VETERINARSKI ARHIV 70 (3), 113-120, 2000

Antigenic variation among Nigerian strains of equine H3 influenza viruses

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ADEYEFA, C. A. O., J. W. MCCAULEY, T. OYEWALE: Antigenic variation among Nigerian strains of equine H3 influenza viruses. Vet. arhiv 70, 113-120, 2000.

ABSTRACT

Antigenic variation among three recent isolations of equine-2 H3N8 influenza viruses from Ibadan, Nigeria is reported. Antigenic analysis with panels of monoclonal antibodies (mAbs) and polyclonal antisera indicated that the three viruses were antigenically divergent, although they were all H3N8 subtype related to other equine 2-viruses isolated between 1963 and 1987. Results of virus protein synthesis investigated by pulse-chase experiments showed heterogeneity among the proteins of the ribonucleoprotein complex and the haemagglutinin glycoproteins, which were not cleaved in tissue culture. The results of this study indicate that equine H3 HAs have evolved by a process of evolutionary divergence and mutational changes, as confirmed by genetic analysis in another study. The results also showed that antigenic variation occurs among equine H3 influenza viruses and that H3 viruses with antigenically different HA molecules could co-circulate in equine populations.

Key words: H3 influenza viruses, antigenicity analysis, equine, Nigeria

Introduction

Two subtypes, H7N7 and H3N8, of influenza A viruses infect equines, although the H7N7 subtype has not been isolated from equines for over 15 years, while H3N8 is predominant in most parts of the world (KAWAOKA et al., 1989: DONATELLI et al., 1991; CHAMBERS, 1992; WOOD and MUMFORD, 1992; GUO et al., 1992; OXBURGH et al., 1993; BINNS et al., 1993; GUPTA et al., 1993; LAI et al., 1994; SHORTRIDGE et

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ISSN 0372-5480
Printed in Croatia

al., 1995). The first known outbreak of equine-2 influenza infection in Nigeria was recently reported and from which three viruses were isolated (ADEYEFA and McCAULEY, 1994). These three viruses were observed to be antigenically divergent, although they were H3N8 subtype viruses and were from the same evolutionary tree as H3 equine influenza viruses isolated in Europe in 1989 and 1991 (ADEYEFA et al., 1996).

Recent equine H3 viruses have been shown to have evolved through a process of antigenic drift (KAWAOKA et al., 1989; ENDO et al., 1992; BINNS et al., 1993; LAI et al., 1994; ADEYEFA et al., 1996). This evolution is thought to have occurred at a 50% faster rate from the mid-1980s than in the 1960s and mid-1970s (LAI et al., 1994). Also, an H3N8 virus was isolated in China in 1989 that was antigenically and genetically different from equine-2 H3N8 viruses (GUO et al., 1992).

The degree of antigenic variation within equine influenza viruses is of considerable interest, since the current commercial equine vaccines which, although trivalent, have been reported to produce very short-lived immunity in vaccinated horses (WOOD and MUMFORD, 1992; CHAMBERS, 1992) and several outbreaks of disease have occurred despite recent vaccinations and strict quarantine measures (LAI et al., 1994). In the light of these facts, it is desirable to know the antigenic variations in equine-2 influenza viruses currently circulating among equine populations in the world, knowledge of which could be utilised in modifying or upgrading currently available equine vaccines.

In the present study, we have determined the antigenic variation among three recently isolated equine-2 influenza viruses from Nigeria by antigenic analysis and pulse-chase experiments and have been able to infer the genetic basis of the variation from studies on molecular characterization of the three viruses reported earlier (ADEYEFA et al., 1996).

Materials and methods

The viruses used in this study were three H3N8 equine viruses recently isolated in Ibadan, Nigeria: A/Eq/Ibadan/4/91 (Ib4), A/EQ/Ibadan/6/91 (Ib6), A/Eq/Ibadan/9/91(Ib9) (ADEYEFA and McCAULEY, 1994), A/Eq/Prague/56 (H7N7), A/Turkey/England/1/77 (H7N7), as well as A/FPV/Rostoc/34 as control. They were grown in 10- to 11-day-old embryonated hen eggs and 25 l/well of clarified allantoic fluid at a multiplicity of infection of >10 was used to infect monolayer cells in flat bottom 96 well tissue culture plates for 30 minutes at room temperature (22 °C). The inoculum was removed, the cells were covered

with 50 l/well of Eagle's medium containing Earle's salt and incubated at 37 °C+5 CO_2 for 6 hours. Virus protein synthesis and cleavability of the HA polypeptides were investigated by pulse-chase experiments by labelling virus-infected monolayer cells at 6 hrs post-infection with ^{35}S-Methionine (1232.7 Ci/mmpl at 100 Ci/ l (New England Nuclear) in Earle's salt for 10 minutes and chasing with medium containing 10 mM methionine for 15-30 minutes (McCAULEY and PENN, 1990), followed by addition of 200 l/well of protein gel buffer (8 M urea, 2% SDS, 2% 3-mecarptoethanol pH 6.8, 10 Mm Tris-NCH, pH 7.4, 0.005% bromophenol blue). Electrophoresis of cell lysates was carried out on 12.5-15% SDS-PAGE (LAEMMLI, 1970) under reducing conditions. To determine the influence of the virus cell systems on HA cleavability in tissue culture, nine different monolayer cell types were used, including chicken embryo fibroblasts (CEF), lamb testes primary cells (Ltp6c) with extended life produced at Pirbright Laboratory, Madin Darby Canine and Bovine Kidney cells (MDCK, MDBK), African green monkey cells (Vero) Baby hamster kidney cells (BHK), Rim suino 2 Swine kidney cells (RS2), rhesus monkey kidney cells (LLCMK$_3$T) and BSC40 cells.

Antigenic cross-reactivity was carried out in haemagglutination inhibition tests (HI) in micro-titer plates with panels of monoclonal antibodies (mAbs) and polyclonal antisera raised against equine H3N8 and H3N7 viruses. Antisera were also raised in outbred adult rabbits against homologous viruses isolated in Ibadan, Nigeria, by standard method and used in antigenic analysis of these viruses. The mAbs used were MH1, MH3, MH4, MH6 against Miami/63 HA, FH1, FH2 against Fontainebleau/79 HA, T2/1, T4/1, T14/1, T22/1 against Tennessee/85 HA, EQ1, EQ5, EQ6, EQ7 against Miami/63 vaccine strain HA, K1/1, K2/2, K2/1, K3/1 and K4/1 against Kentucky/81 HA and I1/1 against Ilowa/86. Polyclonal antisera were raised against the whole viruses. The well-characterised laboratory virus strains against which the mAbs and antisera were raised were not included in the study in order to preclude cross-contamination, because the subsequent strategy used for molecular characterisation of the three viruses involved extensive use of polymerase chain reaction (PCR), which is highly susceptible to cross-contamination.

Results

The HI titers and patterns of reactivity of the three Ibadan virus isolates with mAbs are shown in Fig. 1., while HI titers with polyclonal antisera are shown in Fig. 2. Eq/Miami/63, Eq/Tennessee and Eq/Kentucky mAbs showed greater cross-reactivity with Ib4 and Ib6, than with Ib9 except MH6 which showed lower reactivity with Ib4 and greater

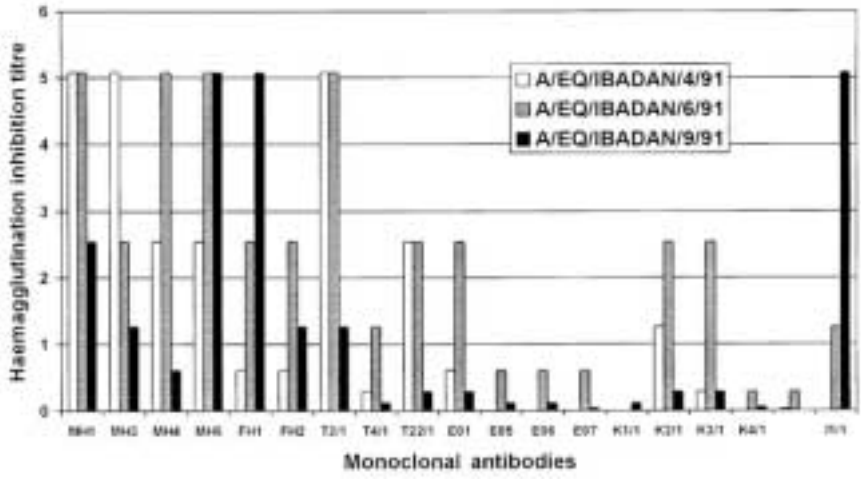

Fig. 1. Comparison of equine H3 influenza virus isolates from Ibadan, Nigeria; use of monoclonal antibodies

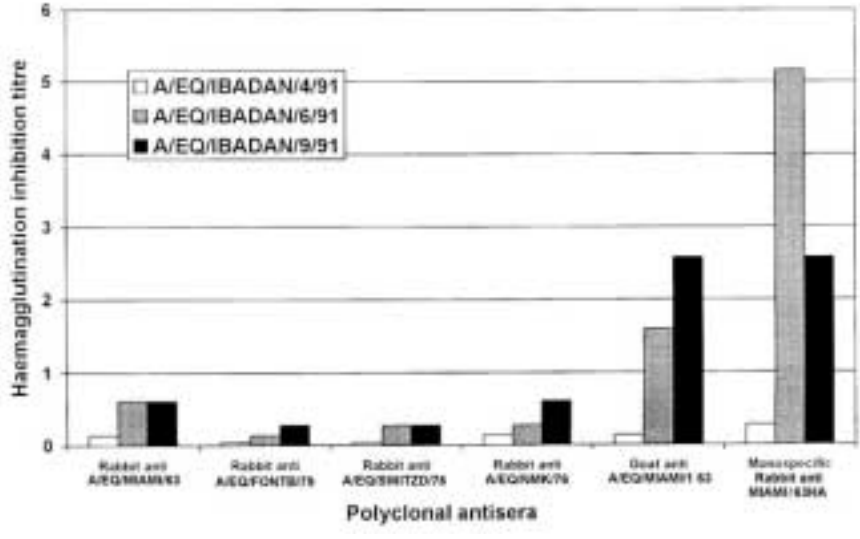

Fig. 2. Comparison of equine H3 influenza virus isolates from Ibadan, Nigeria; use of polyclonal antisera

reactivity with Ib9 while Eq/Fontainebleau/79 and Eq/Iowa Mabs showed greater cross-reactivity with Ib6 and Ib9 than with Ib4, which gave 10-fold lower titers than the former two viruses. Similar reactivity patterns were observed with antisera against homologous and heterologous viruses. Antisera against Eq/Miami/63, Eq/Fontainebleau/79, Eq/Switzerland/76 and E/Newmarket/76 cross-reacted to much lower titers with Ib4 than with the other two, while Ib9 showed much greater cross-reactivity to these antisera.

Comparison between the three viruses in their antigenic cross-reactivity indicated that although they were all H3N8 subtype, they were antigenically distinct from each other. Ib4 and Ib6 seemed to be closer to each other than to Ib9, implying that some degree of antigenic variation exists among the viruses, as shown in Figs. 1 and 2.

The electrophoretic mobility and pattern of virus polypeptides are shown in Fig. 3. Some differences were observed in the migrational

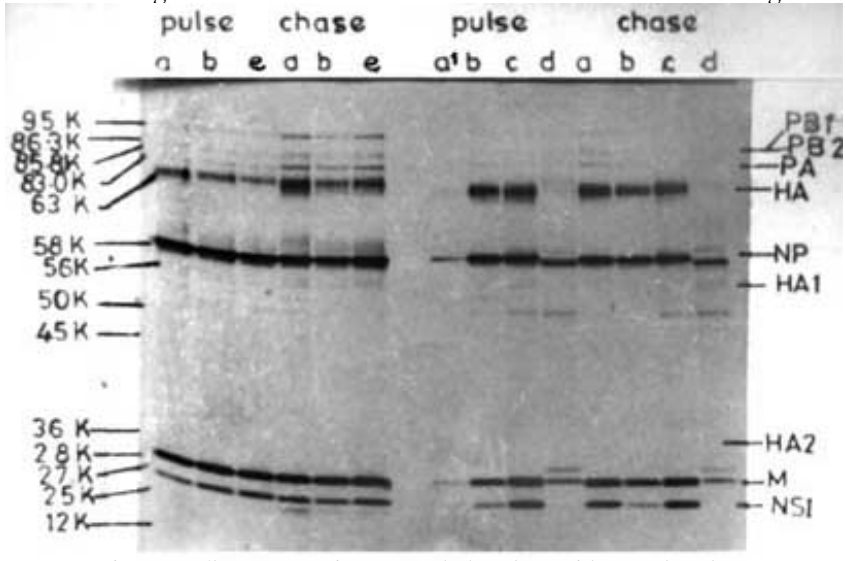

Fig. 3. Influenza A virus encoded polypeptides. Pulse-change labelling of chicken embryo fibroblast monolayer cells infected with influenza A viruses using 35^{s} methionine.
a^{1}=A/FPV/Rostoc/34; a=A/Equine/Ibadan/4/91; b=A/Equine/Ibadan/9/91; c=A/Turkey/England/1/77; d=A/Equine/Prague/56; e=A/Equine/Ibadan/6/91

pattern of the HA, polymerase proteins and the nucleoprotein (NP) of the three viruses, which differ slightly from those of the other two control viruses (Prague/56 and Turkey/England/1/77), particularly the NP of Ib4, which appears to be avian-like.

The HA glycoproteins of the three equine H3 and the non-pathogenic avian H7 viruses were not cleaved in any of the cell types used, in contrast to that of equine-1 H7N7 virus (Prague/56). This implies that cleavability of equine-2 HA polypeptides into HA1 and HA2 in tissue culture does not depend on the virus-cell system but rather on some intrinsic properties of the HA subtypes.

Discussion

Antigenic analysis showed that the three viruses were antigenically divergent, which was further reinforced by the heterogeneity observed in their internal proteins, particularly those of the ribonucleoprotein (RNP) complex. The differences observed in the migrational pattern of the HA, polymerase proteins and the NP have proved to be of genetically and epidemiological significance, as elucidated in molecular studies (ADEYEFA et al., 1996). These observations indicate that antigenic variation occurs among equine H3 HA molecules and that equine H3 viruses with antigenically different HA molecules could co-circulate. KAWAOKA et al. (1989) made similar observations in a study of equine H3N8 viruses isolated between 1963 and 1987. The implication of this is that like human H3 HAs, equine H3 HAs may have evolved by progressive antigenic variation and divergence, probably due to selective pressure or some mutational effect in their HA gens. These intra and inter strain differences have potential significance in the production of high yield influenza virus reassortants that could be used in vaccines against antigenic variants emerging in nature.

Previously, equine H3 viruses have been shown to be antigenically divergent (KAWAOKA et al., 1989; ENDO et al., 1992; BINNS et al., 1993). Although the panels of mAbs and antisera used in the present study are not identical to those used by previous workers, the broad reactivity of the three viruses suggested that they were similar to, but not identical to, the viruses isolated in Europe and USA between 1963 and 1987. The antigenic variation appears to be at the limit of significance with a 10-fold difference in titer with one mAb in Ib4, although none of the 21 mAbs used failed to react with any of the three viruses. This could be taken as an indication of a relationship between these Ibadan viruses and those against which the mAbs and antisera were realised. Variation was observed in the nucleotide and amino acid sequences of the HA gens and HA polypeptides respectively and those of the RNP complex in the three viruses (ADEYEFA et al., 1996), which is a reflection and a confirmation of the antigenic diversity among the viruses as observed in the present study.

The HAs of the three Ibadan viruses and the non-pathogenic avian H7 virus were not cleaved in tissue culture, in contrast to equine H7 HAs (GIBSON et al., 1992). *In vivo* cleaving of the HA polypeptide into HA1 and HA2 is required for virus infectivity in nature (KLENK et al., 1979) and is related to the virulence of avian influenza A viruses, where two structural features are involved: a series of basic amino acids at the cleaving site and an oligosaccharide side-chain nearby (KAWAOKA, 1991). In equine H7 HAs, a tetra basic amino acid cleavage site with an additional nine amino acid insertions has been reported (GIBSON et al., 1992). This has not been demonstrated for equine and human H3 Has, or for those of avirulent avian viruses. It is probable that lack of this series of basic amino acids and the additional amino acid insertion at the cleavage site of equine and human H3 Has, and those of avirulent avian viruses, was responsible for non-cleavability of these HAs in tissue culture. The non-cleavability of the HAs of the three equine and the non-pathogenic avian H7 HAs in any of the nine cell types used in this study implies that cleavability of equine-2 HAs into HA1 and HA2 in tissue culture does not depend on the virus-cell system used but rather on some intrinsic properties of the HA subtype. In-vivo cleavage of equine H3 HAs and other HAs of infectious and virulent influenza viruses occurs in natural infections, probably due to the abundance and ubiquity of cellular proteases capable of cleaving the HAs of virulent viruses.

Acknowledgement
The mAbs and polyclonal antisera used in this study were kindly provided by Mr. Allan Douglas of the WHO Influenza Collaborating Center, National Institute for Medical Research, Mill Hill, London, and Dr. R. G. Webster, St. Jude's Children's Research Hospital, North Lauderdale, Memphis, Tennessee, U.S.A. Dr. Adeyefa was supported by the Commonwealth Academic Staff Fellowship at Pirbright.

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Received: 25 October 1998
Accepted: 10 May 2000

ADEYEFA, C. A. O., J. W. McCAULEY, T. OYEWALE: Raznolikost antigena nigerijskih sojeva virusa influence konja H3. Vet. arhiv 70, 113-120, 2000.

SAŽETAK

Obrađena je raznolikost antigena tri nova izolata nigerijskih sojeva virusa influence konja H3N8 iz Ibadana u Nigeriji. Analiza antigena s pločama monoklonskih protutijela (mAbs) i poliklonskim antiserumima pokazala je da su tri virusa antigenski različiti, iako su svi bili H3N8 podtipa i srodni s drugim virusima influence konja serotipa A_2 izoliranim od 1963. do 1987. Rezultati sinteze virusnog proteina istraženi sa "pulse-chase" pokusima pokazali su heterogenost proteina ribonukleinskog kompleksa i hemaglutininskih glikoproteina, a koji nisu rasli u kulturi tkiva. Rezultati ovog istraživanja naznačuju da su konjski H3 HAs nastali procesom evolucijske divergencije i mutacijama, a to je potvrđeno i genetskim analizama u drugoj studiji. Rezultati također pokazuju da se pojavljuju antigene varijacije među H3 virusima konjske influence i da H3 virusi s antigeno različitim HA molekulama mogu zajedno cirkulirati u populacijama konja.

Ključne riječi: H3 virusi influence, analiza antigena, konj, Nigerija