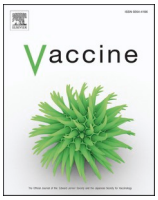
Vaccine 43 (2025) 126496

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Review

Vaccination strategies to protect chickens from fowl adenovirus (FAdV)-induced diseases: A comprehensive review

Carlotta De Luca *, Michael Hess

Clinic for Poultry and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

ARTICLE INFO

Keywords:
Fowl adenovirus
Hepatitis-hydropericardium syndrome
Inclusion body hepatitis
Live vaccines
Inactivated vaccines
Subunit vaccines

ABSTRACT

In recent years, fowl adenovirus (FAdV)-induced diseases became a global problem with considerable impact on chicken health and welfare. This has prompted numerous studies to focus on experimental immunization strategies using whole virus formulations (live or killed vaccines), some of them modified as recombinantly constructed vector vaccines. In addition, FAdV capsid proteins were frequently reported as immunizing antigens (subunit vaccines), with fiber proteins being amongst the most successful candidates. To date, there is no standardized protocol to assess vaccine efficacy in experimental FAdV protection studies, with the consequence that the experimental settings present several degrees of variations even when sharing similar premises. Differences in formulation preparations, route of inoculation, antigen dose, vaccination scheme, choice of challenge strain, or type and age of the birds are capable to greatly influence the magnitude of the immune response and the consequent protective efficacy, altogether addressing remaining challenges. Beyond the antigen composition of a vaccine, the epidemiology of FAdVs with the potential of vertical transmission of virus and/or antibodies from breeders to progenies has a substantial impact on protection strategies. The goal of this review is to outline a broad overview of the findings made thus far regarding immunization strategies against diseases associated to FAdV infections, considering the literature published since the appearance of hepatitis-hydropericardium syndrome (HHS) in the late Eighties, in order to emphasize the current knowledge on FAdV vaccines and highlight fields of future research and intervention.

1. Introduction

Fowl adenoviruses (FAdVs) belong to the family *Adenoviridae*, genus *Aviadenovirus*, and are classified into five species, FAdV-A to -E, according to their genome sequences. FAdV species were very recently renamed by the International Committee on Taxonomy of Viruses (ICTV) by introducing binominal latinized names: *Aviadenovirus ventriculi, A. quintum, A. hydropericardii*, *A. gallinae* and *A. hepatitidis* [1]. However, for the sake of clarity and to avoid confusion with existing literature, the assignment of species to letters A-E will be kept throughout this review (Table 1). A further classification sees these viruses divided into twelve serotypes (FAdV-1 to -8a, and FAdV-8b to -11), determined by serological neutralization test and, subsequently, molecular phenotyping [1–4]. FAdVs exhibit an icosahedral structure, consisting of non-enveloped double-stranded DNA viruses, with capsid composed by 252 capsomers: 240 hexons and 12 pentons, each one structured by a penton base and two fibers [5–7]. The FAdV genome contains a single gene encoding for the fiber protein with the exception of species FAdV-A and -C, where the two fibers refer to separate open reading frames (ORFs), denoted here as fiber-1 and fiber-2 [8,9].

The origin of FAdVs can be traced back to 1949, when a virus was accidentally isolated from embryonated chicken eggs [10]. Since then, FAdVs have been detected in various avian species, in healthy and diseased individuals, indicating a wide distribution and potential impact on poultry health [11]. To this date, three diseases affecting chickens have been associated to different species and serotypes of FAdVs: adenoviral gizzard erosion (AGE), caused by FAdV-A (serotype 1), hepatitis-hydropericardium syndrome (HHS), caused by FAdV-C

Abbreviations: AGE, adenoviral gizzard erosion; AGPT, agar gel precipitation test; EDS, egg drop syndrome; ELISA, enzyme-linked immunosorbent assay; FAdV, fowl adenovirus; HE, hemorrhagic enteritis; HHS, hepatitis-hydropericardium syndrome; IBH, inclusion body hepatitis; MAbs, maternal antibodies; NAbs, neutralizing antibodies; NDV, Newcastle Disease Virus; non-NAbs, non-neutralizing antibodies; ORF, open reading frame; Pb-Dd, penton-dodecahedron; SPF, specific pathogen-free; VLPs, virus-like particles; VNT, virus neutralization test; VP2, viral protein 2; vvIBDV, very virulent infectious bursa disease virus.

* Corresponding author at: Clinic for Poultry and Fish Medicine, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria.
E-mail addresses: carlotta.deluca@vetmeduni.ac.at (C. De Luca), michael.hess@vetmeduni.ac.at (M. Hess).

https://doi.org/10.1016/j.vaccine.2024.126496
Received 14 April 2024; Received in revised form 15 October 2024; Accepted 27 October 2024
Available online 9 November 2024

Table 1
Overview of FAdV-associated diseases.

disease	etiological agent	main host(s)	experimental vaccines
adenoviral gizzard erosion (AGE)	*Aviadenovirus ventriculi* (FAdV-A), serotype 1 (FAdV-1)	broilers, layers, pullets	live vaccines
hepatitis-hydropericardium syndrome (HHS)	*Aviadenovirus hydropericardii* (FAdV-C), serotype 4 (FAdV-4)	broilers	live vaccines inactivated vaccines subunit vaccines
inclusion body hepatitis (IBH)	*Aviadenovirus gallinae* (FAdV-D), serotypes 2 and 11 (FAdV-2, -11) *Aviadenovirus hepatitidis* (FAdV-E), serotypes 8a and 8b (FAdV-8a, -8b)	broilers	live vaccines inactivated vaccines subunit vaccines

(serotype 4), and inclusion body hepatitis (IBH), caused by FAdV-D (serotypes 2 and 11) and -E (serotypes 8a and 8b) [12] (Table 1). As the name suggests, AGE affects mainly the gizzard of the host, whereas HHS and IBH are metabolic diseases that find their main target in young broilers [12]. Currently, FAdVs represent a worldwide concern that continues to pose an ever-growing challenge to the poultry sector [13]. Despite efforts to implement biosecurity measures, there is a current lack of comprehensive approaches to safeguard chickens against these diseases, with research on vaccine development at the forefront. A comprehensive list of protection studies, with associated formulations and experimental settings, can be found as Supplementary Table 1, updated from Schachner et al. [12].

2. Live vaccines

Live vaccines are regarded as highly suitable for mass application, which is favoured in the poultry industry. Considering the faecal-oral transmission of FAdV, such vaccines should be able to trigger a protective immune response in the host after oral or respiratory administration [14]. However, live virus formulations come with the risk of shedding the vaccine strain, with potential spread to wild birds and subsequent dissemination in the environment, an issue that has already been described for aviadenoviruses [15,16]. Moreover, live formulations require a careful integration in the immunization schemes adopted in the field, as interaction with pre-existing vaccines or the presence of subclinical infections in the flock must be considered.

When it comes to FAdVs, live formulations possess the significant feature of being the only successful vaccines described against AGE so far, as they alone are able to induce an adequate local immunity in the gastrointestinal tract of chickens after oral inoculation [17,18].

2.1. Adenoviral gizzard erosion

Unlike HHS and IBH, which exhibit similarities in terms of disease development and protective mechanisms, notable distinctions arise in terms of protection against AGE. In particular, it has been observed that systemic maternal antibodies (MAbs) do not protect chickens from developing the disease after ocular and/or nasal infection with virulent FAdV-1 [17,18]. Conversely, instances have been documented where clinically healthy birds displayed no lesions in the gizzard upon re-infection with the same virulent FAdV-1 [19]. These findings highlight the need for an oral administration of the vaccine in order to allow the onset of a local immune response in the gastrointestinal tract. Therefore, experimental studies in the context of AGE have focused on the administration of apathogenic FAdV-1 strains prior challenge [12]. Grafl et al. demonstrated that oral inoculation of 1-day-old specific pathogen-free (SPF) broiler chicks with apathogenic FAdV-1 prevented the onset of clinical signs and pathological lesions upon challenge with a virulent field strain twenty days later, although infection and shedding of the virulent isolate were still documented [20]. Later on, it was confirmed that the same strategy could be used to protect SPF layer-type chickens with either single or prime-boost vaccination between 14 and 17 weeks of life and challenge at 20-week-old [21]. The levels of circulating neutralizing antibodies (NAbs) induced by such vaccine differ between immunized young broilers and layers in production, with higher and more widely distributed titers in the latter [20,21]. Despite the fact that systemic humoral immunity does not appear to be the crucial mechanism when it comes to horizontal protection against AGE, these observations serve as further confirmation of the influence of age and chicken type in the efficiency of the immune response. On the other hand, data on the local cellular response, thought to be the elective immune pathway against the disease, are still lacking and further research is needed to fully clarify the protective mechanisms of this vaccine approach.

2.2. Hepatitis-hydropericardium syndrome

Live-attenuated FAdV-4 vaccines against HHS primarily owe their standardization and formulation to embryo and cell culture. Schonewille et al. achieved the development of a live attenuated strain derived from a virulent FAdV-4 field isolate after adaptation in a fibroblast cell line (QT-35) through repeated passages [22]. This attenuated strain was effectively employed for the immunization of SPF layer chickens against HHS, after oral administration within the first day of life. However, it was noted that the vaccine could not completely prevent the shedding of the virus after challenge [22,23]. Moreover, birds excreted the vaccine strain up to seven days post vaccination, confirming the potential risk of environmental spillover [22,23]. FAdV-4 attenuation was also achieved through repeated passages on chicken embryos, as well as Vero cell line, and successfully adopted for further experimental vaccines against HHS [24,25]. Ali et al. demonstrated that, even after lyophilisation, the cell culture-attenuated FAdV-4 was more efficient than its inactivated counterpart in protection, antibody development, and phyto-hemagglutinin-P assay [25]. Different routes of administration were also tested for such vaccines, showing that ocular and spray administration are less efficient than oral or intramuscular inoculation [26]. It is worth noting that all the studies revolving around live FAdV-4 vaccines have employed a strain-homologous vaccine/challenge setting, using the original virulent strain for both vaccine development and challenge. As no investigations over the cross-protective potential across serotype boundaries of these vaccines have been pursued, the matter of broad coverage, even within the realm of diverse FAdV-4 strains, remains to be assessed.

Recently, studies on FAdV-4 live formulations focused on designing recombinant strains carrying engineered modifications in their major structural proteins in order to overcome the common issues of live vaccines, such as safety and potential spreading to the environment. In 2021, recombinant FAdV-4 strains were expressed as live-attenuated variants by targeting the region between the tail and shaft of fiber-1 or -2, and generating modified fiber fusion proteins by replacing ORFs non-essential for replication with a cassette for fluorescent proteins [27,28]. These constructs were able to protect intramuscularly immunized SPF chickens from strain-homologous challenge through the development of circulating NAbs, without inducing shedding of the

vaccine strain. Similar results were achieved with the same technology by deletion of the fiber-2 domain responsible for the interaction with the karyopherin alpha 3/4 host protein [29]. It was also shown that the complete deletion of fiber-2 did not affect the production of circulating NAbs against FAdV-4 in birds after immunization, which led to full protection upon strain-homologous challenge [30]. Moreover, replacing the hexon gene of a virulent FAdV-4 with one from an apathogenic strain led to the generation of an attenuated and protective live vaccine that could be used through various inoculation routes (intranasal, intramuscular, or subcutaneous) [31]. Pan et al. developed a recombinant FAdV-4 that could be used as a vector for viral subunits from different viruses, such as the VP2 protein of very virulent infectious bursal disease virus (vvIBDV) [32]. The rescued recombinant virus induced NAbs against both FAdV-4 and vvIBDV, and could protect SPF chickens after intramuscular administration, showing that such technology can be adopted to expand the protective spectrum of FAdV vaccines across viral species [32].

Whilst recombinant live formulations seem to rely on the development of systemic NAbs, the neutralizing activity of vaccine-derived antibodies was shown to be dispensable for other live FAdV-4 vaccines, as first demonstrated by Schonewille et al. [23]. In fact, on top of protecting chickens from the disease in the absence of a systemic humoral response, this oral vaccination with attenuated FAdV-4 prevented challenge-induced depletion of $CD4^+$ and $CD8^+$ T lymphocytes in thymus and B lymphocytes in bursa of Fabricius of immunized birds [22]. This represents the first instance of investigations on the cellular immune response to FAdV vaccines.

2.3. Inclusion body hepatitis

When devising immunization strategies to combat IBH, it is essential to consider the varied aetiology of the disease and the need for a wide-ranging defence against the spread of the causative FAdVs. Therefore, information over broad-spectrum protection between FAdV serotypes and species are of great value for these types of vaccines.

Live FAdV vaccines against IBH were first described in 1993, when a study conducted by Pallister et al. documented a successful spray-vaccination of SPF chickens using a FAdV-E strain obtained from an IBH outbreak and associated with NAbs development [33]. Years later, dual-serotype formulations consisting of two FAdV strains belonging to FAdV-D and -E were assessed against strain-homologous challenges [34,35]. In these studies, broiler breeders were vaccinated orally with a formulation containing FAdV-11 mixed with either FAdV-8a or -8b, which led to the development of NAbs against the cognate serotypes and, additionally, FAdV-2. Subsequently, the transfer and protective efficacy of MAbs was tested *via* virus neutralization test (VNT) and challenge of the progeny, which resulted protected from FAdV-2, -7, -8a, -8b, and -11 [35]. On the other hand, single vaccination with live FAdV-8b within the same setting led to development of NAbs against FAdV-8a and -8b, but their neutralizing activity did not extend to FAdV-2 or -11 [34,35]. Shedding of the vaccine virus by live FAdV-8b immunized-birds led to in-contact naïve broiler breeders developing comparable NAbs levels against FAdV-8a and -8b [35]. However, it was also shown that intramuscular injection of this FAdV-8b vaccine in 29-week-old broiler breeders vertically induced IBH within the first week post vaccination, until an adequate level of circulating NAbs was reached in the breeders, with subsequent MAbs transferred to the progeny [34]. On the contrary, no vertical transmission was noticed when birds were vaccinated orally at 16 weeks of age with a dual serotype formulation (FAdV-8b + FAdV-11). In fact, a FAdV-8b strain identical to the commercial live vaccine was isolated in the field in Australia, where attenuated vaccines derived from virulent FAdV-8b are utilized to this date to immunize breeder flocks, underlining the balance between safety and efficacy [36]. Despite this preventive measure, outbreaks of IBH triggered by FAdV-8b and -11 continue to be documented in Australia. Although this could be attributed to variations in vaccination strategies in terms of dosage, timing, and administration methods in the field, it points towards incomplete protection of progenies when breeders are solely immunized with live vaccines. Furthermore, such findings confirm that certain FAdV live vaccines represent a potential spillover hazard for the environment.

3. Inactivated vaccines

Historically, inactivated vaccines represent the first attempt to immunize birds against FAdV-related diseases [37]. Since then, several protection studies have focused on the inactivation of FAdV strains as a suitable vaccine strategy to protect chickens [24,25,38–40], and most of the available commercial vaccines, as well as autogenous formulations, are inactivated products [12]. Despite their efficacy, such vaccines are bound to a parenteral route of administration, most commonly intramuscular and occasionally subcutaneous, which affects their suitability for mass application in the field, a highly desirable feature in the poultry industry.

3.1. Hepatitis-hydropericardium syndrome

The late 1980s and early 1990s saw the first attempts to control FAdV-related diseases using autogenous vaccines made from inactivated liver homogenates obtained from HHS outbreaks [37,41,42]. In these formulations, still in use in certain regions, the inactivation of the virus is carried out through treatment with variable concentrations of formalin [24,43,44]. However, standardization and characterization of the antigen are hardly possible for these vaccines. Consequently, cell or egg culture-purified and propagated virus were adopted to prepare the antigen prior to inactivation in attempt to standardize vaccine formulations [25,38,45–48]. It is worth noticing, however, that numerous discrepancies between studies persist to this day, especially in regards to vaccine and challenge dosages, as well as type and age of chickens.

Inactivated vaccines against FAdV-4 are linked to the stimulation of a potent humoral immune response, often measured *via* ELISA, although other methods such as AGPT or phyto-hemagglutinin-P assay were used [25,47–52]. Fewer studies have characterized the neutralizing activity of such response, linking the efficacy of inactivated FAdV-4 vaccines to the development of NAbs [28,53,54]. Transmission of MAbs from vaccinated breeders to their progeny and consequent vertical protection has also been demonstrated for inactivated FAdV-4 vaccines [38,49]. In particular, Kim et al. tested the efficacy of such Mabs to protect 1-day-old progeny not only against FAdV-4, but also against various FAdV serotypes, finding that the survival rate of chicks from seropositive breeders was significantly increased compared to the challenge control group against FAdV-4, -8b and -11 [49]. Another study tested the cross-protective potential of FAdV-4 inactivated vaccines by challenging vaccinated broilers with a virulent FAdV-8a strain, observing a significant reduction in the lesions on liver and kidneys of immunized birds [55].

Analogously to live vaccines against HHS, the latest trend for inactivated FAdV-4 formulations relies on recombinant FAdV-4 strains carrying modifications in their structural proteins, such as replacement of the hexon gene with one from an apathogenic strain, or FAdV-4 expressing the vvIBDV VP2 protein. It was in fact shown that the inactivated counterparts of successful recombinant live vaccines were also able to protect SPF chickens from strain-homologous challenge, albeit often not as efficient as the live-attenuated ones due to induction of lower NAbs titers [28,56,57].

3.2. Inclusion body hepatitis

Inactivated dual-serotype formulations represent the first published attempt to counter the issue of broad protection against IBH with inactivated vaccines. In fact, a commercial formulation comprising strains from serotypes 8a and 11 was able to protect chickens not only from homologous isolates, but also from FAdV-8b challenge [39]. In this

case, commercial broilers and broiler breeders from vaccinated grandparents were successfully tested for the presence of MAbs by FAdV-8a ELISA, and subsequently challenged within their first week of life with either FAdV-8a, -11 (homologous vaccine strains), or -8b. The lack of a challenge control group for the FAdV-8a and -11 settings makes it difficult to evaluate protection against these strains, although survival rates ranged from 92 to 98%. However, cross-protection was ensured against FAdV-8b challenge, which caused 100% mortality in the challenge control group (seronegative SPF broilers). It is interesting to notice that the broiler breeder flocks obtained from 30-week-old grandparents (vaccinated at 10 and 17 weeks of life) had significantly lower ELISA-measured MAbs against FAdV-8a compared to the ones hatched from 50-week-old grandparents. This suggests that not only the vertical transmission of MAbs does not wane down with the age of the breeders, but it may even increase due to a possible field infection of the grandparent flocks [39]. Similar results were achieved with a bivalent formulation including FAdV-8b and -11 inactivated strains, which resulted protective for the progeny of vaccinated broiler breeders against strain-homologous challenge and FAdV-2 challenge [34]. Such protection was associated to broadly reactive NAbs (*vs.* serotypes FAdV-2, -7, -8b, -8a, and -11) in serum of the vaccinated breeders, which translated in vertical transmission of MAbs. It must be highlighted that FAdV-2 and FAdV-11 share a high degree of genetic identity and cross-neutralizing potential, to the point that their categorization as two distinct serotypes has been questioned [36,58]; therefore, a certain degree of cross-protection is expected.

Another report of possible cross-protection comes from a study in SPF layer-type chickens, vaccinated at 3-week-old with a formalin-inactivated formulation of either FAdV-8a, -8b, or -11 field isolates, and challenged three weeks later with either homologous strain [59]. The results showed that, aside from the expected homologous protection for FAdV-8b and -11 vaccines, the FAdV-8a vaccine also protected birds against FAdV-8b and -11 infection, representing the first instance of broad-spectrum protection across different viral species from vaccines formulated with IBH-causing serotypes. On the other hand, the results also showed that FAdV-8b-vaccinated birds were not protected from FAdV-11. These data only partially correlate to the observations over the associated humoral response: while all the immunized birds had detectable antibodies on commercial IBH ELISA and NAbs against the respective vaccine strain before challenge (albeit with variable titers), cross-reacting NAbs against FAdV-8b and -11 were present in low titers only in a few FAdV-8a-vaccinated birds [59]. Therefore, in this case, the role of NAbs in cross-protection is unclear and deserves further investigation. Moreover, the effect of such vaccines on the cellular immunity of the birds should also be more widely explored.

Inactivated vaccines against IBH are also dose-dependent, as recently illustrated by Wu et al. [60], who showed that a dose of $3\times10^{4.5}$ $TCID_{50}$ formalin-inactivated FAdV-8a vaccine was not as efficient as higher doses ($3\times10^{5.5}$ $TCID_{50}$ and $3\times10^{6.5}$ $TCID_{50}$) in protecting chickens challenged with the homologous strain from developing clinical signs and lesions in the target organs.

4. Subunit vaccines

The concept of utilizing capsid proteins as subunit antigens for crafting vaccines against FAdVs draws inspiration from prior investigations on other adenoviruses targeting poultry. The immunogenic nature of structural proteins, notably hexon, fiber, and fiber-knob, had previously been established for hemorrhagic enteritis (HE) in turkeys and egg drop syndrome (EDS) in chickens. These studies showcased that vaccination with adenoviral structural proteins could trigger systemic NAbs and decrease the viral load in the spleen following HEV infection [61–63]. This prompted the investigation over FAdV subunit vaccines, which demonstrated that it is feasible to generate specific antibodies against recombinant capsid proteins, including fibers, penton base, hexon, and 100K [64–69]. In general, subunit vaccines and inactivated vaccines share similar drawbacks, including the lack of mass administration due to the requirement for a parenteral route of inoculation. Depending on the expression platform, recombinant proteins can be manufactured in fermenters, eliminating the need for tissue culture (unlike inactivated vaccines), which can have an impact on production costs. FAdV subunits are commonly expressed in *E. coli*, although a few studies have reached equally successful results *via* the baculovirus system.

4.1. Hepatitis-hydropericardium syndrome

The first successful subunit vaccine against FAdV-related diseases, namely HHS, was described in 2012 by Shah et al. [65]. For this, the penton base of FAdV-4 was effectively utilized to vaccinate 2-week-old commercial broiler chickens against challenge by the same strain. However, protection rates reported by further penton base vaccination studies are somewhat inconsistent. Two studies noted a dose-dependent effect, with survival rate of immunized chickens challenged with homologous FAdV-4 strain varying from 35% to 100% according to the quantity of administered subunit [70,71]. In addition, variations in the vaccine dose do not fully explain the discrepancies in survival rates, as higher degrees of protection have been achieved with lower dosages [65,72], and higher subunit quantities have only achieved 50% survival rate on another occasion. It is interesting to note that, in this instance, the FAdV-4 isolate used for challenge did not correspond to the vaccine template [43]. Other factors that seem to be influencing the magnitude of the immune response are type and age of the birds. As a general rule, older birds develop a higher antibody response than 1-day-old birds and have better chances to be protected upon challenge, possibly due to a more mature immune system [12,43,65,70]. Even so, in the case of penton base vaccines, the detection of antigen-specific antibodies on ELISA does not necessarily correlate with clinical protection from HHS [43,70,72,73]. Furthermore, the discrepancies in outcomes observed across different studies suggest a potentially crucial role for conformational epitopes of recombinant penton base proteins and their stability. Structural analysis of this subunit from various FAdV isolates suggested that the penton base could be unstable in extracellular environments [74]. A recent study aimed to address this issue by generating highly stable variants of the FAdV-4 penton base before conducting an *in vivo* protection study. Remarkably, the stable variants achieved 100% protection, in contrast to the full-length protein [73]. Therefore, maintaining the three-dimensional structure of the subunit could play a pivotal role in eliciting a penton base-specific response that offers substantial protection.

To this date, the most effective outcomes in safeguarding chickens against HHS using subunit vaccines have been attained through FAdV-4 fiber-2 vaccination. The effectiveness of this approach was initially demonstrated in 2014, establishing full protection against HHS [67]. Following this proof of concept, subsequent studies have consistently affirmed this outcome. Further investigations explored a range of factors, including fiber-2 vaccine dosages, expression systems, formulations, and immunization schemes [40,53,70,71,75,76]. A positive dose-dependent effect has been described for fiber-2 vaccines as well, with higher quantities of inoculated subunit corresponding to higher antibody titers on ELISA and broader degrees of protection, although the minimum protective dosage seems to depend on the age of the birds and the vaccination scheme [40,54,71,72]. While the levels of ELISA-measured antibodies before challenge typically correlate well with protection, the same does not apply to the presence of NAbs, which seem to be dispensable to prevent the onset of severe HHS [67]. This has been recently confirmed when chickens immunized with the knob region of fiber-2 showed a good antibody response on ELISA but no neutralizing activity, still translating to 100% coverage against strain-homologous challenge [54]. Similarly, immunization with a chimeric fiber protein merging epitopes from FAdV-4 fiber-2 and FAdV-11 fiber was able to grant protection from virulent FAdV-4 even in the absence of systemic

NAbs [77]. Chen et al. reported higher protective efficacy of fiber-2 vaccine compared to the inactivated whole virus formulation derived from the same FAdV-4 isolate [53]. In this case, the protection rate correlated well with antibody titers measured by ELISA, even though the neutralizing activity of such antibodies was higher for the whole virus formulation. The observed development of NAbs following fiber-2 inoculation may be connected to the older age of the immunized birds compared to previous studies, where fiber-2 was unable to stimulate the production of NAbs [53,67,77]. This scenario reflects the reported situation of live FAdV-4 vaccines, straightening the hypothesis over an important role of the non-NAbs and/or the cellular immune response when it comes to protection from HHS [23,77,78]. Moreover, the integration of additional subunits into the recombinant fiber-2 protein, such as short peptides from *Salmonella* Typhimurium, has been shown to enhance the immune response, translating to higher degree of protection when compared to simple fiber-2 immunization [76]. Despite the frequent success of FAdV-4 fiber-2 vaccines against HHS, their broad protective potential still needs to be further explored, not only across serotype boundaries, but also within the growing variety of FAdV-4 strains. In fact, while Schachner et al. and De Luca et al. assessed protection by FAdV-4 fiber subunits against a FAdV-4 challenge strain distinct from the vaccine template, most studies have focused on testing the subunit in strain-homologous vaccine/challenge settings. Zhao et al. recently showed that such protection does not necessarily translate to protection against different FAdV-4 isolates [52], as SPF chickens vaccinated with fiber-2 from an apathogenic FAdV-4 were completely unprotected from challenge with a virulent isolate, as opposed to chickens immunized with fiber-2 derived from the same strain [52].

Contrary to fiber-2, research on FAdV-4 fiber-1 as an immunization antigen has yielded somewhat conflicting results, with varying survival rates observed in vaccinated chickens subsequently exposed to HHS challenge [67,70,72,79]. Higher immunogenicity and 100% survival rate seem to be linked to a prime-booster regimen and an older age of vaccinated birds. In fact, whereas immunization of 1-day-old SPF chickens with 50 μg of fiber-1 was unable to elicit a detectable antibody response and only resulted in partial protection (as opposed to full protection by fiber-2 vaccination in the same setup), birds immunized with 100 μg at 2- and 4-week-old developed high titers of NAbs against FAdV-4 within three weeks post prime vaccination [67,79].

Interestingly, the most abundant capsid protein and the one historically described as the most immunogenic after adenovirus infection, the hexon [80], does not perform as efficient immunogen in chickens. Vaccination with recombinant FAdV-4 hexon loop-1 was unable to grant full protection against HHS, independent from the quantity of administered antigen, although higher survival rates correlated with increased ELISA antibodies [67,70]. More recently, three different epitope regions of FAdV-4 hexon were predicted *in silico* and expressed as recombinant virus-like particles (VLPs), before being used in an *in vivo* protection study [44]. Despite comparable levels of immunogenicity on antigen-specific ELISA, the three epitope regions achieved different levels of protection, with the highest survival rate being 90%, representing the most promising example of FAdV-4 hexon vaccine so far.

Another subunit tested for its efficacy as a protective antigen was the recombinant 100K protein, which could only achieve partial protection despite the high immunogenicity on antigen-specific ELISA [66]. More comprehensive antigens like penton-dodecahedron complexes (Pb-Dd), VLPs comprising the penton base with the attached trimeric structure of fiber-1 and fiber-2, were also tested as vaccine antigens, granting full protection from HHS to immunized SPF chickens [72].

All of these studies demonstrated that different FAdV-4 capsid proteins possess different immunogenic properties, and the 3D structure of such particles may be crucial to ensure a functional epitope presentation to the immune system of the host. Therefore, recent works have been focusing on designing recombinant constructs merging epitopes from different subunits, in order to grant a more comprehensive stimulation of the immune system of the chicken. In 2021, Hu et al. successfully expressed a truncated FAdV-4 fiber-2 protein incorporating a hexon epitope, and this subunit proved to be immunogenic and protective against strain-homologous challenge [81]. More recently, Guo et al. proved the importance of recombinant subunit linear and 3D structure by expressing multiantigenic constructs of *in silico*-predicted epitopes derived from FAdV-4 penton base, hexon, fiber-1, and fiber-2, arranged in five different combinations, and using them in an *in vivo* protection study [82]. This experiment revealed that one specific epitope combination was superior to the others, with protection rates correlating to immunogenicity [82]. Similar findings were associated with successful vaccination with a recombinant chimeric fiber protein merging epitopes from FAdV-4 fiber-2 and FAdV-11 fiber, a construct designed to expand the protective efficacy of fiber immunization across different FAdV species, thus covering multiple FAdV-related diseases [77,78]. Such recombinant subunit proved able to prevent the onset of clinical signs and reduce lesions upon challenge with either virulent FAdV-4 and -11, albeit in the absence of NAbs [77]. Instead, the administration of this vaccine stimulated a rise of splenic $CD8\alpha^+$ T cells. Additionally, the levels of B lymphocytes and cytotoxic T cells in blood, liver and spleen of immunized birds rose due to proliferation in thymus after challenge, while splenic monocytes/macrophages recovered more rapidly in vaccinated birds after experiencing a challenge-induced decline [78]. Another study used solely the terminal part of the fiber protein as vaccine antigen, specifically a fusion knob with mixed FAdV-4 fiber-1 and fiber-2 identities, which provided full protection against homologous-strain challenge [83]. In this case, the protection did correlate with the development of NAbs, which were in fact higher than the titers induced by full FAdV-4 fiber-2 vaccination in the same setting. Overall, these data suggest that the immune mechanisms associated with subunit vaccines against HHS should be explored more in depth, keeping into account both the humoral response, as VNT is often neglected, and the cellular immune response on a systemic and local level.

More recently, FAdV-4 subunits have been incorporated as part of live microbiological vectors in order to become suitable to oral inoculation and potentially expand the protective spectrum of such formulations. However, a recombinant NDV LaSota vaccine strain expressing FAdV-4 fiber-2 afforded parenteral injection, as protection by oral vaccination was not successful [84]. The antigen was able to induce the production of anti-fiber-2 antibodies in SPF layers, with higher immunogenicity and protection when applied in its live formulation as opposed to the inactivated vaccine. Bacteria or yeast have also been engineered to express other FAdV-4 capsid proteins, namely the hexon or fiber-2, and proved to be systemically immunogenic after repeated oral inoculations in SPF chickens, achieving various degrees of protection after intramuscular challenge of strain-homologous FAdV-4 [85–87]. In this context, some vectors have performed better than others, such as *Enterococcus faecalis* and *Saccharomyces cerevisiae* (as opposed to *Lactococcus lactis*), and the highest protection rates were observed when the fiber-2 was used as antigen.

4.2. Inclusion body hepatitis

Similar to HHS, most experimental subunit vaccines against IBH revolve around recombinant fiber proteins. It is noteworthy that the protection conferred by vaccines with a FAdV-D or -E template appears to primarily depend on the stimulation of NAbs [34,35,59,88,89]. The FAdV-8b fiber was first tested as an immunizing antigen in a strain-homologous vaccine/challenge setting, granting a survival rate of 82.7% in progeny of vaccinated breeders, as opposed to 100% achieved by FAdV-8b-derived VLPs, and ~57% by FAdV-8b fiber knob [89]. In this case, higher protection rates were associated with higher level of NAbs in breeders and, subsequently, MAbs in their progeny. Additionally, a decrease in the $CD4^+$:$CD8^+$ T cell ratio was observed following a booster injection of either FAdV-8b VLPs or FAdV-8b fiber in commercial broiler breeders [89]. Despite this first success, the potential cross-protection efficacy of subunit vaccines against different IBH-causing

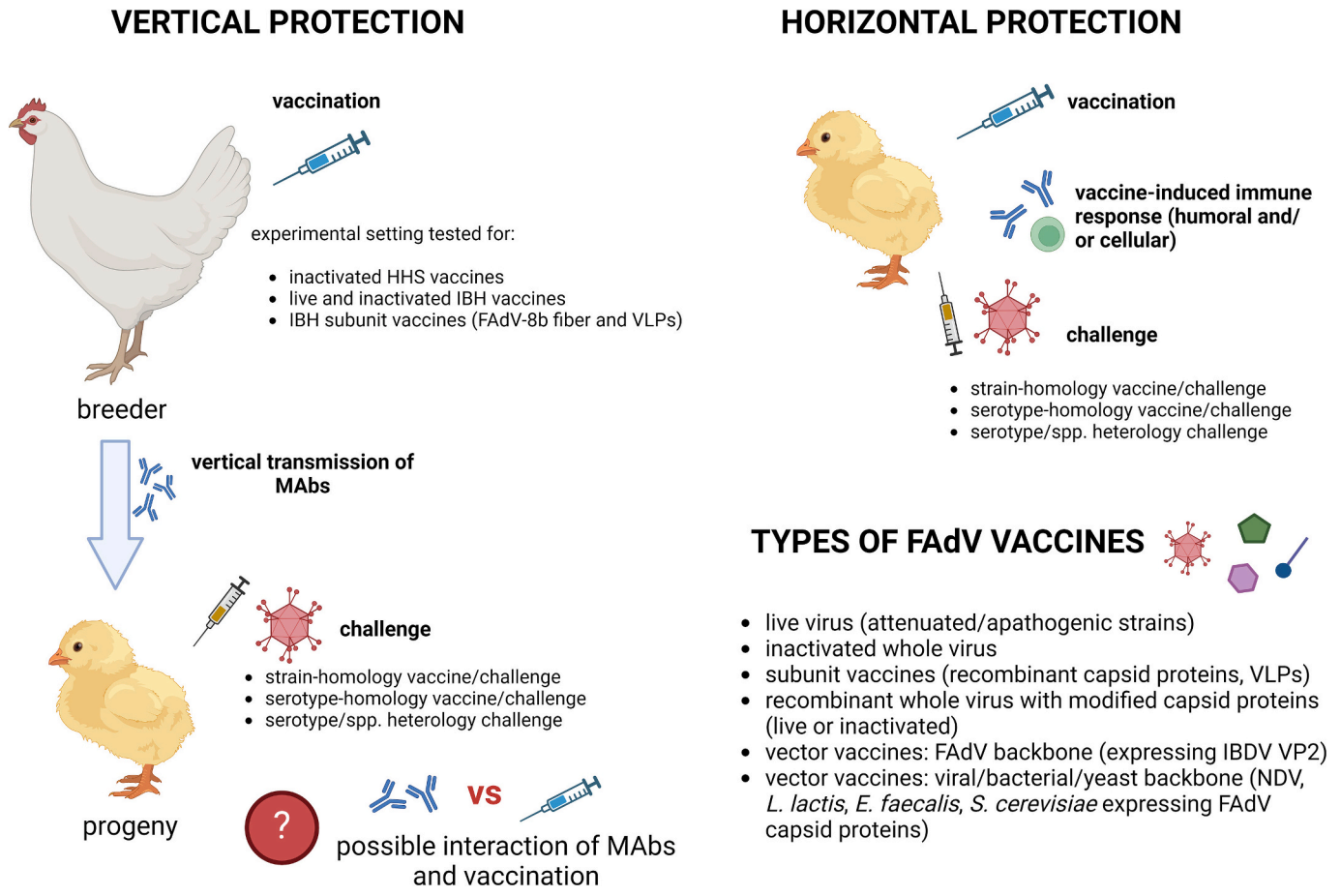


Fig. 1. Dynamics of FAdV-induced diseases with consequences on experimental vaccination strategies. Created with BioRender.com

serotypes represents a crucial point to be investigated. In this regard, De Luca et al. demonstrated that vaccination of SPF broiler chicks with FAdV-8a fiber led to production of serotype-specific NAbs, which translated in protection exclusively against FAdV-8a challenge, but not FAdV-8b [90]. This observation was further corroborated when a recombinant FAdV-4 whole-virus vaccine was engineered to express the FAdV-8b fiber and used in its inactivated form in a protection study: immunized chickens developed NAbs against FAdV-4 and -8b, and were protected from strain-homologous challenge. However, such antibodies were unable to neutralize FAdV-8a, leaving the birds vulnerable to FAdV-8a infection [51,91]. Analogously, vaccination with recombinant FAdV-4 expressing FAdV-8a fiber was able to induce protective NAbs against FAdV-4 and -8a, but not -8b [92]. Therefore, it appears that single fiber vaccination is insufficient to offer comprehensive protection against the diverse causative IBH agents, primarily due to the generation of serotype-specific NAbs. To devise a more encompassing strategy against IBH, Schachner et al. developed chimeric fiber proteins [93]. These proteins maintain the complete fiber structure while combining epitopes from FAdV-8a and -8b serotypes, accomplished through the introduction of a specificity switch in the fiber knob. Similar to results on chimeric constructs merging different FAdV-4 epitopes in different combinations, it was observed that, out of two chimeric fiber proteins with opposite FAdV-8a/8b identity on each side of the specificity switch, one was superior in eliciting bilateral NAbs and thus protecting SPF broiler chickens from both IBH-serotypes [93]. The potential cause for the inadequate development of NAbs by one of the variants could be attributed to structural disparities between the two constructs that led to distinct linear and structural epitope presentation. Consistently with the successful trend of recombinant FAdV vaccines retaining multiple serotype identities, it was demonstrated that vaccination with a newly-designed FAdV-4 expressing both FAdV-8b and -11 fibers (and lacking FAdV-4 fiber-1) was able to trigger antigen-specific antibodies in 7-day-old chickens, protecting them from challenge with any of the three serotypes, although information on NAbs are lacking [94].

Differently from HHS, vaccination with either native or recombinant FAdV-8b hexon protein was able to protect SPF layers after immunization at 1- and 9-day-old, and challenge as early as 16-day-old, although antibody titers measured on whole FAdV-8b ELISA were not detectable before challenge [64]. As for the penton base protein, despite the high degree of aa sequence conservation of the subunit [59,74], which suggests it as a suitable candidate for broad-protection against IBH-causing serotypes, the FAdV-E subunit failed to achieve protection when used to immunize SPF birds against virulent FAdV-8b challenge [95]. The reason for this failure may be due to the absence of vaccine-induced NAbs in immunized birds and the overall low immunogenicity observed on antigen-specific ELISA as well. Because of the possible instability of this protein in extracellular environments, we can hypothesize that ensuring proper folding of the recombinant protein may be necessary to achieve sufficient NAbs coverage [74,95].

These studies demonstrate that an adequate humoral response against IBH-inducing serotypes, and particularly the development of NAbs in FAdV-E vaccine/challenge settings, is fundamental in protecting chickens from the disease in the context of most subunit vaccines. As a consequence, fewer studies have expanded their investigation to explore the cellular response triggered by such formulations. Nevertheless, it was shown that FAdV-8a fiber vaccination was associated with the activation of systemic B and $CD4^+$ T lymphocyte populations, and the vaccine prevented a challenge-induced drop of circulating B cells and monocytes in SPF broilers [90]. These findings highlight the importance of both humoral and cellular immune response

investigations.

5. Conclusion and perspectives

The increasing trend of FAdV-induced diseases poses a significant economic challenge for the global poultry industry [13]. Stringent biosecurity measures, particularly for breeder flocks, coupled with the absence of commercially available broad-protective immunization strategies, have prompted substantial efforts in developing vaccines against FAdVs. Protection studies have demonstrated the efficacy of live, inactivated, and subunit vaccines, elucidating various factors influencing the associated immune response. However, the lack of standardization in experimental design across studies complicates drawing definitive conclusions for devising a comprehensive immunization strategy. While many studies serve as proof of concept for the efficacy of different vaccine models, there remains a need for in-depth analysis of various functional aspects of protection to facilitate their effective application in the field. The level of antibodies transmitted from breeders to progenies seems a crucial parameter for successful protection of broilers against FAdVs [96]. Understanding the dynamics of such immunity is fundamental, as the role of MAbs is to provide protection for the progeny until the age resistance to FAdV-related diseases comes into play (Fig. 1) [12]. Additionally, elucidating the potential interference of vertically transmitted MAbs (possibly due to prior infection in breeders) with antibody development in one-day-old broiler chicks post-vaccination is essential (Fig. 1). Comprehensive information on the immunogenic epitopes of different FAdV subunits needs to be expanded, particularly regarding the breadth of their protective spectrum within and across serotype boundaries. Despite the growing diversity of FAdV isolates, many experimental studies employed a homologous vaccine/challenge strain setting and only partially characterized the strains used. Addressing this plastic diversity in the context of protection stands as a significant challenge for FAdV vaccines as reports from the field indicate a change in strain circulation following introduction of vaccination [97–99].

Overall, significant knowledge about vaccination against FAdV-induced diseases has been accrued through numerous experimental vaccine studies. Nevertheless, the factors outlined above represent the missing pieces in the mosaic of experimental FAdV vaccines, critical for delineating an efficient, broad-protective immunization strategy that is still missing in the field.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 3.5 in order to perform grammar checks on the text of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Carlotta De Luca: Writing – original draft, Formal analysis, Conceptualization. **Michael Hess:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.126496.

Data availability

All data summarized in the present review can be found in the respective referenced studies.

References

[1] Benkő M, Aoki K, Arnberg N, Davison AJ, Echavarría M, Hess M, et al. ICTV virus taxonomy profile: Adenoviridae 2022. J Gen Virol 2022;103:001721. https://ictv.global/report/chapter/adenoviridae/adenoviridae/aviadenovirus. and ICTV Report Consortium [last consulted: 15.10.2024].
[2] McFerran JB, BMCC Adair. Avian adenoviruses – a review. Avian Pathol 1977;6:189–217.
[3] Hess M. Detection and differentiation of avian adenoviruses: a review. Avian Pathol 2000;29:195–206.
[4] Marek A, Günes A, Schulz E, Hess M. Classification of fowl adenoviruses by use of phylogenetic analysis and high-resolution melting-curve analysis of the hexon L1 gene region. J Virol Methods 2010;170:147–54.
[5] Laver WG, Younghusband HB, Wrigley NG. Purification and properties of chick embryo lethal orphan virus (an avian adenovirus). Virology 1971;45:598–614.
[6] Gelderblom H, Maichle-Lauppe I. The fibers of fowl adenoviruses. Arch Virol 1982;72:289–98.
[7] Tan PK, Michou AI, Bergelson JM, Cotten M. Defining CAR as a cellular receptor for the avian adenovirus CELO using a genetic analysis of the two viral fibre proteins. J Gen Virol 2001;82:1465–72.
[8] Hess M, Cuzange A, Ruigrok RWH, Chroboczek J, Jacrot B. The avian adenovirus penton: two fibres and one base. J Mol Biol 1995;252:379–85.
[9] Marek A, Nolte V, Schachner A, Berger E, Schlötterer C, Hess M. Two fiber genes of nearly equal lengths are a common and distinctive feature of fowl adenovirus C members. Vet Microbiol 2012;156:411–7.
[10] Van Den Ende M, Don PA, Kipps A. The isolation in eggs of a new filtrable agent which may be the cause of bovine lumpy skin disease. J Gen Microbiol 1949;3:174–83.
[11] Hess M. Aviadenovirus infections. In: Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair V, Suarez DL, editors. Diseases of poultry. Hoboken, NJ: Wiley-Blackwell; 2020. p. 322–31.
[12] Schachner A, Matos M, Grafl B, Hess M. Fowl adenovirus-induced diseases and strategies for their control – a review on the current global situation. Avian Pathol 2018;47:111–26.
[13] Schachner A, Grafl B, Hess M. Spotlight on fowl adenovirus (FAdV) in chickens and beyond – an unresolved host-pathogen interplay. Avian Pathol 2021;50:2–5.
[14] Collet SR. Principles of disease prevention, diagnosis, and control. In: Swayne DE, editor. Diseases of poultry. Iowa: Wiley-Blackwell, Ames; 2013. p. 1–60.
[15] Devlin JM, Vaz PK, Coppo MJC, Browning GF. Impacts of poultry vaccination on viruses of wild bird. Curr Opin Virol 2016;19:23–9.
[16] Das S, Fearniside K, Sarker S, Forwood JK, Raidal SR. A novel pathogenic aviadenovirus from red-bellied parrots (*Poicephalus rufiventris*) unveils deep recombination events among avian host lineages. Virol 2017;502:188–97.
[17] Okuda Y, Ono M, Yazawa S, Imai Y, Shibata I, Sato S. Pathogenicity of serotype 1 fowl adenovirus in commercial broiler chickens. Avian Dis 2001;45:819–27.
[18] Grafl B, Liebhart D, Günes A, Wernsdorf P, Aigner F, Bachmeier J, et al. Quantity of virulent fowl adenovirus serotype 1 correlates with clinical signs, macroscopical and pathohistological lesions in gizzards following experimental induction of gizzard erosion in broilers. Vet Res 2013;44:38.
[19] Ono M, Okuda Y, Yazawa S, Shibata I, Sato S, Okada K. Outbreaks of adenoviral gizzard erosion in slaughtered broiler chickens in Japan. Vet Rec 2003;153:775–9.
[20] Grafl B, Prokofieva I, Wernsdorf P, Steinborn R, Hess M. Infection with an apathogenic fowl adenovirus serotype-1 strain (CELO) prevents adenoviral gizzard erosion in broilers. Vet Microbiol 2014;172:177–85.
[21] Grafl B, Berger E, Wernsdorf P, Hess M. Successful reproduction of adenoviral gizzard erosion in 20-week-old SPF layer-type chickens and efficacious prophylaxis due to live vaccination with apathogenic fowl adenovirus serotype 1 strain (CELO). Vaccine 2020;38:143–9.
[22] Schonewille E, Singh A, Göbel TW, Gerner W, Saalmüller A, Hess M. Fowl adenovirus (FAdV) serotype 4 causes depletion of B and T cells in lymphoid organs in specific pathogen-free chickens following experimental infection. Vet Immunol Immunopathol 2008;121:130–9.
[23] Schonewille E, Jaspers R, Paul G, Hess M. Specific-pathogen-free chickens vaccinated with a live FAdV-4 vaccine are fully protected against a severe challenge even in the absence of neutralizing antibodies. Avian Dis 2010;54:905–10.
[24] Mansoor MK, Hussain I, Arshad M, Muhammad G. Preparation and evaluation of chicken embryo-adapted fowl adenovirus serotype 4 vaccine in broiler chickens. Tropl Anim Health Prod 2011;43:331–8.
[25] Ali S, Mahmood MS, Hussain I, Khan MN. Preparation and evaluation of lyophilized live attenuated vaccine of inclusion body hepatitis hydro-pericardium

syndrome (IBH-HPS) against challenge in broiler chickens. Int J Agric Biol 2015; 17:658–62.
[26] Yeo J, Lee R, Kim H, Ahn S, Park J, Sung HW. Genetic modification regulates pathogenicity of a fowl adenovirus 4 strain after cell line adaptation (genetic mutation in FAdV-4 lowered pathogenicity). Heliyon 2023;9:e19860.
[27] Mu Y, Xie Q, Wang W, Lu H, Lian M, Gao W, et al. A novel *Fiber-1*-edited and highly attenuated recombinant serotype 4 fowl adenovirus confers efficient protection against lethal challenge. Front Vet Sci 2021;8:759418.
[28] Xie Q, Cao S, Zhang W, Wang W, Li L, Kan Q, et al. A novel fiber-2-edited live attenuated vaccine candidate against the highly pathogenic serotype 4 fowl adenovirus. Vet Res 2021;52:35.
[29] Xie Q, Wang W, Li L, Kan Q, Fu H, Geng T, et al. Domain in Fiber-2 interacted with KPNA3/4 significantly affects the replication and pathogenicity of the highly pathogenic FAdV-4. Virulence 2021;12:754–65.
[30] Xie Q, Wang W, Kan Q, Mu Y, Zhang W, Chen J, et al. FAdV-4 without Fiber-2 is a highly attenuated and protective vaccine candidate. Microbiol Spectr 2022;10. e01436–21.
[31] Zhang Y, Pan Q, Guo R, Liu A, Xu Z, Gao Y, et al. Immunogenicity of novel live vaccine based on an artificial rHN20 strain against emerging fowl adenovirus 4. Viruses 2021;13:2153.
[32] Pan Q, Zhang Y, Liu A, Cui H, Gao Y, Qi X, et al. Development of a novel avian vaccine vector derived from the emerging fowl adenovirus 4. Front Microbiol 2021;12:780978.
[33] Pallister JA, Erny KM, Fahey KJ. Serological relationships within the group E fowl adenoviruses. Intervirology 1993;36:84–90.
[34] Gupta A, Popowich S, Ojkic D, Kurukulasuriya S, Chow-Lockerbie B, Gunawardana T, et al. Inactivated and live bivalent fowl adenovirus (FAdV8b + FAdV11) breeder vaccines provide broad-spectrum protection in chicks against inclusion body hepatitis (IBH). Vaccine 2018;36:744–50.
[35] Popowich S, Gupta A, Chow-Lockerbie B, Ayalew L, Ambrose N, Ojkic D, et al. Broad spectrum protection of broiler chickens against inclusion body hepatitis by immunizing their broiler breeder parents with a bivalent live fowl adenovirus vaccine. Res Vet Sci 2018;118:262–9.
[36] Steer PA, O'Rourke D, Ghorashi SA, Noormohammadi AH (2011) Application of high-resolution melting curve analysis for typing of fowl adenovirus in field cases of inclusion body hepatitis. Aust Vet J 89:184–192.
[37] Chishti MA, Afzal M, Cheema AH. Preliminary studies on the development of vaccine against the "hydropericardium syndrome" of poultry. Revue Scientifique et Technique de l'Office International des Epizooties 1989;8:797–801.
[38] Toro H, González C, Cerda L, Morales MA, Dooner P, Salamero M. Prevention of inclusion body hepatitis/hydropericardium syndrome in progeny chickens by vaccination of breeders with fowl adenovirus and chicken anaemia virus. Avian Dis 2002;46:547–54.
[39] Alvarado IR, Villegas P, El-Attrache J, Jensen E, Rosales G, Perozo F, et al. Genetic characterization, pathogenicity, and protection studies with an avian adenovirus isolate associated with inclusion body hepatitis. Avian Dis 2007;51:27–32.
[40] Yin D, He L, Zhu E, Fang T, Yue J, Wen M, et al. A fowl adenovirus serotype 4 (FAdV-4) Fiber2 subunit vaccine candidate provides complete protection against challenge with virulent FAdV-4 strain in chickens. Vet Microbiol 2021;263: 109250.
[41] Afzal M, Ahmad I. Efficacy of an inactivated vaccine against hydropericardium syndrome in broilers. Vet Rec 1990;126:59–60.
[42] Anjum AD. Experimental transmission of hydropericardium syndrome and protection against it in commercial broiler chickens. Avian Pathol 1990;19: 655–60.
[43] Aziz F, Tufail S, Shah MA, Shah MS, Habib M, Mirza O, et al. *In silico* epitope prediction and immunogenic analysis for penton base epitope-focused vaccine against hydropericardium syndrome in chicken. Virus Res 2019;273:197750.
[44] Tufail S, Shah MA, Zafar M, Asif TA, Shehzad A, Shah MS, et al. Identification of potent epitopes on hexon capsid protein and their evaluation as vaccine candidates against infections caused by members of *Adenoviridae* family. Vaccine 2021;39: 3560–4.
[45] Naeem K, Rabbani M, Hussain M, Cheema AH. Development of cell culture vaccine against hydropericardium syndrome in poultry. Pakistan Vet J 1995;15:150–1.
[46] Kaur A, Oberoi MS, Singh A. Neutralizing antibody and challenge response to live and inactivated avian adenovirus-1 in broilers. Tropl Anim Health Prod 1997;29: 141–6.
[47] Du D, Zhang P, Li X, Tian H, Cheng Y, Sheng D, et al. Cell-culture derived fowl adenovirus serotype 4 inactivated vaccine provides complete protection for virus infection on SPF chickens. Virus Dis 2017;28:182–8.
[48] Pan Q, Yang Y, Gao Y, Qi X, Liu C, Zhang Y, et al. An inactivated novel genotype fowl adenovirus 4 protects chickens against the hydropericardium syndrome that recently emerged in China. Viruses 2017;9:216.
[49] Kim M, Lim T, Lee D, Youn H, Yuk S, Kim B, et al. An inactivated oil-emulsion fowl adenovirus serotype 4 vaccine provides broad cross-protection against various serotypes of fowl adenovirus. Vaccine 2014;32:3564–8.
[50] Meng K, Yuan X, Yu J, Zhang Y, Ai W, Wang Y. Identification, pathogenicity of novel fowl adenovirus serotype 4 SDJN0105 in Shandong, China and immunoprotective evaluation of the newly developed inactivated oil-emulsion FAdV-4 vaccine. Viruses 2019;11:627.
[51] Wang B, Song M, Song C, Zhao S, Yang P, Qiao Q, et al. An inactivated novel chimeric FAdV-4 containing fiber of FAdV-8b provides full protection against hepatitis-hydropericardium syndrome and inclusion body hepatitis. Vet Res 2022; 53:75.
[52] Zhao Z, Song Y, Huang Z, Liu L, Liao H, Sun W, et al. Immunity analysis against fowl adenovirus serotype 4 (FAdV-4) based on Fiber-2 trimer protein with the different virulence. Virus Res 2022;308:198652.
[53] Chen L, Yin L, Zhou Q, Li Q, Luo Y, Xu Z, et al. Immunogenicity and protective efficacy of recombinant fiber-2 protein in protecting SPF chickens against fowl adenovirus 4. Vaccine 2018;36:1203–8.
[54] Song Y, Zhao Z, Liu L, Li Y, Gao Y, Song X, et al. Knob domain of Fiber 2 protein provides full protection against fowl adenovirus serotype 4. Virus Res 2023;330: 199113.
[55] Xia J, Yao K, Liu Y, You G, Li S, Liu P, et al. Isolation and molecular characterization of prevalent fowl adenovirus strains in southwestern China during 2015-2016 for the development of a control strategy. Emerg Microbes & Infect 2017;6:e103.
[56] Zhang Y, Liu A, Jiang N, Qi X, Gao Y, Cui H, et al. A novel inactivated bivalent vaccine for chickens against emerging hepatitis-hydropericardium syndrome and infectious bursal disease. Vet Microbiol 2022;266:109375.
[57] Zhang Y, Liu A, Cui H, Qi X, Liu C, Zhang Y, et al. An inactivated vaccine based on artificial non-pathogenic fowl adenovirus 4 protects chickens against hepatitis-hydropericardium syndrome. Vet Microbiol 2022;264:109285.
[58] Schachner A, Gonzalez G, Endler L, Ito K, Hess M. Fowl adenovirus (FAdV) recombination with intertypic crossovers in genomes of FAdV-D and FAdV-E, displaying hybrid serological phenotypes. Viruses 2019;11:1094.
[59] Steer-Cope PA, Sandy JR, O'Rourke D, Scott PC, Browning GF, Noormohammadi AH. Vaccination with FAdV-8a induces protection against inclusion body hepatitis caused by homologous and heterologous strains. Avian Pathol 2019;48:396–405.
[60] Wu J, Lu X, Song L, Liu L, Gao Y, Li H, et al. Preparation and evaluation of the immune efficacy of an inactivated fowl adenovirus 8a serotype oil emulsion vaccine. Heliyon 2024;10:e26578.
[61] Van den Hurk JV, Littel-van Van Drunen, den Hurk S. Protection of turkeys against hemorrhagic enteritis by monoclonal antibody and hexon immunization. Vaccine 1993;11:329–35.
[62] Fingerut E, Gutter B, Gallili G, Michael A, Pitcovski J. A subunit vaccine against the adenovirus egg-drop syndrome using part of its fiber protein. Vaccine 2003;21: 2761–6.
[63] Pitcovski J, Fingerut E, Gallili G, Eliahu D, Finger A, Gutter B. A subunit vaccine against haemorrhagic enteritis adenovirus. Vaccine 2005;23:4697–702.
[64] Dar A, Tipu M, Townsend H, Potter A, Gerdts V, Tikoo S. Administration of poly[di (sodium carboxylatoethylphenoxy) phosphazene] (PCEP) and avian beta defensin as adjuvants in inactivated inclusion body hepatitis virus and its hexon protein-based experimental vaccine formulations in chickens. Avian Dis 2015;59:518–24.
[65] Shah MS, Ashraf A, Rahman M, Khan MI, Qureshi JA. A subunit vaccine against hydropericardium syndrome using adenovirus penton capsid protein. Vaccine 2012;30:7153–6.
[66] Shah MS, Ashraf A, Khan MI, Rahman M, Habib M, Qureshi JA. Molecular cloning, expression and characterization of 100 K gene of fowl adenovirus-4 for prevention and control of hydropericardium syndrome. Biologicals 2016;44:19–23.
[67] Schachner A, Marek A, Jaskulska B, Bilic I, Hess M. Recombinant FAdV-4 fiber-2 protein protects chickens against hepatitis–hydropericardium syndrome (HHS). Vaccine 2014;32:1086–92.
[68] Feichtner F, Schachner A, Berger E, Hess M. Development of sensitive indirect enzyme-linked immunosorbent assays for specific detection of antibodies against fowl adenovirus serotypes 1 and 4 in chickens. Avian Pathol 2018;47:73–82.
[69] Feichtner F, Schachner A, Berger E, Hess M. Fiber-based fluorescent microsphere immunoassay (FMIA) as a novel multiplex serodiagnostic tool for simultaneous detection and differentiation of all clinically relevant fowl adenovirus (FAdV) serotypes. J Immunol Methods 2018;458:33–43.
[70] Wang X, Tang Q, Chu Z, Wang P, Luo C, Zhang Y, et al. Immune protection efficacy of FAdV-4 surface proteins fiber-1, fiber-2, hexon and penton base. Virus Res 2018; 245:1–6.
[71] Liu W, Liu M, Wang S, Tang W, Liu J, Song S, et al. The development of a novel fiber-2 subunit vaccine against fowl adenovirus serotype 4 formulated with oil adjuvants. Vaccines 2024;12:263.
[72] Wang X, Tang Q, Qiu L, Yang Z. Penton-dodecahedron of fowl adenovirus serotype 4 as a vaccine candidate for the control of related diseases. Vaccine 2019;37: 839–47.
[73] Tufail S, Shah MA, Asif TA, Ullah R, Shehzad A, Ismat F, et al. Highly soluble and stable "insertion domain" of the capsid penton base protein provides complete protection against infections caused by fowl adenovirus. Microb Pathog 2022;173: 105835.
[74] Kumar V, Chandra R, Ralte R, Randhawa SS. Analysis of penton base gene of fowl adenovirus reveals recombination among different species *Aviadenovirus* genus. Int J Curr Microbiol App Sci 2020;9:1045–54.
[75] Ruan S, Zhao J, Yin X, He Z, Zhang G. A subunit vaccine based on fiber-2 protein provides full protection against fowl adenovirus serotype 4 and induces quicker and stronger immune responses than an inactivated oil-emulsion vaccine. Infect Genet Evol 2018;61:145–50.
[76] Li Y, Zhou H, Li B, Li J, Shen Y, Jiang Y, et al. Immunoprotection of FliBc chimeric fiber2 fusion proteins targeting dendritic cells against fowl adenovirus serotype 4 infection. Poult Sci 2024;103:103474.
[77] De Luca C, Schachner A, Heidl S, Hess M. Vaccination with a fowl adenovirus chimeric fiber protein (crecFib-4/11) simultaneously protects chickens against hepatitis-hydropericardium syndrome (HHS) and inclusion body hepatitis (IBH). Vaccine 2022;40:1837–45.
[78] De Luca C, Schachner A, Heidl S, Hess M, Liebhart D, Mitra T. Local cellular immune response plays a key role in protecting chickens against hepatitis-

hydropericardium syndrome (HHS) by vaccination with a recombinant fowl adenovirus (FAdV) chimeric fiber protein. Front Immunol 2022;13:1026233.
[79] Watanabe S, Yamamoto Y, Kurokawa A, Iseki H, Tanikawa T, Mase M. Recombinant fiber-1 protein of fowl adenovirus serotype 4 induces high levels of neutralizing antibodies in immunized chickens. Arch Virol 2023;168:84.
[80] Toogood CI, Crompton J, Hay RT. Antipeptide antisera define neutralizing epitopes on the adenovirus hexon. J Gen Virol 1992;73:1429–35.
[81] Hu J, Li G, Wang X, Cai L, Rong M, Li H, et al. Development of a subunit vaccine based on fiber2 and hexon against fowl adenovirus serotype 4. Virus Res 2021;305:198552.
[82] Guo X, Chang J, Lu S, Hu P, Zou D, Li Y, et al. Multiantigen epitope fusion recombinant proteins from capsids of serotype 4 fowl adenovirus induce chicken immunity against avian Angara disease. Vet Microbiol 2023;278:109661.
[83] Liu S, Dong X, Lei B, Zhang W, Wang X, Yuan W, et al. A novel subunit vaccine based on Fiber1/2 knob domain provides full protection against fowl adenovirus serotype 4 and induces stronger immune responses than a Fiber2 subunit vaccine. Poult Sci 2024;103:103888.
[84] Tian K, Guo H, Li N, Zhang Y, Wang Z, Wang B, et al. Protection of chickens against hepatitis-hydropericardium syndrome and Newcastle disease with a recombinant Newcastle disease virus vaccine expressing the fowl adenovirus serotype 4 fiber-2 protein. Vaccine 2020;38:1989–97.
[85] Jia Z, Ma C, Yang X, Pan X, Li G, Ma D. Oral immunization of recombinant *Lactococcus lactis* and *enterococcus faecalis* expressing dendritic cell targeting peptide and hexon protein of fowl adenovirus 4 induces protective immunity against homologous infection. Front Vet Sci 2021;8:632218.
[86] Jia Z, Pan X, Zhi W, Chen H, Bai B, Ma C, et al. Probiotics surface-delivering fiber2 protein of fowl adenovirus 4 stimulate protective immunity against hepatitis-hydropericardium syndrome in chickens. Front Immunol 2022;13:919100.
[87] Cao H, Hua D, Zhang H, Zhang H, Liu N, Feng Z, et al. Oral immunization of recombinant *Saccharomyces cerevisiae* expressing fiber-2 of fowl adenovirus serotype 4 induces protective immunity against homologous infection. Vet Microbiol 2022;271:109490.
[88] Junnu S, Lertwatcharasarakul P, Jala S, Phattanakulanan S, Monkong A, Kulprasertsri S, et al. An inactivated vaccine for prevention and control of inclusion body hepatitis in broiler breeders. The Thai J Vet Med 2015;45:55–62.
[89] Gupta A, Ahmed KA, Ayalew LE, Popowich S, Kurukulasuriya R, Ojkic D, et al. Immunogenicity and protective efficacy of virus-like particles and recombinant fiber proteins in broiler-breeder vaccination against fowl adenovirus (FAdV)-8b. Vaccine 2017;35:2716–22.
[90] De Luca C, Schachner A, Mitra T, Heidl S, Liebhart D, Hess M. Fowl adenovirus (FAdV) fiber-based vaccine against inclusion body hepatitis (IBH) provides type-specific protection guided by humoral immunity and regulation of B and T cell response. Vet Res 2020;51:143.
[91] Lu H, Xie Q, Zhang W, Zhang J, Wang W, Lian M, et al. A novel recombinant FAdV-4 virus with fiber of FAdV-8b provides efficient protection against both FAdV-4 and FAdV-8b. Viruses 2022;14:376.
[92] Lu Y, Yuan Y, Jiang H, Xu Z, Guo Y, Cao X, et al. Efficient cross-protection against serotype 4/8a fowl adenoviruses (FAdVs): recombinant FAdV-4 with FAdV-8a Fiber. Microbiol Spectr 2023;11. e02462-23.
[93] Schachner A, De Luca C, Heidl S, Hess M. Recombinantly expressed chimeric fibers demonstrate discrete type-specific neutralizing epitopes in the *fowl aviadenovirus E* (FAdV-E) fiber, promoting the optimization of FAdV fiber subunit vaccines towards cross-protection *in vivo*. Microbiol Spectr 2022;10. e02123–21.
[94] Song C, Zhao S, Song M, Qiao Q, Yang P, Wang B, et al. An inactivated novel trivalent vaccine provides complete protection against FAdV-4 causing hepatitis-hydropericardium syndrome and FAdV-8b/-11 causing inclusion body hepatitis. Transbound Emerg Dis 2023:5122382.
[95] De Luca C, Schachner A, Hess M. Recombinant *fowl aviadenovirus E* (FAdV-E) penton base vaccination fails to confer protection against inclusion body hepatitis (IBH) in chickens. Avian Pathol 2023;52:277–82.
[96] Venne D. Field data of the changing clinical picture over time of inclusion body hepatitis in Canada with an emphasis on diagnosis, prevention and trials on supportive treatments. In: Proceedings of the 24th annual Australian poultry science symposium. Sydney, New South Wales, Australia: The poultry research foundation and the World's poultry science association; 2013. p. 222–9. February 2013.
[97] Bertran K, Blanco A, Antilles N, Nofrarías M, Valle RM, Cobos À, et al. A 10-year retrospective study of inclusion body hepatitis in meat-type chickens in Spain (2011−2021). Viruses 2021;13:2170.
[98] Lai VD, Min K, Lai HTL, Mo J. Epidemiology of fowl adenovirus (FAdV) infections in south Korean chickens during 2013-2019 following introduction of FAdV-4 vaccines. Avian Pathol 2021;50:182–9.
[99] Mo J. Historical investigation of fowl adenovirus outbreaks in South Korea from 2007 to 2021: a comprehensive review. Viruses 2021;13:2256.