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Review Article

Fowl Adenoviruses Causing Hydropericardium Syndrome in Poultry

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ABSTRACT

Hydropericardium syndrome (HPS) (also known as Angara disease and Litchi heart disease) is an emerging disease of poultry caused by fowl adenovirus. The disease affects birds between 3–6 weeks of age and is economically important due to significantly high/spiking mortality in young broiler birds that may reach up to 50%. The disease can spread both vertically and horizontally/laterally and has been reported in most of the Asian countries including India, Central and South American countries, Russia and Europe. Concurrent infection with chicken infectious anaemia (CIA) and infectious bursal disease (IBD) viruses aggravates the disease. Clinically, the HPS is characterized by typical hydropericardium, anemia and necrotic hepatitis. The pathological changes may be observed in vital visceral organs viz. liver, kidneys and heart along with characteristic excessive acquisition of fluid in pericardial sac giving it a shape of litchi fruit. Histopathologically, hepatocytes contain intranuclear inclusion bodies that are basophilic in nature. Immunosuppression is mainly due to depletion of lymphocytes in bursa of Fabricius and thymus. Clinical pathology includes decrease in blood cell counts and drastic variation in the serum biochemical profiles. Besides isolation of the virus in SPF egg and cell culture, serological tests like AGID, CIE, ELISA, SNT, HI, molecular techniques like PCR, PCR–RE digestion, real–time PCR and others are used for diagnostic purposes. Sequencing of the short fiber gene can differentiate various serotypes of the fowl adenovirus. Biosecurity at farms may act as an important control measure. Several vaccines have been used for the control of disease, but in India, inactivated oil emulsified vaccine is used in some farms. Even though the disease is an emerging threat to the poultry industry, controlling the disease should not be as challenging due to the availability of rapid diagnostics along with improved vaccines.

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INTRODUCTION

Hydropericardium syndrome (HPS) also known as hydropericardium–hepatitis syndrome/Angara disease (in Pakistan)/Litchi heart disease (in India) or inclusion body hepatitis–hydropericardium syndrome (IBH–HPS), is an emerging poultry disease in recent times characterized by sudde onset with a high death rate (Memon et al., 2006). The disease was named so due to accumulation of fluid in pericardial sac. The disease was first described in young broilers in Angara Goth, Pakistan, during the year 1987. Thereafter, it spread throughout Pakistan (Anjum et al., 1989) as well as neighboring countries like India (Dahiya et al., 2001). Currently, HPS is considered as an economically important endemic disease that can cause huge economic loss to poultry sector, especially in the South Asian region, owing to mortality, reduced productivity and immunodepression (McFerran and Adair, 2003). The disease was recorded subsequently in Iraq; Mexico, Ecuador; Peru and Chile; South as well as Central America; Slovakia; Russia and Japan (Hafez, 2010). During the last decade, several novel diseases, which affect the poultry with variable consequences, have emerged. Among these, IBH–HPS has a significant position (Fernandez, 2003; Kataria et al., 2006). HPS is more dangerous compared to IBH alone as it can cause significant mortality in broiler chicks (McFerran and Adair, 2003; Balamurugan and Kataria, 2004, 2006; Kataria et al., 2006). The HPS is an emerging and immunosuppressive disease of 3–6 week old broilers, with a characteristic rapid onset and spiking mortality reaching up to 60% but more typically 10–30%, with distinctive hydropericardium (Mathew et al., 2002; Kataria et al., 2005, 2006; Balamurugan and Kataria, 2006). Concurrent infection with infectious bursal disease (IBD) or chicken infectious anemia (CIA) viruses might predispose the chickens for HPS (Dhama et al., 2002; Balamurugan and Kataria, 2004; Kataria et al., 2006). A decrease in the antibody levels have been observed when broiler chicks having HPS infection were vaccinated with Newcastle disease virus (NDV) vaccine, thus confirming the immunosuppressive potential of HPS (Manoharan et al., 2004). In chickens less than 6 weeks of age, the mortality usually varies from 2–40 per cent. Under certain conditions however

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mortality up to 80 per cent has been recorded on the basis of the pathogenecity of the virus. Peak mortality usually observed within 3–4 days is followed by cessation within 9–14 days. Lethargy, huddling with ruffled feathers, loss of appetite along with yellowish mucoid droppings may be seen in birds clinically. The feed conversion ratio may be affected in addition to reduction in weight gain (Cowen, 1992). Especially in broilers in the age group of 3–6 weeks, the disease has been detected in certain countries of Asia as well as America and has been characterized by a sudden onset. This is found to be accompanied by mortality as high as 80 per cent in broilers and that as low as below 10 per cent in layers with a course of 7–15 days specifically in these parts of the globe (Asrani et al., 1997; Chandra et al., 2000; Asthana et al., 2013). The present review is an update on HPS in poultry with particular reference to etiology, epidemiology, diagnosis, vaccination and appropriate prevention and control measures to be adopted for combating this economically important disease.

Etiology

Initially, the disease was thought to occur due to either toxicity or nutritional deficiency. However, reproduction of the disease by inoculation of a filtrate of a liver extract from infected bird free from bacteria is indicative of the presence of virus. Viral physical characterization along with detection of intranuclear inclusion bodies (basophilic) in hepatocytes, virions that are hexagonal in nature when detected under electron microscope from a liver homogenate are prominent features of a DNA virus involvement. The etiological agent can be confirmed subsequently by isolating avian adenovirus (AAV) from HPS cases.

Generally all the AAVs belong to three groups. Group I comprises of five *Aviadenovirus* species (A–E) with 12 serotypes isolated from poultry, with largely fowl adenovirus (FAdV) (aviadenovirus C) strains causing HPS. Group II includes turkey haemorrhagic enteritis virus (HEV); virus causing marble spleen disease in pheasants and the splenomegaly adenoviruses of chickens. Group III consist of EDS–76 virus, which causes egg drop syndrome (EDS) in domestic poultry birds (Koncicki et al., 2006).

The FAdV is a non–enveloped icosahedral virus belonging to the *Aviadenovirus* genus of the *Adenoviridae* family (McFerran and Adair, 2003; Balamurugan and Kataria, 2004). The group I FAdVs have been categorized into 12 serotypes (McFerran and Adair, 2003; Rahul et al., 2005) and all have been reported from cases of inclusion body hepatitis. However, only serotype FAdV–4 has been found predominant in HPS (Mazaheri et al., 1998). Under electron microscopy, it was observed that the intranuclear hexagonal FAdV virions have a diameter of 75 nm diameter (McFerran and Adair, 2003). Also, the virus, on sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) showed a total of 12 polypeptides having molecular weight in the range of 13– 110 kDa out of which the Western blot analysis revealed seven immunogenic polypeptides having molecular weight between 16– 110 kDa (McFerran and Adair, 2003; Kumar and Chandra, 2004).

Epidemiology

During 1987 the disease was first reported in Angara Goth, a place close to Karachi, Pakistan (Khawaja et al., 1988; Gowda and Satyanarayana, 1994), and thereafter documented to be significant in certain Asian and American countries (Jaffery, 1988; Shane, 1996; Abe et al., 1998). Besides its presence in some Asian countries like India, Pakistan, Bangladesh, Iraq, Kuwait, Korea and Japan, the disease has also been reported in Central and South American countries such as Mexico, Ecuador, Peru and Chile, (Abdul–Aziz and Al–Attar 1991; Afzal et al., 1991; Abe et al., 1998; Hess et al., 1999; Biswas et al., 2002; McFerran and Adair, 2003; Balamurugan and Kataria, 2004; Rahul et al., 2005; Kim et al., 2008; Park et al., 2011 Thakor et al., 2012), European countries such as Slovakia (Jantosovic et al., 1991) and Greece (El–Attrache and Villegas, 2001) and Russia (Borisov et al., 1997). The AAVs are widespread among all avian species and most of the adenoviruses cause subtle disease. Both the presence of antibodies and virus isolation has been demonstrated in clinically healthy birds. Certain AAVs are however involved in particular clinical conditions and are the causative agents of 2 significant diseases viz., inclusion body hepatitis (IBH) and HPS.

There have been occasional reports in broiler breeders and commercial layers although the problem has been seen predominantly in broilers. Many workers have reported that there was no difference in the susceptibility of various viral strains in field as well as experimental cases. However, the broiler strain Hubbard was significantly more susceptible following Indian River and Lohmann strains (Khan et al., 1995). Characteristic spiking mortality has been reported to occur in broilers between 3 and 6 weeks of age with more than 50 % mortality (McFerran and Adair, 2003). Chandra et al. (2001) has suggested that the mortality rate can even go up to 100%. Reports are suggestive that other viral synergy or earlier immunosuppression is essential for reproducing HPS in poultry. The fatality in flocks may depend on the presence of other immunosuppressive diseases such as chicken infectious anaemia (CIA), infectious bursal disease (IBD), reoviral infections and aflatoxicosis (Singh et al., 1996; Toro et al., 2000). Mycotoxins can act as pre–disposing factor in the development of the disease especially during the months of July– September in India (rainy season). Aflatoxin as well as Ochratoxin– A have been found to be important mycotoxins associated with feed that can lead to development of gross pathological lesions of IBH–HPS (Singh et al., 1996; Goyal et al., 2009; Madhuri and Verma, 2009).

Few epidemiological factors have been implicated in occurrence of HPS. While frequent visits of vaccination crews led to 15 fold increase (Akhtar et al., 1992), the use of electricity as light and heat source than Kerosin oil was reported to be inversely correlated (Memon et al., 2006) with incidence of HPS. Seasonal influence on the occurrence of HPS has been reported with major outbreaks in summer and rainy seasons and sporadic outbreaks in winter season.

Transmission

The disease has been reported to be highly contagious, which can spread quickly from one flock to another and from one farm to another by horizontal and mechanical routes of transmission and with contaminated litter (Abdul–Aziz and Hassan, 1995; McFerran and Adair, 2003; Balamurugan and Kataria, 2004). The FAdV is prolifically excreted through the feces of infected birds and serve as a major source of infection to young broilers. Horizontal transmission of the virus from bird–to–bird occurs in a flock via oro–faecal route followed by further mechanical spread of the disease along with faecal contamination. From one country to another a mechanism of spread may be commercial hatching of eggs (Adair and Smyth, 2008). Anjum and coworkers (1989) could not reproduce the disease by inoculating orally or by affected birds' contact whereas Abdul–Aziz and Hassan (1995) could transmit the disease successfully to birds experimentally by inoculation of contaminated suspension of liver intramuscularly; orally and by contact. Naturally, the horizontal transmission of HPS virus in a flock occurs mainly by feco–oral route. It can also be transmitted by contact or mechanically through the poultry farm workers, vaccinators, contaminated equipments and fomites or by flies and rodents. It has been proven that oral route seems to be most

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vulnerable route of infection as compared to other routes while in experimental conditions the disease can be reproduced by various parenteral routes out of which the subcutaneous route is of prime importance (Khan 2008). Experimentally, the disease can be transmitted by subcutaneous inoculation of healthy birds with 0.25–0.3 ml of liver homogenate of HPS affected birds (Chandra et al., 2001; Balamurugan and Kataria, 2004; Kataria et al., 2006). Chicks contracting the disease at 4 weeks of age may die at 5th week (Shafique and Shakoori, 2002). The property of the fowl adenovirus (FAdV) to get efficiently transmitted both laterally (especially *via* the personnel), and vertically has been documented by some researchers based on animal experiments (Toro *et al*., 2001; McFerran and Adair, 2003; Residbegovic et al., 2003).

Pathogenesis

There is scanty information on the FAdV and its interaction with the immune system of broiler chickens. The growth of both primary and secondary lymphoid organs is depressed significantly in case of HPS. The major predilection sites for FAdV are lymphoid organs viz., spleen, thymus, bursa of Fabricius and caecal tonsil which results in immunosuppression (McFerran and Adair, 2003).

It has been found that for development of the hydropericardium hepatitis syndrome (HHS) immunosuppression prior to concurrent infection with a Fowl adenovirus (FAV) is necessary. The pathogenecity of the infection due to fowl adeno virus is enhanced by infectious bursal disease virus (IBDV) and chicken anaemia virus (CAV); as well as mycotoxins (Toro et al., 1999 and 2002; Shivachandra et al., 2003). Zavala et al. (2002) have done an experiment wherein they infected chickens of 1 day of age (grandparent meat–type) that use to carry FAV specific maternal antibodies along with antibodies to avian leucosis subgroup J (ALV–J); or both FAV as well as ALV–J. On the basis of clinical signs as well as total mortality the effects of either FAV alone or together with ALV–J have been examined. No differences have been found significantly with such criteria in the birds that have been infected dually when compared with birds that received a monovalent challenge with either FAV or ALV–J. Recently, techniques like the flow cytometry and immunohistochemistry have been exploited to acquire knowledge regarding FAdVs and immune system interaction in poultry (Schonewille et al., 2008). Following virulent FAdV infection, a reduction in CD4+ (T–helper) and CD8+ cells (cytotoxic T cells) were reported in the spleen and thymus. In the bursa of Fabricius, an extreme decrease in lymphocytes has also been observed by immunohistochemistry which were inoculated with virulent FAdVs. The atrophy of thymus starts later than bursal atrophy but its magnitude is greater than that in bursa and spleen. It can be assessed altogether that a FAdV–4 infection affects profoundly the cells that play role in generation of humoral and cell–mediated immune responses. This is the major reason for a negative impact on both humoral and cellular immune responses of chicks, resulting in poor vaccine responses. But it is still not clear whether the mortality observed in broilers is a direct result of infection with FAdV or whether the infection results in an immune dysfunction, which may then lead to enhanced secondary infections with concurrent bacterial, viral, and fungal agents resulting in death. Nevertheless, immune dysfunctions in broilers by FAdV seem to correlate with its pathogenicity (Hussain et al., 2012).

Clinical Pathology

Severe anaemia (Niazi et al., 1989; Asrani et al., 1997) has been reported in affected birds (Bhatti *et al*., 1988; Niazi et al., 1989). Gowda (1994) and Gowda and Satyanarayana (1994) reported a decrease in total leukocyte and erythrocyte count; haemoglobin values and an increase in percentage of heterophils in natural as well as experimental cases of infection in broilers. There are also reports of reduced haematocrit valuesin HPS affected birds when compared with control birds (Cowan et al., 1996; Asrani et al., 1997). Bhatti et al. (1989) observed leukocytosis, erythrocytosis and an increased haemoglobin concentration. These contrary reports on blood values appear to be due to variations in the time of blood collection and the presence of other concurrent infections such as chicken anaemia virus.

In HPS–affected birds, a decrease in the concentration of blood glucose and plasma protein and significant rise in the uric acid, potassium, calcium and triglycerides concentrations had been found (Bhatti et al., 1989) along with other biochemical changes (Deepak et al., 2001). A significant decrease in albumin with an increase in β–globulins in serum of affected birds had also been reported (Mahmood et al., 1995). Decrease in total proteins and cholesterol, and increase in creatinine, urea nitrogen and in the activity of serum glutamic pyruvic transaminase (SGPT) and serum glutamic pyruvic transaminase (SGPT) had also been reported (Asrani et al., 1997). The synthesis of albumin is reduced in case of liver damage, which in turn decrease the colloidal plasma osmotic pressure resulting in leakage of fluid into the pericardial sac. The elevation of SGPT activity may be attributed to liver damage and damage to the cardiac muscle, which results in heart failure. Zaman and Khan (1991) reported significantly higher activity of creatinine phosphokinase in affected chickens when compared to healthy due to degeneration and necrosis in the cardiac muscle fibers. The impairment of urea nitrogen excretion due to reduced renal blood flow and glomerular filtration pressure might result in increased urea nitrogen which is one of the factors for heart failure (Benjamin, 1978). Elevated serum potassium and calcium concentrations due to the accumulation of fluid in the pericardial sac and other organs were also reported (Bhatti et al., 1989; Akhtar, 1994).

Gross Lesions

The most consistent gross lesion observed is hydropericardium with an accumulation of nearly 5–20 ml of excessive straw or amber colored fluid in pericardial sac (Nakamura et al., 1999; Chandra et al., 2001; McFerran and Adair, 2003; Tripathi et al., 2004; Meenakshi et al., 2005). Flabby heart with its apex floating in pericardial sac and yellowish discoloration and petechial hemorrhage of pericardial fat are also common gross lesions observed. Pale, enlarged and friable liver, congested and edematous lungs and atrophy of bursa and thymus were also reported in affected birds (Nakamura et al., 1999; Chandra et al., 2000; Rahul et al., 2003; Shivachandra et al., 2003; Kumar et al., 2004). Other gross lesions such as presence of urate deposits in kidney tubules and ureters, congestion and necrotic foci in kidneys, pancreatic necrosis and gizzard erosions were reported by various workers (Nakamura et al., 2002; McFerran and Adair, 2003; Deepak et al., 2004).

Histopathology

Histopathologically, mononuclear cell infiltration, multiple necrotic areas on the ventricles, shrunken, eosinophilic, fragmented and calcified myocardial fibers, vascular changes and edema leading to disintegrated cardiac muscle bundles, vacuolar disintegration of myocardial blood vessels, loss of cross striations, sarcoplasmic vacuolations, fragmentation and disintegration of cardiac fibres were reported by Venkatesha et al. (2005) and Cheema et al. (1989). Infiltrations with mononuclear cells in hepatic tissues, multifocal and centrilobular necrosis with diffuse degeneration and presence of intranuclear inclusion bodies (basophilic) in hepatocytes were commonly observed in liver (Singh et al., 2004; Balamurugan and Kataria, 2004). Electron microscopic

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examination of hepatocytes revealed intranuclear adenovirus–like particles distributed in paracrystalline arrangement (Kumar et al., 2004). Kumar and Grewal (2002) reported swelling of tubular epithelium, necrosis and presence of extensive hemorrhage along with glomerulonephritis in kidneys. In lungs, congestion, haemorrhages, oedema, catarrhal changes in the secondary bronchi along with infiltration of macrophages, were reported by Venkatesha et al. (2005). Degeneration and erosion of lymphocytes in bursa of Fabricius and thymus were reported by various researchers (Kumar and Grewal, 2002; Shivachandra et al., 2003; Rahul et al., 2003; Balamurugan and Kataria, 2004). In some cases of HPS, multifocal necrosis of acinar cells of pancreas with intranuclear inclusions and focal necrosis of the ventricular koilin layer have been reported (Nakamura et al., 1999; Nakamura et al., 2002; Sawale et al., 2012). Ivanics et al. (2010) observed hepatitis and hydropericardium which is of severe acute type in farms of Hungary that maintains goslings on large scale basis and in such cases necrotic areas that are multifocal in nature have been observed. Two different types of inclusion bodies have been observed in the liver adjacent to the necrotic areas. These are inclusion bodies that take up basophilic stain filling completely the nucleus (enlarged); other is eosinophilic occupying the nucleus at its centre.

Diagnosis

Diagnosis of the disease largely depends on observing typical gross and histopathological lesions, isolation of the causative virus, serological tests and molecular diagnostic tools like polymerase chain reaction (PCR). Diagnosis of HPS clinically before onset of mortality is difficult due to absence of specific clinical signs in natural outbreaks owing to its acute nature. Occurrence of high mortality suddenly among broiler chicks (young) with hydropericardium and the detection of basophilic intranuclear inclusions in hepatocytes are crucial in diagnosing the infection (McFerran and Adair, 2003).

Serological tests for the detection of antibodies to FAdV like indirect immunoflourescent assay (IIFA), enzyme linked immunosorbent assay (ELISA), Dot ELISA and double immuno–diffusion are hard to interpret as antibodies against these viruses are found in healthy as well as infected birds. However, these conventional tests are laborious, time consuming and are also less sensitive and specific (Shamim et al., 2009). Even though IIFA takes only few hours, but it is reported to be poorly sensitive and specific. For detection of serotype specific antibodythe serum neutralization test (SNT) has been used but has been found to be labour intensive as well as expensive. The interpretation of serological test in general is difficult to assess as anti– fowl adeno virus antibodies can be detected both in healthy as well as diseased birds (Mc Ferran and Adair, 2003). Hussain et al. (2003) performed indirect haemagglutination (IHA) test for detecting the seroprevalence of HPS in commercial broilers.

Virus isolation in chicken embryonic liver (CEL) cell cultures is generally followed. Cytopathic changes like cell rounding and degeneration have been reported within 24 hours to 4 days (Kumar et al., 2004). When the tissue extracts were inoculated in to 7–8 days old specific–pathogen–free (SPF) eggs via the yolk sac and/or chorioallantoic membrane routes, mortality, hemorrhages, intestinal sloughing, enlarged green livers with intranuclear inclusion bodies, poor feathering and stunting were observed (Cheema et al., 1989) and El–Attrache and Villegas (2001) reported 100% mortality in specific–pathogen–free (SPF) chickens inoculated intramuscularly with the tissue extracts from infected birds within 72 hours post inoculation.

Use of chicken embryonic fibroblast cell culture apart from chicken embryo liver (CEL) in order to identify and determine the pathogenecity of the virus are significant as there may be wide range of variation in the pathogenecity involving the isolates. It is necessary to employ the cross neutralization tests as well as molecular biological tools for serotyping the virus following determination of new serotype (McCracken and Adair, 1993; Kumar et al., 2003; Lüschow et al., 2007; Steer et al., 2009). Mahmood and Hassan (1995) had made efforts to propagate the HPS virus in embryonated duck eggs inoculated via yolk sac and chorioallantoic sac route with observation of hemorrhages, stunting and death of embryos in both the routes with few variations in severity of lesions. Intranuclear inclusion bodies in hepatocytes of the inoculated embryos were seen in both the groups. The CEL cell culture is considered as a method of choice for virus isolation as it is more sensitive than chicken embryo inoculation.

Negative staining electron microscopy of purified liver homogenate samples revealed isometric, roughly spherical particles of 83–93 nm diameter inside the hepatocyte nucleus similar to morphology of adenovirus (Chandra et al., 1997). Typical adenovirus particles aggregated in crystalline arrays in the nuclei of the hepatocytes were observed by transmission electron microscopy.

Agar gel immunodiffusion (AGID), counter immuno–electrophoresis, ELISA and serum neutralization test are commonly used for immunodiagnosis (McFerran and Adair, 2003). Indirect ELISA has been found more sensitive than AGID (Gupta et al., 2005). Balamurugan et al. (1999) had reported the use of dot–ELISA for the detection of group I fowl adenoviruses. Identification of serotype of the isolate is done by SNT using specific sera against all the known serotypes (Jadhao et al., 1997; McFerran and Adair, 2003; Singh et al., 2004). The fluorescent antibody technique (FAT) for the detection of FAdV–4 in liver, spleen, bursa of Fabricius, thymus and kidney tissues from cases of experimental infection in SPF layer chicks has been reported by many workers (Rahul et al., 2003; Balamurugan and Kataria, 2004; Kataria et al., 2006).

Tests based on antibodies do not provide any information regarding active infection (Najib et al., 2011) and therefore polymerase chain reaction (PCR) is used to detect FAdV for confirming the infection status (Rahul et al., 2004). The PCR is usually targeted against the variable region of hexon gene flanked by conserved primer sites (Ganesh et al., 2002; Rahul et al., 2003; Parthiban et al., 2004; Thakor et al., 2012). Parthiban et al. (2005) identified that majority of the FAdV–4 isolates existing in the poultry environment have a homology of 96–98% by sequencing. Loop –mediated isothermal amplification (LAMP) has been standardized to detect the viral antigen from cloacal swab followed by subsequent confirmation by conventional PCR (Xie et al., 2011). Phylogenetic analysis and high–resolution melting–curve (HRM) analysis of the hexon L1 gene region to classify fowl adenovirus are also useful (Cowen et al., 1997; Marek et al., 2010; Dar et al., 2012).

Interestingly, Ivanics et al. (2010) detected a goose adenovirus involvement in severe acute disease in association with hepatitis and hydropericardium by a combination of PCR and DNA sequencing. Combination of CsCl–gradient centrifugation and PCR was used for confirmation of FAdV isolates by Park et al. (2011). There is 100 per cent specificity along with high sensitivity when duplex PCR assay is used for diagnosis of the disease caused FAdV infection, simultaneously detecting avian adenovirus and chicken anemia virus in poultry, which increases the accuracy of molecular diagnosis. This assay not only saves time while detecting the type of the virus but also saves foreign exchange that is spent for confirming the

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diagnosis from separate nations which is precious (Hess, 2000; Rehman et al., 2011). Detection and quantitation of FAV DNA in chicken tissues by a real–time PCR assay holds potentials to be an excellent research and diagnostic tool with regards to higher sensitivity, specificity and quick analyses post–PCR (Romanova et al., 2009). Use of a SYBR Green based real–time PCR targeting conserved nucleotide sequences within the 52K gene for detection and quantification of all 5 FAdV species (FAdV–A to FAdV–E) was reported recently by Gunes et al. (2012). Hexon based PCR followed by RE–analysis was used to confirm the FAV–4 in HPS disease outbreaks and differentiate all 12 serotypes (Ganesh 1998; Raue and Hess 1998). Differentiation of various FAdV–4 strains by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis targeting the short fiber gene using particular restriction enzyme like *Alu* I was reported by Mase et al. (2010). Recently, Ohizumi et al. (2012) detected FAV from formaline fixed tissue using simple PCR based reaction and serotyping.

Prevention and Control

The primary and essential steps for preventing infection are the biosecurity practices against HPS infection, which gives due regard to strict cleaning and disinfection procedures. Following the managemental practices properly along with cleaning as well as disinfection of premises as well as equipments; restriction to entry of visitors as well as crews involved in vaccination in the poultry houses significantly play crucial role in preventing the disease (Kataria et al., 2005; Fitzgerald, 2008). Isolation of flocks accompanied by good sanitary procedures usually helps to prevent the spread of infection. The mortality may also be reduced by proper management and treatment of the diseased flocks. Diuretics like sodium citrate and furosemide could be used to reduce the intensity of fluid pressure and liver tonics, haematinics and Vitamin E could be used to regenerate and improve general health status of birds (Kataria et al., 2006). Proper disinfections of the poultry farm premises and equipments, controlled admission of visitors and crews for vaccination purposes, along with sufficient poultry house ventilation and lighting play crucial role in preventing disease (Abdul Aziz and Hasan, 1996).

Vaccines

Numerous attempts have been made to develop inactivated vaccines of embryo and cell culture origin (Naeem et al., 1995). Prophylactic vaccination of birds at the age of 10–15 days using formalin–inactivated vaccine (0.1% formalin) prepared from liver homogenate (20% suspension) of infected birds, protected broilers under field condition in Pakistan (Chishti et al., 1989). The birds are usually vaccinated at a dose rate of 0.25 ml by subcutaneous route. However, some researchers have suggested the use of 10% liver suspension homogenate vaccine that could give best results by preventing mortality during challenge infection (Ahmad et al., 2003). Although this type of autogenous vaccine has been used with considerable success, batch–to–batch variation in quantum of viral antigen, incomplete inactivation and contamination by adventitious avian viruses are the major disadvantages and might compromise biosecurity. The farmers are importantly advised to use quality vaccines as they can provide 100 per cent protection against HPS provided they are used at the proper age (Mughal et al., 2011).

An avian adenovirus belonging to serotype 4 has been proved to be a mainstay while vaccinating the broiler birds in Pakistan against HPS. It has also been found that polyether ionophore compound acts as a necessary component of medication programme in broiler in major parts of India as well as Pakistan. An investigation involving 1600 birds divided into eight groups consisting of 200 birds in each group has been carried out to determine the protective immunity that develops due to use of polyether ionophores in vaccines. 800 birds were vaccinated and rest 800 remained unvaccinated. The first three groups each from the vaccinated as well as unvaccinated lots received salinomycin and monensin concomitantly (a total of 1200 birds) and the remaining group each from vaccinated as well as unvaccinated lots did not receive the ionophore compounds (a total of 400 birds).Concomitant administration of salinomycin and monensin enhances the protective immunity status while using ionophore compound in vaccines. Such study thereby supports the use of salinomycin and monensin in broiler vaccination programmes in connection to HPS (Munir et al., 2007). In chickens that are vaccinated as well as challenged against the virus, humoral as well as cell mediated immunity is stimulated in such birds together by arginine in the diet (Munir et al., 2009a; Wang et al., 2013). Similarly salinomycin has been reported to show beneficial effect on cell–mediated immunity (Munir et al., 2009b).

Ahmad (1999) was able to prove that formalinized vaccine including Aqua Base Liver Organ (ABLO) vaccine and Oil Base Tissue Culture (OBTC) vaccine are effective for the control of HPS. The ABLO vaccine gives an early immune response as compared to OBTC vaccine, but OBTC vaccine gives a better immune response. Reddy *et al*. (2004) observed that the HPS vaccine containing mineral oil and Tween–80 elicited greater and sustained immune response. The vaccination against HPS positively influences immune responses against Newcastle disease virus.

On proper vaccination of breeders there is transmission of antibodies to the progeny chicks providing protection against infection in field as well as clinical form of the disease. A live vaccine has been given in Australia through drinking water particularly in case of 10–14 weeks age group of breeders. In countries like Mexico and Peru there has been routine use of inactivated vaccines for vaccinating breeders as well as broilers.

In the United States use of autogenous inactivated vaccines are in vogue most often in primary breeder flocks wherein biosecurity measures are followed strictly. In other countries like Mexico and Peru both breeders as well as broilers are vaccinated with inactivated vaccines. The progeny properly receives the maternal antibodies in case of breeders provided proper vaccination is done. A substantial number of unvaccinated birds are observed in less than 10 days old age group which either do not receive anti–adenovirus antibodies that are serotype specific or if the transmission is erratic due to vaccination done improperly (Roy et al., 1999; www.merckmanuals.com).

The recent advances in diseases diagnosis (Mansoor et al., 2009; Romanova et al., 2009; Rehman et al., 2011; Deb et al., 2013; Dhama et al., 2011, 2013a, 2013b), developing effective vaccines (Kataria et al., 2005; Dhama et al., 2008, 2013c), novel therapeutics (Mahima et al., 2012; Dhama et al., 2013d, 2013e; Tiwari et al., 2013) along with appropriate prevention and control strategies (Kataria et al., 2005) need to be exploited to their full potentials for reducing economic impacts due to this important pathogen of poultry.

Indian Scenario

The HPS was called as "Leechi" disease in India as the heart of the affected birds surrounded by hydropericardium resembled a peeled Indian Leechi fruit. The disease was first detected in Jammu and then in Punjab and Delhi in 1994 (Gowda and Satyanarayana 1994). Subsequently, the disease was continuously recorded with an average mortality of 34.6 % in broilers in Uttar Pradesh and Haryana. Serum neutralization test and polymerase chain reaction assay coupled with restriction enzyme analysis confirmed that the FAdV belonged

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to serotype 4. In 28–day–old broilers, inoculation of isolated FAdV–4 subcutaneously and orally has led to reproduction of the disease (Shukla et al., 1997; Dahiya et al., 2002). Sequence analysis of the variable region hexon gene confirmed that the virus belonged to avian adenovirus serotype 4. The Pakistani and Indian isolates shared 94% to 98% homology (Mansoor et al., 2009). Phylogenetic analysis on the basis of nucleotide sequences (complete) of the short fiber genes revealed that the FAdV–4 strains from HPS in Japan, India, and Pakistan formed a separate cluster from FAdV–4 strains not originating from HPS (Mase et al., 2010). Phylogeny of the hexon gene sequences of two FAdV isolates from Anand, Gujarat revealed that both the viruses grouped with Fowl adenovirus 12 (strain 380) and Fowl adenovirus 11 (strain C2B) and formed a minor branch of upper group indicating evolution of a new fowl adenovirus genotype (Thakor et al., 2012). An anti–IBH–HPS inactivated vaccine (oil emulsified) prepared from fowl adenovirus propagated in cell culture that provided protection within first week of vaccination was reported by Kataria et al. (1997). In 2–3 week old chicks, the vaccine with 0.5 ml dose ($10^{5.5}$ $TCID_{50}$/0.1 ml) afforded 100% protection till vaccination after 6 weeks. Similarly, Gupta et al. (2005) has developed a chicken embryo kidney (CEK) cell culture inactivated vaccine against the HPS virus. The vaccine gave 100% protection in broiler chickens, when challenged with virulent FAdV–4. In India, only inactivated oil emulsion vaccines are used for control of HPS although only when the outbreaks are suspected and not on a regular basis. Recently, FAdV has been reported to play role as a primary respiratory pathogen in chickens (Gowthaman et al., 2012).

Conclusion and Future Perspectives

Currently, HPS is considered an emerging disease of poultry. Concurrent infections of other immunosuppressive viruses like CIAV, IBDV and MDV significantly increase the severity of infection in field conditions. Clinical diagnosis is based on the characteristic hydropericardium in association with hepatomegaly and hepatic necrosis. Recently, molecular detection using PCR has emerged as the method of choice for detection of HPS in birds. Good hygiene, management practices, strict biosecurity and effective vaccination programme are of paramount importance in checking the disease. Administration of inactivated vaccines prepared from the prevalent serotype of FAdV is practical and financially justifiable in endemic areas for prevention and control of the disease. But the vaccines must be tested for safety and efficacy prior to its use in the field conditions. In recent years, outbreaks of IBH along with hydropericardium had caused higher morality in birds in contrast to milder outbreaks that occurred earlier leading to severe economic losses to the poultry sector. Hence it could be possible that the virus might have changed its virulence causing HPS along with IBH leading to high mortality. With the emergence of chicken infectious anemia virus having a potent immunosuppressive effect, there is possibility of re–emergence of IBH–HPS in poultry flocks, and this could pose a significant threat to the rapidly growing poultry industry. Hence constant vigilance and preparedness will go a long way towards prevention and control of HPS.

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