Deciphering the Influenza Riddle: A Moving Target

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Abstract

Objective:
Determine if the annual formulated Influenza A (H3N2) vaccine has been an effective deterrent to worldwide outbreaks of orthomyxoviruses over the last six years.

Materials:
Computer with Internet access and browser
Word Processing Program (Microsoft-Word)
Spreadsheet Program (Microsoft-Excel)
Various Databases (Reference Bibliography for website addresses)
The Influenza Sequence Database (ISD)
US Centers for Disease Control and Prevention (CDC) Flu Home
National Center for Biotechnology Information (NCBI) Databases and Programs
CLUSTAL-W Multiple Sequence Alignment Tool

Procedure:
For years 1999-2005, identify Influenza vaccine strains selected by WHO and CDC organizations. Document statistics on worldwide flu outbreaks categorized by Influenza subtypes using the Influenza Sequence Database. Locate the related nucleotide for each virus strain identifying its ISD Nucleotide Record and GenBank accession numbers, including raw DNA sequences for the protein. Using the NCBI's BLAST program, compare amino acids of the yearly vaccine with viruses isolated by the CDC. Highlight the effectiveness of the vaccine using pair-wise alignment. Analyze the hemagglutinin (HA) protein antigens of actual virus strains that occurred during period under investigation. Capture isolated viruses for each flu season along with their ISD Nucleotide Record and GenBank accession numbers, including raw DNA sequences in FASTA format. Using isolated viruses encountered in yearly flu outbreaks, compare yearly viruses with chosen vaccines using BLAST program. Using a multiple sequencing alignment program, CLUSTAL-W, compare viruses by arranging them into phylogenic trees. Repeat analysis for the neuraminidase (NA) protein antigens. Determine if an alternate candidate virus strain shares a higher degree of similarity to the HA and NA proteins that occurred during flu seasons under review.

Results:
In our research we were able to use the CDC databases to locate data summarizing the flu activity for the years 1999-2005. We were then able to tabulate the data and produce a chart that clearly documents the severity of Influenza outbreaks during those years. This data indicates that Influenza A (H3N2) was the prevalent virus in circulation during those six years. In the 2003-2004 season the H3N2 cases increased dramatically indicating a possible

antigenic shift might have occurred with the H3N2 and B viruses. Using the NCBI's BLAST program, we were able to make sequences producing significant alignment graphs for each selected vaccine. Using the ISD Database, we isolated 42 Influenza A H3N2 viruses that occurred between the 2002-2003, 2003-2004 and 2004-2005 seasons, capturing their accession numbers and DNA sequences. After analyzing the sequences, we were able to produce a phylogenic tree which implied that there was significant drift in the HA antigent from 2002-2004. Neither the Fujian/411/2002 nor the Wellington/1/2004 viruses were at the base of the tree. We believe from looking at the tree and the sequences that produced significant alignments that the A/Netherlands/220/2003 would have stimulated HA antibodies in a higher. Using the ISD Database, we isolated 19 Influenza A H3N2 viruses where the NA segment had been mapped that occurred between the 2002-2003, 2003-2004 and 2004-2005 seasons, capturing their accession numbers and DNA sequences. Refer to Table 8 for these details. After analyzing the sequences, we were able to produce a phylogenic tree, which implied that there was no drift in the NA antigent from 2002-2004. Wyoming/3/2003, which is an antigentically equivalent strain to Fujian/411/2002, would have been as good as a selection for this vaccine based on the NA gene.

Conclusions:

From phylogenic trees using H3N2 virus strains occurring during the 2002-2004 seasons, we was able to isolate another virus strain candidate that appears to be as good or better match for the outbreaks that occurred during these years. Our data indicates that the A/Netherlands/220/2003 would have stimulated HA antibodies in a higher frequency than the A/Fujian/411/2002 based vaccine therefore providing better protection to the population. We understand there are limitations with our data though we looked at many viruses. Optimizing the phylogenic trees or choosing different viruses might have produced a slightly difference results.

Question

Has the annually formulated Influenza A (H3N2) vaccine been an effective deterrent to worldwide outbreaks of orthomyxoviruses over the last six years?

Hypothesis

We believe that if a different H3N2 vaccine strain were selected, it would have stimulated a higher antibody response to the Influenza virus during the 2003-2005 flu seasons.

Review of Literature

Influenza, also known as the flu, is a viral disease that infects the upper respiratory tract. This illness is capable of causing epidemics and pandemics. Epidemics and pandemics are generally referred to as widespread diseases that affect many individuals in a population. Table 1 lists the Influenza A pandemics of the last century. These flu pandemics have killed millions of people. For example, the "Spanish Flu" (H1N1) pandemic of 1918-1919 is believed to have killed more people than those who died in World War I. The second largest pandemic called the "Asian flu" (H2N2) in 1957 resulted in seventy thousand deaths in the United States alone. In regions similar to Asia, where large concentrations of people and animals live together in close proximity, cross-species infections enable genetic material to be exchanged between the various strains of flu. There are two flu seasons each year that occur during the winter season of the Northern and Southern hemispheres. Cold and dry weather enables the virus to survive longer outside the body than in other climates, which is why seasonal epidemics in temperate areas appear in winter. Constant genetic changes in influenza viruses mean that the vaccines' virus composition must be adjusted annually to include the most recent circulating influenza A (H3N2) and A (H1N1) and the influenza B viruses.

Influenza belongs to the RNA (ribonucleic acid) family of orthomyxoviruses. Instead of using DNA (deoxyribonucleic acid) as their genetic material, this virus uses RNA. There are three types of Influenza virus: A, B, and C. Influenza A infects humans, avian, and swine. Influenza B and C infects humans exclusively. Gene sequence analyses indicate that human influenza viruses might have originated either directly or indirectly from avian virus ancestors.

Currently, there are three different influenza strains in circulation worldwide: two type A viruses and one type B. Influenza A can be broken down into two subtypes: A (H3N2) and A (H1N1/H2N1), a combination currently associated with most worldwide pandemics.

The two major surface proteins of Influenza viruses, hemagglutinin (HA) and neuraminidase (NA), negotiate and release the Influenza virus into the host cell. The virus is arranged into eight different RNA segments. Table 2 highlights the fourteen (now sixteen) HA subtypes and nine NA subtypes identified in birds, but note only a limited number of combinations have been observed in humans. The HA gene contributes to the major immune response to the virus. A person's immunity to the surface antigens reduces the likelihood of infection. Furthermore, antibodies to one antigenic variant of influenza virus may not protect the host against a new antigenic variant of the same type or subtype. Antigenic drift is the natural mutation over time of known strains of influenza where two different strains of influenza combine to form a new subtype, and is characterized by a mixture of the surface antigens of the two original strains. A rare occurrence of an antigenic shift can produce different strains of influenza where there is no natural immunity as in the case of the emergence of the H3N2 subtype in 1968 also known as the "Hong Kong flu".

Frequent development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual incorporation of new strains every year. The emergence of the " Hong Kong flu" in 1968-69 marked the beginning of the type A (H3N2) era. When this virus first emerged, it was associated with lower mortality than that caused by the two previous pandemic viruses. Since 1977, both Influenza A H3N2 and H1N1 viruses have coexisted and been in circulation.

The National Institute of Health (NIH), World Health Organization (WHO) and the Center of Disease Control (CDC) have monitoring stations around the world that analyze Influenza outbreaks as they occur. Constant genetic changes in the annual vaccines' virus composition include the most recent circulating Influenza A (H3N2), A (H1N1) and the B DNA. Tables 3 and 4 document the virus strains selected for the annual vaccines for the past six years from the Northern and Southern hemispheres. Annual Influenza production is a complex process taking ten months that can yield a less effective vaccine for the following season's outbreak. Our experiment will examine the potential effectiveness of these and other candidate vaccines.

Materials

Computer with Internet access and browser
Word Processing Program (Microsoft-Word)
Spreadsheet Program (Microsoft-Excel)
Various Databases (Reference Bibliography for website addresses)
The Influenza Sequence Database (ISD)

Flu viruses sequences and information on vaccines

US Centers for Disease Control and Prevention (CDC) Flu Home Page

Flu activity by type and subtype

National Center for Biotechnology Information (NCBI) Databases and Programs

Nucleotides and Protein Sequences

BLAST

FASTA

CLUSTAL-W Multiple Sequence Alignment Tool

Procedure

1) For years 1999-2005, identify Influenza vaccine strains selected by WHO and CDC organizations using CDC Databases.
2) Document statistics on worldwide flu outbreaks categorized by Influenza subtypes using the Influenza Sequence Database.
3) Using the Influenza Sequence Database, locate the related nucleotide for each virus strain identifying its ISD Nucleotide Record and GenBank accession numbers, including raw DNA sequences for the protein.
4) Using the NCBI's BLAST program, compare amino acids of the yearly vaccine with viruses isolated by the CDC. Highlight the effectiveness of the vaccine using pair-wise alignment.
5) Analyze the hemagglutinin (HA) protein antigens of actual virus strains that occurred during period under investigation. Capture isolated viruses for each flu season along with their ISD Nucleotide Record and GenBank accession numbers, including raw DNA sequences in FASTA format.
6) Using isolated viruses encountered in yearly flu outbreak, compare yearly viruses with chosen vaccines using BLAST program.
7) Using a multiple sequencing alignment program, CLUSTAL-W, compare viruses by arranging them into phylogenic trees.
8) Repeat steps 5-7 analyzing the neuraminidase (NA) protein antigens.
9) Determine if an alternate candidate virus strain shares a higher degree of similarity to the HA and NA proteins that occurred during flu seasons under review.

Results

The US Influenza season runs between September to May of the following year. In our research we were able to use the CDC databases to locate data summarizing the flu activity for the years 1999-2005. We were then able to tabulate the data and produce a chart that clearly documents the severity of Influenza outbreaks during those years, see Figure 1. This data indicates that Influenza A (H3N2) was the prevalent virus in circulation during those six years. Overlapping the data, Figure 2, also shows that the 2003 and 2004 flu seasons peaked earlier during the season in November and December rather than in January thru March as in earlier years. Transmission of disease and mortality rates also appears to be more drastic during 2003 and 2004 seasons, which might imply that vaccines used during those years may not have been as effective. In Table 4, comparing the Northern and Southern hemispheres Influenza A H3N2, we documented virus strains selected.

From Figure 1, we were able to document the following trends. In the 1999-2000 season we saw that Influenza A of unknown type and Influenza A H3N2 were common. During the 2000-2001 season the Moscow/10/99 vaccine was used and H3N2 virus disappeared. In 2001-2002 season, the Panama/2007/99 strain was used and H3N2 virus reappeared. Then in the following two seasons the Panama/2007/99 strain was used again and the H3N2 cases initially decreased but the B virus re-emerged. Then in the 2003-2004 season the H3N2 cases increased dramatically indicating a possible antigenic shift might have occurred with the H3N2 and B viruses. The mortality rate for the 2003-2004 season was also very high. The CDC selected a new vaccine strain called Fujian/411/2002 for the 2004-2005 season, but it also appears to not have been extremely effective.

From using the NCBI's BLAST program, we were able to make sequences producing significant alignment graphs for each selected vaccine. Tables 5-7 show how effective the yearly vaccine was for the viruses isolated during each season. The Moscow/10/99 virus strain had two columns that were hard to match which were amino acids groupings 541 and 661. In the Panama/2007/99 vaccine strain the chart had two columns that did not align closely which were 61 and 541. Then in the Fujian/11/2002 virus strain there were three columns that did not align well. They were 61, 421, and 721. These figures were able to show us how closely related the actual vaccines were to the virus strain. Each grouping represented 60 amino acids. Now we knew what to look for in getting a better match for the H3N2 outbreaks during the 2003-2004 and 2004-2005 seasons where we will focus the rest of our research. We split the following analysis of the HA and NA antigents between us: Kathryn focused on HA and Krystina focused on NA.

HA Antigents

Using the ISD Database, we isolated 42 Influenza A H3N2 viruses that occurred between the 2002-2003, 2003-2004 and 2004-2005 seasons, capturing their accession numbers and DNA sequences. Refer to Table 8 for these details. After analyzing the sequences, we were able to produce a phylogenic tree which implied that there was significant drift in the HA antigent from 2002-2004. Neither the Fujian/411/2002 or the Wellington/1/2004 viruses were at the base of the tree. We believe from looking at the tree and the sequences that produced significant alignments that the A/Netherlands/220/2003 would have stimulated HA antibodies in a higher frequency as shown in Figure 3. It is also clear from the tree that the H3N2 virus has migrated away from the Panama and Moscow vaccines used in previously flu seasons/ The tree has developed a new branch with the A/Netherlands/220/2003 at the base.

NA Antigents

Using the ISD Database, we isolated 19 Influenza A H3N2 viruses where the NA segment had been mapped that occurred between the 2002-2003, 2003-2004 and 2004-2005 seasons, capturing their accession numbers and DNA sequences. Refer to Table 8 for these details. After analyzing the sequences, we were able to produce a phylogenic tree, which implied that there was no drift in the NA antigent from 2002-2004. Wyoming/3/2003 which is a antigentically equivalent strain to Fujian/411/20012 would have been as good as a selection for this vaccine based on the NA gene as shown in Figure 4.

Conclusions

From phylogenic trees using H3N2 virus strains occurring during the 2002-2004 seasons, we were able to isolate another virus strain candidate that appears to be as good or better match for the outbreaks that occurred during these years. Our data indicates that the A/Netherlands/220/2003 would have stimulated HA antibodies in a higher frequency than the A/Fujian/411/2002 based vaccine therefore providing better protection to the population. We understand there are limitations with our data though we looked at many viruses. Optimizing the phylogenic trees or choosing different viruses might have produced a slightly different result.

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Tables and Figures

Table 1 – Influenza A Pandemics of the Last Century

Year of Origin	Subtype in Circulation	Source	Impact
1890	H2N8		
1900	H3N8		
1918	H1N1 (Spanish Flu)	Possible emergence from swine or avian host of a mutated H1N1 virus	21M deaths globally 500K deaths US
1957	H2N2 (Asian Flu)	Posible mixed inflection of an animal with human H1N1 and avian H2N2 virus in Asia	70K deaths US H1N1 virus disapears
1968	H3N2 (Hong Kong)	High probability of mixed inflection of an animal with human H2N2 and avian H3Nx virus strains in Asia	34K deaths US H2N2 virus disappeared
1977	H3N2 and H1N1 (Russian Flu)	Source unknown but virus almost identical to 1950 virus	Benign panademic involving persons born after 1950 Coexistence of H3N2 and H1N1 in humans since 1977

Table 2 – Influenza Subtypes of Influenza A Virus

Hemagglutinin and Neuraminidase
Subtypes of Influenza A Virus
Species of Origin

Subtype	Human	Swine	Horse	Bird
H1	PR/8/34	Sw/Ia/15/30		Dk/Alb/35/76
H2	Sing/1/57			Dk/Ger/1215/73
H3	HK/1/68	Sw/Taiwan/70	Eq/Miami/1/63	Dk/Ukr/1/63
H4				Dk/Cz/56
H5				Tern/S.A/61
H6				Ty/Mass/3740/65
H7			Eq/Prague/1/56	FPV/Dutch/27
H8				Ty/Ont/6118/68
H9				Ty/Wis/1/66
H10				Ck/Ger/N/49
H11				Dk/Eng/56
H12				Dk/Alb/60/76
H13				Gull/Md/704/77
H14				Mall/Gurjev/263/82
N1	PR/8/34	Sw/Ia/15/30		Ck/Scot/59
N2	Sing/1/57	Sw/Taiwan/70		Ty/Mass/3740/65
N3				Tern/S.A/61
N4				Ty/Ont/6118/68
N5				Sh/Austral/1/72
N6				Dk/Cz/56
N7			Eq/Prague/1/56	FPV/Dutch/27
N8			Eq/Miami/1/63	Dk/Ukr/1/63
N9				Dk/Mem/546/74

Table 3 – Influenza Strains Contained in US Trivalent Vaccines

	A/H3N2	ACCESSION #	A/H1N1 & H1N2	ACCESSION #	B	ACCESSION #
1999-2000	Sydney/5/97 A/Panama/2007/99	ISDNASYD97	A/Beijing/262/95	ISDNX127	B/Beijing/184/93 B/Yamanashi/166/98	ISDNYAM98
2000-2001	A/Panama/2007/99 A/Moscow/10/99	ISDNCDA001	A/New Caledonia/20/99	ISDNAU0001	B/Yamanashi/166/98 B/Beijing/184/93	ISDNYAM98
2001-2002	A/Moscow/10/99 A/Panama/2007/99	ISDN13277	A/New Caledonia/20/99	ISDNAU0001	B/Sichuan/379/99 B/Johannesburg/5/99 B/Guangdong/120/2000 B/Victoria/504/2000	ISDN13281
2002-2003	A/Moscow/10/99 A/Panama/2007/99	ISDN13277	A/New Caledonia/20/99	ISDNAU0001	B/Hong Kong/330/2001 B/Hong Kong/1434/2002	ISDN13279
2003-2004	A/Moscow/10/99 A/Panama/2007/99	ISDN13277	A/New Caledonia/20/99	ISDNAU0001	B/Hong Kong/330/2001 B/Hong Kong/1434/2002 B/Shangdong/7/97	ISDN13279
2004-2005	A/Fujian/411/2002 A/Wyoming/3/2003 A/Kumamoto/102/2002	ISDN38157	A/New Caledonia/20/99	ISDNAU0001	B/Shanghai/361/2002 B/Jilin/20/2003 B/Jiangsu/10/2003	ISDN80784

INDENTED STRAINS REPRESENT ANTIGENICALLY EQUIVALENTS

Table 4 – Influenza Strains Contained in Northern and Southern Hemisphere H3N2 Vaccine

	A/H3N2 Northern Hemisphere	ACCESSION #	A/H3N2 Southern Hemisphere	ACCESSION #
1999-2000	Sydney/5/97 A/Panama/2007/99	ISDNASYD97	A/Moscow/10/99	ISDN13277
2000-2001	A/Panama/2007/99 A/Moscow/10/99	ISDNCDA001	A/Moscow/10/99	ISDN13277
2001-2002	A/Moscow/10/99 A/Panama/2007/99	ISDN13277	A/Moscow/10/99 A/Panama/2007/99	ISDN13277
2002-2003	A/Moscow/10/99 A/Panama/2007/99	ISDN13277	A/Moscow/10/99 A/Panama/2007/99	ISDN13277
2003-2004	A/Moscow/10/99 A/Panama/2007/99	ISDN13277	A/Fujian/411/2002 A/Wyoming/3/2003 A/Fujian/330/2002 A/Kumamoto/102/2002	ISDN38157
2004-2005	A/Fujian/411/2002 A/Wyoming/3/2003 A/Kumamoto/102/2002	ISDN38157	A/ Wellington/1/2004	ISDN69596
2005-2006	A/California/7/2004	ISDN110647/8		

INDENTED STRAINS REPRESENT ANTIGENICALLY EQUIVALENTS

Figure 1 – US Influenza Trends between 1999-2005

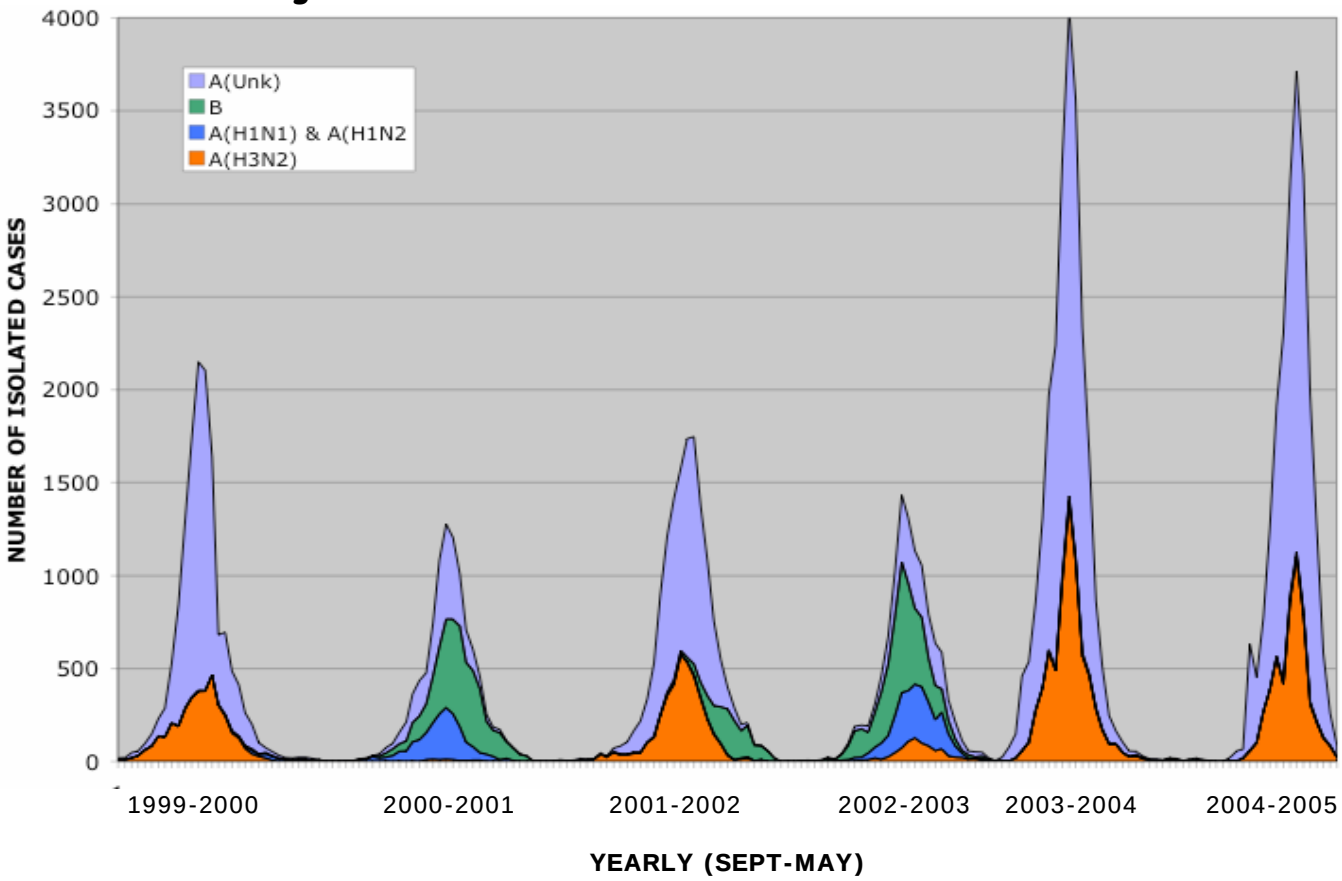

Figure 2 – Yearly Influenza Trends

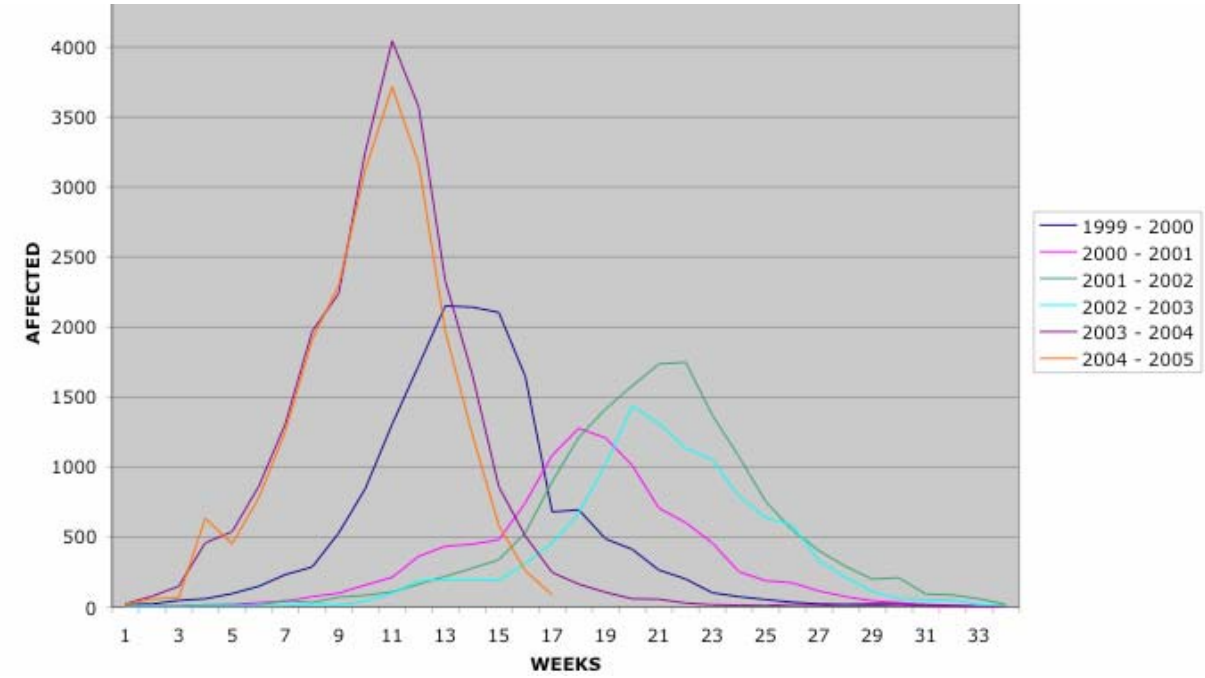

Table 5 – Fujian/411/2002 Vaccine Strain

FUJIAN411/2002 VACCINE STRAIN (2004-2005)
SEQUENCES PRODUCING SIGNIFICANT ALIGNMENTS

ACCESSION	VIRUS	SCORE	MATCHING IDENTITIES	%	1	61	121	181	241	301	361	421	481	541	601	661	721	781	841	901	961	1021
AY589661.1	A/KYON/347/2002	2042	1039/1042	0.997		1						1					1					
AY589654.1	A/CHUN/447/2002	2034	1038/1042	0.996		1						1					1				1	
AY589649.1	A/CHEJU/311/2002	2034	1038/1042	0.996		1						1					1				1	
AY589653.1	A/CHEON/432/2002	2026	1037/1042	0.995		1						1		1			1				1	
AY589647.1	A/PUSAN/504/2002	2026	1037/1042	0.995		1						1		1			1				1	
AY589656.1	A/DAEJE/39092002	2018	1036/1042	0.994		1						1	1	1			1				1	
AY589658.1	A/KWANG/219/2002	2010	1035/1042	0.993		1						2				1	1				1	
AY589648.1	A/CHEJU/274/2002	2010	1035/1042	0.993		1				1		1	1	1			1				2	
AY589659.1	A/KWANG/243/2002	2002	1034/1042	0.992		1				1		2				1	1				1	1
AY661036.1	A/NETH/217/2003	1925	983/987	0.996	1	1	1										1					
AY661032.1	A/FINL/170/2003	1925	983/987	0.996	1	1						1					1					
AY661035.1	A/NETH/213/2003	1917	982/987	0.995	1	1	1					1					1					
AY589651.1	A/CHEON/338/2002	1915	1023/1042	0.982		1						1		3	2	4	1	3	1		3	
AY589650.1	A/CHEON/323/2002	1907	1022/1042	0.981		1			1			1		3	2	4	1	3	1		3	
AY695087.1	A/ZHEJI/95/2003	1901	980/987	0.993		1	1			1		1		1		1	1					
AY661037.1	A/NETH/222/2003	1901	980/987	0.993	1	1	1					3					1					
AY589652.1	A/CHEON/340/2002	1899	1021/1042	0.980		1				1		2		3	2	4	1	3	1		3	
AY714347.1	A/ZHEJI/92/2003	1893	979/987	0.992		1				2		1				1	2			1		
AY695089.1	A/NINGB/198/2003	1893	979/987	0.992		1						1		1	1	1	1			2		

Table 6 – Panama/2007/99 Vaccine Strain

PANAMA/2007/99 VACCINE STRAIN (2001-2004)
SEQUENCES PRODUCING SIGNIFICANT ALIGNMENTS

ACCESSION	VIRUS	SCORE	MATCHING IDENTITIES	%	1	61	121	181	241	301	361	421	481	541	601	661	721	781	841	901
AF501517.1	A/VIRG/21833/99	1941	985/987	0.998		1								1						
AF297097.1	A/CHAR/69/99	1941	985/987	0.998		1								1						
AF534058.1	A/CORD/1007333/01	1935	982/984	0.998								1								1
AF534037.1	A/MISI/195/99	1935	982/984	0.998		1								1						
AF501530.1	A/VIRG/21735/99	1933	984/987	0.997		1								1	1					
AF501529.1	A/UK/26554/99	1933	984/987	0.997		1				1				1						
AF501528.1	A/VIRG/21828/99	1933	984/987	0.997		1		1						1						
AF501523.1	A/VIRG/21799/99	1933	984/987	0.997		1								2						
AF501522.1	A/VIRG/21845/99	1933	984/987	0.997		1								1		1				
AF501521.1	A/VIRG/G1/99	1933	984/987	0.997		1								2						
AF297095.1	A/CHAR/73/99	1933	984/987	0.997		1		1						1						
AF297094.1	A/CHAR/10/99	1933	984/987	0.997		1								1			1			
AY661026.1	A/NETH/301/99	1931	992/998	0.984		1			1			1	1	1			1			
AF501519.1	A/VIRG/21754/99	1929	983/987	0.996		1								1	1		1			
AF534042.1	A/SAFE/9/99	1927	981/984	0.997	1	1								1						
AF534040.1	A/MADEPL/267/99	1927	981/984	0.997		1					1			1						
AF534035.1	A/CHARC/140/99	1927	981/984	0.997		1								1			1			
AF501527.1	A/UK/34300/99	1925	983/987	0.996		1							1	1				1		
AF534039.1	A/NEUG/102/99	1919	980/984	0.996	1	1						1		1						
AF534033.1	A/BUEARES/M7/99	1919	980/984	0.996	1	1						1		1						
AY634010.1	A/FRAN/15/00	1917	982/987	0.995		1							1	1		1		1		
AF501516.1	A/CAND/33312/99	1917	982/987	0.9949		2		1						1			1			

Table 7 – Moscow/10/99 Vaccine Strain

MOSCOW/10/99 VACCINE STRAIN (2000-2001)
SEQUENCES PRODUCING SIGNIFICANT ALIGNMENTS

ACCESSION	VIRUS	SCORE	MATCHING IDENTITIES	%	1	61	121	181	241	301	361	421	481	541	601	661	721	781	841	901
AF255029.1	A/CNIC/149/98	1943	986/988	0.998										1		1				
AF255024.1	A/CNIC/121/98	1943	986/988	0.998										1		1				
AF255023.1	A/CNIC/109/98	1943	986/988	0.998										1		1				
AF255022.1	A/CNIC/97/98	1943	986/988	0.998										1		1				
AF255021.1	A/CNIC/96/98	1943	986/988	0.998										1		1				
AF501526.1	A/PENN/20109/99	1941	985/987	0.998										1		1				
AF442481.1	A/FINL/680/99	1935	982/984	0.998										1		1				
AY661019.1	A/MOSCOW/10/99	1935	984/988	0.996								1		2			1			
AF255027.1	A/CNIC/145/98	1935	985/988	0.997										1		1	1			
AF255026.1	A/CNIC/130/98	1935	985/988	0.997				1						1		1				
AF255025.1	A/CHIC/125/98	1935	985/988	0.997										1		1		1		
AF255019.1	A/CHIC/3/98	1935	985/988	0.997	1									1		1				
AY035588.1	A/HK/1143/99	1933	984/987	0.997	1									1		1				
AF382320.1	A/HK/1143/99	1933	984/987	0.997	1									1		1				
AF501534.1	A/IND/2817/99	1933	984/987	0.997				1						1		1				
AY138513.1	A/ZHEJ/6/99	1933	984/987	0.997						1				1		1				
AF442469.1	A/FINL/616/99	1927	981/984	0.997									1	1		1				
AY035590.1	A/HK/1179/99	1927	984/988	0.996	1			1						1		1				
AY035589.1	A/HK/1179/99	1927	984/988	0.996					1		1			1		1				
AF382324.1	A/HK/1179/99	1927	984/988	0.996	1			1						1		1				
AF442473.1	A/FINL/645/99	1919	980/984	0.996	1									2		1				
AY035592.1	A/HK/1182/99	1919	983/988	0.995	1					1	1			1		1				

Table 8 – HA and NA Accession Numbers for Isolated Viruses

Year	Virus Strain	Accession # HA	Accession # NA
2005	A/Norway/70/2005	ISDN119864	ISDN119865
2004	A/Brazil/1759/2004		ISDN68695
2004	A/California/7/2004 (cell-passaged)	ISDN110647	
2004	A/California/7/2004 (egg-passaged)	ISDN110648	
2004	A/Charlottesville/03/2004	AY947474	
2004	A/Hong Kong/2982/2004		ISDN68696
2004	A/Jiangsu/131/2004	AY854046	
2004	A/Jiangsu/91/2004	AY854047	
2004	A/Johannesburg/2/04	ISDN110773	
2004	A/Nepal/1732/2004	AY945270	
2004	A/New York/52/2004	CY000257	
2004	A/New York/69/2004		CY000563
2004	A/Norway/807/2004	ISDN69439	ISDN69440
2004	A/Singapore/36/2004		ISDN68697
2004	A/Wellington/1/2004	ISDN69596	ISDN68699
2003	A/Denmark/70/03		AY531018
2003	A/Denmark/92/03	AY531055	
2003	A/Finland/170/03	AY661032	
2003	A/Jiangsu/Children67/2003	AY851473	
2003	A/Johannesburg/64/03	AY389355	
2003	A/Middleburg/45/03	AY389357	
2003	A/Netherlands/222/03	AY661037	
2003	A/New York/54/2003		CY000099
2003	A/Ningbo/198/03	AY695089	
2003	A/Taiwan/1523/2003	AY479982	
2003	A/Taiwan/7100/2003	AY604830	
2003	A/Wyoming/3/2003	ISDN38156	ISDN68694
2003	A/Zhejiang/92/03	AY714347	
2002	A/Cheju/311/2002	AY589649	
2002	A/Chungnam/447/2002	AY589654	AY589668
2002	A/Daejeon/390/2002	AY589656	AY589670
2002	A/Fujian/411/2002	ISDN38157	
2002	A/Gyeongbuk/2/02	AY377129	
2002	A/Incheon/260/2002	AY589657	
2002	A/Ireland/1092/2002		AJ457947
2002	A/Kumamoto/102/2002E	ISDN69739	
2002	A/Kwangju/243/2002	AY589659	
2002	A/Kyongbuk/320/2002	AY589660	AY589674
2002	A/Kyongnam/347/2002	AY589661	
2002	A/Netherlands/120/02	AY661030	
2002	A/New York/135/2002	CY000337	CY000339
2002	A/New York/95/2002	CY000217	
2002	A/ningbo/17/2002	AY138518	
2002	A/ningbo/25/2002	AY138517	
2002	A/Oslo/669/2002	ISDN13294	ISDN13295
2002	A/Pusan/504/2002	AY589647	AY589676
2002	A/Sapporo/304/02		U42776
2002	A/Taiwan/5153/2002	AY604816	
2002	A/Texas/131/2002	AY947476	AY947477
2002	A/zhejiang/11/2002	AY138516	
2002	A/zhejiang/8/2002	AY138519	
1999	A/Moscow/10/99	ISDN13277	
1999	A/Panama/2007/99	ISDNCDA001	

Figure 3 – Influenza A H3N2 HA Antigent Phylogenic Tree

Figure 4 – Influenza A H3N2 NA Antigent Phylogenic Tree

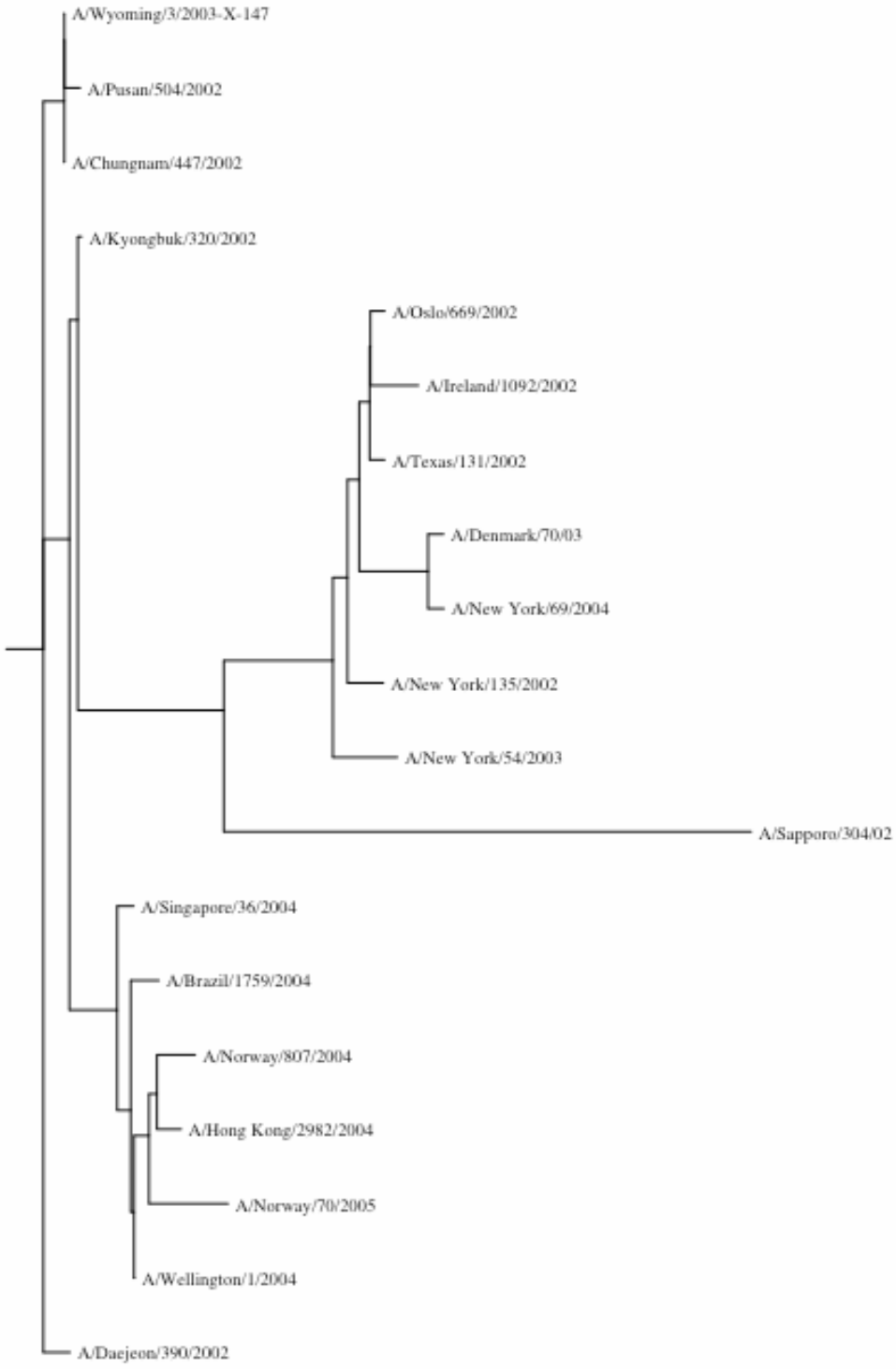

Figure 5 – Picture of Kathryn's Display at the Synopsys Championship Science Fair