

## Hepatitis E Infections in Poultry-An Overview

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### Abstract

With an attempt to review, the causes, epidemiology, symptoms, diagnosis, treatment, prevention and control of Hepatitis E virus in poultry are briefly discussed. Hepatitis E virus causes hepatitis-splenomegaly syndrome or big liver and spleen disease in poultry. It is one of the neglected diseases of poultry causing mild clinical symptoms of egg production loss and hepatitis. The epidemiological, pathological and prevalence study of the disease is important to certain the current status of the disease in Indian context and improve the production of Indian poultry industry caused by this disease.

**Key words:** Hepatitis E, Big liver and spleen disease, Hepatitis-splenomegaly syndrome, Poultry.

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Received: 12/09/2015

Revised: 26/09/2015

Accepted: 28/09/2015

### 1. Introduction

Hepatitis E infection in poultry causes Hepatitis-Splenomegaly (HS) Syndrome in USA, Big liver and spleen disease (BLS) in Australia which is an infectious, transmissible disease of poultry characterized by sudden drop in egg production, increased mortality and enlargement of the liver and spleen of mature chickens, especially broiler breeders and less commonly, egg layers (Vasickova *et al.*, 2007).

Avian Hepatitis E virus (HEV) was first recognized in Australia in 1980 as BLS while same is named as primary feather and drop syndrome in the United States. There are minor differences in the clinical signs and genomes of these viruses. Later related viruses have been reported to cause a hepatitis/splenomegaly syndrome in poultry, sub-clinical infection in pigs and type E Hepatitis in man (Clarke *et al.*, 1990). The HEV of pig origin can cause disease in man, but viruses of avian are believed not to be zoonotic. Only chickens, most commonly broiler parents are known to be naturally affected. The syndrome is considered economically important for poultry industry.

### 2. Virion Properties

HEV is small non-enveloped, single-strand, positive-sense RNA virus. The genome of avian HEV is only about 6.6 kb in length, which is approximately 600 bp shorter than that of mammalian HEV. The

complete genome of avian HEV contains 3 ORFs (Open reading frames) of which ORF2 gene encodes capsid protein containing the primary epitopes of viral particles and is target gene for sero-diagnostic antigen and vaccine candidate (Chandra *et al.*, 2008). Although avian HEV have shown high degree of divergence (66.49–82.99%) but the genomic organization and functional motifs are relatively conserved between avian and mammalian HEV (Haqshenas *et al.*, 2001; Huang *et al.*, 2004; Oliveira-Filho *et al.*, 2013). Antigenic epitopes in the capsid protein that are unique to avian HEV as well as common between mammalian HEV and avian HEV have been identified (Guo *et al.*, 2006).

### 3. Viral Classification

Avian Hepatitis E Virus is classified under free floating genus *Hepevirus* and family *Hepeviridae* (<http://www.ncbi.nlm.nih.gov/9threportICTV/>). The HEV genomes of several geographically distinct isolates show a high degree of sequence conservation and classified into four recognized major genotypes that infect humans and other animals. Genotype 1 includes Asian and African HEV strains, genotype 2 includes the single Mexican HEV strain and few variants identified from endemic cases in African countries, genotype 3 includes human and swine HEV strains from industrialized countries, and genotype 4 includes human and swine HEV strains from Asia,

particularly China, Japan and Taiwan (Chandra *et al.*, 2008).

Phylogenetic analyses indicated that avian HEV is distinct from mammalian HEV, and thus likely belongs to a separate genus within the family (Meng, 2010). Avian HEV is tentatively classified as genotype 5 while additional genera have been proposed for rat HEV (12) and bat HEV (Smith *et al.*, 2013). Additional sequences of avian HEV isolates from different geographic regions of the world will help more definitively classify avian HEV as a separate genus (Huang *et al.*, 2002; Smith *et al.*, 2013).

#### 4. Epidemiology

Transmission of avian HEV within and between chicken flocks appears to occur readily which is mainly via the fecal-oral route, with higher levels of virus in feces. Fecal contamination of drinking water is likely to be an efficient means of spread of this infection. Embryos inoculated intravenously become persistently antigen positive, experimental avian HEV infection has been successfully reproduced via oro-nasal route inoculation of SPF chickens (Billam *et al.*, 2005), although other routes of transmission cannot be ruled out. In addition to chickens, young turkeys experimentally inoculated with avian HEV also became infected. However, attempts to experimentally infect rhesus monkeys and mice with avian HEV were unsuccessful (Meng, 2010). There is no known carrier or vector implicated in the transmission of avian HEV, although rodents in the chicken farms might serve as a mechanical carrier.

Natural infections have only been demonstrated in chickens over 24 weeks of age though it is possible that vertical transmission and/or infection in rear occur with a subsequent period of latency. In chickens, the morbidity and mortality of avian HEV infection in the field are relatively low, although subclinical infections are very common in chicken flocks in the United States, Italy, Spain and perhaps in other countries as well including India (Huang *et al.*, 2002; Sun *et al.*, 2004; Massi *et al.*, 2005; Peralta *et al.*, 2009). Molecular epidemiologic investigations have shown that avian HEV infection in chickens is endemic to the United States and Spain (Meng, 2010). Disease has been reported in Leghorn hens, broiler breeders, and dual-purpose hens (Chandra *et al.*, 2008).

In chickens, HS syndrome was first reported in western Canada in 1991, but the disease has now been reported in eastern Canada and the United States (Meng, 2010). BLS associated with avian HEV infection has been reported from chickens in Australia, and serological evidence of avian HEV infection was also reported in the United Kingdom and Spain (Peralta

*et al.*, 2008). Leghorn hens in cages are typically affected although the disease has also been recognized in broiler breeder hens. In the United States, avian HEV infection is enzootic in chicken flocks. Like human and swine HEV, the sero-prevalence in chickens appears to be age dependant and adults are more susceptible to the infection (Huang *et al.*, 2002).

Payne *et al.* (1999) reported a HEV-related virus associated with big liver and spleen disease (BLS) in chickens from Australia. The BLS disease affects commercial broiler breeder flocks and causes decreased egg production and slight increase in mortality. Based upon the sequence of a 523 bp fragment of the BLS virus (BLSV), it was found that BLSV shared approximately 62% nucleotide sequence identity with human HEV (Payne *et al.*, 1999). A variant strain of same virus has been reported from North America having approximately 80% nucleotide sequence identity with the Australian BLSV (Haqshenas *et al.*, 2001). Haqshenas *et al.* (2001) first isolated and characterized a virus genetically and anti-genetically related to human HEV from bile samples of chickens with HS Syndrome in the United States. Phylogenetic comparison using helicase and capsid gene sequences of completely sequenced avian HEVs and four mammalian HEVs revealed three genotypes within the avian HEV. These genotypes tend to be differentiated geographically. This may be important to understand the epidemiology of avian HEV infections in chickens and can give new insight into the phylogenetic relationships between isolates (Marek *et al.*, 2010).

#### 5. Clinical Signs and Symptoms

Avian HEV infection is characterized by above normal mortality in broiler breeder hens and laying hens of 30-72 week of age, with the highest incidence occurring between 40-50 weeks of age. Prior to death, clinical sign is generally not recognized in chickens with HS syndrome. In some (but not all) cases, a drop in egg production of up to 20% was observed. Weekly mortality increases to approximately 0.3% for several weeks during the middle of the production period and may sometimes exceed 1.0%. Similar to HS syndrome, the clinical signs for BLS in Australia also vary from subclinical infection to egg drops that may reach 20% with up to 1% mortality per week over a period of 3-4 weeks (Crerar *et al.*, 1994; Billam *et al.*, 2005; Meng, 2010). Affected flocks in the, Canada Europe and United States appear to have milder or subclinical infections compared to those in Australia. Affected birds are anemic and premature moulting takes place in some of the flocks.

## 6. Postmortem Lesions and Histopathology

Dead chickens associated with avian HEV infection usually have regressive ovaries, yolk peritonitis, congested lungs, red fluid in the abdomen, and enlarged liver and spleen. Livers are enlarged with hemorrhage and may have subcapsular hematomas. Spleens from affected birds are mild to severely enlarged (over 1 gm/kg bodyweight, often with pale foci). Histologically, lymphocytic periphlebitis and phlebitis with amyloid are prominent within the liver of affected birds. Liver lesions varied from multifocal hemorrhage to extensive areas of necrosis and hemorrhage and infiltration of heterophils and mononuclear inflammatory cells around portal triads (Meng, 2010). Under experimental conditions, gross lesions characteristic of HS syndrome including subcapsular hemorrhages and slightly enlarged right intermediate lobe of the livers were reproduced in approximately one-fourth of the SPF chickens experimentally infected with avian HEV (Billam et al., 2005). Foci of lymphocytic periphlebitis and phlebitis were the characteristic histological lesions in livers. There was no significant elevation of serum levels of liver enzymes AST, albumin/globulin (A/G) ratios or bile acids. However, LDH levels behaved differently over time (Billam et al., 2005).

## 7. Diagnosis

A presumptive diagnosis is made on the basis of clinical signs, and gross and microscopic lesions which must be confirmed by laboratory tests. Definitive diagnosis is made by visualization of 30-35 nm virus particles in bile by negative-stain electron microscopy or isolation of virus in embryonated chicken eggs inoculated intravenously. Since avian HEV does not replicate in cell culture systems (Song et al., 2010) hence RT-PCR and indirect-ELISA based on ORF 2

## References

- Billam P, Huang FF, Sun ZF, Pierson FW, Duncan RB, Elvinger F, Guenette DK, Toth TE and Meng XJ. (2005). Systematic pathogenesis and replication of avian hepatitis E virus in specific-pathogen-free adult chickens. *Journal of Virology*, 79: 3429-3437.
- Chandra V, Taneja S, Kalia M and Jameel S (2008). Molecular biology and pathogenesis of hepatitis E virus. *Journal of Bioscience*, 33: 451-464.
- Clarke JK, Allan GM, Bryson DG, Williams W, Todd D, Mackie DP and McFerran JB (1990). Big liver and spleen disease of broiler breeders. *Avian Pathology*, 19: 41-50.
- Crerar SK and Cross GM (1994). Epidemiological and clinical investigations into big liver and spleen disease of broiler breeder hens. *Australian Veterinary Journal*, 71(12): 410-413.
- Guo H, Zhou EM, Sun ZF, Meng XJ and Halbur PG (2006). Identification of B-cell epitopes in the capsid protein of avian hepatitis E virus (avian HEV) that are common to human and swine HEVs or unique to avian HEV. *Journal of General Virology*, 87: 217-223.
- Guo H, Zhou EM, Sun ZF and Meng XJ (2007). Protection of chickens against avian hepatitis E virus (avian HEV) infection by immunization with recombinant avian HEV capsid protein. *Vaccine*, 25(15): 2892-2899.
- Haqshenas G, Shivaprasad HL, Woolcock PR, Read DH and Meng XJ (2001). Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. *Journal of General Virology*, 82: 2449-2462.

recombinant protein are used most commonly for the detection of avian HEV infection (Zhao et al., 2013). Real time PCR test have also being developed for viral quantification (Troxler et al., 2011). Virus-specific antibodies can be detected by agarose gel immunodiffusion test or enzyme linked immunosorbent assay.

## 8. Prevention and Control

There is no specific treatment and vaccine available for the disease so thorough cleaning and disinfection after depletion of an affected flock along with good biosecurity and all-in/all-out policy are methods of prevention and control. Certain vaccine development process is going on includes immunization of chickens with avian HEV recombinant ORF2 capsid protein with aluminum as adjuvant can induce protective immunity against avian HEV infection (Guo et al., 2007).

## 9. Conclusion

Although avian HEV has not been studied extensively in India but since our country is endemic for mammalian HEV so the occurrence of avian HEV cannot be ruled out. So the diseases with symptoms of hepatitis and egg drop with a low mortality should be diagnosed with postmortem examination and advance molecular tools. A detailed phylogenetic study of different Indian isolates is required to characterize and classify them. Development of tools for rapid diagnostic methods for this viral disease will be helpful to predict the prevalence of this disease in our country. Hence further research is required to study pathogenesis and development of molecular diagnostics and effective vaccines to prevent economic losses due to this disease in future.

- Huang FF, Haqshenas G, Shivaprasad HL, Guenette DK, Woolcock PR, Larsen CT, Pierson FW, Elvinger F, Toth TE and Meng XJ (2002). Heterogeneity and seroprevalence of a newly identified avian hepatitis E virus from chicken in the United States. *Journal of Clinical Microbiology*, 40: 4197-4202.
- Huang FF, Sun ZF, Emerson SU, Purcell RH, Shivaprasad HL and Pierson FW (2004). Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. *Journal of General Virology*, 85: 1609-1618.
- Marek A, Bilic I, Prokofieva I and Hess M. (2010). Phylogenetic analysis of avian hepatitis E virus samples from European and Australian chicken flocks supports the existence of a different genus within the Hepeviridae comprising at least three different genotypes. *Veterinary Microbiology*, 145(1-2): 54-61.
- Massi P, Tosi G, Bassi D, Gelmetti D, Lavazza A, Lombardi G and Torcoli G (2005). Big liver and spleen disease in broiler breeders in Italy. *Italian Journal of Animal Science*, 4: 303-305.
- Meng XJ (2010). Hepatitis E virus: Animal Reservoirs and Zoonotic Risk. *Veterinary Microbiology*, 140(3-4): 256.
- Oliveira-Filho EF, Konig M and Thiel H (2013). Genetic variability of HEV isolates: Inconsistencies of current classification. *Veterinary Microbiology*, 165: 148-154.
- Payne CJ, Ellis TM, Plant SL, Gregory AR and Wilcox GE (1999). Sequence data suggests big liver and spleen disease virus (BLSV) is genetically related to hepatitis E virus. *Veterinary Microbiology*, 68: 119-125.
- Peralta B, Biarnés M, Ordóñez G, Porta R, Martín M, Mateu E, Pina S and Meng XJ (2009). Evidence of widespread infection of avian hepatitis E virus (avian HEV) in chickens from Spain. *Veterinary Microbiology*, 137(1-2): 31-36.
- Smith DB, Purdy MA and Simmonds P (2013). Genetic variability and the classification of Hepatitis E Virus. *Journal of Virology*, 87(8): 4162-4169.
- Song YJ (2010). Studies of hepatitis E virus genotypes. *Indian Journal of Medical Research*, 132(5): 487-488.
- Sun ZF, Larsen CT, Dunlop A, Huang FF, Pierson FW, Toth TE and Meng XJ (2004). Genetic identification of avian hepatitis E virus (HEV) from healthy chicken flocks and characterization of the capsid gene of 14 avian HEV isolates from chickens with hepatitis-splenomegaly syndrome in different geographical regions of the United States. *Journal of General Virology*, 85: 693-700.
- Troxler S, Marek A, Prokofieva I, Bilic I and Hess M (2011). TaqMan real-time reverse transcription-PCR assay for universal detection and quantification of avian Hepatitis E virus from clinical samples in the presence of a heterologous internal control RNA. *Journal of Clinical Microbiology*, April 2011: 1339-1346.
- Vasickova P, Psikal I, Kralik P, Widen F, Hubalek Z and Pavlik I (2007). Hepatitis E virus: a review. *Veterinarni Medicina*, 52(9): 365-384.
- Zhao Q, Sun Y, Zhao J, Hu S, Zhao F, Chen F, Clavijo A, Zhou E and Xiao Y (2013). Development and application of an indirect ELISA for detection of antibodies against avian hepatitis E virus. *Journal of Virological Methods*, 187: 32-36.