective and cross-reactive immune responses originates from studies evaluating recombinant hexon or fiber proteins, inactivated vaccines, chimeric constructs, or viral-vector systems rather than fully assembled FAdV VLPs. Consequently, conclusions concerning broad serotype coverage, durability of immunity, and long-term protective efficacy of FAdV VLP vaccines should remain cautious until supported by comprehensive *in vivo* investigations using well-characterized multicomponent VLP formulations.

Recent studies describing multivalent and chimeric adenoviral vaccine technologies provide important structural, immunological, and translational information relevant to FAdV vaccine engineering. Nevertheless, many advanced candidates remain at the experimental or preclinical stage, and large-scale field evidence is limited. These studies should therefore be used to identify promising design principles and research directions rather than to establish the clinical or commercial effectiveness of FAdV VLP vaccines.

Differences among experimental studies also limit direct comparisons. Vaccine composition, antigen dose, adjuvant, administration route, vaccination schedule, age and immune status of birds, challenge strain, challenge dose, and definitions of protection vary considerably among investigations. Standardized experimental and reporting approaches would improve comparison of candidate vaccines and help determine whether VLP formulations provide measurable advantages over established platforms.

Publication bias is another potential limitation of the available literature because studies reporting successful particle assembly, favorable immunogenicity, or protective efficacy may be more likely to be published than studies reporting unsuccessful assembly or limited protection. Because this review was narrative rather than a formal systematic review with a predefined risk-of-bias assessment, the magnitude of publication bias cannot be quantified. The evidence was therefore interpreted cautiously, with particular attention to distinguishing experimentally demonstrated findings from emerging concepts and extrapolations from other vaccine platforms [13, 23, 69].

CONCLUSION

FAdV VLPs represent a promising experimental platform for poultry vaccine development because they can present viral structural antigens in an organized particulate form without containing a complete infectious viral genome. Current structural knowledge of hexon, fiber, and penton base provides an important foundation for understanding capsid organization, antigen presentation, and potential particle assembly. However, direct experimental evidence for fully assembled FAdV VLP vaccines remains substantially more limited than the broader evidence available for conventional, recombinant, subunit, chimeric, and multivalent FAdV vaccines.

The modular characteristics of VLPs could potentially support incorporation of antigenic determinants from epidemiologically important FAdV serotypes and thereby facilitate development of multivalent vaccine candidates. Successful translation to poultry production would require vaccines that can be manufactured economically and reproducibly, remain stable during storage and distribution, and provide reliable protection under commercial conditions. Improvements in formulation, delivery, and manufacturing may further increase their practical applicability.

This review integrates evidence concerning FAdV capsid architecture, structural-protein function, self-assembly, recombinant expression, vaccine immunology, production technologies, and emerging antigen engineering strategies. It also considers findings from related adenovirus and VLP systems where these provide relevant mechanistic or technological context. Importantly, the review distinguishes fully assembled FAdV VLPs from recombinant capsid proteins, chimeric antigens, inactivated vaccines, viral vectors, and other vaccine platforms.

The principal limitation of the current evidence base is the relatively small number of studies directly evaluating fully assembled FAdV VLP vaccines. Heterogeneity in vaccine formulations, experimental models, immunization protocols, challenge conditions, and efficacy endpoints further complicates comparison among studies. Evidence from other adenoviruses and unrelated VLP platforms can provide mechanistic guidance but cannot be assumed to apply directly to FAdV. In addition, the narrative nature of this review does not permit quantitative assessment of publication bias or formal comparison of vaccine efficacy across platforms.

Future research should prioritize well-characterized FAdV VLP constructs evaluated through standardized *in vitro* characterization and controlled *in vivo* poultry studies. Important objectives include improving capsid-protein expression and assembly efficiency, defining protective and conserved epitopes, developing multivalent constructs, improving thermostability and storage characteristics, optimizing adjuvants and delivery systems, and establishing scalable manufacturing and quality control processes. Large-scale field trials will ultimately be required to determine vaccine performance under commercial production conditions. Structural biology, computational modeling, protein engineering, and synthetic biology may accelerate candidate development when integrated with rigorous experimental validation.

FAdV VLP technology provides a scientifically compelling framework for next-generation poultry vaccine development, but its practical value must ultimately be established through direct experimental and field evidence. Continued multidisciplinary collaboration among researchers, veterinary vaccine manufacturers, poultry industry stakeholders, and regulatory authorities will be important for translating structural and immunological advances into reproducible and commercially feasible vaccines. Addressing current limitations in assembly, stability, cross-serotype protection, manufacturing, and field validation will determine whether FAdV VLPs can progress from promising experimental candidates to practical tools for sustainable control of FAdV-associated diseases.

DATA AVAILABILITY

No new data were generated or analyzed in this review article. All information discussed is derived from previously published studies, which are cited in the manuscript.

GENERATIVE AI DECLARATION

The authors declare that no generative AI tools were used in the preparation of this manuscript.

AUTHORS' CONTRIBUTIONS

MM, MIN, and MHB: Conceptualization. MM, MIN, MHB, and NMS: Methodology. MM and MIN: Project administration. MIN, MHB, and NMS: Supervision. MM: Writing-original draft. MM, MIN, MHB, and NMS: Writing

and review. MFA: Writing original draft, supervision, review, and editing. All authors have read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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