

Physicochemical characterization of fowl adenovirus serotype 4 isolate from Himachal Pradesh*

POOJA KANWAR¹, MANDEEP SHARMA², PRASENJIT DHAR³, VIPIN KATOCH⁴ and R K ASRANI⁵

Received: 11 November 2009; Accepted: 16 April 2010

CSK Himachal Pradesh Krishi Vishwavidyalaya, Palampur, Himachal Pradesh 176 062 India

Key words: Hydropericardium syndrome, FAV 4, Physico-chemical characterization

Hydropericardium syndrome (HPS) is a newly introduced lethal disease of 3- to 6-week-old broiler chicks which causes significant economic losses to the poultry farmers. HPS is caused by a fowl adenovirus (FAV serotype 4), a non-enveloped, icosahedral virus belonging to adenovirus genus of the DNA containing virus family adenoviridae and is characterized by hydropericardium and hepatitis with characteristic intranuclear inclusion bodies in hepatocytes (Ahmad *et al.* 1989, Anjum *et al.* 1989). The disease has also been reported from Himachal Pradesh (Asrani *et al.* 1997). The present study reports physicochemical characterization of FAV4 isolate recovered from natural outbreak of disease in Himachal Pradesh.

FAV 4 isolate was obtained from Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Palampur, who recovered the same from an outbreak of hydropericardium syndrome at a private poultry farm in Kangra district of Himachal Pradesh. The broilers aged between 3 and 6 weeks were affected. Fast-growing broilers were the most affected, mortality peaking on third or fourth day, followed by an almost constant death rate for 5–7 days with an average mortality of 15–60%. Post-mortem examination of dead birds revealed hydropericardium with accumulation of jelly like, straw colored fluid in the pericardial sac.

The liver samples from affected birds were collected in PBS for further virus isolation.

The liver tissue was washed with Hank's Balanced Salt Solution (pH 7.4). Then the tissue was triturated in sterile pestle and mortar using sterile sand to make 30% (W/V) suspension in HBSS. The antibiotics were then added at the

rate of penicillin (10 000 IU/ml), streptomycin (10mg/ml) and gentamicin (250 microgram/ml). The suspension was centrifuged at 10 000 rpm for 30 minutes. The supernatant was collected and filtered through membrane filter of pore size 0.22 micronmeter. The inoculum was then stored at –20°C until further use. The virus inoculum was then for physicochemical characterization studies.

Sensitivity of the virus isolate at different temperatures (56°C for 15 min, 56°C for 30 min, 56°C for 1 h, 60°C for 15 min, 60°C for 30 min, 60°C for 1 h, 80°C for 10 min, 100°C for 5 min) was determined by method used by Mc Ferran *et al.* (1972). Sensitivity of the isolate to chloroform was determined as per Feldman and Wang (1961) and to ether was determined as per Andrews and Hortsman (1949).

Sensitivity of the virus isolate at different pH was determined as described by Mc Ferran *et al.* (1972). After such treatments using different physical and chemical agents, the infectivity of virus sample was assayed in day old chicks as described by Nakamura *et al.* (1999) and 8–9 days old embryonated chicken eggs via yolk sac route.

Day-old broiler chicks (100) were randomly divided in 20 groups with 5 chicks in each group. The birds were then inoculated with 0.2 ml of 30% liver homogenate treated with various ranges of physicochemical parameters by intramuscular route.

Positive controls infected with untreated FAV4 showed signs like dullness, depression and anorexia. Birds huddled in the corners and had ruffled feathers. Greenish diarrhoea was also present in some birds. In terminal stage of the disease, birds assumed a characteristic posture with their chest and beak resting on the ground and were depressed with their eyelids completely closed in the later stages. The predominant and most consistent gross lesion at necropsy was hydropericardium, which was present in 99% of the affected birds. Hydropericardium was characterized by the accumulation of colourless, watery or jelly-like fluid in the pericardial sac with pH ranging from 7.5 to 8.0. The heart

*M.V.Sc Thesis of the first author.

Present address: ¹Veterinary Officer, I/C, Government Rabbit Breeding Farm, Kandbari, Kangra, Himachal Pradesh.

²Professor and Head, ³Department of Veterinary Microbiology, (e-mail: mandeepsharma289@hotmail.com); ⁵Department of Veterinary Pathology, Dr G.C. Negi College of Veterinary and Animal Sciences.

appeared misshapen with its apex floating in the pericardial sac. Other changes observed were a discoloured, pale yellow, swollen, friable and mottled liver. In some cases, petechial or ecchymotic hemorrhages were present in liver. Lungs and kidneys were congested. Negative controls did not show any mortality.

In present study, physical characterization of FAV 4 was done at different temperatures and different ranges of pH. The untreated virus showed mortality in day old chicks as well as in embryonated eggs, whereas virus exposed to 56°C for 1 h, 60°C for 1 h, 80°C for 10 min and 100°C for 5 min did not result in any mortality thus indicating that FAV 4 is sensitive to these temperatures. Afzal *et al.* (1991) demonstrated that agent withstand heating at 60°C for 30 min and 50°C for 1 h. He also demonstrated that heating at 60°C for 1 h, 80°C for 10 min or 100°C for 5 min inactivated the virus. Kumar *et al.* (1997) also reported that the etiological agent was inactivated by heat treatment at 56°C for 1 h and 80°C for 10 min. Treatment of virus isolate at different ranges of pH (from pH 3 to pH 10) did not inactivate the virus. Afzal *et al.* (1991) recorded that the sensitivity of the agent to pH 3 and pH 10 was variable.

For chemical characterization of FAV 4, virus isolate was treated with ether and chloroform. It was found that non-enveloped FAV 4 was not sensitive to lipolytic agents such as chloroform and ether and virus was not inactivated by them. 5% chloroform and 10% ether have been reported to be partially detrimental to the causal agent of HPS (Afzal *et al.* 1991).

Control on viral diseases is of utmost importance rather than going for the treatment after an outbreak has been occurred. The information on the physicochemical characterization of a virus can help in prevention of mechanical and horizontal spread of virus. Therefore in present study attempts were made to know the physicochemical characters of FAV 4 which may help for its proper management through biosecurity measures.

SUMMARY

Physicochemical characterization of FAV-4 isolate

recovered from natural outbreak of Hydropericardium syndrome in poultry in Himachal Pradesh was done. FAV-4 virus was inactivated at 56°C for 1 h, 60°C for 1 h, 80°C for 10 min and 100°C for 5 min. The virus was not found to be sensitive to the different pH ranges (pH 3 to pH 10) and lipolytic agents, viz. ether and chloroform.

REFERENCES

- Afzal M, Muneer R and Stein G. 1991. Studies on the etiology of hydropericardium syndrome (Angara disease) in broilers. *Veterinary Record* **128**: 591–93.
- Ahmad I, Afzal M, Malik M I, Hussain Z and Hanif W. 1989. Studies on the disease pattern and etiology of hydropericardium syndrome (Angara disease) in broiler chickens in Pakistan. *Pakistan Journal of Agriculture Research* **10**: 195–99.
- Andrews C H and Hortsman D M. 1949. The susceptibility of viruses to ethyl ether. *Journal of General Microbiology* **3**: 290–96.
- Anjum A D, Sabri M A and Iqbal Z. 1989. Hydropericarditis syndrome in broiler chicken in Pakistan. *Veterinary Record* **124**: 247–48.
- Asrani R K, Gupta V K, Sharma S K, Singh S P and Katoch R C. 1997. Hydropericardium hepatopathy syndrome in Asian poultry. *Veterinary Record* **141**: 271–73.
- Feldman H A and Wang S S. 1961. *Proceedings of Society of Experimental Biology Medicine* **106**. (fide Pradhan S K, Kataria J M, Verma K C and Jadhao S J. 1996. Physicochemical and biological characterization of an Indian isolate of Quail pox virus. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* Vol.17 (2) (July-Dec).)
- 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 disease by an autogenous inactivated vaccine. *Tropical Animal Health and Production* **29**: 158–64.
- Mc Ferran J B, Clarke J K and Connor T J. 1972. Serological classification of avian adenovirus. *Arch. ges. Virusforsch* **39**: 132–39.
- Nakamura K, Mase M, Yamaguchi S and Yuasa N. 1999. Induction of hydropericardium in one day old specific pathogen free chicks by adenoviruses from inclusion body hepatitis. *Avian Diseases* **44**: 192–96.