

Different pattern of haemagglutinin immunoreactivity of equine influenza virus strains isolated in Poland

Małgorzata Kwaśnik, Ilona Marcelina Góra, Wojciech Rożek

Department of Virology
National Veterinary Research Institute, 24-100 Pulawy, Poland
malgorzata.kwasnik@piwet.pulawy.pl

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Abstract

The immunoreactivity of haemagglutinin (HA) polypeptides of equine influenza virus was compared among the strains isolated in Poland, using H3 monoclonal antibody. A stronger signal in immunoblot reaction was observed for A/equi/Pulawy/2008 HA polypeptides compared to A/equi/Pulawy/2006, despite the fact that both strains are phylogenetically closely related and belong to Florida clade 2 of American lineage. The strongest signal, observed in the case of A/equi/Pulawy/2008, seemed to be connected with the presence of G135, I213, E379, and/or V530 instead of R135, M213, G379, and I530 present in A/equi/Pulawy/2006 HA sequence. This implies that point mutations within amino acid sequences of HA polypeptides of equine influenza virus may change their immunoreactivity even when they are not located within five basic antigenic sites.

Keywords: equine influenza virus, haemagglutinin, immunoreactivity, horses.

Introduction

The haemagglutinin (HA), a surface glycoprotein of influenza virus, is synthesised as a precursor HA0, which is proteolytically cleaved into two subunits: HA1 and HA2. The HA is responsible for binding virions to sialic acid containing cell surface receptors mediating fusion of viral and cellular membranes (6, 7, 8). The analysis of HA structure defined five antigenic sites. Amino acid changes occurring either in the antigenic sites or adjacent positions may affect antibody recognition. Equine influenza virus (EIV) undergoes antigenic drift and it is suggested that even one or two substitutions in key sites may be sufficient to cause antigenic changes (3). We would like to report the unusual pattern of HA reactivity of three Polish EIV isolates with the monoclonal antibody for H3 serotype. The viruses were isolated from horses in three different studs. At isolation time, mild clinical signs were observed. However, since the time of isolation we have monitored the clinical status of horses in these studs and have not found any further symptoms of respiratory diseases to date. It seems that

the reactions observed do not influence the pathogenic properties of the strains.

Material and Methods

Purification of virions. Three strains of EIV: A/equi/Pulawy/2005, A/equi/Pulawy/2006, and A/equi/Pulawy/2008 were multiplied in embryonated chicken eggs. Virions were purified from allantoic fluids showing HA titre above 64 by ultracentrifugation through glycerol layer as described previously (4). Protein concentration was determined using DC Protein Assay (Bio Rad) according to manufacturer's instructions.

Electrophoresis and western blot analysis. SDS PAGE in 15% polyacrylamide gel under reducing or non-reducing conditions (+DTT, -DTT) and western blot were performed as described previously (2). An amount of 5 µg of viral proteins was used for electrophoretic and western blot analysis. Two monoclonal antibodies were used: a haemagglutinin H3 mouse monoclonal antibody

(InA227, Novus Biologicals) and a mouse monoclonal anti influenza A matrix protein (MCA401 AbD Serotec).

Sequence analysis. Amino acid sequences translated from full length HA genes of A/equi/Pulawy/2005 (GenBank Ac. no GU066395), A/equi/Pulawy/2006 (GenBank Ac. no GU066396) (5), and A/equi/Pulawy/2008 (GenBank Ac. no KT429521) were aligned against A/Shangdong/9/93 (immunogen used for InA227 antibody preparation, GenBank Ac. no ACL12129) using BioEdit software (Ibis Pharmaceuticals Inc.).

Results

Western blot analysis of A/equi/Pulawy/2005, A/equi/Pulawy/2006, and A/equi/Pulawy/2008 revealed differences in HA polypeptides immunoreactivity with H3 monoclonal antibody InA227. Under reducing conditions, signals were observed for HA0 (80 kDa), HA1 (50 kDa), and HA2 (30 kDa) for A/equi/Pulawy/2005 and A/equi/Pulawy/2008. HA0 and HA1 signals for A/equi/Pulawy/2008 were stronger. In the case of A/equi/Pulawy/2006 a weak signal on the level of HA2 only was detected (Fig. 1). Under non-reducing conditions the signal corresponding to HA0 (80kDa) and the signals for multimeric HA forms (dimers, trimers) were observed for all strains; however, the signals obtained for A/equi/Pulawy/2006 antigen were weaker than the signals for A/equi/Pulawy/2005 and A/equi/Pulawy/2008 antigens (Fig. 1). M1 was used as a loading control.

Amino acid sequences of full length haemagglutinin of three Polish EIV isolates and A/Shangdong/9/93 (used for antibody preparation) were compared. Sequence of A/equi/Pulawy/2006 differed from A/equi/Pulawy/2005 and A/equi/Pulawy/2008 in 6 and 4 positions, respectively (Table 1). All analysed strains had different amino

acids in position 213. In positions 135 and 530, A/Shangdong/9/93 and A/equi/Pulawy/2008 had the same amino acids (G and V) contrary to A/equi/Pulawy/2006 (R and I, respectively).

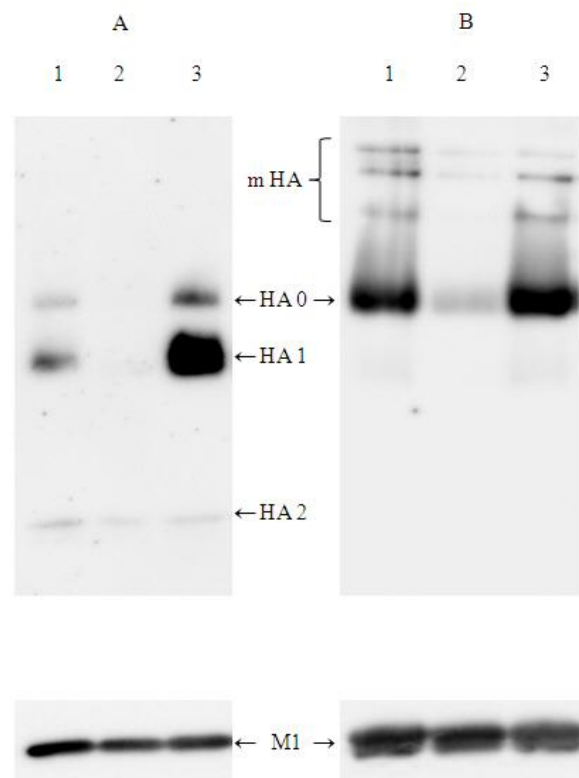


Fig. 1. Western blot analysis of EIV strains isolated in Poland: 1- A/equi/Pulawy/2005, 2- A/equi/Pulawy/2006, 3- A/equi/Pulawy/2008, A- SDS PAGE under reducing conditions (+ DTT), B - SDS PAGE under non-reducing conditions (- DTT), m HA – multimeric forms of haemagglutinin, HA0, HA1, HA2 – polypeptides of haemagglutinin. Immunodetection was carried out using monoclonal antibody for H3 – upper panel. Bottom panel represents reactions of M1 protein with mouse anti Influenza A matrix protein performed on the same samples and used as a loading control

Table 1. Amino acid positions of HA sequence distinguishing three Polish EIV isolates from A/Shangdong/9/93 (immunogen used for InA227 antibody preparation)

Amino acid position	7	31	59	79	135	213	379	530
A/equi/Pulawy/2005	G	T	V	D	R	V	G	V
A/equi/Pulawy/2008	N	S	I	V	G	I	E	V
A/equi/Pulawy/2006	N	S	I	V	R	M	G	I
A/Shangdong/9/93	N	T	I	G	G	V	G	V

Discussion

Immunoblotting analyses revealed different patterns of HA immunoreactivity of three tested EIV strains isolated from horses in Poland with H3 monoclonal antibody. The highest differences in immunoblot signals were observed between A/equi/Pulawy/2006 and A/equi/Pulawy/2008, which are phylogenetically closely related, belonging to Florida clade 2 of American lineage. Levis *et al.* (3) postulated that single amino acid substitution may likely be the cause of antigenic differences among phylogenetic clusters. Amino acid HA sequences of A/equi/Pulawy/2006 and A/equi/Pulawy/2008 differ in four positions (135, 213, 379, and 530). The strongest signal in western blot analysis, which was observed in the case of A/equi/Pulawy/2008, seemed to be connected with the presence of G135, I213, E379, and/or V530 instead of R135, M213, G379 and I530, present in A/equi/Pulawy/2006 HA sequence. The intermediate signal was obtained for A/equi/Pulawy/2005. Two positions out of four were shown to differ between A/equi/Pulawy/2006 and A/equi/Pulawy/2008 (R135 and G379) strains, whereas A/equi/Pulawy/2005 had the same amino acid as A/equi/Pulawy/2006. The differences observed in the reaction of EIV strains with H3 monoclonal antibody seemed to be connected with the substitution in the position of four amino acids; one is situated within antigenic site D (position 213). Amino acid changes within HA1 domain are mainly clustered between residues 137 and 257 (1). Woodward *et al.* analysed antigenically significant substitutions of EIV H3 (9). The authors noted differences in positions 159, 189, 227, and in at least eight other amino acids, affecting the antigenic sites. The authors also observed that I213V substitution was characteristic for Euroasian lineage, without influence on HA antigenicity. Generally, recent reports have postulated that substitutions within regions adjacent to antigenic sites may be significant for antigenic changes. Point mutations within sequences of immunologically important proteins, such as haemagglutinin of equine influenza virus, may change their immunoreactivity even when they are not located within well-characterised antigenic sites. Such changes, influencing protein conformation, may be located throughout the

whole HA sequence. Changes in conformational and nonlinear epitopes on the protein surface or in antigen spacing result in weaker or even lack of antibody binding.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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