

suggested. Affinity to a dispersed pollen taxon *Matanomadhiasulcites* is based on gross morphological characters as one of us (KPN) had observed similar pollen grains in the West African sediments. Further investigation of materials and critical

comparison with extant forms could suggest its affinity with a liliaceous taxon.

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Characterization of structural proteins of A/Equine-2/Ludhiana/87

A/Equine/Ludhiana/87 (H3N8) equine influenza virus isolated from horses during the 1987 epizootic in India¹, resembled recent origin A/Equine-2 viruses by monoclonal antibody typing and sequencing of the HA-1 gene². Since characterization of the structural polypeptides of this virus is not reported earlier, efforts were made to study these by polyacrylamide gel electrophoresis (PAGE).

The virus under study was grown in allantoic cavity of 9-10-day-old chick embryonated eggs. It was concentrated and purified by gradient centrifugation³. A/Equine-2/Kentucky/81 (H3N8) virus used as standard strain for comparison was similarly grown, concentrated and purified.

For polyacrylamide gel electrophoresis (PAGE), the technique of Oxford *et al*⁴ was used with slight modification. It comprised a separating gel of 20% polyacrylamide, above which was a 5% polyacrylamide stacking gel. The upper and lower surfaces of the gels were in contact with a buffer containing glycine, Tris HCl and sodium dodecyl sulphate (SDS) which gave low conductivity and low ionic strength. Virus samples were boiled with SDS and mercaptoethanol to split the virus into constituent polypeptides and to provide a net negative charge. The treated virus samples were then applied to the wells in the stacking gel and an electric current of 24 milliamps was applied across the gel. Virus polypeptides migrated rapidly through the stacking gel and were then separated in the 20% gel according to their molecular weights. After 18 h of run, the separating gel was removed and the polypeptides were stained with Coomassie Blue dye (0.1%). The relative amounts of the virus polypeptides were analysed by scanning the gels with a densitometer (Biorad 620 Video Densitometer)

Polypeptide bands as revealed by densitometric analysis of PAGE of Ludhiana/87 and Kentucky/81 viruses are shown in Figure 1. The structural polypeptides seen were nucleoprotein (NP), matrix protein (M) and haemagglutinin (HA) which was dissociated into HA1 and HA2 under the reducing conditions of the gel system. Ludhiana virus contained 17% NP, 52% M and 31% HA (HA1 plus HA2) which were almost similar to 17% NP, 47% M and 34% HA of Kentucky virus. Also present was a high molecular weight aggregate which was composed of NP, M and HA (detected by immunoblotting in separate experiment). Both Ludhiana and Kentucky virus polypeptides were found to have similar molecular

weights. The PAGE results are in agreement with antigenic⁵ and genetic analysis² performed elsewhere which indicate the similarity of the Ludhiana/87 virus with other equine influenza viruses circulating in America and Europe during the 1980s. The results will form the basis of attempts to produce and standardize an equine influenza vaccine in India.

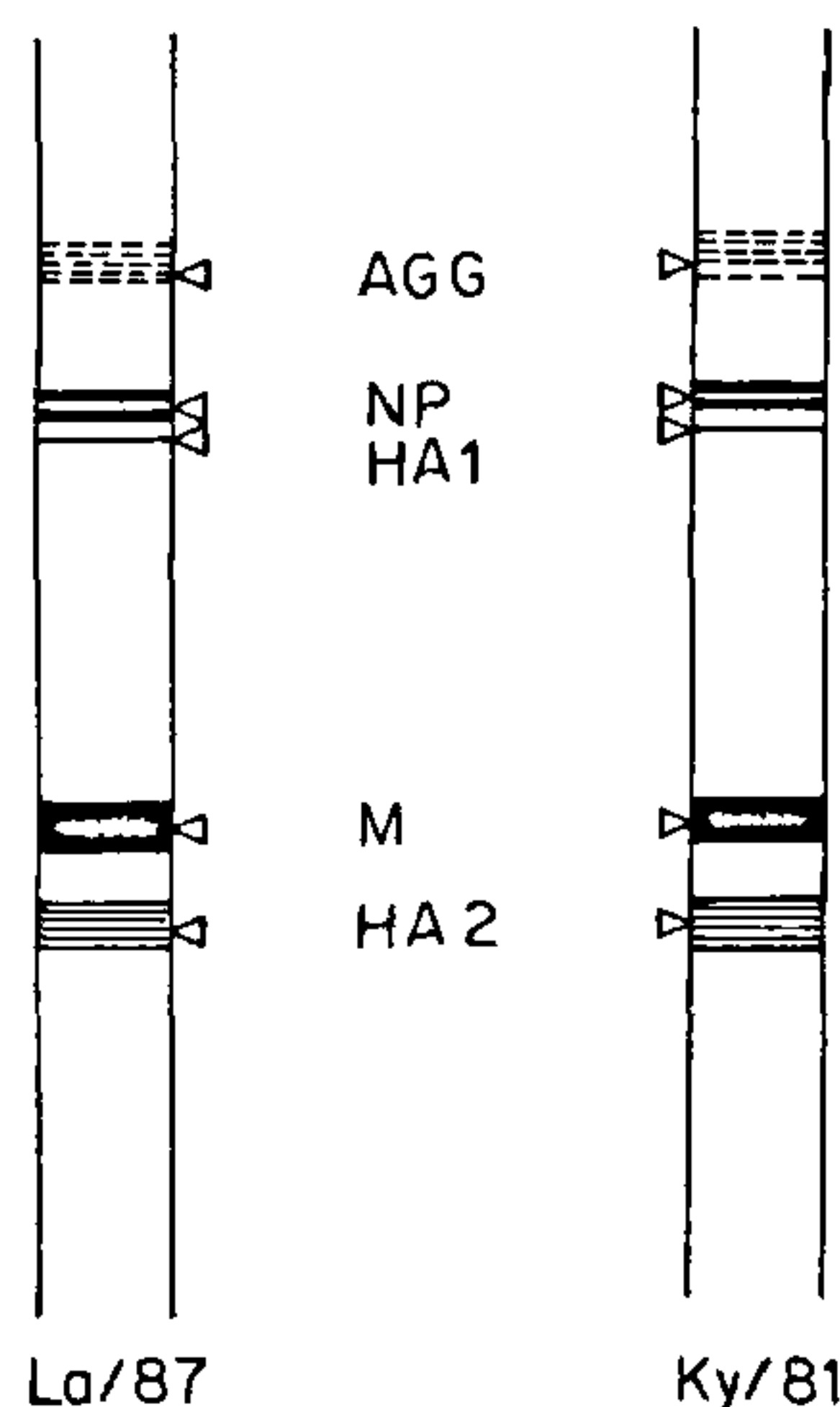


Figure 1. PAGE analysis of Ludhiana/87 (La/87) and Kentucky (KY/181) equine influenza virus A/Equine-2 (H3N8) isolates showing nucleoprotein (NP), matrix (M), haemagglutinin (HA1, HA2) polypeptide bands. A high molecular weight aggregate is shown on the top (AGG)

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