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Review Paper

The Hydropericardium Syndrome in Poultry – A Current Scenario

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ABSTRACT

Inclusion-body hepatitis–hydropericardium syndrome (IBH-HPS) is an important, recently emerged, disease of poultry, particularly of 3- to 6-week-old broiler chicks, characterized by its sudden onset, with high mortality ranging from 20% to 70%, typical hydropericardium and enlarged mottled and friable livers, with intranuclear inclusion bodies in the hepatocytes. The causative agent is a non-enveloped icosahedral fowl adenovirus (FAV) serotype 4, belonging to the *Adenovirus* genus of the family *Adenoviridae*, which can be propagated or cultivated in chicken embryo liver and kidney primary cell cultures. The transmission of disease occurs vertically and laterally by the oral–faecal route. The liver of infected birds shows necrotic foci and basophilic intranuclear inclusion bodies in the hepatocytes. The disease can be diagnosed from its gross and histopathological changes in the liver and by various serological tests, such as agar gel immunodiffusion, counterimmunoelectrophoresis, indirect haemagglutination, the fluorescent antibody technique, enzyme-linked immunosorbent assay and the polymerase chain reaction. The disease has been brought under control by the use of formalin-inactivated vaccines, prepared from infected liver homogenate, and of inactivated cell culture vaccines. The vaccines are effective in the face of natural outbreaks or experimental challenge and significantly reduce the mortality.

Keywords: aetiology, chickens, diagnosis, fowl adenovirus, hydropericardium, pathogenesis, vaccines

Abbreviations: AGID, agar gel immunodiffusion; AGPT, agar gel precipitation test; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CAM, chorioallantoic membrane; CAV, chicken anaemia virus; CEK, chicken embryo kidney; CEL, chicken embryo liver; CIA, chicken infectious anaemia; CMI, cell-mediated immunity; CPK, creatinine phosphokinase; DIG-11-DUTP, digoxigenin-labelled deoxyuridine triphosphate; DPI, days post infection; DPV, days post vaccination; ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate; FAV, fowl adenovirus; HA, haemagglutination; HHS, hydropericardium–hepatitis syndrome; HPS, hydropericardium syndrome; IBD, infectious bursal disease; IBH, inclusion-body hepatitis; IBH-HPS, inclusion-body hepatitis–hydropericardium syndrome; INIB, intranuclear inclusion body; LD_{50}, dose of virus that kills 50% of birds on average; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; PARC, Pakistan Agricultural Research Council; RBC, red blood cell; RE, restriction enzyme; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; SPF, specific pathogen free; $TCID_{50}$, tissue culture infective dose 50%; TLC, total leukocyte count

INTRODUCTION

Avian adenoviruses are a very diverse group of pathogens causing a variety of problems for poultry production. Among the various emerging diseases in general, and avian adenoviruses in particular, they have been incriminated as the aetiological agents for various clinical conditions in poultry (Fadly and Winterfield, 1973; Rosenberger *et al.*, 1974). Even though various serotypes of fowl adenovirus (FAV) produce inclusion-body hepatitis (IBH) in broiler chicks, IBH along with the hydropericardium syndrome (IBH-HPS), popularly called litchi heart disease, has recently been reported to be particularly important in some countries in Asia and America (Jaffery, 1988; Shane, 1996; Abe *et al.*, 1998). Hydropericardium syndrome (HPS) is a recently emerged disease, primarily of broilers, affecting the 3- to 6-week-old age group and causing a severe hazard to poultry producers, particularly in the broiler industry in India and Pakistan (Gowda and Satyanarayana, 1994). It is caused by FAV serotype 4, a non-enveloped, icosahedral virus belonging to the *Adenovirus* C species of the *Adenovirus* genus of the *Adenoviridae* family, and is characterized by hydropericardium and hepatitis, with intranuclear inclusion bodies (INIBs) in hepatocytes (Ahmad *et al.*, 1989; Anjum *et al.*, 1989). The overall current situation of FAV-4 associated with hydropericardium syndrome in chicken is reviewed in this paper.

THE DISEASE

Jaffery (1988) and Khawaja and colleagues (1988a) reported a peculiar disease condition, with involvement of both the heart and liver, in broilers of 3 to 7 weeks of age, that was characterized by the accumulation of a transparent straw-coloured watery or jelly-like fluid in the pericardium, swollen, discoloured and friable livers, with eosinophilic or basophilic inclusions, congested kidneys, and a mortality of 70% (Ahmad *et al.*, 1989; Anjum *et al.*, 1989; Cheema *et al.*, 1989; Hasan, 1989). The syndrome, which had some clinical similarities to IBH but characteristically caused severe hydropericardium in broilers, was then reported in various parts of the world. Although liver damage is also seen in IBH, the severe hydropericardium in 3-week-old chicks had not previously been reported as due to an adenovirus infection. To distinguish HPS from classical IBH and to emphasize its infectious nature, the new disease was named 'infectious hydropericardium' (Abdul-Aziz and Hasan, 1995; Mazaheri *et al.*, 1998). The disease is variously called 'Angara disease' in Pakistan, after the place Angara Goth, near Karachi (Akhtar, 1994), 'leechy disease' or 'litchi disease' in India, after the peculiar appearance of the heart floating in pericardial fluid, which appears similar to the deshelled leechy (lichee) fruit (Gowda and Satyanarayana, 1994; Gowda, 1994) or inclusion-body hepatitis–hydropericardium syndrome (IBH-HPS) (Abdul-Aziz and Al-Attar, 1991; Jadhao *et al.*, 1997; Balamurugan *et al.*, 2001, 2002) and also the hydropericardium syndrome (Naeem *et al.*, 1995a), the hydropericardium hepatopathy syndrome (Asrani *et al.*, 1997) or the hydropericardium hepatitis syndrome (HHS) (Shane, 1996; Ganesh *et al.*, 2001a). The disease is characterized by its sudden occurrence and high morbidity, with a high mortality of

up to 80% in broilers and a low mortality of below 10% in layers, associated with hydropericardium (Shane, 1996).

PREVALENCE OF THE DISEASE

The first epidemic of HPS in broiler chicks was reported from Angara Goth near Karachi, Pakistan, in late 1987 (Jaffery, 1988; Khawaja *et al.*, 1988a; Cheema *et al.*, 1989; Hasan, 1989), although sporadic cases were recorded as early as 1985 (Cheema *et al.*, 1989). It has been subsequently recorded in Iraq (Abdul-Aziz and Al-Attar, 1991). India (Gowda and Satyanarayana, 1994), Mexico, Ecuador, Peru, Chile (cited by Voss *et al.*, 1996; Cowen *et al.*, 1996), South and Central America (Shane, 1996), Slovakia (Jantosovic *et al.*, 1991), Russia (Borisov *et al.*, 1997) and Japan (Abe *et al.*, 1998). In India, IBH-HPS was first reported in some parts of Jammu and Kashmir, Punjab and Delhi during 1994 (Gowda and Satyanarayana, 1994), though some cases had been seen prior to 1994 (Singh *et al.*, 1996). Later, the disease was reported to have spread to other parts of the country. Indian states that have recorded HPS include Uttarpradesh, Andhrapradesh, Tamilnadu, Himachalpradesh, Karnataka, Maharashtra and Haryana (Gowda and Satyanarayana, 1994; Bhowmik, 1996; Kataria *et al.*, 1996; Nighot *et al.*, 1996; Asrani *et al.*, 1997; Kumar *et al.*, 1997; Shukla *et al.*, 1997b). During 1996, a study group on poultry diseases reported the mortality rate due to HPS as ranging from 10% to 80% (Anonymous, 1996) and identified HPS as an emerging problem of the broiler industry.

EPIDEMIOLOGY

HPS has been observed in broiler chickens (Agsar-Hasan, 1989; Cheema *et al.*, 1989), of either sex (Kumar *et al.*, 1997; Singh *et al.*, 1997), aged 3 to 6 weeks (Khawaja *et al.*, 1988a; Anjum *et al.*, 1989; Niazi *et al.*, 1989; Abdul-Aziz and Al-Attar, 1991; Gowda and Satyanarayana, , 1994; Javeed *et al.*, 1994; Kumar *et al.*, 1997) or over 5 weeks of age (Ahmad *et al.*, 1989; Muneer *et al.*, 1989; Akhtar and Cheema, 1990) and occasionally in layers and breeder pullets aged to to 20 weeks (Jaffery, 1988; Ahmad *et al.*, 1989; Cheema *et al.*, 1989; Akhtar, 1992; Javeed *et al.*, 1994; Asrani *et al.*, 1997; Shukla *et al.*, 1997a). Rare outbreaks of HPS in older birds/broilers (Asrani *et al.*, 1997), and in other species of poultry, including pigeons (Naeem and Akram, 1995) and quail (Karunamoorthy and Manickam, 1998) have also been recorded. Most of the researchers have reported that different strains of broilers are equally susceptible in the field (Anjum *et al.*, 1989; Akhtar and Cheema, 1990) and in experimental cases (Afzal and Hussain, 1993). However, Khan and colleagues (1995) found the Hubbard strain of broilers to be relatively most susceptible, followed in order by the Indian River and Lohmann strains.

The course of the disease under natural conditions or following oral inoculation ranged from 7 to 15 days (Anjum *et al.*, 1989; Cheema *et al.*, 1989; Abdul-Aziz and Al-Attar, 1991; Akhtar, 1995). However, a liver homogenate prepared from infected birds

and inoculated by the parenteral route caused disease within 2–5 days (Anjum, 1990; Kumar *et al.*, 1997). Fast-growing broilers were the most affected, mortality peaking on the third or fourth day, followed by an almost constant death rate for 5–7 days, before it declined, giving an average mortality of 15–60% (Asrani *et al.*, 1997). IBH is also seen in young broilers, but high rates of mortality of 60–70% in Pakistan, 10–30% in Iraq and 10–60% in India are only characteristic of HPS (Abe *et al.*, 1998). Studies on the epidemiological factors associated with the development and spread of HPS in broiler flocks in Pakistan indicated that flocks that were visited frequently by vaccination crews were 15 times more likely to be affected by the syndrome than flocks that had no visits (Akhtar *et al.*, 1992), and also that the use of electricity as the source of light and heat entailed a much lower risk of HPS than when kerosene oil was used for these purposes.

The presence of aflatoxins in the feed at higher concentrations than 20 ppb is commonly associated with a large number of outbreaks of IBH, causing heavy mortality among 3- to 5-week old broiler chicks, which displayed typical lesions of IBH in addition to hydropericardium (Singh *et al.*, 1996). In Russia, HPS was recorded in chickens aged between 2 and 13 weeks, with mortality ranging from 3.5% to 30% in broilers and from 2.6% to 15.29% in layers (Borisov *et al.*, 1997). It was also recorded in a number of poultry farms in Russia as an acute disease with hydropericardium in 3- to 5-week-old broilers (Aliev *et al.*, 1997). The mortality rate in various outbreaks in broiler farms in Pakistan ranged from 20% to 75% (Khawaja *et al.*, 1988a; Anjum *et al.*, 1989; Cheema *et al.*, 1989), while in India it ranged from 30% to 80%, with an average of 61.62% (Kumar *et al.*, 1997; Singh *et al.*, 1997). Occasional outbreaks in older broilers (32 weeks old) and commercial layers (17 weeks old) have also been recorded, with 5–8% mortality (Asrani *et al.*, 1997).

TRANSMISSION EXPERIMENTS

Although the aetiology of the disease could not be ascertained initially, the syndrome was reproduced in broilers by inoculation of a bacteria-free liver homogenate from infected birds (Khawaja *et al.*, 1988a; Anjum *et al.*, 1989; Cheema *et al.*, 1989; Afzal *et al.*, 1991; Gowda and Satyanarayana, 1994; Asrani *et al.*, 1997; Chandra *et al.*, 1997). Afzal and colleagues (1990) showed that the pellet obtained after ultracentrifugation of a liver homogenate from affected birds was infectious, but not the supernatant. However, Shafique and Shakoori (1994) found that mortality was only higher in chicks injected with the resuspended pellet obtained after centrifugation of a liver homogenate than in those receiving the supernatant fraction. The disease was transmitted to broiler chicks by liver homogenate filtered through 0.22 μm and 0.1 μm membrane filters, indicating that a virus is the aetiological agent of HPS (Afzal *et al.*, 1991); these authors speculated that, in addition to the virus, some other agents may also be involved but required co-infection with an adenovirus to produce the typical signs of the syndrome.

The disease can be reproduced in broiler chicks by subcutaneous inoculation of 0.25–0.3 ml per bird of a 10% or 20% suspension of liver homogenate from naturally or experimentally infected birds (Cheema *et al.*, 1989; Anjum, 1990; Kumar *et al.*, 1997).

However, none of the chicks inoculated with pericardial fluid by the intraperitoneal route or fed infected liver for 3 days died of HPS (Shafique and Shakoori, 1994). Moreover, the disease could not be reproduced experimentally by parenteral inoculation of pericardial fluid or homogenates from bursa, lung, heart or kidney (Anjum *et al.*, 1989). When infected liver suspensions were inoculated intramuscularly or orally into day-old broiler chicks, the mortality rates were 100% and 30%, respectively (Abdul-Aziz and Hasan, 1995). However, these authors did not observe any changes in the heart and lungs, which may have been due to the difference in the severity of the infection in experimentally produced and natural cases. Subsequent studies have shown that the virus has a predilection for lymphoid organs, since a homogenate from these organs reproduced the disease in healthy chicks (Naeem *et al.*, 1995a). Mortality was observed 3 to 5 days after experimental infection. Generally, broilers dying less than 60 h after infection did not show any clinical signs (Kumar *et al.*, 1997). Chandra and colleagues (1997) demonstrated, by electron microscopy, the presence of adenovirus particles in the liver homogenate and in the hepatocytes of broilers experimentally infected with the HPS agent, and reproduced the disease by inoculation of the liver homogenate of birds that had died of HPS. IBH-HPS was also reproduced experimentally in broiler chicks aged 1 to 3 weeks by inoculating FAV-4 propagated in a CEL cell culture (Kataria *et al.*, 1997a; Deepak, 1998; Balamurugan *et al.*, 2001).

The syndrome was experimentally reproduced in broilers by using either liver homogenate or virus prepared from the field samples (Ganesh *et al.*, 2001a). The disease was also reproduced experimentally in 28-day-old broilers by subcutaneous or oral inoculation of isolated FAV-4, with typical hydropericardium and basophilic INIBs in hepatocytes being observed at 4 DPI (Dahiya *et al.*, 2002).

Gowda and Satyanarayana (1994) experimentally reproduced the condition in broilers by inoculating liver homogenate from infected birds, the disease being characterized by hydropericardium, with the accumulation of about 12 ml of straw-coloured fluid (giving the heart the 'litchi fruit' appearance), a pale enlarged liver, oedematous and congested lungs, pale and tumified kidneys and an icteric carcase. Other investigators have also described lesions of hydropericardium, splenomegaly, necrotic foci on the myocardium, hypertrophy of the bursa of Fabricius and poorly developed musculature in experimentally induced cases of HPS (Cheema *et al.*, 1988; Muneer *et al.*, 1989; Abdul-Aziz and Al-Attar, 1991). While studying the pathogenicity of the disease, Mazaheri and colleagues (1998) reproduced the condition in day-old SPF chicks by oral inoculation of purified virus from field outbreaks of HHS in Ecuador, and subsequently re-isolated the agent. Thus, overall, studies have shown that some strains of FAV-4 could cause HHS following oral or intramuscular infection.

AETIOLOGY

Initially, it was suspected that HPS was caused by toxicity or a nutritional deficiency. The possible causative factors investigated were mycotoxins, toxic fat agents, sodium chloride, polychlorinated biphenyls, chlordane and phytotoxins, all of which were associated with hydropericardium (Jaffery, 1988; Qureshi, 1988, 1989). However, all

attempts failed to reproduce the disease experimentally with feed samples from infected farms, feed containing mycotoxins, or both (Anjum, 1988, 1990). The search for an infectious agent was initiated by several diagnostic laboratories seeking to isolate a possible pathogen. An infectious agent was suggested as a possible cause on the basis of transmission experiments in which young broilers were infected using a bacteria-free liver homogenate from affected birds (Khawaja *et al.*, 1988a; Ahmad *et al.*, 1989; Anjum *et al.*, 1989). Subsequent studies confirmed that an adenovirus was associated with the condition, this being incriminated by the demonstration of either basophilic or eosinophilic inclusion bodies in the hepatocytes from the affected cases (Niazi *et al.*, 1989; Abdul-Aziz and Al-Attar, 1991; Afzal *et al.*, 1991; Gowda, 1994; Gowda and Satyanarayana, 1994) and by the demonstration of discrete icosahedral virions in purified liver extracts by negative staining electron microscopy (Cheema *et al.*, 1989). However, Afzal and colleagues (1991) suggested the possibility that another agent is involved that requires co-infection by an adenovirus to produce the classical signs of this syndrome.

In another study, with a Mexican liver homogenate, an RNA virus was implicated as an additional agent, on the basis of lack of inhibition by 5-bromodoexyuridine (Shane and Jaffery, 1997). As FAVs have been isolated from both healthy and diseased chickens, associating FAVs with this specific disease has been difficult. However, evidence grew showing FAV as the primary pathogen in HPS. In India, Kataria and colleagues (1995) isolated adenoviruses from cases of HPS from various parts of the country, these reacting in an AGID test with antiserum against FAV-1 (Verma *et al.*, 1971). Subsequently, isolation of FAV has been reported from many of the poultry farms in the country, which were experiencing HPS (Kataria *et al.*, 1996; Oberoi *et al.*, 1996; Vairamuthu *et al.*, 2002). All the isolates from field outbreaks of IBH-HPS have been serotyped as FAV-4 using standard antisera to the 12 different serotypes of FAV (Jadhao *et al.*, 1997) and identified as HPS virus (Vairamuthu *et al.*, 2002), and it was shown that some biotypes of FAV-4 caused the HHS (Mazaheri *et al.*, 1998).

In Pakistan and in several South American countries, inclusion-body hepatitis (IBH-HPS) virus, in many cases associated with hydropericardium, was predominant, and the condition was caused by FAV-4 (Voss *et al.*, 1996). An adenovirus (K31/89), isolated from field cases of HPS in Pakistan (Voss, 1989), was identified as adenovirus serotype 4 and as the causative agent of HPS (Voss *et al.*, 1996). European serotype 5 (FAV-8), itself associated in the reproduction of the hydropericardium syndrome with IBH in Mexico, has also been reported to be involved (Shane and Jaffery, 1997). The liver damage seen in HPS is also seen in IBH, but hydropericardium in 3-week-old chicks, due to an adenovirus infection, was not a feature noted until recently. FAV isolated from liver samples from cases of HPS was neutralized by antisera against the KR5 strain (FAV-4 reference strain) and the failure of attempts to isolate other viral agents from these liver samples in chicken kidney, chicken embryo and MDCC-MSB-1 cells confirmed the causative agent (Abe *et al.*, 1998).

The HPS was reproduced in SPF chicks using isolated and purified virus from field cases of HPS (Cowen *et al.*, 1996; Mazaheri *et al.*, 1998), thus proving the association of FAV-4 with HPS as the sole agent responsible for causing the disease. The experimental reproduction of the disease in susceptible chicks, accompanied by the

serological and electron-microscopic evidence also indicated that FAV-4 is solely responsible for causing HPS (Ganesh *et al.*, 2001a).

CHARACTERIZATION OF THE HPS AGENT

FAVs were isolated and characterized from several cases of HPS in Ecuador and Pakistan. FAV-4 isolates recovered from different outbreaks of HPS in Pakistan were found to differ in their pathological characteristics, although no significant differences were recorded regarding their biological properties or their protein profiles (Rabbani and Naeem, 1996). Studies on the protein profile analysis of the purified HPS agent from Pakistan by SDS-PAGE revealed eight polypeptides, ranging in molecular weight from 15.7 to 119 kDa. The extracted nucleic acid was found to be DNA, with a molecular weight of 23 kDa (Izhar-ul-Haq *et al.*, 1997). On analysis of purified PARC-1 isolates of HPS by SDS-PAGE, Rabbani and colleagues (1998a) found seven bands of polypeptides with molecular weights ranging from 24 to 120 kDa. Similarly, three field isolates of FAV-4 from HPS from India, subjected to SDS-PAGE, showed eight similar polypeptides, with molecular weights ranging from 20 to 107 kDa but differing from FAV-1, particularly in their 24.2 kDa protein (Balamurugan *et al.*, 2002). Immunoblot analysis of HPS virus with other FAV serotypes, using type-specific or polyclonal serum antibodies directed against proteins of FAVs, indicated the presence of common bands, which revealed serological relatedness among the FAV serotypes, with a close relationship between serotypes 4 and 9 in group I of FAV (Rabbani *et al.*, 1998a; Balamurugan *et al.*, 2002).

Indian isolates from field outbreaks of IBH-HPS in poultry from different geographical locations were typed as FAV serotype 4 (Jadhao *et al.*, 1997), and the agent was characterized by restriction enzyme (RE) analysis of its genomic DNA (Jadhao, 1998). Other studies showed that antibodies against serotypes 4 and 10 (FAV strains KR 5 and 2B) neutralized new isolates of FAV characterized from several cases of HPS (Mazaheri *et al.*, 1998). However, cross-neutralization tests and RE analysis strengthened their grouping under serotype 4. It has been shown by immunological and molecular analytical methods that FAV-4 and FAV-10 are very closely related (Erny *et al.*, 1995). The classification of HPS field isolates as FAV-4 strains was confirmed by RE digestion with *Bam*HI, *Dra*I and *Eco*RI, generating similar restriction fragment patterns between known strains of FAV-4 and the field isolates (Mazaheri *et al.*, 1998). However, although RE analysis with field isolates and reference strains (KR5) revealed identical patterns between KR5 and some field isolates, *Pst*I digestion differentiated field isolates from KR5. This study concluded that only some specific strains of FAV-4 cause the disease (Mazaheri *et al.*, 1998). Three field isolates of FAV from IBH-HPS cases in chicks were characterized by virus neutralization tests and RE analysis of a DNA fragment and were also identified as FAV-4 resembling the KR5 strain (Toro *et al.*, 1999).

Comparison of a predicted KR95 strain hexon sequence and eight mammalian and avian adenovirus hexon sequences revealed the highest homology between KR95 and FAV-10 and FAV-1 (91.1% and 80.1%, respectively) (Lobanov *et al.*, 2000). A similarly

variable region of hexon gene, encoding 728 bp of L1 and part of P1, was amplified and both the nucleotide and the derived amino acid sequences were compared with FAV-1, FAV-8 and FAV-10 (Ganesh *et al.*, 2001b). There was variability of 8.2%, 28.1% and 40.3% in the nucleotide sequence from FAV-10, FAV-1 and FAV-8, respectively. However, the derived amino acid sequence showed variability as high as 28.8%, 38% and 45.1% with FAV-10, FAV-1 and FAV-8, respectively. Although the nucleotide sequence showed only an 8.2% difference between HHS and FAV-10, the amino acid sequences differed by 28.8%. Such a high degree of variability has been found to be due to the shift in the reading frame caused by deletions, indicating that the FAV-4 associated with HHS is unique and is different from FAV-10 (Ganesh *et al.*, 2001b).

PHYSICOCHEMICAL PROPERTIES OF THE VIRUS

The causative agent of HPS is filterable through a Seitz filter (Khawaja *et al.*, 1988a; Kumar *et al.*, 1997) and through a membrane filter of 0.1 μm average pore diameter (Kumar *et al.*, 1997). The sensitivity of the HPS agent to pH 3 and pH 10 is variable. Unlike other adenoviruses, the agent withstands heating to 60°C for 30 min and to 50°C for 1 h. However, heating to 60°C for 1 h, 80°C for 10 min or 100°C for 5 min inactivates the virus. Treatment with chloroform (5%) or ether (10%) appears to be detrimental to the HPS virus (Afzal *et al.*, 1991). The virus has also been reported to agglutinate red blood cells in a similar manner to adenovirus (Khawaja *et al.*, 1988a), but this property has not been confirmed by other researchers.

CULTIVATION OF THE VIRUS

The HPS agent can be isolated or propagated in primary cell cultures of chicken kidney (Khawaja *et al.*, 1988a) or chicken embryonic liver cells (Naeem *et al.*, 1995a; Kataria *et al.*, 1995, 1996, 1997a; Oberoi *et al.*, 1996; Jadhao *et al.*, 1997). The virus can also be passaged or isolated in embryonated chicken eggs, in which it causes stunted growth, haemorrhages and death of the embryo (Cheema *et al.*, 1989; Shafiq *et al.*, 1993; Naeem *et al.*, 1995a; Jadhao, 1998). Khawaja and colleagues (1988a) isolated a virus from the liver, heart and kidneys of chickens affected by HPS by inoculating embryonated chicken eggs via the yolk sac route and also in CEK cell cultures. The virus produced INIBs in CEK cell cultures and also agglutinated the red blood cells of rats. The cytopathic effects produced in the cell cultures were characterized by round cell degeneration within 3–4 days, detachment of the cells from the surface and the presence of INIBs in the infected cells (Khawaja *et al.*, 1988a; Oberoi *et al.*, 1996; Balamurugan, 1999; Balamurugan *et al.*, 2001, 2002). Inoculation of filtered liver homogenate from affected cases into the CAM or yolk sac of 8-day-old SPF embryos resulted in embryonic death within 4–7 days. The agent produced small syncytia on both chick kidney and liver monolayers (Shane, 1996).

TRANSMISSION

HPS is a contagious disease (Abdul-Aziz and Hasan, 1995) and is transmitted horizontally among broilers by mechanical means (Akhtar *et al*., 1992) and by contamination with infected faeces (Shafique and Shakoori, 1994). The HPS agent is highly pathogenic (Khawaja *et al*., 1988a), spreading rapidly from flock to flock and farm to farm (Cowen, 1992). The bird-to-bird transmission of the virus in a flock occurs horizontally (Akhtar, 1995) by the oral–faecal route (Abdul-Aziz and Hasan, 1995). Anjum (1990) failed to reproduce the disease by oral inoculation or by contact with naturally affected birds. However, Cowen and colleagues (1996) showed that the oral–faecal route is a possible mechanism for the spread of the disease under field conditions. The disease can be produced in broiler chicks by inoculating a liver extract from naturally infected pigeons, but the role of wild birds in spreading the disease under natural conditions needs confirmation (Naeem and Akram, 1995), which might help in identifying a specific reservoir for the infection. Experimentally, broiler chicks kept in close contact with infected birds, at stocking densities of 1.0, 0.83 or 0.71 ft^2/bird, became infected (Abdul-Aziz and Hasan, 1995; Akhtar, 1995). A study on the transmission of HPS in broiler and layer chicks kept on infected litter revealed that the mortality was higher in the broilers than in the layers (Shafique and Shakoori, 1994), which may be due to factors associated with the increased growth rate of broilers. The dissemination pattern of avian adenovirus after experimental infection with HPS has also been described (Naeem *et al*., 2001). Toro and colleagues (2001) found, from their experiment, that an association of FAV and CAV is necessary for the successful induction of the IBH-HPS in chickens when transmitted vertically.

PATHOGENESIS

The causative agent seems to have a special affinity for hepatic and endothelial cells. The incubation period varies from 5 to 18 days (Akhtar, 1992). There are reports showing the simultaneous presence of infectious bursal disease (IBD) and chicken infectious anaemia (CIA) viruses in areas where HHS occurs frequently (Shane and Jaffery, 1997). IBD and CIA are known for their immunosuppressive effects (Todd, 2000) and FAVs require impairment of the immune response to produce their pathogenic potential (Monreal, 1996). These viruses could be the other agents that Afzal and colleagues (1991) speculated were required in addition to the adenovirus for the production of typical signs of the IBH-HPS syndrome. The HPS agent also has a predilection for lymphoid tissues, which can result in immunosuppression (Naeem *et al*., 1995a; Deepak, 1998). Therefore, the presence of IBD or CIA viruses may predispose for HPS, or HPS may predispose for these other viral infections. The role of IBD in precipitating HPS in layer flocks has been well documented by Shukla and colleagues (1997a). Studies on the pathogenicity of FAV isolates have also suggested a synergism with CIA or other viruses, or that prior immunosuppression is necessary to produce IBH-HPS in chickens (Deepak, 1998; Toro *et al*., 2000), and the susceptibility of chickens to oral infection with FAV, resulting in IBH-HPS, varies throughout the course of CIA infection (Toro *et al*., 2000).

INCUBATION PERIOD AND SIGNS

A range in incubation period from 5 to 18 days, with a mean of 10 days, has been reported in controlled experiments (Akhtar, 1992). However, this depends upon the route and dose of infection, on the type of exposure and on the age of the birds. Another study (Akhtar, 1995) reported an incubation period of 9.5 to 14.5 days, with the duration of the disease being 10–15 days. However, Aliev and colleagues (1997) recorded the incubation period as 48–72 h in experimental infection, while the duration was 7 days. In another study, the incubation period varied between 2 and 4 days after inoculation of homogenate or purified virus (Ganesh *et al.*, 2001a). Experiments conducted by several researchers have shown that clinical signs or sudden deaths due to HPS were observed from 2 to 5 DPI (Anjum, 1990; Afzal *et al.*, 1991; Gowda and Satyanarayana, 1994). However, Voss and colleagues (1996) observed mortality from 6 to 17 DPI in SPF chicks, following intramuscular inoculation of a liver homogenate. The reasons for such variations in the incubation period have not been satisfactorily explained by any of the workers.

Generally, there are no specific clinical signs associated with the disease. In natural outbreaks of HPS, the affected birds may not exhibit any signs (Jaffery, 1988), other than a heavy mortality of up to 75% and of sudden onset in well-grown healthy broiler flocks of 3 to 5 weeks of age (Muneer *et al.*, 1989; Abdul-Aziz and Al-Attar, 1991; Gowda and Satyanarayana, 1994; Voss *et al.*, 1996; Asrani *et al.*, 1997; Kumar *et al.*, 1997). However, in the terminal stages, the individual birds may become dull and depressed, huddle in corners and have ruffled feathers, showing a characteristic posture with their chest and beak resting on the ground and with closed eyelids (Asrani *et al.*, 1997). Hydropericardium is a prominent sign of HHS and has never been noted in many reports of field and experimental cases of HPS (Gallina *et al.*, 1973).

GROSS LESIONS

The predominant and consistent gross lesion at necropsy is hydropericardium, which occurs in more than 90% of the affected birds (Anjum *et al.*, 1989; Qureshi, 1988, 1989; Cheema *et al.*, 1989; Gowda and Satyanarayana, 1994). This is characterized by the accumulation of colourless or amber-green-coloured, watery or jelly-like fluid in the pericardial sac, ranging from 3 to 20 ml in quantity and with a pH of 7 (Cheema *et al.*, 1989; Gowda and Satyanarayana, 1994; Abdul-Aziz and Hasan, 1995; Asrani *et al.*, 1997; Kumar *et al.*, 1997). The heart appears misshapen and flabby (Anjum *et al.*, 1989), with its apex floating in the pericardial sac (Kumar *et al.*, 1997). The pericardial fat may exhibit yellowish discoloration and petechial haemorrhages (Asrani *et al.*, 1997). Other changes observed were a discoloured, pale yellow, swollen, friable and mottled liver, with large areas of focal necrotic patches and petechial or ecchymotic haemorrhages; oedamatous and congested lungs; and pale yellow, swollen and friable kidneys, containing deposits of urates in the tubules and ureters (Anjum *et al.*, 1989; Cheema *et al.*, 1989; Gowda and Satyanarayana, 1994; Abdul-Aziz and Hasan, 1995; Asrani *et al.*, 1997; Kumar *et al.*, 1997; Nakamura *et al.*, 1999). Similar gross lesions

have also been described in various organs from experimentally infected broilers (Figure 1) (Anjum, 1990; Deepak, 1998). A yellowish discoloration of the subcutis and abdominal fat was also recorded in a few birds, along with bursal and thymic atrophy in 10% of the birds examined (Asrani *et al.*, 1997). In addition to the characteristic hydropericardium Nakamura and colleagues (2002) observed pinpoint white foci in the pancreas and ventricular erosions in broilers. The mortality and the severity of the lesions may be greater in immunosuppressed birds, as was evident in colchicine-treated chicks (Shafiq *et al.*, 1993). Nakamura and colleagues (2000) indicated that IBH strains of adenovirus can also reproduce the lesions of HPS and mortality in day-old SPF chicks and that IBH and HPS strains may have similar pathogenicities, except for the difference in their virulence against older chickens.

Figure 1. Gross lesions observed at 5 days after infection in 2-week-old chicks orally infected with FAV-4 (0.3 ml/bird, titre 2×10^5 $TCID_{50}$/ml). Severe hydropericardium with accumulation of straw-coloured fluid in the pericardial sac. The liver is congested and enlarged and there are pinpoint haemorrhagic and necrotic foci. The heart is shrunken and cone-shaped

MICROSCOPIC LESIONS

The liver was identified as the major organ showing histological changes, such as small multifocal areas of coagulative necrosis, mononuclear cell infiltration and the presence of basophilic INIB, almost always in the hepatocytes and surrounded by a clear halo, or as a filling in the entire enlarged nucleus (Anjum *et al.*, 1989; Cheema *et al.*, 1989; Gowda and Satyanarayana, 1994; Abdul-Aziz and Hasan, 1995; Asrani *et al.*, 1997; Kumar *et al.*, 1997; Nakamura *et al.*, 1999). In some birds, centrilobular or diffuse degeneration and necrosis of hepatocytes, swelling of the cells, with partial clearing of the cytoplasm or rupture of the cell membrane, and shrunken hepatocytes with

pyknotic nuclei were noticed (Abdul-Aziz and Hasan, 1995). These changes were confirmed by transmission electron-microscopic observation of the hepatocytes (Chandra *et al.*, 1997). The lungs showed congestion, oedema and infiltration by inflammatory cells (Cheema *et al.*, 1989; Asrani *et al.*, 1997) and there was haemorrhagic exudate in the bronchi and alveoli (Kumar *et al.*, 1997) and a moderate diffuse infiltration of macrophages into the pulmonary parenchyma (Nakamura *et al.*, 1999). Histopathological examination of the heart revealed mononuclear cell infiltration, severe vascular changes (Anjum *et al.*, 1989; Cheema *et al.*, 1989), massive oedema, haemorrhages (Asrani *et al.*, 1997; Kumar *et al.*, 1997) and degenerative changes (Gowda and Satyanarayana, 1994). The kidneys showed marked swelling of the tubular epithelium, necrosis and extensive haemorrhages (Abdul-Aziz and Hasan, 1995; Asrani *et al.*, 1997). Macrophages, containing erythrocytes and prominent yellow pigment in the red pulp, were also recorded in the spleen (Nakamura *et al.*, 1999). The gastrointestinal tract showed catarrhal inflammation of the mucosae, particularly of the villi of the intestine (Kumar *et al.*, 1997). Other changes observed were lymphocytolysis and cyst formation in the bursa of Fabricius, thymus and spleen (Gowda and Satyanarayana, 1994; Asrani *et al.*, 1997), which leads to depletion of lymphocytes in the medullae of the follicles in the bursa of Fabricius (Abdul-Aziz and Hasan, 1995; Kumar *et al.*, 1997). Deepak (1998) also observed similar histological changes in various organs. The presence of INIBs in the cells of the gizzard, pancreas, proventriculus, duodenum, caecum, kidney and lungs of chicks experimentally inoculated at 1 day of age was also observed (Nakamura *et al.*, 1999). In another experimental infection, Nakamura and colleagues (2002) observed that affected chickens had multifocal hepatic necrosis, with INIBs in the hepatocytes, a marked increase in macrophages in the spleen and lung, mild epicardial oedema, multifocal necrosis of pancreatic acinar cells, with intranuclear inclusions, focal necrosis of the ventricular koilin layer, and degeneration of the ventricular glandular epithelium, with intranuclear inclusions.

CLINICAL PATHOLOGY

HPS-affected birds showed severe anaemia, with significant reductions in all the haematological parameters, except the mean corpuscular volume (MCV) and the percentages of heterophils and eosinophils (Niazi *et al.*, 1989; Asrani *et al.*, 1997). However, Bhatti and colleagues (1989) observed leukocytosis, erythrocytosis and an increased concentration of haemoglobin. Loss of fluid from the blood and its accumulation in the pericardial sac and other organs, and the resultant haemoconcentration, may be responsible for the increased haematological values. There is also an increase in the potassium and calcium concentrations in the serum (Bhatti *et al.*, 1989), which also appears to be due to the accumulation of fluid in the pericardial sac and other organs. The lymphopenia may be due to lymphocytolysis, as reported by Gowda and Satyanarayana (1994) and Abdul-Aziz and Hasan (1995). Conflicting reports may be due to the presence of concurrent infections and to variations in the times of blood collections. Thus, other haematological studies conducted on HPS cases and normal

birds indicated a significant decrease in TLC, RBCs, haemoglobin, haematocrit, ESR and lymphocyte values and an increase in heterophils and MCV compared to normal birds (Bhatti *et al.*, 1989; Niazi *et al.*, 1989; Gowda and Satyanarayana, 1994). These findings indicated a severe degree of anaemia and alterations in the heterophil and lymphocyte counts due to the infection.

Decreased synthesis of protein due to extensive liver damage could possibly alter the albumin and globulin ratio. The resultant decrease in the colloid osmotic pressure of the plasma may cause the leakage of fluids into the pericardial sac. Alexander and colleagues (1962) reported a drastic reduction in the total serum protein in birds affected with hydropericardium. The serum protein profile revealed a decrease in albumin, with an increase in β-globulins and no change in α_1- and α_2-globulins (Mahmood *et al.*, 1995). Asrani and colleagues (1997) reported a decrease in total protein and cholesterol and increases in creatinine and urea nitrogen and in the activity of ALT and AST. There was a decrease in the concentration of blood glucose and plasma protein, whereas the uric acid, potassium, calcium and triglyceride concentrations were significantly raised in birds affected by HPS (Bhatti *et al.*, 1989).

Any damage to the liver, kidney or heart results in the release of AST, ALT, AP and LDH enzymes into the circulation (Benjamin, 1978). The activities of ALT, AST and CPK serum enzymes were lowest in normal birds, intermediate in vaccinated birds and highest in HPS-affected birds, while the AP and LDH activities were similar in normal and vaccinated birds but raised in birds affected by HPS (Iqbal *et al.*, 1994). The elevation of AST activity may be attributed to the damage to the liver and to the cardiac muscle, which results in heart failure. Heart failure may also be one of the factors for the increased urea nitrogen values in HPS-affected birds as, when this occurs, renal blood flow and glomerular filtration pressure are reduced and the excretion of urea nitrogen is impaired (Benjamin, 1978). Similar observations of increases in the concentrations of organ-specific enzymes were reported in experimental infections (Deepak, 1998).

ELECTRON MICROSCOPY

The demonstration of discrete icosahedral or hexagonal viral particles by negative staining electron microscopy of purified liver homogenates from HPS cases supported the view that the disease was caused by avian adenovirus, although the role of an enhancing factor could not be ruled out (Cheema *et al.*, 1989). Isometric, roughly spherical particles, resembling the morphology of adenovirus and measuring 83–93 nm in diameter, were demonstrated by transmission electron microscopy inside the nucleus of hepatocytes from cases of HPS and by negative staining electron microscopy of virus pellets obtained from liver extracts (Chandra *et al.*, 1997; Ganesh *et al.*, 2001a).

IMMUNOLOGICAL ASPECTS

Eight per cent of vaccinated birds undergo seroconversion by day 7 after vaccination, as evidenced by the gel diffusion test, and this correlates well with protection. Recovered birds are immune to subsequent attacks of HPS (Cowen, 1992). The percentage of responders in a single vaccination group decreased by 45% and 20% by the 28th and 45th day after vaccination, respectively, whereas all the birds in a double vaccination group remained seropositive with higher indirect HA titres. However, ELISA using an adenovirus-free antigen failed to exhibit significant titres, nor did the CMI response, as evidenced by a delayed hypersensitivity reaction in the wattles, show a significant increase following vaccination (Noor-ul-Hassan *et al.*, 1994). Deepak (1998) also observed a CMI response, shown by the lymphocyte transformation assay, from 7 days following vaccination with an inactivated cell culture vaccine in 2-week-old broilers. Further work is needed to determine the role of CMI in affording protection against HPS. The PARC-1 isolate of HPS caused immunosuppression, resulting in a reduced serological response to Newcastle disease vaccination compared to that in the controls (Naeem *et al.*, 1995a).

DIAGNOSIS

Clinical diagnosis of the disease before the occurrence of mortality is difficult since the birds do not show specific clinical signs. Clinical diagnosis of HPS is, therefore, hardly possible in spontaneous outbreaks owing to its acute nature. However, the sudden occurrence of high mortality among broiler chicks aged 3–6 weeks (Kumar *et al.*, 1997) with hydropericardium (Anjum *et al.*, 1989; Cheema *et al.*, 1989; Abdul-Aziz and Al-Attar, 1991) and the demonstration of basophilic INIBs in hepatocytes are pathognomonic (Akhtar, 1994). Diagnosis of IBH-HPS infection has been carried out on the basis of gross lesions, histopathological lesions, particularly of INIBs in hepatocytes (Gowda and Satyanarayana, 1994, Kumar *et al.*, 1997), demonstration of adenovirus particles in the nuclei of infected liver cells by transmission electron microscopy (Cheema *et al.*, 1989; Chandra *et al.*, 1997; Ganesh *et al.*, 2001a) or isolation of virus, either in cell culture or in embryonated eggs (Kataria *et al.*, 1996, 1997a); and confirmation has been done by neutralization tests using serotype-specific sera (Rabbani and Naeem, 1996; Jadhao *et al.*, 1997). Since the aetiology of the condition has almost been confirmed, immunodiagnosis by various serological tests, such as the indirect haemagglutination test (Rahman *et al.*, 1989; Hassan *et al.*, 1993), AGPT (Hassan *et al.*, 1993; Kumar *et al.*, 1997), counterimmunoelectrophoresis (Oberoi *et al.*, 1996; Kumar *et al.*, 1997), the fluorescent antibody technique (Deepak, 1998; Balamurugan, 1999) and various modifications of ELISA (Hassan *et al.*, 1993; Oberoi *et al.*, 1996; Balamurugan *et al.*, 1999, 2001) and the dot immunobinding assay (Naeem *et al.*, 1995a; Rabbani *et al.*, 1998b), is considered specific and reliable.

Deepak (1998), while studying the pathogenesis of HPS in 2-week-old chickens, detected an immunofluorescent FAV antigen in the thymus, spleen and bursa from 3 to 10 DPI, in the liver from 3 to 14 DPI and in the heart up to 5 DPI. In an attempt to

develop a laboratory method for diagnosing HPS in chickens, Balamurugan and colleagues (2001) detected viral antigen in various tissues (liver, kidney, bursa of Fabricius, spleen and thymus) from experimentally infected chickens by antigen capture (sandwich) ELISA, using guinea-pig and chicken hyperimmune sera for trapping and tracing the viral antigen, respectively.

The polymerase chain reaction (PCR) has also been developed for the diagnosis of HPS (Toro *et al.*, 1999; Dahiya *et al.*, 2002; Ganesh *et al.*, 2002). The DNA isolated from either infected liver tissue or purified virus was subjected to PCR using hexon gene specific primers, which amplified a 700 bp section of the variable region of HPS viral DNA (Ganesh *et al.*, 2002). PCR-amplified DNA was labelled with digoxigenin (DIG-11-DUTP) and used as a probe for the detection of virus by dot blot hybridization of the viral genome (Ganesh *et al.*, 2002).

PREVENTION AND CONTROL

As the epidemiological factors associated with the development and spread of HPS are unclear (Akhtar *et al.*, 1992), 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. The addition of iodophor solution (2.5%) to drinking water at 0.07–0.1% has been found to be efficacious in reducing the course and mortality of HPS in broilers (Abdul-Aziz and Al-Attar, 1991; Abdul-Aziz and Hasan, 1996). However, vaccination of the birds against IBD did not affect the outcome of the disease, as vaccinated birds were equally susceptible to the HPS agent (Khawaja *et al.*, 1988b). HPS has been controlled with the use of an autogenous formalin-inactivated vaccine prepared from a suspension of an infected liver homogenate (Chishti *et al.*, 1989; Cheema *et al.*, 1989; Afzal and Ahmad, 1990; Ahmad *et al.*, 1990; Anjum, 1990; Mashkoor *et al.*, 1994b; Shane, 1996; Kumar *et al.*, 1997). A dose of 0.25 ml/bird at 10–15 days of age provided satisfactory results (Chishti *et al.*, 1989; Afzal and Ahmad, 1990; Anjum, 1990; Kumar *et al.*, 1997). However, a single vaccination at 15–18 days of age was more effective in preventing relapses at 35–40 days of age (Ahmad *et al.*, 1990). In an experimental challenge study, protection in a double vaccinated group was 90–100% in comparison to 80–100% in a single vaccinated group, while 70–100% of the birds in the unvaccinated control died (Noor-ul-Hasan *et al.*, 1994). In two field trials, involving 570 000 birds on 128 farms, the overall mortality ranged from 0.77% to 3.8% in vaccinated and from 11.11% to 30% in unvaccinated birds (Ahmad *et al.*, 1990). In another trial, the mortality in vaccinated birds was 0.52% compared to 5.34% in unvaccinated birds kept on the same premises. Vaccination was also effective when carried out in the face of an outbreak, mortality in the vaccinated infected birds being 2.33% compared with 10.27% in unvaccinated infected birds (Afzal and Ahmad, 1990). In India, birds were vaccinated on various farms experiencing severe outbreaks of HPS; 100% protection was observed following a single dose of 0.25 ml/bird of the vaccine given at 10–12 days of age (Kumar *et al.*, 1997). Inactivation of the vaccine using 0.1% β-propiolactone (Ahmad *et al.*, 1990), or 0.5% formalin for 72 h (Anjum, 1990) or heat treatment at 56°C for 1 h

followed by overnight inactivation with 0.1% formalin (Kumar *et al.*, 1997) were found to be superior to use of the vaccine inactivated with 0.1% formalin alone. An inactivated and freeze-dried liver homogenate vaccine, resuspended in saline, provided 100% protection and was more effective than vaccines prepared from a pellet obtained after ultracentrifugation and resuspended in saline (Mashkoor *et al.*, 1994b). Oil-emulsified vaccines induced greater antibody titres (Mashkoor *et al.*, 1994a) than did formalinized vaccine. On challenge at 30 DPV with an oil-emulsion vaccine, there was 90–100% protection (Hussain *et al.*, 1996). An inactivated vaccine, prepared from liver homogenate extracted with chloroform, inactivated with formalin and adjuvanted with liquid paraffin, was highly effective against challenge with infected liver homogenate in chickens aged 4, 5 and 7 weeks (Roy *et al.*, 1999).

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, with strict biosecurity and high standards of hygiene and management. More recently, vaccines propagated in cell cultures and embryonated eggs have been developed. Experimentally, a vaccine was developed by propagating the virus in chicken liver cells and embryonated eggs by the allantoic route (Naeem *et al.*, 1995b). These preparations were inactivated and standardized to a titre of $10^{4.5}$ LD_{50}/0.5 ml and used subcutaneously at $10^{3.5}$ LD_{50}/dose per bird. Antibodies were detectable by AGPT using the PARC-1 isolates as antigen from 7 to 28 DPV (Naeem *et al.*, 1995a). Both vaccines provided protection against subcutaneous challenge with 1 ml of a 20% liver homogenate at a biological titre of 2×10^5 LD_{50}/0.5 ml.

An inactivated, oil-adjuvanted vaccine prepared from the adeno-Pak strain isolated in cell culture and given to broilers of 20 days of age also provided 100% protection against challenge at 42 days of age (Khusi *et al.*, 1996). In India, a killed, oil-emulsified vaccine was prepared using HPS virus (FAV-4) in cell culture. Vaccination of 3-week-old chicks with 0.5 ml doses of vaccine prepared from $10^{5.5}$ $TCID_{50}$/0.1 ml provided 100% protection against challenge with HPS virus at 1, 2, 3, 4 or 6 weeks after vaccination (Kataria *et al.*, 1997b). Studies on the comparative efficacy of different kinds of vaccines against HPS have been carried out by several researchers (Shane, 1996; Zia *et al.*, 2001). Shane (1996) evaluated five inactivated vaccines used in Mexico by injecting 0.5 ml of each vaccine into SPF chicks. Complete protection, with an absence of histological changes in chicks challenged with $10^{3.5}$ LD_{50} of the DCV-94 adenovirus strain, was observed. Icochea and colleagues (2001) evaluated the efficacy of three inactivated vaccines against IBH and HPS in Peru in two different experiments and concluded that the protective effect of a commercial oil-adjuvanted cell culture IBH vaccine was superior to the autogenous vaccines and that the mortality rates were not dose-dependent. Toro and colleagues (2002) reported that effective protection of the progeny of chickens against IBH-HPS could be achieved by dual vaccination of breeders with FAV-4 and CAV. In this study, induction of IBH-HPS in the progeny was evaluated by challenging them with the virulent strains FAV-341 and CAV 10343, following various strategies. Dual vaccination of breeders with FAV and CAV provided maximum protection of the progeny, with only 7% hepatic inclusion bodies (HIBs) and no mortality, whereas single vaccination with FAV-4 alone produced a less protective effect in the progeny, with 26% HIB and 7.4% mortality. In general, the control of HPS

can be achieved by the use of an autogenous formalin-inactivated vaccine prepared from a homogenate of an infected liver, inactivated cell culture vaccine or a killed oil-emulsified vaccine.

Safe vaccines that will transmit a strong active immunity that could protect broiler chicks throughout the whole growing period may be established in the future. In this context, recombinant DNA technology may have advantages over other approaches.

SUMMARY

There is a strong likelihood that avian adenovirus infections will be recognized as causing an additional disease in avian species, including commercial poultry. Should this be the case, this new type of adenoviral infection or disease in general, and HPS in particular, with which poultry veterinarians and avian disease experts are not versed, will prove to be a challenge. From the economic and welfare standpoints, it is a threat to the poultry industry, particularly the broiler industry, causing heavy mortality. As the HPS agent is highly pathogenic, it rapidly spreads horizontally among broilers, either by the oral–faecal route or by mechanical means, which leads to potential spread to other geographical areas, as when the disease emerged in India by spreading from neighbouring Pakistan through Jammu and Kashmir in 1993. It may also be possible to propagate the HPS virus in a continuous cell line for vaccine production. This ability would almost certainly mean that the application of recombinant DNA-based methodologies will have major roles in both the diagnosis of adenovirus infection and the development of vaccines for controlling the disease.

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