

## CHARACTERIZATION OF A/EQ-1 VIRUS ISOLATED DURING THE EQUINE INFLUENZA EPIDEMIC IN INDIA

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**Summary.** – The equine influenza virus, Ludhiana/5/87, isolated from the clinical material during the epidemic of equine influenza in India in 1987 was inhibited in haemagglutination-inhibition test by the antiserum against the prototype A/eq/Prague/1/56 (H7N7) virus and by post-epidemic horse sera. In haemagglutinin and neuraminidase analysis, the A/eq/Ludhiana/5/87 isolate appeared similar to the prototype A/eq/Prague/1/56 virus and was characterized as the H7N7 subtype.

**Key words:** equine influenza virus; epidemic; India

The epidemic of equine influenza which has occurred in India in 1987 was due to A/eq-2 (Uppal *et al.*, 1987; Singh *et al.*, 1987) and A/eq-1 viruses (Singh, 1992). This communication describes the isolation and characterization of the A/eq-1 virus involved.

The nasal swabs were obtained from a number of sick horses and ponies at different places in the Punjab State and Union Territory of Chandigarh and placed directly into the transport medium with antibiotics. The swab extracts were injected into 10–11 days-old chick embryos usually by combined amniotic and allantoic cavity routes. The recovered haemagglutinating agents were propagated in the allantoic cavity of embryonated eggs.

The haemagglutination and haemagglutination-inhibition (HI) tests for viral identification were performed as described by Kendal *et al.* (1982) using antisera against prototype A/eq/Prague/1/56 (H7N7), A/eq/Miami/1/63 (H3N8) and A/eq/Fontainebleau/1/79 virus strains, and a panel of six monoclonal antibodies (Ascites fluid) against A/eq/Miami and A/eq/Font virus strains. The neuraminidase-inhibition test was used as described (Aymard-Henry *et al.*, 1973).

The viral isolates identified as A/eq-2 viruses have been reported elsewhere (Singh *et al.*, 1987).

The isolated viral agent, Ludhiana/5/87, was found to contain haemagglutinating antigens inhibited by A/eq/Prague antiserum, thus identifying the agent as A/eq-1 virus. It should be mentioned that no live or inactivated equine influenza virus or other influenza virus was ever brought to the virus laboratory or the institute when the isolation and confirmation experiments were under way.

Table 1. HI tests with the Ludhiana isolate

Antigens	Hyperimmune rabbit sera against		
	A/eq/Prague 1/56	A/eq/Miami 1/63	A/eq/Font 1/79
A/eq/Ludhiana/5/87	320	< 40	< 40
A/eq/Prague/1/56	640	40	40
A/eq/Miami/1/63	40	640	320
A/eq/Fontainebleau/1/79	< 40	320	640

The A/eq/Ludhiana/5/87 isolate did not react with hyperimmune rabbit sera against A/eq-2 virus strains (Table 1). Similarly, it failed to react with four monoclonal antibodies (MoAbs) (HC1, HC3, HC4 and HC6) raised against Miami/63 virus and two MoAbs (HC1 and HC2) raised against Font/79 virus. The prototype A/eq/Prague virus did not react with a panel of six MoAbs as well.

The neuraminidase reaction with the Ludhiana isolate and prototype strains, A/eq/Prague, A/eq/Font, A/Hong Kong/1/68 and A/Puerto Rico/8/34 are shown in Table 2. It can be seen that the Ludhiana/5/87 isolate reacted as typical of N7 equine influenza virus.

The A/eq/Ludhiana/5/87 isolate was examined in HI test with serum specimens from horses taken 5 months after natural infection with of equine influenza viruses (Table 3). All the 8 sera had antibodies against the Ludhiana/5/87 virus, while only 5 of them against the A/eq-2 (A/eq/Ludhiana/8/87) virus. The titers with the Ludhiana isolate

Table 2. Neuraminidase-inhibition tests with the Ludhiana isolate

Viruses	Rabbit sera against			
	A/PR/8/34 (H1N1)	X31 (H3N2)	Vic/75-eq-1 (H3N7)	A/eq/Font/1/79 (H3N8)
A/eq/Ludhiana/5/87	< 20	< 20	200	20
A/Puerto Rico/8/34	1000	< 20	< 20	< 20
A/Hong Kong/1/68	20	10 000	100	100
A/eq/Prague/1/56	< 20	20	500	20
A/eq/Fontainebleau/1/79	20	20	20	2000

Table 3. HI tests with sera from horses from Hoshiarpur, Punjab

Serum No.	Antigens <sup>†</sup>		
	A/eq/Ludhiana 5/87	A/eq/Prague 1/56	A/eq-2/Ludhiana 8/87
1	160 <sup>†</sup>	80	20
2	80	80	< 20
4	160	80	< 20
6	320	80	20
7	160	160	20
10	80	80	40
11	160	80	40
12	320	160	< 20

In HI test, four HA units of virus were used.

Antigens were treated with ether as described (Berlin *et al.*, 1956).

Sera were periodate treated.

<sup>†</sup>Reciprocal titers

and Prague strain were higher than those with A/eq-2/Ludhiana/8/87 virus.

These results indicate that the A/eq/Ludhiana/5/87 isolate is antigenically similar to the prototype A/eq/Prague/1/56 virus and belongs to the H7N7 subtype. Therefore, it is typed as an A/eq-1 virus.

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