

Hydropericardium syndrome: current state and future developments

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Abstract Hydropericardium syndrome (HPS) is a highly infectious disease caused by fowl adenovirus serotype 4 (FAV-4) affecting poultry, especially broiler birds. The disease was initially reported from Angara Goth, Pakistan, and then from India during 1994, in the poultry belt of Jammu and Kashmir, and thereafter, from almost all parts of the country, causing heavy economic losses to the poultry industry. The disease occurs predominantly in broilers of the age group of 3–5 weeks, characterized by sudden onset of high mortality up to 80 %. The causative agent of HPS is fowl adenovirus 4, which is a member of the species *Fowl Adenovirus C*, genus *Aviadenovirus*, family *Adenoviridae* [60]. FAV-4 is non-enveloped and icosahedral in shape, measuring 70–90 nm in size and containing a linear dsDNA of approximately 45 kb in size as its genome. The livers of affected birds show necrotic foci and basophilic intranuclear inclusion bodies in the hepatocytes. The disease can be diagnosed from its gross and microscopic changes in the liver and by various serological tests, such as agar gel immunodiffusion, counter-

immuno-electrophoresis, indirect haemagglutination, fluorescent antibody techniques, and ELISA. In the past few years, PCR has been used as a rapid diagnostic tool for the detection of fowl adenoviruses. The disease has been brought under control by the use of formalin-inactivated, attenuated or live vaccines in experimentally infected birds. Advancement in the field of computational immunology accelerates knowledge acquisition and simultaneously reduces the time and effort involved in screening potential epitopes, leading toward the development of epitope-based vaccines.

Abbreviations

AGID	Agar gel immunodiffusion
AGPT	Agar gel precipitation test
CAV	Chicken anemia virus
CEK	Chicken embryo kidney
CEL	Chicken embryo liver
CIE	Counterimmuno-electrophoresis
DPI	Days postinfection
DPV	Days post-vaccination
ELISA	Enzyme-linked immunosorbent assay
FAT	Fluorescent antibody technique
HHS	Hydropericardium-hepatitis syndrome
HPS	Hydropericardium syndrome
HPSV	Hydropericardium syndrome virus
IBD	Infectious bursal disease
IBH	Inclusion-body hepatitis
IBH-HPS	Inclusion-body hepatitis-hydropericardium syndrome
INIB	Intranuclear inclusion body
LD ₅₀	Lethal dose 50 %
PCR	Polymerase chain reaction
SPF	Specific pathogen free
TCID ₅₀	Tissue culture infective dose 50 %

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History and distribution

In late eighties, a new disease in broiler birds with some clinical similarities to classical inclusion-body hepatitis (IBH) was reported from Pakistan [28, 52, 56], India [112, 114], South America [104] and several other countries [21, 53, 59]. The disease was mainly characterized by accumulation of fluid in the pericardial sac and hepatitis, and hence named hydropericardium syndrome. The disease was first reported in broiler birds of 3 to 5 weeks of age from Angara Goth, near Karachi, Pakistan, in 1987 and is therefore commonly known as ‘Angara Disease’ in Pakistan [7, 28, 44, 56]. Since then, the disease has spread to Iraq [1], Mexico, Peru, Chile [125], South and Central America [104, 106], Russia [21], Slovakia [53] and Korea [59]. In India, HPS was first noticed in the poultry belt of Jammu and Kashmir, Punjab and Delhi during April–July 1994 [114], although some cases were reported prior to that time [112]. After a few months, the disease spread to Terai of Uttarakhand in November 1994 [65]. Several outbreaks were recorded in and around Haldwani in the Nainital district of Uttarakhand [62, 64, 65, 108], followed by spread of disease to other parts of the country, *viz.*, Uttar Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala, resulting in huge economic losses [13, 20, 84]. In India, the disease is commonly known as “leechi disease” due to the characteristic hydropericardium, giving the heart the appearance of the peeled Indian leechi fruit. This condition also has various other names, like inclusion-body hepatitis-hydropericardium syndrome [17, 18, 51], hydropericardium syndrome [79], the hydropericardium hepatopathy syndrome [13] or hydropericardium hepatitis syndrome [40, 104].

Epidemiology and transmission

Hydropericardium syndrome (HPS) has been observed in broiler birds of 3 to 5 weeks of age [28] of either sex [65, 113], and occasionally in layers and breeder pullets aged 10 to 20 weeks [52]. Rare outbreaks of HPS in older birds [13] and in other species of poultry, including quails, pigeons [78] and wild black kites [62] have also been recorded. Most of the investigators have reported that different strains of broilers are equally susceptible under field conditions [12]. The course of disease under natural conditions or after oral inoculation ranged from 7 to 15 days [12]. The mortality rate in various outbreaks in broiler farms in Pakistan ranged from 20 % to 75 % [56], while in India it ranged from 30 % to 80 %, with an average of 61.62 % [65]. Although the disease is more prominent in summer and rainy seasons, sporadic outbreaks do occur in winter as well.

Hydropericardium syndrome is a contagious disease and is transmitted horizontally among broilers from flock to flock

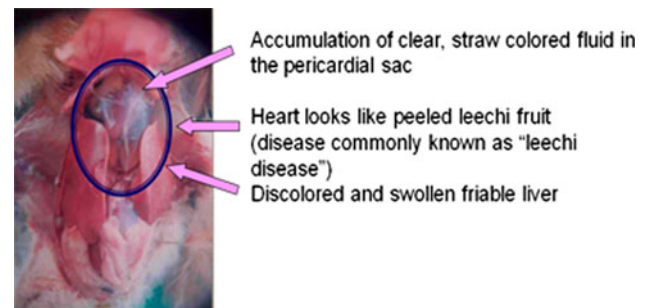


Fig. 1 Disease characteristics of hydropericardium syndrome

and farm to farm [31] by the oral-faecal route [2]. The disease can be reproduced in susceptible broiler chicks by subcutaneous inoculation of liver homogenate extract from naturally or experimentally infected birds [11, 28, 65]. Recovered birds are immune to subsequent attacks of hydropericardium syndrome [31]. Vertical transmission of FAV is also well documented [38]. The existence of a carrier state has not been established in FAV infection, but the occurrence of more than one serotype on a farm might contribute to prolonged persistence and excretion of FAV [72].

Gross pathology

The most predominant and consistent gross lesion of HPS infection is hydropericardium, *i.e.*, accumulation of clear or amber-coloured, watery or jelly-like fluid in the pericardial sac, with the quantity of fluid ranging from 3 to 20 ml and a pH of 7.0. It gives a flabby appearance to the heart (Fig. 1), which is found floating in the pericardial sac [1, 12, 13, 28, 29, 65, 114]. Other pathological changes include a discoloured and swollen reticulated friable liver with focal hepatic necrosis, petechial and ecchymotic haemorrhages in the heart musculature and other organs, congestion and oedema in the lungs, and pale kidneys with the deposition of urates in the kidney tubules and ureters [2, 28, 87, 94]. Some of the diseased birds also showed an enlarged bursa of Fabricius [65] with congestion of intestinal blood vessels [114]. Similar lesions in various organs have also been described by other workers after experimental infection of broiler birds [11].

HPS pathogenesis

The course of the disease studied under natural conditions or following experimental oral inoculation ranged from 7 to 15 days [6]. The HPS agent is highly pathogenic and spreads rapidly from flock to flock and farm to farm [31]. It shows a high affinity towards hepatic,

endothelial and lymphatic cells. Systemic hyperplasia of mononuclear phagocytic cells and the marked destruction of erythrocytes (especially in spleen and lungs) gave been observed in natural and experimental cases [83]. The inclusion bodies were found to be distributed in a wide variety of organs, *viz.*, liver, pancreas, gizzard, proventriculus, duodenum, cecum and kidney, in the case of experimentally infected 1-day-old chicks than in natural cases (liver only) [1]. In India, the presence of intranuclear inclusion bodies (INIBs) in hepatocytes of naturally and experimentally infected broiler birds has been reported by several workers [13, 26, 65]. The detection of numerous INIBs in hepatocytes of the chicks inoculated when they were 1 day old suggest that the HPS adenovirus infects the hepatocyte more intensely than do other strains of adenovirus. HPS adenovirus antigens were detected in the bursa of Fabricius and thymus of chickens between 12 and 48 h after subcutaneous inoculation, and the chicken affected with HPS adenovirus showed immunosuppression [79]. "Nephritis" was observed in chickens inoculated with HPS adenovirus from Ecuador and Pakistan [71]. Some reports show extensive hemorrhages and nephrosis [2].

Etiology

HPS was initially thought to be caused by toxicity or nutritional deficiency [52, 112]. The possible causative factors investigated were mycotoxins, toxic fat agent, polychlorinated biphenyl, sodium chloride, chlordane and phycotoxins, all of which were associated with hydropericardium syndrome [52, 93]. However, none of the attempts to reproduce the disease experimentally using these agents were successful [11, 12]. Subsequent studies confirmed the association with a virus, and an adenovirus was identified as the etiological agent after the demonstration of characteristic hexagonal virions by electron microscopy of a purified liver homogenate [28]. Later, the HPS agent was purified, propagated in chicken embryo liver cells, and passaged in chicken embryos, and it reproduced the disease in susceptible birds [80]. In India also, intranuclear adenovirus particles measuring 80-90 nm in diameter were demonstrated in hepatocytes by transmission electron microscopy [26, 27], and the virus was isolated in CEL cell culture and typed as FAV-4 using standard anti-FAV sera to 12 serotypes [51]. The classification of field isolates of HPS was confirmed by restriction enzyme analysis by several workers [36, 71, 121].

FAV-4 is non-enveloped, icosahedral particle measuring 70-100 nm in diameter [25]. The virion has 252 capsomeres, of which 240 are hexons and 12 are pentons (vertex capsomeres). The virus is heat stable at 60 °C for 30

minutes and for 1 h at 50 °C, unlike the other adenoviruses. The virus sensitivity varies for pH change from 3 to 10. Treatment with 5 % chloroform and 10 % ether inactivates the HPS agent [4].

Virus propagation

Primary cell cultures of chicken kidney [56] and chicken embryo liver cells [51, 64, 80, 87] have been used for propagation of FAV-4. The cytopathic effects include rounding and swelling of cells, detachment of cells from the surface within 3-4 days, and the presence of basophilic INIBs [3, 56, 87]. The virus can also be passaged in embryonated chicken eggs [79], and it causes stunted growth of embryos [28, 103], haemorrhages [28] and 100 % mortality [103].

Viral proteins and antigens

Antigens on the surface of the virion are mainly type specific. Hexon and the fibre are the major structural proteins, of which the fibre is non-covalently linked to the penton base [123]. Hexon is the major protein of the adenovirus capsid known to have a region related to virus neutralization and serotype specificity [86, 98, 118]. It consists of a trimer of polypeptide II and a central core; VI, VIII and IX are minor polypeptides associated with the hexon and are thought to be involved in stabilization and assembly of the particle. The hexon and penton fibres are responsible for type-specific neutralization. Studies on the protein profile analysis of HPS agent from Pakistan by SDS-PAGE has revealed eight polypeptides, ranging in molecular weight from 15.7 to 119 kDa in a 10 % resolving gel [49]. In India, studies on the protein profiles of three field isolates of FAV-4 showed eight polypeptides with molecular weight ranging from 20 to 107 kDa [17], while another study reported 12 polypeptides in FAV-4, ranging from 13.8 to 110 kDa in a 12.5 % resolving gel, of which seven polypeptides, ranging in molecular weight from 15.8 to 110 kDa, were found to be immunogenic in western blot analysis [63].

The pentons are more complex and consist of a pentamer of peptide III together with five molecules of IIIa, which are also associated with the penton base. A trimeric fibre protein extends from each of the 12 vertices and is responsible for recognition and binding to the cellular receptors. A globular domain at the end of the adenovirus fibre is responsible for recognition of the cellular receptors. The members of the species *Fowl adenovirus C* bear 2 fibers of nearly equal length [68].

The viral genome

The genome of FAV-4 consists of a linear dsDNA molecule of 43–45 kb in size, and at the 5' end of each DNA strand, a virus-encoded 55-kDa protein is covalently linked, and inverted repeats are present at the terminal ends of each strand [9]. Recently, the genome of a non-pathogenic isolate (ON1) of FAV-4 was fully sequenced and found to be of 4,5667 bp in length with a G + C content of 54.6 % [42]. Of the fowl adenoviruses, the complete genome sequence has been determined for FAV-1, FAV-4, FAV-8 and FAV-9 [30, 42, 89]. The genome of this FAV-4 isolate is larger than that of FAdV-8 (45,063 bp) [89] and FAdV-1(43,804 bp) [30], making it the largest adenoviral genome reported so far. A total of 46 potential protein-coding open reading frames (ORFs) were identified on both strands (57 % on the sense strand and 43 % on the anti-sense strand). Among these 46 ORFs identified in the FAV-4 genome, 18 represented genus-common genes, and 28 represented genus-specific genes. Phylogenetic analysis shows clustering of the FAV-4 ON1 isolate with members of species FAdV-C, closer to an IBH/HPS-associated strain of FAV-4.

The genome is transcribed in both directions by RNA polymerase II with alternative splicing, which allows generation of multiple transcripts from the same sequence. The genome carries five early transcriptional units (E1A, E1B, E2, E3 and E4), two intermediate units and one late unit (major late), which has been proposed to generate families of late mRNA (L1 to L5), all of which are transcribed by RNA polymerase II [91].

The adenoviral genome was also extracted from infected cells [14, 69] as well as from purified virus preparation or cell culture [40, 71] and used for restriction endonuclease (RE) analysis and subsequent cloning and sequencing [14]. The DNA was extracted from the viral suspension by initial treatment with SDS and proteinase K and subsequent extraction with phenol and chloroform [23]. A method for DNA isolation from a small volume of FAV-infected cultures or allantoic fluid was also developed, which yielded DNA pure enough for RE analysis [50].

The immunosuppressive nature of the virus

All FAdVs commonly infect liver cells, resulting in IBH occurring mainly in broiler birds [126]. The possible influence of the immune system on the pathogenicity of FAdV-4, FAdV-8 and FAdV-1 has been shown in some studies. The predilection of HPS agent for lymphoid tissues can result in immunosuppression [2, 71, 79]. Several reports have shown the coexistence of infectious bursal disease (IBD) and CIA viruses in areas where HPS occurs

frequently [104]. The immunosuppressive effects of IBD and CIA are well documented [107, 117], and impairment of the immune response is required by FAdVs to produce their pathogenic potential [74]. The role of IBD virus in precipitating HPS in layer flocks has been well studied [109]. Pathogenesis studies of FAV isolates have also suggested that there is a synergism with CIA virus or other viruses, or that prior immunosuppression is necessary to produce IBH-HPS in chickens [120]. A virulent strain of serotype 8 fowl adenovirus was isolated from an outbreak of IBH in broiler flocks. These findings suggest that the damage caused by replication of this virulent strain of FAV in lymphoid tissues compromises the immunological capability of infected chickens [99]. The effects of a simultaneous and/or a subsequent coinfection with CAV isolate 10343 and FAV isolate 341 in SPF light chickens were evaluated. The results of that study corroborate previous reports on the pathogenicity of Chilean FAV isolates, which suggest that synergism with other viruses or prior immunosuppression is necessary to produce IBH/HPS in chickens. These results also suggest that the susceptibility of chickens to oral infection with FAV resulting in IBH/HPS varies throughout the course of CAV infection [120].

The virulence of the HPSV and presence of other immunosuppressive factors in host birds affects the mortality of HPS-infected birds, which can reach up to 80 % [2, 16, 76, 77, 79, 82, 103]. The avian immune system may be affected by several factors, including immune status, type of infectious agent, and environmental, genetic, physiological, toxicological and dietary factors. There have been several studies reporting the effects of dietary arginine on various aspects of immune functions in animals [19, 61, 65, 88, 90, 98, 128]. The immunomodulatory effects of arginine in animals include increased nitric oxide production by macrophages [10, 47, 122], improved thymic weight and function [19], enhanced lymphocyte response to mitogens such as concanavalin A and phytohemagglutinin [88], improved immunity against tumors [24], enhanced wound healing [129], and stimulatory effects either on the production or function of cytokines and other cells of the immune system [88, 129]. Arginine is also one of the factors required for differentiation and release of B lymphocytes from the bone marrow [35, 90]. Chickens are unable to synthesize arginine due to the incomplete urea cycle they possess [32, 58]. Two recent reports have indicated that arginine significantly improves the capacity of chickens to surmount immunosuppression induced by vaccine strains of IBD virus [115, 116]. However, research evaluating the effects of arginine on the responsiveness of the immune function of chickens to other infectious agents is still sparse, especially if this amino acid is to be used as a common immunomodulator in poultry flocks. The immunomodulatory effect of arginine on protective responses

against HPSV has been demonstrated [76]. The effects of dietary arginine on the protective humoral and cellular immune responses in broiler chickens vaccinated against and challenged with HPSV have been assessed. It has also been reported earlier that HPSV causes immunosuppression by damaging lymphoid tissues of birds [15]. It has been suggested that arginine supplementation could prove crucial in overcoming the immunosuppressive effects of HPSV in chickens, because arginine helps in the differentiation of pro-B cells to pre-B cells, and also in the release of these cells from bone marrow [35, 90]. The dietary supplementation of arginine was not only found to enhance HPSV-specific humoral and cell-mediated immune responses but also to provide complete protection against virulent HPSV challenge in HPS-vaccinated broiler chickens. Thus, arginine could act as a valuable immunoregulator, not only against HPSV but also against other poultry pathogens. In 2008, for the first time, flow cytometric analysis was applied in combination with immunohistochemistry to study the effect of FAdV-4 infection on lymphocyte subpopulations in SPF chickens. These studies show that FAdV-4 causes depletion of B cells and T cells in lymphoid organs of SPF chickens [102]. Suppression of the humoral and cellular immune response could be a common phenomenon of virulent fowl adenoviruses, as it has also been reported for other serotypes [110].

Diagnosis

The disease may be suspected on the basis of a sudden occurrence of high mortality among broiler birds of 3 to 6 weeks of age [65], with hydropericardium as the predominant lesion [12]. However, diagnosis of HPS has been carried out on the basis of gross and histopathological lesions, particularly the detection of basophilic INIBs in hepatocytes [65, 114], demonstration of virus particles in the infected tissues by transmission electron microscopy [27, 28] or isolation of virus in cell culture or embryonated eggs [54], and neutralization test using serotype-specific sera [95]. Various serological and molecular techniques have also been used for detection of viral antigen/DNA and identification of the FAV serotypes involved.

Serological techniques

Serological tests are mainly used for detecting the presence of viral antigens in different organs of the infected birds. Fowl adenoviruses, besides a type-specific antigen, also possess a group-specific antigen, which has broad reactivity in all the serological tests other than neutralization with specific serum and/or type-specific monoclonal antibodies [22]. Various serological tests, *viz.*, agar gel

immunodiffusion, counterimmunoelectrophoresis, fluorescent antibody techniques, immunoperoxidase assays and various modifications of ELISA are used for diagnosis of fowl adenoviral infection in poultry [55, 57, 64, 66, 81, 100].

An agar gel immunodiffusion (AGID) test has been developed for the diagnosis of disease caused by Indian isolates of group I fowl adenovirus [124]. In addition, an ELISA system has been adopted for detection of antibodies against FAV [34], and indirect ELISA and dot-ELISA have been developed for detection of FAV antigen in chicken tissues using antiserum against FAV-1 [81]. Viral neutralization test, ELISA and AGID tests have also been developed and compared for their ability to detect antibodies to FAV [75]. An improved dot immunobinding assay to detect FAV-1 antigen in field samples, which reacted weakly or gave doubtful reactions in conventional dot-ELISA has been developed using different blocking agents, *i.e.*, 5 % acetic acid or 5 % skimmed milk powder, and fixation of antigen-antibody complex with 50 % methanol or 0.25 % glutaraldehyde, or 0.2 % tannic acid and two substrates simultaneously (3, 3'-diaminobenzidine and alpha-chloronaphthol). It has been reported that acetic acid, when used as a blocking agent, increases sensitivity by fourfold and intensifies the color developed by both substrates [65].

A laboratory method has been developed for the diagnosis HPS in chickens [18] in which the presence of viral antigen in various tissues, *i.e.*, liver, kidney, bursa of Fabricius, spleen and thymus, from experimentally infected birds was detected by sandwich ELISA, using guinea pig and chicken hyperimmune sera. The comparative evaluation of various serological tests, *viz.*, AGID, CIE and ELISA for detection of FAV in chicken tissues has been carried out using antiserum to FAV-1. Out of a total of 50 samples tested by AGID, CIE and ELISA, 9, 20 and 31 samples were found positive by the respective tests. The specificity was confirmed by isolation of virus in CEL cell cultures and demonstration of INIB in the hepatocytes [57]. A simple dot immunobinding assay (DIA) for the rapid detection of viral antibodies and quantification of viral antigen present in HPS vaccines was also developed [95].

An indirect immunofluorescence test can also be used to detect avian adenovirus in different tissues of affected birds [43]. In India, indirect FAT was performed to detect the HPS virus in infected cells, blood smears and cryosectioned tissues collected at different time intervals from experimentally infected birds. The infected cells exhibited intense greenish yellow intranuclear fluorescence in various organs of chicks after experimental inoculation [64].

Indirect haemagglutination (IHA) has also been used to detect the antibody titre after vaccination [70, 85, 96]. The test was further used for the detection of antibodies against

HPS virus for studying the seroprevalence of the disease in commercial broilers [48].

Molecular techniques

Recent progress in diagnosis of avian adenoviruses has mainly been made at the molecular level. There have been several reports dealing with nucleic acid technology for detection and differentiation of avian adenoviruses. This includes restriction endonuclease assay (REA), *in situ* hybridization using DNA probes, and polymerase chain reaction (PCR).

Polymerase chain reaction and restriction endonuclease analysis

In the past few years, the polymerase chain reaction for *in vitro* amplification of target gene sequences has been applied as a rapid diagnostic tool for the detection of avian viral pathogens [46, 121]. This method is not only more rapid but also more sensitive and specific than other diagnostic procedures for demonstrating infection with all serotypes or groups of avian adenovirus. In addition, the avian adenovirus PCR assay can be used to confirm the identity of the isolates, thus avoiding the need for doing fluorescent antibody staining and double-immunodiffusion tests, and sometimes virus neutralization [130]. Several PCRs have been reported for the detection of FAVs [39, 62, 97, 130]. The usefulness of PCR combined with RE analysis for detection and typing of FAV isolates has been demonstrated, which can be used to monitor virus excretion and persistence after experimental infection [46, 121].

PCR is now well established for detection of FAV-4 [33, 36, 39, 111, 127]. The majority of the published PCR techniques for detection of avian adenovirus have used the hexon gene for primer design. A rapid *in situ* DNA hybridization test has also been developed for detection/diagnosis of avian adenovirus hepatitis in chickens [41]. Viral DNA was detected in the sections of liver and pancreas from field and experimental birds, using a digoxigenin-labeled virus-specific probe [41]. The use of two sets of primers, H1/H2 and H3/H4, hybridizing to three conserved regions of the hexon gene, revealed that PCR and RE analysis were suitable to detect all avian adenoviruses infecting birds, to distinguish all 12 FAV reference strains, and to differentiate FAVs from egg drop syndrome-76 virus (EDS-76 virus). They could detect variation between the isolates of FAV-4 from India, Pakistan, and several other countries, after digestion of H3/H4 PCR products with *HpaII* enzyme [97].

Recently, a full-length hexon gene was amplified followed by cloning and sequencing from Indian isolates of

FAV-4 by using the primer pair MA1F and MA3R (14). The 0.7-kb PCR-amplified DNA was labeled and used as a probe for the detection of virus by dot-blot hybridization of the viral genome [39]. In Japan, FAV isolated from chickens showing HPS were genetically analyzed. PCR and REA of the 800-bp hexon gene were used for their differentiation, and phylogenetic analysis showed that FAV-4 strains from Japan were identical to each other but were distinct from the isolates from India and Pakistan [69].

Initially, REA was used to differentiate isolates and strains, as it can detect more differences than can be identified by serological methods. Based on restriction enzyme analysis of FAV DNA using *BamHI* and *HindIII* enzymes, the 11 recognized serotypes of FAV were placed into five groups, designated A-E [131]. The genomes of FAV serotypes 4 and 10 were also analysed using nine different enzymes, *HindIII*, *BglIII*, *DraI*, *NaeI*, *XbaI*, *NotI*, *SfiI* and *SmaI*, and it was reported that although these serotypes were found to have considerable similarities in cross-neutralization and cross-protection, RE analysis revealed few differences [37]. The restriction profile of avian adenoviruses associated with IBH isolated from psittacine birds using *BamHI* and *HindIII* enzymes also revealed that the isolates were similar to fowl adenovirus serotype 3 in chickens [23]. RE analysis and pairwise co-migrating restriction fragment analysis of FAV genomes could differentiate between hypervirulent and mildly virulent field isolates of inclusion body hepatitis [36].

Prevention and control

Effective immunization against HPSV through the use of inactivated vaccines prepared from liver homogenates of infected chickens is the major practice employed to control HPS [5, 16, 77, 121]. However, HPS vaccines often fail to provide the desired level of protection under field conditions. Field observations suggest that the concurrent presence of infectious immunosuppressive agents such as IBD and CAV as well as noninfectious factors such as stress and aflatoxins probably interfere negatively with the desired outcome of HPS vaccination [15, 107, 121]. Thus, the success of a vaccination program against an infectious disease depends not only upon the use of efficacious vaccines, immunocompetency of chickens, and better management practices but also on the use of immunostimulants that can amplify the specific immune responses.

Recently, a live vaccine against HPS was developed by adapting a virulent FAdV-4 isolate to the fibroblast cell line QT35, and it was found to be capable of reducing the immunopathology induced by a severe challenge [100].

Epidemiological safeguards associated with the development and spread of HPS [8] such as proper disinfection

of premises and equipment, restricted entry of visitors and vaccination crews, and ventilation and proper lighting in the poultry houses, play a significant role in prevention of the disease [16]. The disease has been brought under control using a formalin-inactivated vaccine prepared from a 20 % (w/v) suspension of infected liver homogenates in PBS (pH 7.4) and inactivated with 0.1 % formalin for 24 h – against experimental challenge or natural outbreak of disease – or by oil-emulsified inactivated cell culture vaccine [65, 105]. In India, a killed, oil-emulsified vaccine was prepared using FAV-4 grown in cell culture. Vaccination of 3-week-old chicks with 0.5-ml doses of vaccine ($10^{5.5}$ TCID₅₀/0.1 ml) provided 100 % protection against challenge with HPS virus at 1, 2, 3, 4 or 6 weeks post-vaccination [54]. Owing to the contagious nature of the disease, the development of a suitable vaccine in SPF chickens and cell culture systems seems to be the best answer apart from strict biosecurity and high standards of hygiene and management [16]. An inactivated vaccine propagated in chicken liver cell culture and embryonated eggs, used subcutaneously at $10^{3.5}$ LD₅₀/dose/bird, provided protection against challenge with 1 ml of a 20 % liver homogenate at a biological titre of 2×10^5 LD₅₀/0.5 ml [80]. The effective protection of the progeny of chickens against IBH-HPS could be achieved by dual vaccination of breeders with FAV-4 and CAV [119]. Very recently, a new chicken-embryo-adapted FAV serotype 4 vaccine was developed [67], which was serially passaged (12 times) to get complete attenuation. Groups of broiler chickens that were free from maternal antibodies against HPS virus at the age of 14 days were immunized either with 16th-passage attenuated HPS virus vaccine or commercially formalized liver organ vaccine. Vaccination with the 16th-passage attenuated HPS virus gave 94.73 % protection, while the liver organ vaccine showed significantly low ($p < 0.05$; 55 %) protection based on clinical signs, gross lesions in the liver and heart, histopathological lesions in the liver, and mortality. Birds in the unvaccinated control group showed high morbidity and mortality and gross and histopathological lesions, with only 10 % protection. This newly developed HPS virus vaccine has proved to be immunogenic and has potential for controlling HPS virus infections in chickens.

Future development

The development of a safe vaccine that can transmit strong passive immunity and protect broiler chicks throughout their growing period is an urgent need for the future. A new concept of vaccine design is emerging with the combination of immunology and the development of bioinformatics tools for prediction of T-cell and B-cell epitopes from

protein sequences. With the advent of computational immunology and immune-informatics, it is possible now to drastically reduce the time and effort required for identification of promiscuous epitopes. These can be designed to be broadly reactive (across HLA) and broadly conserved (across variant strains) sequences.

Recently, the use of different bioinformatics tools was suggested for prediction of promiscuous B-cell epitopes in FAV-4 as a component of peptide-based vaccine [14]. Epitopes recognized by seven hexon-specific monoclonal antibodies were mapped in the case of chimpanzee adenovirus 68 (AdC68) [92]. Similarly, a combination of T-cell epitope prediction and classical immunization experiments can be a useful strategy to speed research in this area. Bioinformatics prediction is extremely cheap and may help to restrict the number of peptides that need to be screened. The expansion of computational immunology methods, coupled with the availability of more than 100 complete and partial genome sequences, raises the exciting possibility of developing epitope-based vaccines by scanning the sequences of the proteins of a pathogen. Some of these proteins have not previously been isolated or cloned, being unique to the pathogen, and may be excellent candidates for vaccine development.

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