Haemorrhagic enteritis of turkeys – current knowledge

Kuldeep Dhama a, Vasudevan Gowthaman b, Kumaragurubaran Karthik c, Ruchi Tiwari d, Swati Sachan e,
M. Asok Kumar f, M. Palanivelu g, Yashpal Singh Malik h, Raj Kumar Singh i and Muhammad Munir j

a Avian Diseases Section, Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, India;
bPoultry Disease Diagnosis and Surveillance Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India;
cCentral University Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India;
dDepartment of Microbiology, DUVASU, Mathura, India; eDivision of Biological Standardization, ICAR-Indian Veterinary Research Institute, Izatnagar, India; fDirector, ICAR-Indian Veterinary Research Institute, Izatnagar, India; gAvian Viral Diseases Programme Compton Laboratory, Berkshire, UK

ABSTRACT
Haemorrhagic enteritis virus (HEV), an adenovirus associated with acute haemorrhagic gastro-intestinal disease of 6–11-week old turkeys predominantly hampers both humoral and cellular immunity. Affected birds are more prone to secondary complications (e.g. colibacillosis and clostridiosis) and failure to mount an effective vaccine-induced immune response. HEV belongs to the new genus Stadenovirus. Feco-oral transmission is the main route of entry of the virus and it mainly colonizes bursa, intestine and spleen. Both naturally occurring virulent and avirulent strains of HEVs are serologically indistinguishable. Recent findings revealed that ORF1, E3 and F6 genes are the key factors affecting virulence. The adoption of suitable diagnostic tools, proper vaccination and biosecurity measures have restrained the occurrence of disease epidemics. For diagnostic purposes, the best source of HEV is either intestinal contents or samples from spleen. For rapid detection highly sensitive and specific tests such as quantitative real-time PCR based on Taq man probe has been designed. Avirulent strains of HEV or MSDV can be effectively used as live vaccines. Novel vaccines include recombinant hexon protein-based subunit vaccines or recombinant virus-vectored vaccines using fowl pox virus (FPV) expressing the native hexon of HEV. Notably, subunit vaccines and recombinant virus vectored vaccines altogether offer high protection against challenge or field viruses. Herein, we converse a comprehensive analysis of the HEV genetics, disease pathobiology, advancements in diagnosis and vaccination along with appropriate prevention and control strategies.

1. Introduction
Turkey enteritis is predominantly responsible for retarding the growth of birds by reducing growth rates and feed-conversion efficiency. It is also the leading cause of losses due to morbidity and mortality. A number of viruses like haemorrhagic enteritis virus (HEV), turkey astrovirus type 2 (TAsTV-2), turkey coronavirus (TCoV), rotavirus, revirus, and bacterial causes viz. Salmonella spp. and Lawsonia intracellularis (Li) are accountable for causing enteric diseases in turkeys (Barnes et al. 2000; Woolcocka & Shivaprasad 2008; Moura-Alvarez et al. 2013; Mettifogo et al. 2014; Moura-Alvarez et al. 2014; Ongor et al. 2015). Of note, some of these pathogens continuously persist in turkey flocks for the entire life (Pantin-Jackwood et al. 2007; Beach et al. 2009a). The state-of-the-art molecular biological techniques have contributed significantly in understanding the causative agents in depth and enabling diagnosis and prevention more feasible and rapid (Pantin-Jackwood et al. 2008; Jindal et al. 2010a; Jindal et al. 2010b; Day & Zsak 2013).

Haemorrhagic enteritis (HE) is an economically significant acute gastro-intestinal disorder caused by virulent strains of a group II adenovirus. More commonly, acute infection is seen in turkeys of more than 4 weeks of age, which cause depression, intestinal damage and haemorrhages, followed by death of infected birds (Suresh & Sharma 1996; Saif 1998; Pierson & Fitzgerald 2013). The ubiquitous HEV has immunosuppressive nature, and hence may give rise to secondary bacterial infections in affected flocks. The clinical course of disease usually persists in the flock for 7–10 days and the losses could extend for an additional 2–3 weeks in the case of super imposed complications. HEV has been found highly related to the other members of group II avianadenovirus such as Marble spleen disease virus (MSDV) affecting pheasants and splenomegaly virus of chickens (Fitzgerald & Reed 1989; Pierson & Fitzgerald 2013). Besides, pheasant MSDV isolates can infect turkeys and turkey HE isolates may infect pheasants, showing their close relationship. Regarding HE in turkeys, it has been suggested that birds younger than 2–4 weeks of age are generally resistant to the infection. However, 2–4-week-old birds can be infected when the maternal antibody levels and virus pressure are high in the environment (Sharma 1991).
A sequence of events occurred in last few decades in understanding the HE pathobiology and virus genetics. The ways of HEV diagnosis changed from traditional methods to the molecular, with higher sensitivity and specificity. New generation vaccines have also paved the way for controlling the epidemics. In this review, a comprehensive analysis of the HE virus genetics, disease pathobiology, along with the advancements in disease diagnosis and vaccination has been conversed.

2. History

HE was first described in turkey poult in 1937 by Pomeroy and Fenstermacher at Minnesota, USA (Pomeroy & Fenstermacher 1937). The flock had experienced mortality up to 10%, the affected birds were well nourished, and had prominent haemorrhagic lesions in the small intestine. Microbiological examination and animal inoculation tests were conclusive to determine a specific etiological agent. Gale and Wyne reported similar kind of outbreak in a turkey flock at Ohio, USA in 1957. The causative agent was not identified on both occasions. In the year 1967, Gross and Moore identified that the etiological agent, responsible for HE lesions, was filterable through a 0.22 micron filter (Gross & Moore 1967). These findings led to the speculation that the etiological agent might be a virus (Domermuth & Gross 1971). In the year 1974, Carlson et al. (1974) demonstrated adeno-like virus particles in tissues from affected birds and determined the etiology. Since then outbreaks of HE are reported from various turkey producing regions of the world such as the United States of America, Canada (Itakura et al. 1974), the United Kingdom (Arbuckle et al. 1979; McDougall 1980), Germany (Winterol 1977), Australia (Tham & Critchley 1981), Spain (Gomez-Villamandos et al. 1994), Italy (Mandelli et al. 1977) and Japan (Fujiwara et al. 1975).

3. Etiology

The HEV belongs to the group II Aviadenovirus within the family Adenoviridae and is the etiological agent of intestinal haemorrhages associated with immunodepression in 4–12-week-old turkeys. Electron microscopy revealed that HEV are non-enveloped icosahedrons 70–90 nm in diameter. The capsid mainly comprises of three major structural proteins (hexon, penton, and fiber). Morphometrically, one fibre structure attaches non-covalently to the penton protein at each vertex (van den Hurk 1992) while two fibres are present in other members of the genus Aviadenovirus (Pierson & Fitzgerald 2013). The uniform sized virions are made up of 252 capsomers. The virus replicates in the nucleus and forms basophilic inclusions. Virions are observed in the nucleus of the cell, in diffuse array as well as in tight crystalline pattern (Carlson et al. 1974; Itakura & Carlson 1975; Ossa et al. 1983). Also, DNA hybridization experiments and gene sequence data has revealed that HEV is distinct from other aviadenoviruses (Jucker et al. 1996). The whole DNA genome sequence of the virus is now available and the information suggests it contains a linear double standard DNA of 26 Kbp, which is shorter than the genomic DNA of other adenoviruses (Jucker et al. 1996). The genome consists of eight open reading frames (ORFs) in two clusters; one that includes ORFs 1, 2, 3 and 4, and other as ORFs 7 and 8 and at least 13 genes including 52K, Illa, Penton base, pVI, Hexon, EP,100K, pVIII, Fiber,IVa2, POL, pTP, and DBP. The genome of HEV has very low ratio of the overall G + C content (34.93%) when compared to other adenoviruses (Pitcovski et al. 1998). Genes of penton, hexon, fiber, core proteins and polymerase have been identified and the amino acid sequences have been predicted (Pitcovski et al. 1998). Recent findings revealed that ORF1, E3 and fib genes are the key factors affecting virulence (Beach et al. 2009).

Similarly, during antigenic characterization of HEV, a total of 11 polypeptides ranging in molecular weight from 14 kDa to 97 kDa were detected. Monoclonal antibodies (MAbs) against HEV were chosen for identification of neutralizing epitopes and they were found to react with the 97kDa hexon protein (Nazerian et al. 1991). Based on recent genomic studies, the HEV has been placed under new genus, Siaadenovirus. This is due to the presence of sialidase homologue genes, which clearly demarcate them from other viruses of the family (Marek et al. 2014). Both naturally occurring virulent and avirulent strains of HEVs are serologically indistinguishable and the avirulent strains of HEVs have been found to efficiently replicate in turkeys without producing any clinicopathological disease (Beach et al. 2009b).

Complete genome sequences of 12 HEV strains in USA revealed that at least one missense mutation in ORF1 revealed the existence of 11 different HEVs (Beach et al. 2009b). The virion fibre contains C-terminal region which do not have any similarity with other adenovirus fibre heads, moreover there are differences of about two amino acid sequences identified between virulent and avirulent TAdV-3 (Singh et al. 2015). Proteomic analysis revealed involvement of at least 13 viral proteins and 18 host proteins in cell-to-cell spread, cytoskeleton dynamics and virus replication (Kumar et al. 2015).

Further, the physico-chemical features of the HEV have also been studied. Owing to the fact that since HEV lacks an envelope, virions are capable of withstanding a wide range of temperatures and environments. The virus remains infective for 6 months and 4 years at 4°C and −20°C, respectively (Domermuth & Gross 1984). The virus is resistant to chloroform, quaternary ammonium compounds, ethyl ether and zephrin chloride (Domermuth & Gross 1971; Smith 1981).
The infectivity of the virus is destroyed when it is exposed to a high temperature (70 °C) for 1 hour; or by treatment with sodium hypochlorite, 1% sodium laurel sulphate or 1% Lysol (Pierson & Fitzgerald 2013) after proper removal of all organic material. On evaluating zoonotic ability of this virus, it was found that HEV does not cause infections in humans (Kayali et al. 2009). Thus, HEV carries no zoonotic threats to turkey handlers and reapers.

4. Viral replication

The HEV interacts and enters the susceptible host cell receptors (integrins) using receptor-mediated endocytosis mechanism. The HEV uses certain amino acid motifs present on the base of penton proteins of fibres. Subsequent to the virus entry uncoating and release of virion into the cellular cytosol occurs. The viral proteases are activated with translocation of viral nucleocapsid into the host cell nucleus (Greber et al. 1997; Shenk 1996). This is followed by activation of the host cell transcription machinery and expression of essential genes for initiation of replication kinetics. At first step, interaction of viral proteins occurs with cellular transcription factors (Parker et al. 1997; Fax et al. 2000), followed by proteins responsible for viral genome replication such as viral DNA polymerase (AdPOL), pre-terminal protein (pTP), and DNA binding protein (DBP) (Liu et al. 2003). The activation of IVa2 transcription helps to elicit the major late promoter (MLP) and by this way IVa2 mediates the infection (Jansen-Durr et al. 1988, 1989, 1990; Lutz & Kedinger 1996; Pardo-Mateos & Young 2004 a, b). This is followed by transcription of late genes from MLP through alternative splicing of a single polycistronic mRNA which marks buildup of late gene products containing structural proteins and instigation of progeny virions assembly. Moreover, IVa2 further enables packaging through binding with target sequences of the genome, and specific proteins needed for assembly (Ostapchuck & Hearing 2003; Singh et al. 2005; Zhang & Arcos 2005). Consequently, virions start accumulating inside the infected cells nucleus and in the end mature virions disrupt the infected cell and are released.

5. Epidemiology

This disease in turkeys has been earlier found to attain epidemic proportions in some of the states of the USA during 1960s (Gross & Moore 1967). HE has been encountered in confined as well as free-range turkey flocks and the infection has the potential to infect successive flocks on the same premises, in a rapid manner. Besides affecting turkey flocks in many states of the USA, the HE, based on serologic surveillance, has been observed throughout the world wherever turkeys are raised (Iancovescu et al. 1985; Gomez-Villamandos et al. 1994; Pierson & Fitzgerald 2013). Considerable variations have been found in the pathogenicity of the HEV isolates, naturally occurring avirulent HEV strains produce either no clinical signs nor death (Beasley & Clifton 1979; Beasley & Wisdom 1978), and the virulent strains produce varying degree of mortality ranging between <1 to over 60% and clinical disease (Gale & Wyne 1957; Gross & Moore 1967; Pomeroy & Fenstermacher 1937).

The natural host of HEV are turkeys, but serological evidence also reported its presence in commercial chickens (Domermuth et al. 1979). In addition, it could infect other gallinaceous birds such as peafowl, bobby white quail and chukars (McFerran & Smyth 2000). The absence of vertical transmission is an unique feature of HEV, and differs from other adenoviruses such as egg drop syndrome and fowl adenovirus.

Based on the available virus sequence information and phylogenetic analysis adenoviruses are clearly divided into Mastadenovirus, Atadenovirus, Siadenovirus, Aviadenovirus and Ichtadenovirus (Benkö et al. 2005) (Figure 1(A)). It has been postulated that siadenoviruses initially originated in amphibian hosts, subsequently they evolved and infected avian hosts (Davison et al. 2003). In a phylogenetic analysis study of the entire hexon gene and partial DNA polymerase study, it was established that the TAdv-3 is in close relationship with that of penguin adenovirus (Lee et al. 2016). Limited information is available on the genetic characterization of the Siadenovirus in which HEV are recently classified. Phylogenetic analysis of eight sequences of HEV available in the GenBank (NCBI) indicates that vaccine strains clustered differently than the field strains (Figure 1(B)). However, the available sequence information is biased toward scarcity and thus requires future studies before any reliable phylogenetic or epidemiological interpretations are made.

6. Transmission

HEV is transmitted from infected to susceptible flocks via movement of virus laden faecal or litter material. The virus enters the host via the oral route. Also, if protected from drying, the HEV can remain infectious for seven weeks in carcasses or faeces (Pierson & Fitzgerald 2013). Latest findings suggest that the recovered birds may act as a source of persistent infection to the flocks as a result of latent shedding (Beach et al. 2009a). The environmental persistence of the virus has been proven beyond doubt as the HEV could be easily recovered from contaminated litter. This factor makes it feasible for the disease to reappear in poultry houses. However, unlike group I adenoviruses, the HEV has not been implicated in any instances of vertical transmission and carriers or vectors have not been identified as a reason for the transmission of the virus in flocks.
HEV enters into the GI tract through the feco-oral route, get absorbed from the intestine and enters into the blood circulation leading to primary viremia then transported to bursa and spleen which are the primary replication sites. HEV is considered to be lymphocytotropic with the B-lymphocytes are the primary target. HEV induced lesions are mainly immune mediated and disease causes economic impact mainly due to the immunosuppression produced by the virus and also the secondary bacterial infection may cause further additional production losses for the next 2–3 weeks even after the clinical disease subsides (Saif1998; Rautenschlein & Sharma 2000; Pierson & Fitzgerald2013; Cervantes 2015). It has been reported that an intact bursa is essential for the induction of HEV infection in turkeys (Fadly & Nazerian 1982). Similarly, the role of macrophages in supporting the replication of the virus has also been suggested. After exposure, the HEV may replicate in B-lymphocytes located in the intestine and bursa, or may travel directly to the spleen via peripheral blood (Pierson & Fitzgerald 2013), infecting more macrophages, B cells and replicates efficiently. This intern leads to influx of macrophages and CD4+ T cells in the white pulp, which results in hyperplasia of white pulp as sequelae to clear the virus. High levels of HEV can be detected in the bursa, during 2 to 7 days post infection (DPI) and in the intestinal tract during 4–7 DPI and remains detectable up to 15 DPI. However, the virus could be first detected in spleen at 2 DPI, which reaches peak levels on 6 DPI (Hussain et al. 1993) and the spleen remains the major site of replication. Also, HEV has the ability to induce apoptosis in spleen cells at 3–4 DPI, during increased virus replication (Rautenschlein et al. 2000). This has been suggested due to the induction of IL-6 like cytokine secretion in the spleen as observed under in vitro conditions (Rautenschlein et al. 2000). Activation of macrophages by HEV leads to production of cytokines such as IL6, TNF and interferon type I and II (IFN). The IFN-type II, stimulates nitric acid production which lead to antiviral and immunosuppressive properties. In addition to this, IFN type-I is also produced to limit the replication of HEV. Apoptosis and necrosis of target cells result in exhaustion of IgM bearing B-lymphocytes. These series of events lead to transient immunosuppression during clinical phase of HEV infection (Rautenschlein et al. 2000). Following splenomegaly and viremia, high levels of HEV are present in the lamina propria of the small intestine, which lead to intestinal congestion and haemorrhage. It has been suggested that initiation of intestinal pathology in HEV attributed due to release of prostaglandins and histamines from increased mast cells and also the immune mediated reactions (Hussain et al. 2013). Flowcytometric analysis revealed an increase in T and B cells subpopulations as well as CD3+CD4+ T cells subpopulation in HEV infected turkeys, on the contrary CD3+CD8+ T cells, IgM+ B cells subpopulations were decreased (Koncicki et al. 2012).

Intestinal haemorrhage occurs as diapedesis without vascular damage and appears to be initiated by response to the inflammatory mediators. Further, it has been identified that HEV replicates first in the lymphoid cells of intestinal tract, followed by bursa, and

![Figure 1. Phylogenetic tree of adenoviruses and HEV.](http://www.lirmm.fr/recherche/equipes/mab)
8. The Disease

8.1. Clinical Signs

HE generally occurs in birds of 6–11 weeks of age, and pouls younger than 3–4 weeks of age are considered refractory to infection, which could be due to the presence of maternal antibodies that may last for about 6 weeks of age (Fadly & Nazerian 1989). In HE, mortality occurs in about 5–6 days and if the infected bird survives, the clinical signs usually subside within 6–10 days after the first appearance of blood droppings. In field conditions, the mortality due to HE generally falls between 10% and 15% whereas during experimental infections, about 80% mortality can be seen as a result of the chances of gaining a 100% infection (Pierson & Fitzgerald 2013). The major clinical manifestations of HE are depression and the presence of frank blood in droppings, and a final culmination to fatal results. Bloody faeces may be frequently present on the skin and feathers around the vent region in dead or infected birds. Reduced levels of glucose, albumin, and total protein in blood may also be observed in cases of HE (Norton et al. 1993).

The birds which survive the acute phase of infection undergo immunosuppression, which manifests in the form of secondary infections such as colibacillosis, mycoplasmosis, bordetellosis, clostridial infections, coccidiosis, etc., so again a second peak of mortality overlap the first which often continue for 2–4 weeks (Giovanardi et al. 2014).

8.2. Gross lesions, histopathology and electron microscopical studies

The gross lesions observed in dead birds in general are paleness and distension of small intestine with blood-mixed contents. Death usually results from massive GI tract haemorrhage of an estimated 60%–70% of the total blood volume of the bird (Gross & Moore 1967). The mucosa of intestine is congested, contains petechial haemorrhages, and in some birds yellowish fibro-necrotic membranes are also seen. The intestinal lesions are more distinct at duodenal part and extended towards caecum in severe cases. Enlarged spleen with marbled or mottled appearance is a characteristic lesion of HE (Saif 1998; Pierson & Fitzgerald 2013; Cobb & Smith 2015). There may be congestion in lungs while other organs are often found pale. Similar to spleen, the liver of the affected birds may also get enlarged. Aside to these lesions, petechial haemorrhages can also be observed in various tissues. Duodenal wall thickening, mild catarrhal enteritis and enlarged mottled spleen may persist in surviving birds for several weeks as these are often evidenced at slaughter. Such spleens may be 1.5 to 4 times larger than normal size (Carlson et al. 1974; Itakura & Carlson 1975).

Histopathologic changes are highly evident in the lymphoreticular and gastrointestinal systems. In spleen, hyperplasia of white pulp, lymphoid necrosis, apoptosis in the germinal centres and intranuclear inclusions in lympho-reticular cells can be seen as sequelae to transient immunosuppression (Pierson & Fitzgerald 2013) which results in increased susceptibility to Colibacillosis. In thymus and bursa, lymphoid depletion can be noticed in both the cortical and medullary regions. Lesions in gastro-intestinal tract include congestion of intestinal mucosa, degeneration of villus epithelium, and presence of haemorrhage in the tip of villus. Infiltration of mast cells, plasma cells, heterophils and intranuclear inclusions are seen in lamina propria, especially more prominent in duodenum. Also, intranuclear inclusions have been observed in liver, lungs, pancreas, brain, and renal tubular epithelium.

Electronic microscopy (EM) was used for better visualization of the viruses in tissues and cell culture (Alavarez et al. 2014). EM of field cases revealed numerous affected cells with several virus particles seen intranuclearly scattered in splenocytes and enterocytes. Three forms of viral particle distribution has been reported viz., loose virus particles throughout the nucleus, fibrous inclusions seen in the perinuclear area, and large intranuclear and perinuclear accumulations of virus particles (Carlson et al. 1974).
9. Immunosuppressive interactions

Furthermore, the infection with HEV has been found to predispose birds to enteropathogenic *E. coli* infection in either natural condition or during experimental studies (Larsen et al. 1985; Sponenberg et al. 1985; van den Hurk et al. 1994; Saif 1998; Palya et al. 2007; Giovanardi et al. 2014) and clostridial dermatitis (Thachil & Nagaraja 2013). HEV infection also depresses the response to vaccines, as they were shown to produce low antibody titres during Newcastle disease (ND) vaccination (Nagaraja et al. 1985). Similarly, the avian metapneumovirus (AMPV), being an immunosuppressive respiratory pathogen of turkeys, has been found to reduce the efficacy of HEV vaccines in turkey flocks (Chary et al. 2002). Thus, owing to its immunosuppressive effect, a good antibody level against HEV throughout the reproductive cycle is necessary to combat the secondary infections (Ceruti et al. 2007).

10. Diagnosis

Diagnosis of HEV is carried out based on a combination of the clinical signs, gross and histological lesions, and demonstration of viral antigen/antibodies by conventional and molecular methods. For diagnostic purposes, the best source of HEV is either intestinal contents or samples from spleen (Gross & Moore 1967). For isolation of HEV, lymphoblastoid B-cell line of turkey origin (MDTC-RP19) can be employed. If the cell line is not available the virus can be prerogated in 6-week-old naïve turkeys by inoculating the intestinal contents or splenic material by oral or intravenous (IV) route. Death usually occurs approximately three days after the intravenous injection and five or six days after oral infection. The surviving birds have prominent splenomegaly after 6 DPI. The presence of HEV will be conformed in a double immunodiffusion test from splenic material. Diagnosis can be made by histopathological examination and the observation of adenovirus in splenic cells (Gomez-Villamandos et al. 1994). For detection of the virus, agar gel precipitation test and microdiffusion tests are commonly employed by which the splenic tissue sample will be precipitated with anti-HEV sera (Domermuth et al. 1972; Domermuth et al. 1973; Pierson & Fitzgerald 2013). Likewise, the presence of viral antigen in tissues can be identified by immunofluorescence or immunoperoxidase methods. An immunohistochemical staining technique for the detection of HEV has been developed using a mixture of monoclonal antibodies against HEV and a streptavidin-biotin system (Fitzgerald et al. 1992). Also, the distribution of HEV in various tissues can be studied using immunofluorescence or immunoperoxidase techniques (Silim & Thorsen 1981; Fasina & Fabricant 1982). Aside to these techniques, highly sensitive enzyme linked immunosorbent assays (ELISA) have been developed to detect HEV antibodies, by using virus-infected RP19 cells as the coating antigen (Nazerian & Fadly 1987). ELISA can also be used to detect and quantify the HEV antibodies in sera and viral antigens in tissue extracts (van den Hurk 1986; Nazerian et al. 1990). A recombinant hexon protein (N-terminal) based ELISA test was found to be cost efficient and sensitive technique to detect specific antibodies against HEV in turkey sera (Lobová and Celer 2016). Further, by using monoclonal antibodies, various group II viruses can be differentiated (van den Hurk & van Drunnen Little-van den Hurk 1988).

Recently, the availability of whole genome sequence data has enabled the development of standard (Hess et al. 1999), nested (Beach et al. 2009b) and real-time (Beach 2006) PCR assays for detection of viral DNA in splenic tissue samples (Hess et al. 1999). PCR is more sensitive than agar gel immunodiffusion test in diagnosing HEV infection in cases where pathological lesions were mild (Palya et al. 2007). Also, DNA from group II viruses like MSDV or splenomegalgy virus can be differentiated by using different restriction endonucleases (RE) like *Bgl* II, *EcoR* I, *Hind* III, *Hha* I and *Xho* I. Hence RE analyses should be employed for distinguishing genetically different and serologically similar strains of type II avian adenoviruses (Zhang & Nagaraja 1989). For rapid detection of this virus in tissues of affected turkeys, highly sensitive and specific tests such as quantitative real-time PCR based on Taq man probe has also been designed using hexon gene to detect and quantify HEV (Shah et al. 2013). This test can be very helpful in studying pathogenesis, different phases of viral infection in birds and for epidemiological purposes. Such methods are useful in the case of an outbreak where large numbers of samples are required to be processed in less time. Thus, these nucleotide-based techniques will be very helpful in epidemiological study of HEV infection in turkey due to their ability to distinguish closely related viruses and also because of their speed of detection.

An overview on pathogenesis, clinical signs and diagnosis of HEV is presented in Figure 2.

11. Differential Diagnosis

HEV should be differentiated from various bacterial diseases such as *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *E. coli*, *Salmonella* sp. and viral diseases including reticuloendotheliosis, lymphoproliferative disease, avian influenza and ND, which induce similar splenic and gastrointestinal lesions. Although diagnostic assays are developed for identification of individual viral or bacterial agents, it would be of immense value to consider multiplexed assays for differential diagnosis of HEV from other secondary bacterial or viral infections.
12. Prevention and control

For an effective prevention of HE, good biosecurity measures such as complete physical cleaning, disinfection, maintaining good ventilation and decontamination of feeding equipments have to be implemented (Poss 1998). This is a crucial factor because contaminated litter or environment accounts for the major mode of transmission between flocks. An efficient manure management system should be implemented to reduce the ammonia emission and dust generation as low as possible. The manure should be totally removed and fresh litter should be placed during placement of every new flock in order to provide cleaner environment. Documented biosecurity guidelines should be strictly adapted on all the premises of the farm. The visitors should be minimized; all personal should wear clean head cape, outer clothing and footwear. Shower-in-and-shower-out facilities are advised in breeder and commercial operations. Implementation of an integrated pest management including an efficient rodent and insect control program will minimize the mechanical transmission of HEV. Provision of vitamin E, vitamin C, selenium and zinc in starter feeds will improve the immunity of poults. If infection is noticed in a particular poultry shed, then the facility should be thoroughly cleaned and disinfected with sodium hypochlorite or phenolic compounds, followed by complete drying for 7 days at 25 °C. However, in commercial operations, having multiple age groups of birds, total eradication of HEV is difficult and in such instances, vaccination remains the most viable option for preventing the infection. Also, antiserum obtained from recovered flocks can be administered to obtain passive protection (Pierson & Fitzgerald 2013). Virus-neutralizing monoclonal antibodies against multiple epitopes of hexon protein of HEV have the ability to confer passive protection and at a high dose it can prevent clinical disease by reducing the replication of virus in tissues. Antibiotics may be used to treat the secondary bacterial complications, the choice should be chosen based on susceptibility profiles of locally obtained E. coli strains.

13. Vaccination

Regarding vaccination against HE, avirulent strains of HEV or MSDV can be effectively used as live vaccines (Sharma 1994; Pierson & Fitzgerald 2013; Fadly et al. 1985). The currently available vaccines are either
prepared from spleen homogenate obtained from 6-week-old turkeys inoculated with the avirulent HEV strain or produced in vitro using RP19 cells commercially available (Fadly et al. 1985; Pierson & Fitzgerald 2013). These vaccines have been found to generate adequate protection, but commercially, the cell culture-based vaccines are only available. Cell culture propagated liquid vaccines are more effective than frozen vaccines in eliciting seroconversion and antigen clearance from splenic tissue (Barbour et al. 1993). However, in some countries, vaccines have been developed by propagating avirulent HEV in peripheral blood leukocytes (van den Hurk 1990). These live vaccines can be administered either in ovo in 18–19-day old embryos or via drinking water in 3–6-week-old birds. In birds, if a booster is given one week after the initial vaccination, then complete protection can be elicited.

Further, HE vaccine virus obtained by propagating in turkey origin Marek’s disease induced B-lymphoblastoid cell line have also proved effective in combating HE infection without any untoward reactions (Fadly & Nazerian 1984). The vaccination protocols are developed based on age susceptibility of HEV strains because the maternal antibodies may interfere with the vaccine virus. Therefore, it is imperative to vaccinate pouls against HEV after the maternal antibody titres are reduced, but earlier to prevent exposure to virulent field strains. The vaccinated flocks showing 60% or higher seroconversion with splenic homogenate indicate full protection. The second vaccination is repeated one week after first vaccination in the flocks with low seroconversion especially when cell culture based vaccines are used. Presence of immunosuppressive agents such as aMPV (Chary et al. 2002) or presence of residual water sanitizers in the pipeline will reduce the efficiency of vaccination.

Besides, exploiting the advances in the genetic engineering techniques, novel vaccines are also being developed to prevent HE in turkeys. Such vaccines include recombinant hexon protein-based subunit vaccines or recombinant virus-vectored vaccines using fowl poxvirus (FPV) expressing the native hexon of HEV (Cardona et al. 1999). The advantage of such advanced vaccines is that they would not produce even the slightest immunosuppression when compared to the conventional tissue culture vaccines (Pierson & Fitzgerald 2013). However, while developing recombinant hexon protein-based subunit vaccines, it has to be kept in mind that the developed protein should not be denatured and also it should retain its native structure in order to provide the desired results (van den Hurk & van Drunnen Little-van den Hurk 1993). Further, during the development of virus-vectored vaccines, the hexon requires a 100 kDa folding protein to retain its native form. Hence, the recombinant FPV construct should co-express the hexon and a 100 kDa folding protein to obtain the best humoral response in birds (Cardona et al. 2001). Pitcovski et al. (2005) have developed a subunit vaccine utilizing the capsid protein (knob protein) of HEV expressed in E. coli. A vaccination trial with this recombinant protein showed adequate protection against virus challenge, suggesting the use of knob protein for delivering safe and efficient vaccine against HEV in turkeys. Also, combined vaccination of turkey with HE and ND virus vaccines appeared to be a failure in terms of protection against both the diseases (Rautenschlein & Sharma 1999). The disadvantages of recombinant vaccines include that they may not completely protect against a broad range of field viruses. Most subunit vaccines are not appropriate for oral delivery or vaccination of a large number of birds. It is also difficult to determine the proper dosage of vaccination which could lead to failure of the generation of desired protective immunity to counter virulent challenge. However, in ovo vaccination with such combined live viral vaccines produced an entirely opposite effect, providing adequate protection from the challenge of the two viruses in young pouls (Ahmad & Sharma 1993).

An overview on viral structure, vaccines and control strategies of HEV is presented in Figure 3.

### 14. Conclusion and future directions

HE is an economically important viral disease of turkeys. Prior to the development of effective cell culture vaccines, the disease has created several havoc and incurred huge financial losses especially in countries such as USA and Canada. Such financial losses were primarily due to the immunosuppressive effects of the HEV, which resulted primarily from secondary bacterial infections. However, as per the current scenario, due to the extensive use of vaccines and following strict biosecurity, highly pathogenic outbreaks of HE are rare in commercial operations. But, insufficient protection in vaccinated birds may result in secondary bacterial infections, especially colibacillosis, which is considered as a disease of major economic significance in commercial turkey production units. Immunosuppression is an important problem caused by the virus and leading to secondary bacterial infection causing mortality. Hence, besides vaccination, the flocks should be administered with appropriate feed based antimicrobials, based on culture and sensitivity testing. Live vaccines are used to control the infection in several areas of the world and research in the field of vaccine design led to development of several recombinant subunit vaccines that showed good result. Currently, novel vaccines based on recombinant technology have been developed for prevention of HE. Such vaccines have to be commercialized in the near future for negating the drawbacks of conventional vaccines. Aside to these vaccines, feed grade transgenic plants expressing the...
HEV proteins could also be developed that could function as a novel and prospective mode of vaccination.

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ORCID

Kuldeep Dhama http://orcid.org/0000-0001-7469-4752
Kumaragurubaran Karthik http://orcid.org/0000-0002-9215-6306
M. Palanivelu http://orcid.org/0000-0002-1221-5231

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Figure 3. Structure, vaccines and control strategies of HEV.
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