

Influence of dose of inocula on outcome of clinical disease in highly pathogenic avian influenza (H5N1) infections - An experimental study

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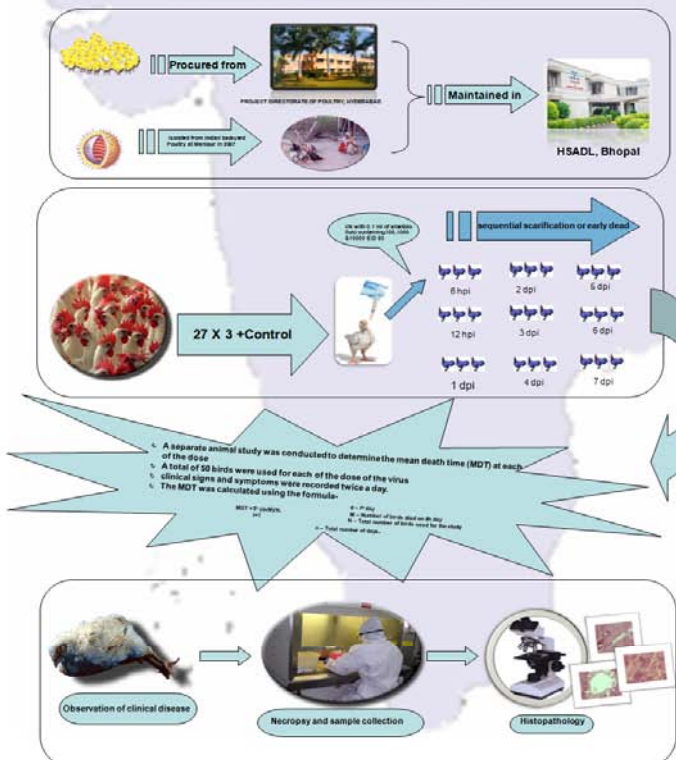
Abstract

Twelve week old *Vanaraja* (an Indian native dual purpose breed) chickens were inoculated intranasally with different doses (100, 1000 and 10000 EID₅₀) of H5N1 virus and the clinical disease and pathological changes were compared. Although the overall severity of clinical signs was more in 100 EID₅₀ group, the progression of clinical disease was slower with delayed onset of mortality when compared with other two groups. The MDT of 100 EID₅₀ group (4.57 days) differed significantly from that of 10000 EID₅₀ group (3.60 days) and 1000 EID₅₀ group (3.33 days). Similarly, overall severity of gross lesions was more pronounced in 100 EID₅₀ group. The histopathological lesions were more of haemorrhagic and necrotic nature in 100 EID₅₀ group, inflammatory/ proliferative nature in 1000 EID₅₀ group and tendency for intravascular coagulopathy was observed in 10000 EID₅₀ group. These differences may be assigned to the influence of dose in the outcome of disease.

Introduction

Highly pathogenic avian influenza (HPAI) or fowl plague is an acute, generalized, highly infectious and dynamically evolving disease of birds resulting in huge morbidity and mortality. HPAI is caused by H5 or H7 subtypes of influenza A viruses of the family *Orthomyxoviridae*. In natural conditions, the birds are exposed to different doses of viruses, and documentary evidence regarding the correlation between the influence of dose and outcome of clinical disease is scanty. An outbreak of H5N1 HPAI was detected in a single backyard poultry farm with chickens of *Vanaraja* breed in East Imphal district of Manipur State of India in 2007 (4). In this paper we report the results of an experimental study to elucidate the influence of dose of inocula on the outcome of clinical disease in HPAI infections using one of the H5N1 viruses isolated from this outbreak.

Materials & Methods



Results

- The clinical signs similar to that of HPAI infection in chickens have been reported earlier (3).
- The onset of symptoms was slow and overall severity of clinical signs were more severe in 100 EID₅₀ group.
- In 1000 EID₅₀ group, the overall severity of clinical signs were less pronounced and the time of onset of mortality was rapid
- Time of onset of clinical symptoms was more rapid and mortality was intermediate in 10000 EID₅₀ group compared to other groups.
- Fifty percent of the experimental birds did not show any clinical signs and found dead on 3 DPI in both 1000 and 10000 EID₅₀ groups.

Table 1: Day-wise score card and percentage of birds showing clinical signs in different dosage groups post challenge

Clinical Signs	Dosage groups	Days of post infection						
		1	2	3	4	5	6	7
Depression	100 EID ₅₀	-	-	++ to +++ (55%)	+++ (44%) ++ (55%)	--- (100%)	---	ND
	1000 EID ₅₀	-	+ to ++ (27%)	++ to +++ (100%)	ND	ND	ND	ND
	10000 EID ₅₀	-	++ (57%)	+++ (100%)	ND	ND	ND	ND
Torticoxity & head twitching	100 EID ₅₀	-	-	-	++ (30%)	+++ (55%)	+++ (100%)	ND
	1000 EID ₅₀	-	-	-	ND	ND	ND	ND
	10000 EID ₅₀	-	-	+++ (44%)	ND	ND	ND	ND
Swollen head, eyes & hock	100 EID ₅₀	-	-	++ to +++ (55%)	++ to +++ (55%)	+++ (100%)	+++ (100%)	ND
	1000 EID ₅₀	-	± (15%)	++ (100%)	ND	ND	ND	ND
	10000 EID ₅₀	-	± (24%)	++ (100%)	ND	ND	ND	ND
Cyanotic comb & wattle	100 EID ₅₀	-	-	++ to +++ (55%)	++ to +++ (55%)	+++ (100%)	+++ (100%)	ND
	1000 EID ₅₀	-	± (16%)	++ (100%)	ND	ND	ND	ND
	10000 EID ₅₀	-	± (25%)	++ (100%)	ND	ND	ND	ND
Hasal bleeding	100 EID ₅₀	-	-	-	-	-	-	ND
	1000 EID ₅₀	-	-	-	ND	ND	ND	ND
	10000 EID ₅₀	-	++ (23%)	+++ (55%)	ND	ND	ND	ND
Greenish diarrhoea/eggs vert	100 EID ₅₀	-	-	++ (44%)	+++ (100%)	+++ (100%)	+++ (100%)	ND
	1000 EID ₅₀	-	++	-	ND	ND	ND	ND
	10000 EID ₅₀	-	-	+++ (55%)	ND	ND	ND	ND

The MDT differed significantly between 100 EID₅₀ (4.57 days) and the other two doses of inoculum tested. Even though the MDT of 1000 EID₅₀ (3.33 days) was marginally higher than 10000 EID₅₀ (3.60 days), the difference between the two was statistically insignificant. Similar findings were also reported in SPF chickens inoculated with H5N1 virus (2).

The overall severity of gross lesions was more in 100 EID₅₀ group. In 1000 and 10000 EID₅₀ groups, the gross lesions were slightly less severe

Table 2: Day-wise score card for gross lesions in different dosage groups post challenge with H5N1

Gross lesions	Dosage groups	Hours/Days of PI								
		6	12	1	2	3	4	5	6	7
Brain congestion	100 EID ₅₀	-	-	±	±	±	+	++	+++	+++
	1000 EID ₅₀	-	±	±	±	++	+++	ND	ND	ND
	10000 EID ₅₀	-	±	±	++	+++	+++	ND	ND	ND
Tracheal congestion & mucous exudation	100 EID ₅₀	-	-	±	±	±	++	+++	+++	+++
	1000 EID ₅₀	-	-	±	±	++	++	ND	ND	ND
	10000 EID ₅₀	-	-	±	±	++	++	ND	ND	ND
Congestion & edema of lung	100 EID ₅₀	-	±	±	++	+++	+++	+++	+++	+++
	1000 EID ₅₀	±	±	±	++	+++	+++	ND	ND	ND
	10000 EID ₅₀	±	±	++	+++	+++	+++	ND	ND	ND
Pale pancreas with necrotic foci	100 EID ₅₀	-	-	±	±	++	+++	+++	+++	+++
	1000 EID ₅₀	-	±	±	++	+++	+++	ND	ND	ND
	10000 EID ₅₀	-	±	±	++	+++	+++	ND	ND	ND
Congestion of heart, spleen & liver	100 EID ₅₀	-	-	±	±	++	+++	+++	+++	+++
	1000 EID ₅₀	-	±	±	++	+++	+++	ND	ND	ND
	10000 EID ₅₀	-	-	±	±	++	+++	+++	ND	ND
Muscular congestion	100 EID ₅₀	-	-	±	±	++	+++	+++	+++	+++
	1000 EID ₅₀	-	-	±	±	++	+++	+++	ND	ND
	10000 EID ₅₀	±	±	++	++	+++	+++	ND	ND	ND
Kidney congestion & enlargement	100 EID ₅₀	-	-	±	±	++	+++	+++	+++	+++
	1000 EID ₅₀	-	-	±	±	++	+++	+++	ND	ND
	10000 EID ₅₀	-	-	±	±	++	+++	+++	ND	ND
Thymus congestion, petechiae & atrophy	100 EID ₅₀	-	-	±	±	++	+++	+++	+++	+++
	1000 EID ₅₀	-	±	±	++	+++	+++	ND	ND	ND
	10000 EID ₅₀	-	-	±	±	++	+++	+++	ND	ND
Intestinal & mesenteric congestion	100 EID ₅₀	±	±	++	+++	+++	+++	+++	+++	+++
	1000 EID ₅₀	+	++	+++	+++	+++	+++	ND	ND	ND
	10000 EID ₅₀	+	++	+++	+++	+++	+++	ND	ND	ND
Subcutaneous edema of head & breast	100 EID ₅₀	-	-	-	-	++	+++	+++	+++	+++
	1000 EID ₅₀	-	-	-	-	++	+++	+++	ND	ND
	10000 EID ₅₀	-	-	-	-	++	+++	+++	ND	ND

- No Lesions; ± Minimal; + Mild; ++ Moderate; +++ Severe, ND - Not done as all birds died

Histopathology

The tissue lesions were more of haemorrhagic and necrotic nature in 100 EID₅₀ group, inflammatory/ proliferative nature in 1000 EID₅₀ group.

- Disseminated intravascular coagulation (DIC) was the unique lesion observed in blood vessels of brain at 12 HPI and in kidney and liver at 1 DPI of 10000 EID₅₀ group and persisted up to 2 DPI. This finding correlates with the previous study reported by (1)

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