

PATHOGENICITY OF AVIAN INFLUENZA VIRUS STRAIN H₇N₃ IN CHICKEN

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ABSTRACT

Pathogenicity of avian influenza virus A/Chick/Pakistan/1/95(H₇N₃) was studied on 30 to 63 days old cockerels. The virus strain was found to be highly pathogenic killing inoculated birds within 2 to 7 days and produced distinct pattern of organ involvement. Extensive proliferation of lining epithelium of larynx and trachea forming tall villi and lymphoid depletion in germinal centers in spleen were the important histological changes in addition to clinical findings and lesions usually attributed to avian influenza infection in chicken.

Keywords: Avian influenza virus, strain H₇N₃, Pathogenicity.

INTRODUCTION

Avian influenza (AI) is caused by type A influenza virus belonging to family Orthomyxoviridae. These viruses are classified currently into 15 distinct haemagglutinin (H) and 9 neuraminidase (N) types. The influenza infection in poultry ranges from sub-clinical mild upper respiratory tract disease to acute generalized fatal infection, however, outbreaks of highly pathogenic AI have been infrequent (Easterday *et al.*, 1997).

In January, 1995 a virulent form of AI was recorded in Pakistan (Naeem and Hussain, 1995). It affected broiler breeders of all ages (7-65 weeks) and strains. A total of 799 thousand broiler breeder birds were affected and mortality was as high as 60%. The virus was identified as A/Chick/Pakistan/1/95(H₇N₃). The outbreak resulted into an estimated economic loss of 814.4 million rupees. The disease was controlled by culling the infected flocks and vaccinating the birds in the surrounding area using homologous AI virus strain.

The pathogenicity of AI viruses is extremely variable and cannot be predicted based on the host of origin and antigenic subtype of the virus. Subtypes H₅ and H₇ have been associated with severe disease outbreaks in chicken and other birds but many H₅ and H₇ isolates are either non-pathogenic or only moderately pathogenic (Beard and Easterday, 1973, Easterday *et al.*, 1997). Signs and lesions of avian influenza caused by various subtypes are also variable. The disease may be predominantly respiratory, enteric or reproductive; and gross and histopathological changes may be limited to respiratory system, gastrointestinal tract and reproductive organs and may or may not involve immune system (Alexandar *et al.*, 1986). Thus assessing the *in vivo* pathogenicity of avian influenza isolates is very important. The present study reports the experimental pathogenicity of the avian influenza virus A/Chick/Pakistan/1/95(H₇N₃) in chicken.

MATERIALS AND METHODS

Experimental Birds

Day-old broiler parent cockerels (Avians breed) were purchased from M/S Quality Poultry Breeders, Rawalpindi. The birds were brooded using electric brooders and were given feed and water *ad libitum* during course of the experiment. The birds were vaccinated against Newcastle disease (Nobilis Lasota, Intervet Int., Holland) on day-7 and 21 and Gumboro disease (Nobilis E-228, Intervet Int., Holland) on day 12 via drinking water.

Virus Strain

AI virus serotype H₇N₃ isolated from the local outbreak was used for the experimental challenge. The virus was propagated in 9-days old embryos via chorioallantoic route. The harvested material was stored in aliquots at -70°C. The ELD₅₀ was determined following Reed and Muench (1938) and was found to be 10^{-6.83} per 0.1 ml of the chorioallantoic fluid.

Experimental Approach

After the age of 30 days, five birds were challenged weekly (Table 1). On each challenge day, the birds were bled and taken to a separate room where these were inoculated with AI virus. Haemagglutination inhibition titre were determined in microtitre plates using 4HA units (OIE, 1996). The birds were examined daily for clinical signs and mortality. All dead birds necropsied and gross lesions were noted. Tissues from lungs, trachea, spleen, liver, cerebrum, small intestine, heart and kidneys of selected birds were processed for histopathology. Paraffin sections were prepared and stained with hematoxylin and eosin.

RESULTS

None the birds had significant hemagglutination inhibition (HI) titres against AI before experimental challenge. Unchallenged control birds remained healthy throughout the experiment.

AI virus proved to be highly pathogenic for broiler-breeder cockerels when given at 30 days of age or later. It killed all the birds in 2 to 7 days when inoculated with 10^4 ELD₅₀ or higher dose per chick. Maximum deaths were seen on day 3, 4 and 5 post-challenge (Table 1).

Clinical picture and postmortem lesions were uniform in most of the birds. Clinical signs included swollen and cyanosed comb, wattles and head. Shanks were also cyanosed. On pressing the head, a catarhal nasal discharge was seen in many birds. Postmortem lesions included congested trachea and oedematous lungs. Proventriculus had haemorrhages on the villi in many cases. Liver was discoloured and friable, kidneys were haemorrhagic and swollen and larynx had excessive mucous in most of the birds.

Significant histopathological changes were seen in lungs, trachea and spleen. Trachea and larynx showed acute non-suppurative laryngotracheitis. The lining epithelium underwent extensive proliferation forming tall villi and glandular structures. There was considerable infiltration by lymphocytes and macrophages in the lamina propria and submucosa. Several areas in the mucosa have been destroyed by necrosis and filled with erythrocytes, inflammatory cells and fibrinous material. Lung tissue showed congestion and oedema. The alveolar walls were slightly thickened due to congestion and mild leucocytic infiltration. Blood vessels were congested and there was mild oedema. In spleen, there was lymphoid depletion. The germinal centres were acellular and contained a few fragmented cells and proteinous material.

Severe congestion and fatty change were seen in the liver. A few hepatocytes showed karyopyknosis. Mild acute non-suppurative encephalitis was seen in the cerebrum. There was mild microglial cell proliferation and mild mononuclear cell infiltration in the wall of ventricle and at a few other places in the cerebral tissue.

The small intestine and myocardium did not reveal any significant pathological change. The blood vessels in kidneys were congested. Renal epithelial cells were usually swollen, indistinct and separated from basement membrane (oedema).

DISCUSSION

There has been differences in defining the highly pathogenic AI viruses (Alexander, 1986). The strict criteria is 75 to 100 % mortality in 4 to 6 weeks old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of 1:10 dilution of bacteria free infectious allantoic fluid. The other strains of H₅ and H₇ type AI virus which have amino acid sequence at the cleavage site compatible with highly pathogenic AI virus; and AI isolates which can cause 12.5 to 62.5% mortality in chickens and grows in cell culture in the absence of trypsin have also been classified as highly pathogenic AI virus (Report of the Committee on Transmissible Diseases of Poultry and Other Avian Species, 1994). The strain used in the study i.e. A/Chick/Pakistan/1/95 (H₇N₃) qualifies the strict criteria and thus can be classified as highly pathogenic strain.

Clinical symptoms, gross and microscopic lesions seen in experimental infection with AI strain A/Chick/Pakistan/1/95 (H₇N₃) have reported in different studies using other AI strains (Kobayashi *et al.*, 1996). Cyanotic head, comb, wattles and shanks had been seen in infection with other pathogenic AI strains (Acland *et al.*, 1984).

Table 1: Pathogenicity of avian influenza H₇N₃ in chicken

Age on Challenge (Days)	Dose and route *	Deaths post – challenge (Days)							Dead/Total birds
		1	2	3	4	5	6	7	
30	$10^{6.23}$ ELD ₅₀ , I/M	0	0	0	4	0	0	1	5/5
37	$10^{6.23}$ ELD ₅₀ , I/M	0	0	1	3	0	0	0	4/5
44	$10^{6.23}$ ELD ₅₀ , I/M	0	0	2	3	0	0	0	5/5
51	$10^{6.23}$ ELD ₅₀ , I/M	0	2	1	2	0	0	0	5/5
58	10^6 ELD ₅₀ , I/M	0	2	1	1	0	1	0	5/5
58	10^5 ELD ₅₀ , I/M	0	0	2	2	1	0	0	5/5
58	10^4 ELD ₅₀ , I/M	0	0	1	1	3	0	0	5/5
63	5×10^3 ELD ₅₀ , I/M	0	1	0	2	0	0	0	3/5
63	10^4 ELD ₅₀ , I/M	1	0	1	2	3	1	2	10/10
	Uninoculated control	0	0	0	0	0	0	0	0/10

* ELD = egg lethal dose; I/M = intramuscular; I/N = intranasal

Similarly gross lesions in liver, trachea, lungs and kidneys have also been reported (Easterday *et al.*, 1997). Histopathological changes in AI infection vary with virus strain. Respiratory changes are usually seen in mild to moderately pathogenic AI virus infection (Cooley *et al.*, 1989) but were consistently seen in this experimental infection of highly pathogenic type. Lymphoid changes seen in this study have previously been reported in some studies on highly pathogenic AI viruses (Mo *et al.*, 1997). However, Campen *et al.* (1989) reported that effect on lymphocytes and macrophages was limited to a few AI virus isolates. Similarly mild non-suppurative encephalities seen in this infection has been previously reported by some workers (Hooper *et al.*, 1995; Kobayashi *et al.*, 1996).

Many of the lesions seen in experimental infection of AI virus A/chick/Pakistan/1/95 (H₇N₃) have been reported with AI infections associated with different serotypes. However, pattern of organs involved in this infection is distinctive

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REFERENCES

- Acland, H.M., L.A.S. Bachin and R.J. Eckroads, 1984. Lesions in broilers and layer chickens in an outbreak of highly pathogenic avian influenza virus infection. *Vet. Pathol.*, 21: 564-569.
- Alexander, D.J., 1986. Criteria for the definitions of pathogenicity of avian influenza viruses, In: B.C. Easterday (Ed), *Proceedings of Second International Symposium on Avian Influenza*, U.S. Animal Health Association, Richmond. pp: 228-242.
- Alexander, D.J., G. Parsons and R.J. Manvell, 1986. Experimental assessment of the pathogenicity of eight avian influenza A viruses of H₅ subtype for chickens, turkeys, ducks and quails. *Avian Pathol.*, 15: 647-662.
- Beard, C.W. and B.C. Easterday, 1973. A turkey/Oregon/71 avirulent influenza isolate with the hemagglutinin of plague virus. *Avian Dis.*, 17: 173-181.
- Campen, H.V., B.C. Easterday, V.S. Hinshaw and H. Van-Campen, 1989. Virulent avian influenza A viruses: Their effect on avian lymphocytes and macrophages in vivo and in vitro. *J. Gen. Virol.*, 70: 2887-2895.
- Cooley, A.J., H.V. Campen, M.S. Philpott, B.C. Easterday, V.S. Hinshaw and H. Van-Campen, 1989. Pathological lesions in the lungs of ducks infected with influenza A virus. *Vet. Pathol.*, 26: 1-5.
- Easterday, B.C. V.S. Hinshaw and D.A. Halvorson, 1997. *Influenza In: Calnek B.W., H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (eds). Diseases of Poultry, 10th Ed. Iowa State University Press, Ames. pp: 583-605.*
- Hooper, P.T., G.M. Russell, P.W. Selleck and W.I. Stanislawek, 1995. Observations on the relationship in chickens between the virulence of some avian influenza viruses and their pathogenicity for various organs. *Avian Dis.*, 39: 458-464.
- Kobayashi, T., T. Horimoto, Y. Kawaoka, D.J. Alexander and C. Itakura, 1996. Pathological studies of chickens experimentally infected with highly pathogenic avian influenza viruses. *Avian Pathol.*, 25: 285-304.
- Mo, I.B., M. Brugh, D.J. Fletcher, G.N. Rowland and D.E. Swayne, 1997. Comparative pathology of chicken experimentally inoculated with avian influenza viruses of low and high pathogenicity. *Avian Dis.*, 41: 125-136.
- OIE (Office International des Epizooties), 1996. Highly pathogenic avian influenza (fowl plague). In: *Manual of Standards for Diagnostic Tests and Vaccines*. OIE, Paris. pp: 155-160.
- Naeem, K. and M. Hussain, 1995. An outbreak of avian influenza in poultry in Pakistan. *Vet. Rec.*, 137: 439.
- Reed, L.J. and H. Muench, 1938. A simple method of estimating fifty percent end points. *Amer. J. Hyg.*, 27: 493-497.
- Report of the Committee on Transmissible Diseases of Poultry and other Avian Species, 1994. *Proceeding of 98th Annual Meeting of the US Animal Health Association*. Spectrum Press, Richmond. pp: 522.