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Molecular analysis of an outbreak of influenza in the United Kingdom

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Abstract. The first outbreak of influenza virus in Europe in 1995/1996 occurred in a school in the south of England. RT-PCR-restriction analysis was performed on the HA1 portion of influenza haemagglutinin gene amplified directly from clinical samples. These were taken at the onset of the outbreak and indicated co-circulation of two distinct genetic variants of influenza virus which were antigenically identical. Investigations of this nature demonstrate the genetic diversity of circulating variants of influenza and have the potential to provide information on molecular evolution of strains within closed populations.

Key words: HA sequence, Influenza virus, RT-PCR-restriction analysis

Introduction

Acute infections of the respiratory tract are amongst the most common affliction of the human host. In developed countries infections of the upper and lower respiratory tract are important causes of disability and days lost from school or work. Early identification of the organism responsible for outbreaks of respiratory infection is important to facilitate distribution of specific drugs if appropriate, and provide reliable public health information. Investigation of community outbreaks of respiratory illness is often hampered by inability to culture the organism. However, the use of molecular techniques directly on respiratory samples facilitates the investigation of respiratory outbreaks and can provide rapid identification of an organism and its relationship to current vaccines, as we show here for an outbreak of influenza virus in England in 1995.

Influenza surveillance

Influenza virus is a major cause of upper respiratory tract infection in young adults. Influenza A & B viruses undergo gradual antigenic variation resulting from point mutations in the surface antigens of the virus (haemagglutinin [HA] and neuramindase). These antigenic changes allow evasion of the host immune response and the accumulation of mutations means that the influenza vaccine components require updating at regular intervals. Continual surveillance of circulating viruses is essential to ensure a good match between vaccine and prevailing strains. The 1994/1995 epidemic in the UK was caused predominantly by influenza B viruses [1]. Influenza A H3N2 viruses were first isolated in mid February in 1995 in England. Between February and September 1995 43 influenza A H3N2 isolates were identified. These were antigenically closely related to the H3N2 vaccine strain for 1995/1996, A/Johannesburg/33/94 e.g. A/England/282/95 (Table 1). Sequence analysis of the HA1 portion of haemagglutinin gene of 12 of 43 influenza H3N2 isolates using previously described methods [2], indicated that there were two major sequence variants distinguished by eleven or more amino acid changes, which could be grouped by their relatedness to reference strains, either A/Johannesburg/33/94 (Group I) or A/Thessaloniki/1/95 (Group II) (Figure 1). These variants were however, antigenically very closely related e.g. A/England/282 and A/England/286/96 (Table 1). There were approximately equal numbers in each group in England between February and September 1995, and there was no obvious correlation between sequence group and geographical location. Clearly, therefore, in England during spring and summer 1995 there was co-circulation of two different variants of influenza A H3N2 which were antigenically similar but genetically distinct.

RT-PCR-restriction analysis

In order to facilitate the genetic analysis of England A H3N2 strains, we developed RT-PCR Restriction Fragment Length Polymorphism analysis [RT-PCR restriction analysis] for use directly on clinical material. RNA extraction from clinical material (nasopharyngeal aspirates or combined nose and throat swabs) and cDNA synthesis was performed as previously described for egg grown influenza viruses [2]. Amplicons generated from a nested PCR

Table 1. Post infection ferret antisera

Virus strain	A/Beij/353/89 [1]	A/Shand/9/93 [2]	A/Johburg/33/94 [3]	A/Thess/1/95 [4]
A/Beijing/353/89 [1]	**2560**	40	< 10	< 10
A/Shandong/9/93 [2]	160	**1280**	2560	1280
A/Johannesburg/33/94 [3]	160	640	**5120**	2560
A/Thessaloniki/1/95 [4]	320	640	2560	**5120**
A/England/282/95	80	80	5120	2560
A/England/286/95	80	320	2560	2560
A/England/301/95	40	320	2560	2560
A/England/309/95	40	320	2560	2560
A/England/313/95	40	640	2560	2560
A/England/658/95	40	1280	2560	2560

Haemagglutination inhibition using post infection ferret antisera treated with receptor destroying enzyme. HI tests were carried out using 8 HA units of virus and 0.5% v/v turkey red blood cells. A virus which shows a four fold or less reactivity with earlier antigens or antisera or both is considered to show significant antigenic drift.

using primers described previously for HA1 of influenza A H3N2 viruses [3] were digested with two restriction enzymes, *Bst*N1 and *Hpa*II, both of which were capable of distinguishing A/Johannesburg/33/94 like strains from A/Thessaloniki/1/95 like strains (Figure 2a). Cleavage of A/Johannesburg/34/94 like strains with *Bst*N1 gave two fragments (82, 508) whereas cleavage of A/Thessaloniki/1/95 like strains gave three fragments (82, 197, 311). Cleavage of A/Johannesburg/34/94 like strains with *Hpa*II gave four fragments (37, 72, 145, 336) and cleavage of A/Thessaloniki/1/95 like strains gave three fragments (37, 147, 408). Results from genetic analysis were available within 48 hours of receipt of specimen and did not require virus isolation. This approach was applied to specimens received from the first influenza outbreak in Europe in the winter season 1995/96.

Case histories

The first outbreak of influenza in the 1995/96 season in Europe began in September 1995 in a boarding school in the South of England within the first week of the autumn term. At least 307 out of a total of 643 boys aged 13–18 years were affected by an upper respiratory illness with fever or complaints of feverishness over a period of five weeks. The first three boys who presented had nasopharyngeal aspirates taken, from which influenza A virus was identified by direct immunofluorescence, RT-PCR restriction analysis and subsequently by culture (A/England/301/95, A/England/309/95, A/England/313/95). A further six boys with respiratory illness also had nasopharyngeal aspirates taken during the outbreak which were analysed in a similar way. In addition, the only five influenza A H3N2 isolates from the surrounding area in 1995 were also analysed antigenically and by RT-PCR restriction analysis (A/England/293/95, A/England/395/95, A/England/654/95, A/England/658/95, A/England/15/96).

Results

Eight out of nine respiratory samples taken from boys presenting with acute respiratory infection showed evidence of influenza A H3N2 infection by RT-PCR. This was subsequently confirmed by virus isolation in primary Rhesus monkey kidney cells and antigenic typing. All of these strains were antigenically closely related to A/Johannesburg/33/94. Interestingly, two out of three of the boys sampled in the first 48 hours of the outbreak showed evidence of A/Johannesburg/33/94 like strains by RT-PCR restriction analysis, whereas the third boy showed evidence of an A/Thessaloniki/1/95 like strain (Figure 2b). This was subsequently confirmed by sequence analysis of HA1 portion of the haemagglutinin gene of the isolated viruses (data not shown). The five boys who were sampled later in the epidemic also showed evidence of infection with A/Thessaloniki/1/95 like strains by RT -PCR restriction analysis (Figure 2b). The ninth boy who was sampled, had no evidence of influenza virus infection either by PCR or by culture, but a rhinovirus (untyped) was isolated from a nasopharyngeal aspirate. RT-PCR restriction analysis of the influenza strains isolated between July and December 1995 from the locality of the school indicated that these were all A/Thessaloniki/1/95 like viruses (Figure 2a), but antigenically closely related to A/Johannesburg/33/94 e.g. A/England/658/95 (Table 1).

Discussion

The results described here indicate the difficulty of attributing outbreaks of acute respiratory illness to a single agent. In this outbreak of respiratory illness in a boarding school in the South of England in autumn 1995 we have evidence of the simultaneous introduction of two distinct variants of influenza virus A H3N2 along with co-circulation of a rhino-

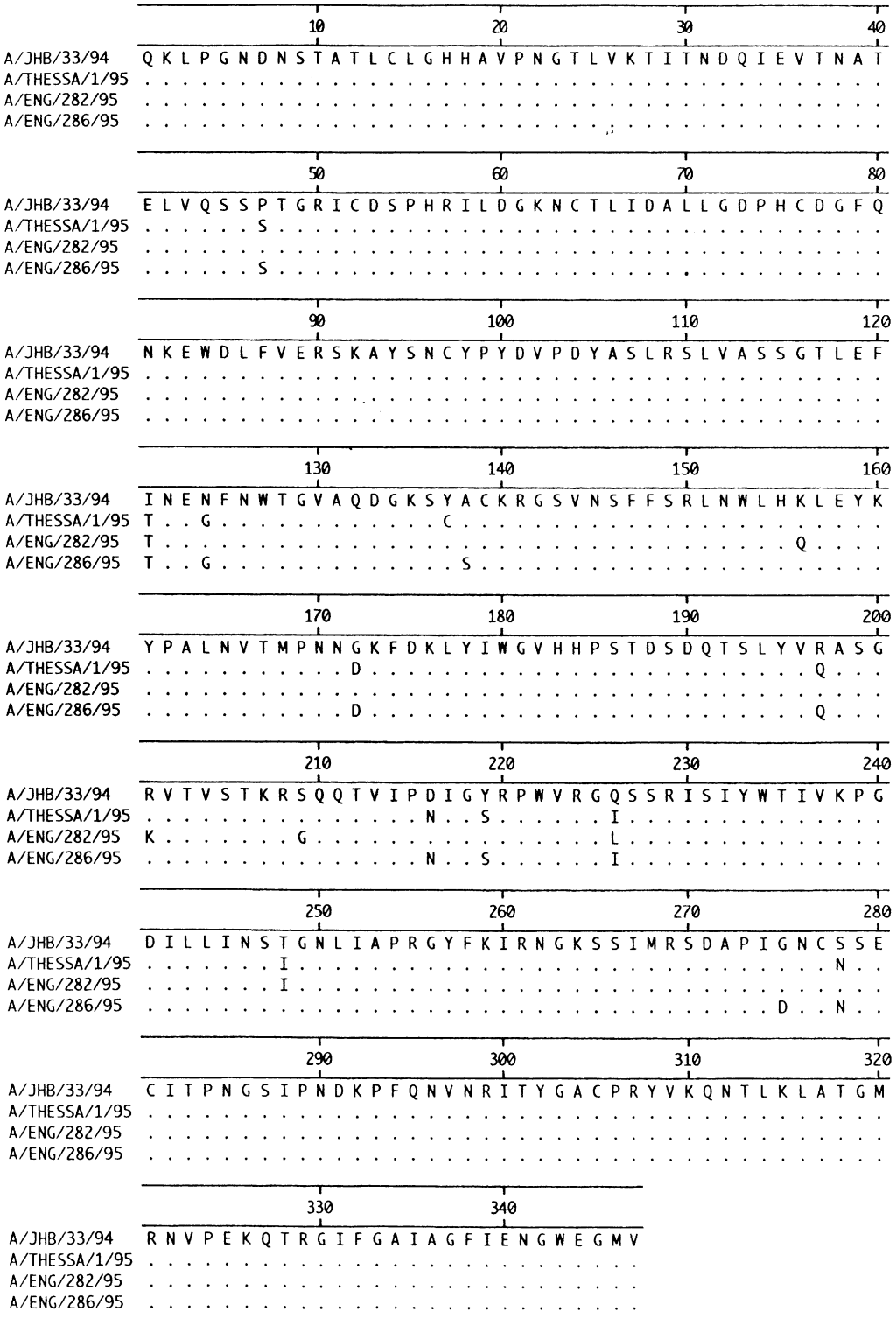

Figure 1. Comparison of amino acid changes in the HA 1 region of the HA gene of influenza A H3N2 variants. Sequences were aligned using the clustal method (Megalign, version 1.03) [5].

virus. This highlights the problems of public health surveillance of influenza where most cases of 'flu like illness' are not investigated virologically and many viruses, e.g., respiratory syncytial virus, rhino-viruses or coronaviruses can cause a similar syndrome. It is clear from the fact that two influenza virus variants were isolated from different individuals on day 1 of the outbreak that there cannot have been a single index case for the outbreak in this school. It is probable that two or more children returning to boarding school from different areas of the country introduced the two variants of influenza into the boarding school community. The A/Johannesburg/33/94 like strains were not reflected in the isolates from the local area of the school (Figure 2b). As only a limited number of respira-

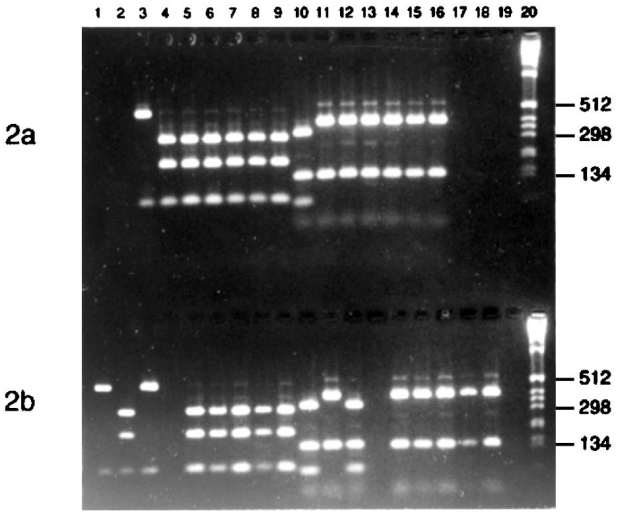

Figure 2. Ethidium-bromide stained agarose gel of RT-PCR restriction patterns of influenza A H3N2 viruses. Profiles obtained after amplification of 590bp region of the HA1 subunit and digested with *Bst*N1 (lanes 1–9) or *Hpa*II (lanes 10–18). Lane 20 contains DNA molecular size marker (sizes shown in base pairs). (a) Lanes 3 and 10, A/Johannesburg/33/94; Lanes 4 and 11, A/Thessaloniki/1/95; Influenza A H3N2 viruses from surrounding area to the outbreak: Lanes 5, 12 A/England/293/95; Lanes 6, 13 A/England/395/95; Lanes 7, 14 A/England/654/95; Lanes 8, 15 A/England/658/95; Lanes 9, 16 A/England/15/96. (b) Lanes 1–18, specimens obtained from boys attending school; Lane 1, 10: A/England/301/95 isolated; Lane 2, 11: A/England/309/95 isolated; Lane 3, 12: A/England/313/95 isolated; Lane 4, 13: Rhinovirus isolated; Lane 5, 14: A/England/772/95 isolated; Lane 6, 15: A/England/554/95 isolated; Lane 7, 16: A/England/653/95 isolated; Lane 8, 17: A/England/674/95 isolated; Lane 9, 18: A/England/658/95 isolated.

tory samples were taken from the total affected population it is not possible to comment on the overall distribution of A/Johannesburg/33/94 to A/Thessaloniki/1/95 like influenza viruses circulating within the school during the period of the outbreak.

This report is the first demonstration of the use of RT-PCR restriction analysis in the investigation of an outbreak of influenza. Although RT-PCR restriction digestion has been described for the analysis of candidate influenza vaccine strains the technique has not been applied directly to respiratory secretions [4], nor indeed has it been widely used to analyse genetic diversity of respiratory viruses. RT-PCR restriction analysis directly on respiratory material provides a method for rapid genetic analysis, with a minimum of material, in absence of cultured virus. Furthermore, RT-PCR restriction is a technique which can be applied easily to a relatively large number of samples. However, investigation of the antigenicity of the viruses requires isolation and further serological characterisation which remains invaluable in assessing the importance of genetic drift in influenza. Further investigations of this nature have the potential to dissect respiratory outbreaks and provide information on genetic diversity and molecular evolution of strains in closed populations.

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