

## EPIDEMIOLOGICAL STUDIES ON INCLUSION BODY HEPATITIS-HYDROPERICARDIUM SYNDROME IN BROILER CHICKEN IN HARYANA STATE

D. MITTAL\*, N. JINDAL and R.S. KHOKHAR

Department of Veterinary Public Health and Epidemiology,

College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004

Received: 27.05.2013; Accepted: 10.12.2013

### ABSTRACT

A total of 480 flocks of commercial broiler chicks were affected with inclusion body hepatitis-hydropericardium syndrome (IBH-HPS) during the period from January 1997 to December 2006 in Haryana. Overall morbidity, cumulative mortality and case fatality rate due to IBH-HPS were 6.69% (111548/1666230), 3.98% (66320/1666230) and 59.45% (66320/111548), respectively. The disease was diagnosed on the basis of history, clinical signs and gross pathological findings. The highest number of outbreaks were recorded in birds of 21-30 days of age followed by 31-40 days, 1-10 days and >50 days of age. Distribution and proportionate incidence of fowl adenovirus outbreaks revealed that the disease occurred throughout the year with more prevalence in winter and rainy seasons followed by summer season. Occurrence of HPS-IBH even after vaccination warrants continuous surveillance for this syndrome in poultry flocks.

**Key words:** Inclusion body hepatitis, hydropericardium syndrome, broiler flocks, epidemiology

Fowl adenoviruses (FAdVs) belong to the family *Adenoviridae* and genus *Aviadenovirus*. The FAdVs are a very diverse group of pathogens and have been incriminated as etiological agents for a number of clinical conditions in broiler chickens, breeder flocks and layers; inclusion body hepatitis (IBH) and hydropericardium syndrome (HPS) are the important ones. The disease has been reported from many countries including India. All 12 serotypes of group I FAdVs have been incriminated in the field outbreaks of IBH and group I FAdV serotype 4 has been implicated in HPS (Chandra *et al.*, 1998).

The disease conditions produced by FAdVs have been named depending upon the presentations and/or pathology involved viz. leechi or litchi disease, infectious hydropericardium, inclusion body hepatitis-hydropericardium syndrome (IBH-HPS), hydropericardium syndrome, hydropericardium hepatopathy syndrome and hydropericardium hepatitis syndrome (HHS) (Ganesh *et al.*, 2001). In this paper, we present the epidemiological analysis of data pertaining to IBH-HPS in broiler chickens in Haryana state.

### MATERIALS AND METHODS

**Collection and Analysis of Data:** The data of IBH-HPS in commercial broiler chicken flocks were collected

\*Corresponding author: mittalvet@yahoo.com

from January 1997 to December 2006. The information regarding total flock population, number of birds affected and died, age of the affected birds, vaccination status, type of vaccines used, presence of concurrent diseases, time of occurrence of disease was recorded for each affected flock during the visit of the farmers to the department for disease diagnosis in their flocks. The farmers mostly belonged to Hisar, Bhiwani, Fatehabad, Sirsa and Jind districts of Haryana. Clinical findings and gross pathological changes in birds of each of the affected flocks were also recorded. Each year was divided into 4 quarters viz. January.-March (A), April-June (B), July- September (C) and October- December (D) to study temporal distribution of disease. Quarters A and D comprised the winter season, B the summer season and C the rainy season in this part of India. The data so collected were statistically analyzed using Z test to draw the inferences following the procedure of Snedecor and Cochran (1980).

**Diagnosis of the Disease:** The tentative diagnosis for IBH-HPS in these flocks was based on clinical and necropsy findings. The clinical findings included of sudden onset of mortality, anorexia, ruffled feathers, dullness, depression, decreased appetite, yellow greenish diarrhea and death. Gross pathological changes were enlarged, mottled, swollen and pale liver with pin point or diffuse white necrotic foci and/or hemorrhages; and

enlarged and congested kidneys exhibiting necrotic foci and punctiform hemorrhagic areas. There was accumulation of straw colored fluid in pericardial sac in birds affected with HPS. Since both the diseases are caused by FAdVs, we present the epidemiological data collectively as IBH-HPS syndrome. Other infectious and non-infectious diseases in IBH-HPS-affected flocks were diagnosed on the basis of clinical findings, gross pathology and/or laboratory examination.

## RESULTS AND DISCUSSION

**Occurrence of IBH-HPS:** A total of 480 broiler flocks were affected with IBH-HPS during the period from January 1997 to December 2006 (Fig. 1). Year-wise analysis of data revealed that per cent morbidity, cumulative mortality and case fatality rate (CFR) varied between 2.92-7.82, 1.66-5.32 and 47.38-77.14, respectively (Fig. 1). Overall 6.69% (111548/1666230) birds in these flocks were affected with IBH-HPS with mortality and CFR of 3.98% (66320/1666230) birds and 59.45% (66320/111548), respectively (Table 1).

Overall morbidity and/or CM recorded in the present study have the support of earlier reports (Kumar *et al.*, 1997; Shukla *et al.*, 1997a; Kumar *et al.*, 2003; Vairamuthu *et al.* 2004; Nakamura *et al.*, 2011). Contrary to the prevalence recorded in this study, Akhtar *et al.* (1992) reported a higher prevalence rate of 46.6% of HPS in parts of Pakistan from July 1989 to Aug 1990. In the present study, no follow up of the affected flocks was carried out and all parameters were calculated on the basis of one time data provided by the poultry farmers. Therefore, overall morbidity, mortality and CM due to this syndrome may be higher. The follow up study is essential to get exact information regarding the morbidity/mortality.

The number of IBH-HPS outbreaks was less up to 2001 and from the year 2002 onwards, there was an upward trend in its occurrence. The decline started again in 2004-2005. The decrease in its prevalence after 2004 could be due to regular vaccination in breeder flocks against this disease.

**Temporal Distribution:** The IBH-HPS syndrome was recorded in all quarters, affecting maximum number of flocks (n=207) in quarter C followed by 123 flocks in quarter D, 86 flocks in quarter A and 64 flocks in quarter B (Table 1). However, percent morbidity was higher in quarter B (7.67%; 16802/218950) followed by

quarters C (7.48%; 52515/702430), D (6.37%; 26836/421250) and A (4.76%; 15395/323600). Percent morbidity in quarters B, C and D was significantly higher than quarter A ( $P \leq 0.05$ ). Percent CM in all quarters varied from 3.02-4.38%. Similar to morbidity, percent CM in quarters B, C and D was also significantly higher than that in quarter A. The CFR in all quarters ranged from 49.74-68.78% and was significantly higher in quarters D and A than that in quarters B and C. Similarly, CFR in quarter C was significantly higher than quarter B (Table 1). Thus, more number of broiler flocks were affected with IBH-HPS in winter and rainy seasons (209 and 207

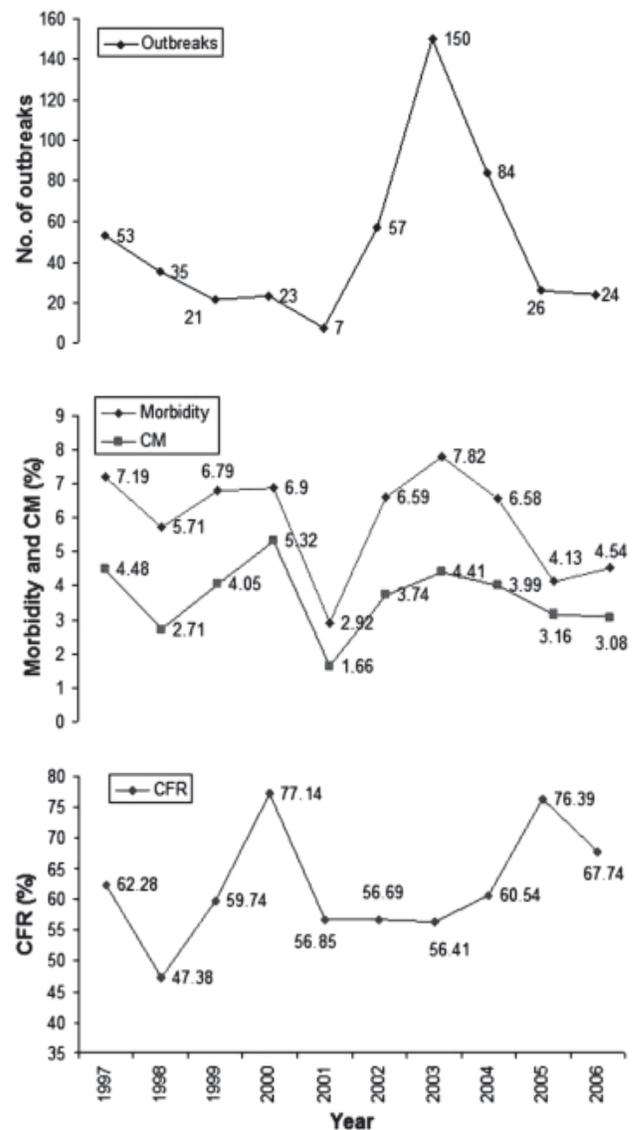


Fig 1. Epidemiological indices (year-wise) due to IBH-HPS in broiler chickens from Jan. 1997-Dec. 2006 (CM= cumulative mortality; CFR= case fatality rate).

**Table 1**  
**Epidemiological indices due to IBH/HPS in broiler chickens during the period from Jan. 1997-Dec. 2006**

Parameter		No. of flocks affected	Total population	Morbidity (%)	Cumulative mortality (%)	CFR (%)
Temporal distribution	Jan.-March (A)	86	323600	15395 (4.76 <sup>b</sup> )	9772 (3.02 <sup>b</sup> )	63.48 <sup>a</sup>
	April-June (B)	64	218950	16802 (7.67 <sup>a</sup> )	8358 (3.82 <sup>a</sup> )	49.74 <sup>c</sup>
	July-Sept. (C)	207	702430	52515 (7.48 <sup>a</sup> )	29733 (4.23 <sup>a</sup> )	56.62 <sup>b</sup>
	Oct.-Dec. (D)	123	421250	26836 (6.37 <sup>a</sup> )	18457 (4.38 <sup>a</sup> )	68.78 <sup>a</sup>
	Total	480	1666230	111548 (6.69)	66320 (3.98)	59.45
Vaccination status	Vaccinated	39	168050	11647 (6.93 <sup>a</sup> )	7307 (4.35 <sup>a</sup> )	62.74 <sup>a</sup>
	Unvaccinated	441	1498180	99901 (6.67 <sup>a</sup> )	59013 (3.94 <sup>a</sup> )	59.07 <sup>a</sup>
	Total	480	1666230	111548 (6.69)	66320 (3.98)	59.45
Age group (days)	1-10	7	28500	1500 (5.26 <sup>b</sup> )	1198 (4.20 <sup>b</sup> )	79.87 <sup>b</sup>
	11-20	58	215380	10277 (4.77 <sup>bc</sup> )	6417 (2.98 <sup>c</sup> )	62.44 <sup>c</sup>
	21-30	197	681500	52769 (7.74 <sup>a</sup> )	29470 (4.32 <sup>b</sup> )	55.85 <sup>d</sup>
	31-40	161	537050	38007 (7.08 <sup>a</sup> )	23867 (4.44 <sup>ab</sup> )	62.80 <sup>c</sup>
	41-50	54	193800	8440 (4.36 <sup>c</sup> )	4865 (2.51 <sup>c</sup> )	57.64 <sup>d</sup>
	>50	3	10000	555 (5.55 <sup>b</sup> )	503 (5.03 <sup>a</sup> )	90.63 <sup>a</sup>
	Total	480	1666230	111548 (6.69)	66320 (3.98)	59.45

Different lower case superscripts within a column for a parameter indicate significant difference ( $P \leq 0.05$ )  
CFR= Case fatality rate

flocks, respectively) followed by 64 flocks in summer. Percent morbidity was higher in summer season followed by rainy and winter seasons, whereas, CM (%) was maximum in rainy season followed by summer and winter seasons. Case fatality rate was higher during winter season followed by rainy and summer seasons.

Shukla *et al.* (1997b) also observed HPS to be prevalent throughout the year in broiler chicks, however, Yunus *et al.* (2009) reported its more prevalence during June-Sept. months in poultry in Pakistan. Vairamuthu *et al.* (2004) reported more prevalence during June-Sept. months and Oct-Dec months that correlated with southwest monsoons and northeast monsoons, respectively. More prevalence of disease in rainy season in the present study could be attributed to the variations in environmental temperature and humidity. Increased contents of mycotoxins in feed invariably suppress the immune system of the birds thereby making them susceptible to FAdVs infections. High wind velocity might also favor the horizontal spread of disease among affected flocks as also opined by Vairamuthu *et al.* (2004).

**Effect of Age:** Maximum numbers of flocks (41.04%) affected with IBH-HPS were recorded in broiler chicks of 21-30 days of age followed by 31-40 days (33.54%),

11-20 days (12.08%) and 41-50 days (11.25%) of age. Only 7 and 3 flocks affected with IBH-HPS had birds of 1-10 days and >50 days of age, respectively (Table 1). Maximum age of the broiler birds affected with IBH-HPS was 64 days, whereas the youngest birds affected with this syndrome were 4 days old. Percent morbidity in birds of 21-40 days of age was significantly higher than that in other age groups. However, CM was significantly higher in birds of age >50 days than the other age groups (except 31-40 days) (Table 1). The CFR was significantly higher in birds of >50 days of age than the other age groups. The CFR in birds of 11-20 and 31-40 days of age was also significantly higher than that in birds of 21-30 days and 41-50 days of age.

The age of 2-6 weeks has been considered as the most susceptible age of broiler chickens for IBH and/or HPS (Kumar *et al.*, 1997). Though the incubation period of disease is about 10 days, however, occurrence of disease in birds during 1<sup>st</sup> and 2<sup>nd</sup> week of age could be due to the presence of virulent strains of FAdVs in the environment. Pilkington *et al.* (1997) also reported IBH in four-day old breeder chicks. It could also be possible that the day-old chicks have been kept in the farm (without proper cleaning) where the previous flock suffered from HPS and/or IBH. Improper cleaning or keeping multiple age group flocks in a farm can also

help in maintenance and persistence of FAdVs. High mortality in broiler chicks of age 3-6 weeks has been reported earlier (Kumar *et al.*, 1997).

**Vaccination Status:** Of the 480 flocks affected with IBH-HPS, only 39 flocks were vaccinated against this disease during second week of age (Table 1). Percent morbidity, CM and CFR were slightly higher in vaccinated flocks than in unvaccinated flocks (6.67 and 3.94%), and the differences were not statistically significant. Of the 39 flocks, the exact information regarding the type of vaccine used could be obtained only from 26 flocks. On the basis of 26 flocks, commonly used vaccines were liver tissue homogenized vaccines of two different private manufacturers. Vaccination against IBH-HPS was carried out at 7-14 days of age via the subcutaneous route in recommended doses.

Occurrence of disease even after vaccination could be due to improper virus concentration in some lots of the vaccine depending upon severity of lesions in liver that served as source of vaccine virus. The vaccine strains that are currently in use contain only FAdV-4 and FAdV-8 serotypes. Other serotypes of FAdV alone or along with FAdV-4 or FAdV-8 have also been detected from field cases of IBH-HPS (Rahul *et al.*, 2005; Ojkic *et al.*, 2008; Mittal *et al.*, 2014). The vaccine strains might have not accorded full protection when challenges involved more than one FAdV serotypes. It could also be possible that vaccination might have been carried out in broiler chicks that were in incubatory phase of the disease, as incubation period ranges from 5-18 days (Akhtar, 1994). Another possibility could be the involvement of more virulent strains of FAdVs that might cause disease even after vaccination. Role of immunosuppressive agents that interfere with HPS-IBH vaccination cannot be ruled out (Shivachandra *et al.*, 2003). The effective protection of the commercial chicks against IBH-HPS could be achieved by regular vaccination of breeder birds with FAdV-4 (Toro *et al.*, 2002); low levels of maternal antibody titres could also be one of the reasons.

**Concurrent Infections:** Concurrent diseases were recorded in 312 flocks and these included: mycotoxicosis (124 flocks), *E. coli* infections (33 flocks), infectious bursal disease (IBD; 26 flocks), coccidiosis (24 flocks), chronic respiratory disease (CRD; 22 flocks), ascites (16 flocks), pneumonia (11 flocks), heat stroke (9 flocks), and Ranikhet disease (8 flocks). Mycotoxicosis,

*E. coli* infections, IBD, CRD and coccidiosis were the predominant concurrent infections. Singh *et al.* (1996) identified presence of aflatoxins in feed as one of the most important risk factors for IBH. Similarly, Choi *et al.* (2012) reported IBD and chicken infectious anaemia to be the complications in about 57% of FAdV cases.

Thus, the present study reveals occurrence of IBH-HPS in commercial broiler chicks of 21-40 days of age in Haryana state with considerable morbidity and mortality. The application of PCR followed by restriction enzyme analysis and sequencing etc would be helpful in generating molecular epidemiological information which would help in better understanding of the disease.

## REFERENCES

- Akhtar, S., Zahid, S. and Khan, M.I. (1992). Risk factors associated with hydropericardium syndrome in broiler flocks. *Vet. Rec.* **131**: 481-484.
- Akhtar, S. (1994). Hydropericardium syndrome in broiler chickens in Pakistan. *World Poult. Sci. J.* **50**: 177-182.
- Balamurugan, V. and Kataria, J.M. (2004). The hydropericardium syndrome in poultry: A current scenario. *Vet. Res. Commun.* **28**: 127-148.
- Chandra, R., Dixit, V.P. and Kumar, M. (1998). Inclusion body hepatitis in domesticated and wild birds: review. *Indian J. Virol.* **14**: 1-12.
- Choi, K.S., Kye, S.J., Kim, J.Y., Jeon, W.J., Lee, E.K., Park, K.Y. and Sung, H.W. (2012). Epidemiological investigation of outbreaks of fowl adenovirus infection in commercial chickens in Korea. *Poult. Sci.* **91**: 2502-2506.
- Ganesh, K., Suryanarayana, V.V.S., Raghavan, R. and Gowda, S. (2001). Nucleotide sequence of L1 and part of P1 of hexon gene of fowl adenovirus associated with hydropericardium hepatitis syndrome differs with the corresponding region of other fowl adenoviruses. *Vet. Microbiol.* **78**: 1-11.
- Kumar, R., Chandra, R., Shukla, S.K., Agarwal, D.K. and Kumar, M. (1997). Hydropericardium syndrome (HPS) in India: a preliminary study on the causative agent and control of the disease by adenovirus serotype-4 (FAV-4) associated with hydropericardium inactivated autogenous vaccine. *Trop. Anim. Hlth. Prod.* **29**: 158-164.
- Mittal, D., Jindal, N., Tiwari, A.K. and Khokhar, R.S. (2014). Molecular characterization of fowl adenoviruses associated with inclusion body hepatitis and hydropericardium syndrome in broiler chickens. *Virus Dis.* **25** (1): 114-119.
- Ojkic, D., Martin, E., Swinton, J., Vaillancourt, J-P., Boulianne, M. and Gomis, S. (2008). Genotyping of Canadian isolates of fowl adenoviruses. *Avian Pathol.* **37**(1): 95-100.
- Pilkington, P., Brown, T., Villegas, P., McMurray, B., Page, R.K., Rowland, G.N. and Thayer, S.G. (1997). Adenovirus induced inclusion body hepatitis in four day old broiler breeders. *Avian Dis.* **41**: 472-474.

- Rahul, S., Kataria J.M., Senthilkumar, N., Dhama, K., Sylvester, S.A. and Uma, R. (2005). Association of fowl adenovirus serotype 12 with hydropericardium syndrome of poultry in India. *Acta Virol.* **49**: 139-43.
- Shivachandra, S.B., Sah, R.L., Singh, S.D., Kataria, J.M. and Manimaran, K. (2003). Immunosuppression in broiler chicks fed aflatoxin and inoculated with fowl syndrome. *Vet. Res. Commun.* **27**: 39-51.
- Shukla, S.K., Chandra, R. and Kumar, M. (1997a). Outbreaks of hydropericardium syndrome in layer flocks of poultry in India. *Indian J. Vet. Med.* **17**: 61-64.
- Shukla, S.K., Chandra, R., Kumar, M. and Dixit, V.P. (1997b). Hydropericardium syndrome in India: A report. *Indian J. Anim. Sci.* **67**: 28-29.
- Singh, A., Oberoi, M.S., Jand, S.K. and Singh, B. (1996). Epidemiology of inclusion body hepatitis in poultry in northern India from 1990 to 1994. *Rev. Sci. Tech. Off. Int. Epiz.* **15**: 1053-1060.
- Snedecor, G.W. and Cochran, W.G. (1980). *Statistical Methods*. 8<sup>th</sup> edn, Iowa State College Press, Iowa, USA.
- Toro, H., Gonzalez, C., Cerda, L., Morales, M.A., Dooner, P. and Salamero, M. (2002). Prevention of inclusion body hepatitis/hydropericardium syndrome in progeny chickens by vaccination of breeders with fowl adenovirus and chicken anemia virus. *Avian Dis.* **46**: 547-554.
- Vairamuthu, S., Manohar, B.M. and George, V.T. (2004). Seasonal occurrence of hydropericardium syndrome in broiler chickens in Tamilnadu. *Indian. Vet. J.* **81**: 340-341.
- Yunus, A.W., Nasir, M.K., Aziz, T. and Bohm, J. (2009). Prevalence of poultry diseases in district Chakwal and their interaction with mycotoxicosis: 2. Effects of season and feed. *J. Anim. Plant Sci.* **19**: 1-5.