

Forecasting pandemic influenza: periodic re-appearance of highly virulent H1N1 viruses and emergence of new recombinant viruses capable of crossing interspecies barriers as a prologue to pandemics; new approaches for design of geographical and polyvalent vaccines of new generation

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Number of nucleotide sequences of different influenza serotypes available in NCBI in 2006

	<i>N1</i>	<i>N2</i>	<i>N3</i>	<i>N4</i>	<i>N5</i>	<i>N6</i>	<i>N7</i>	<i>N8</i>	<i>N9</i>
<i>H1</i>	1195	555	5	-	-	3	4	1	11
<i>H2</i>	25	399	64	-	8	-	2	5	70
<i>H3</i>	8	4859	37	2	2	229	-	307	-
<i>H4</i>	11	7	1	3	3	172	-	11	2
<i>H5</i>	2188	327	31	-	-	3	2	10	11
<i>H6</i>	122	167	-	8	20	3	3	59	6
<i>H7</i>	128	229	177	2	1	-	138	1	-
<i>H8</i>	-	-	-	11	-	-	-	-	-
<i>H9</i>	25	1514	1	-	2	1	1	1	3
<i>H10</i>	-	-	-	3	4	-	15	2	2
<i>H11</i>	7	8	3	2	-	9	-	7	10
<i>H12</i>	1	-	-	3	7	-	-	1	-
<i>H13</i>	-	4	1	-	-	37	2	8	3
<i>H14</i>	-	-	-	-	1	-	-	-	-
<i>H15</i>	-	-	-	-	-	-	-	1	1
<i>H16</i>	-	-	20	-	-	-	-	-	-

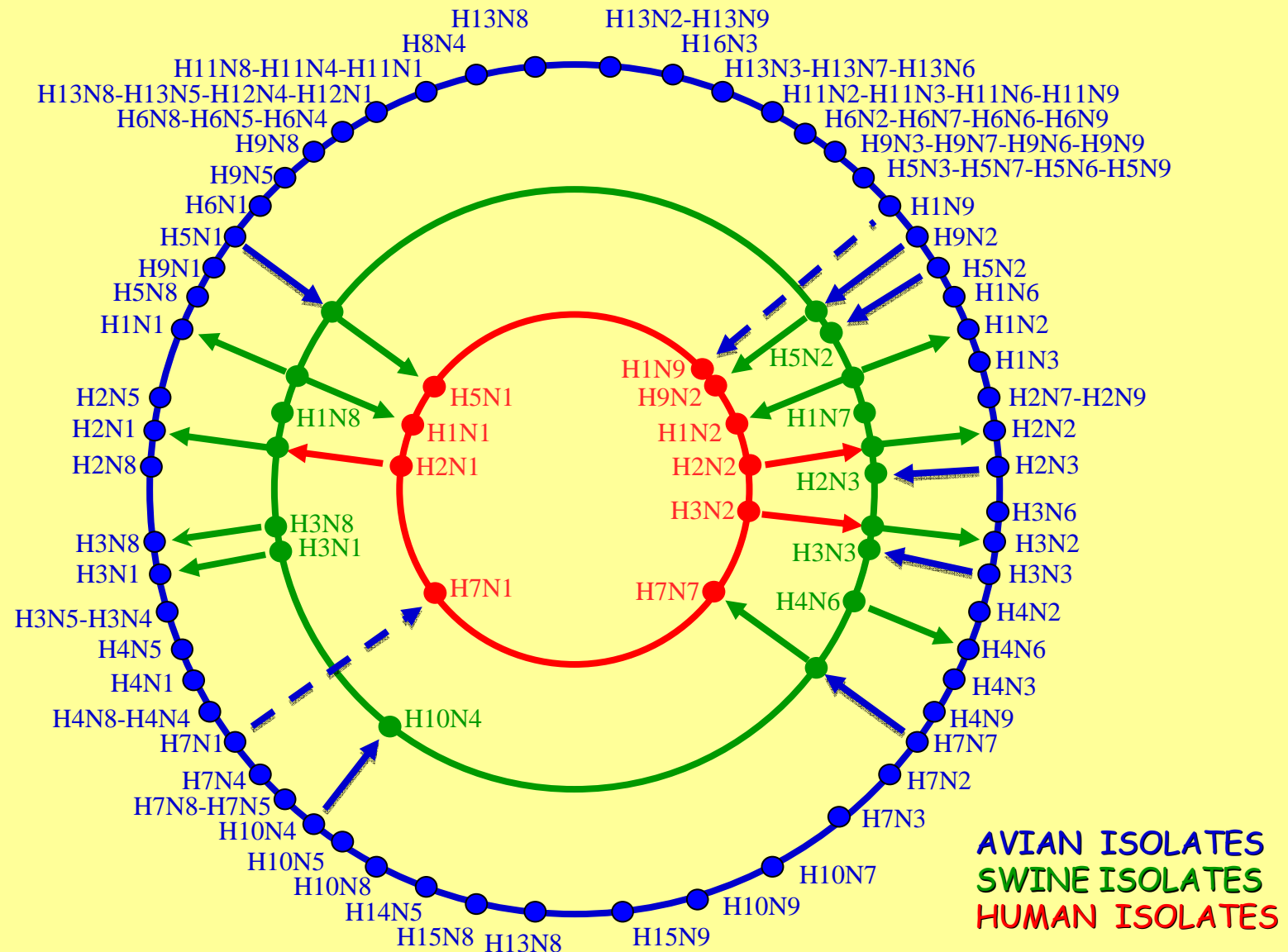
Total: 13,358

Number of nucleotide sequences of different influenza serotypes available in NCBI in 2009

	<i>N1</i>	<i>N2</i>	<i>N3</i>	<i>N4</i>	<i>N5</i>	<i>N6</i>	<i>N7</i>	<i>N8</i>	<i>N9</i>
<i>H1</i>	12611	1457	42	29	14	40	5	17	25
<i>H2</i>	107	1076	329	32	91	1	36	27	153
<i>H3</i>	268	27218	113	14	63	571	29	1910	14
<i>H4</i>	53	142	40	34	32	1158	15	302	35
<i>H5</i>	14624	1475	401	5	11	12	120	30	64
<i>H6</i>	1630	932	52	62	137	115	3	360	22
<i>H7</i>	591	2396	1004	32	17	10	410	33	33
<i>H8</i>	-	3	1	147	1	-	2	-	-
<i>H9</i>	75	6395	9	14	40	25	4	12	7
<i>H10</i>	15	10	52	26	30	20	460	47	20
<i>H11</i>	50	85	58	19	-	37	-	26	436
<i>H12</i>	9	5	1	14	253	24	-	1	17
<i>H13</i>	-	39	12	-	-	97	10	9	20
<i>H14</i>	-	-	1	-	12	4	-	-	-
<i>H15</i>	-	3	-	-	-	1	-	8	25
<i>H16</i>	-	-	52	-	-	-	-	-	-

Total: 81,427

Radial classification of influenza A viruses

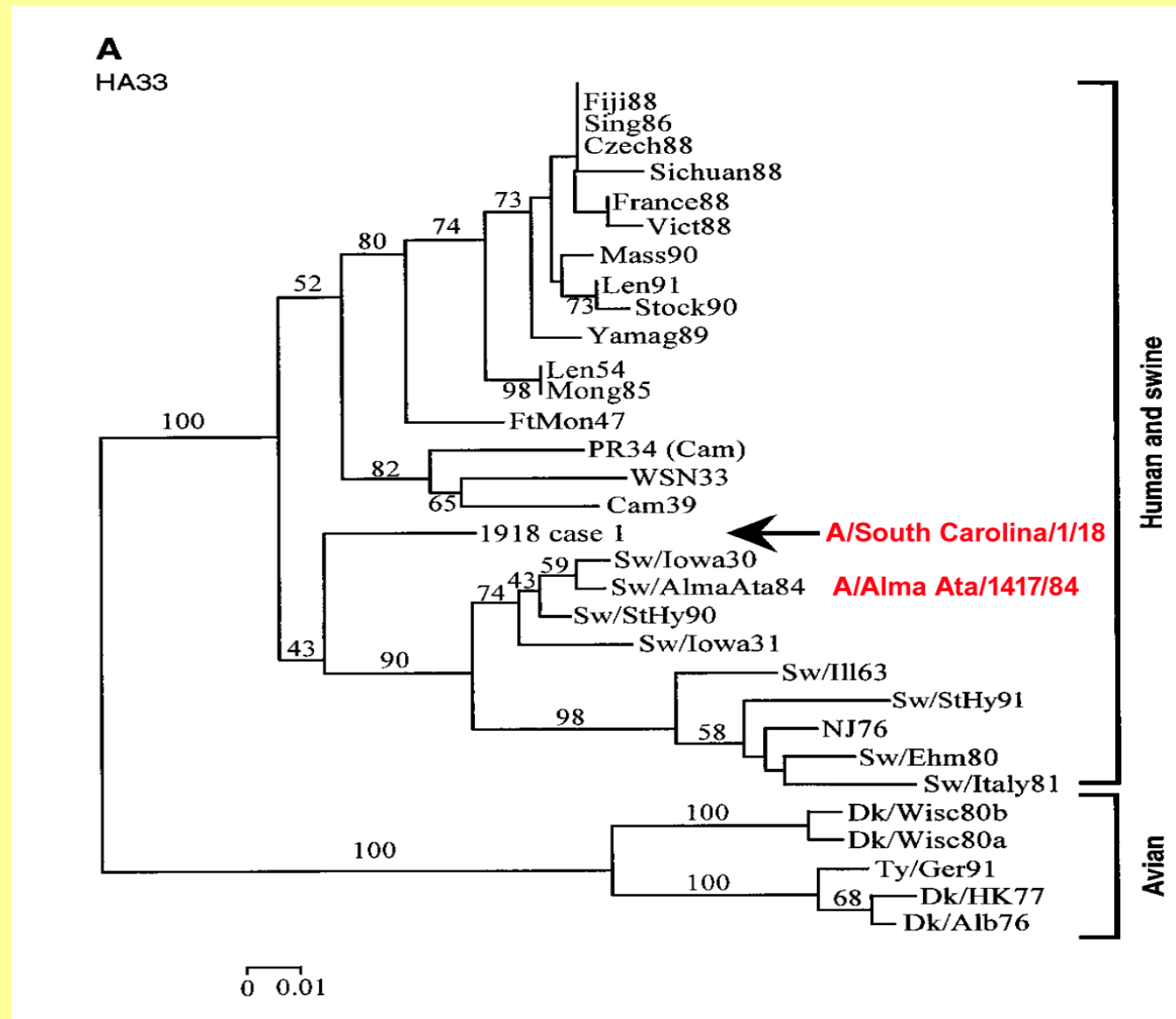


Swine-like influenza viruses isolated from humans in the Almaty region

Isolate	Source	Outcome	Serotype	Contact with swine	Comments
A/Almaty/1044/83	Male, 65, lung sample	Fatal	Hsw1N1	Yes	Related to A/swine/Iowa/15/30, A/NJ/8/76 by HA; to A/WS/33 by NA; 35% coworkers seropositive to Hsw1
A/Almaty/1196/83	Male, 27, nasal wash	Non-fatal	H1N2-Hsw1N2	No	Related to A/Bangkok/1/79 (H3N2) by NA
A/Almaty/1135/83	Female, 10, nasal wash	Non-fatal	A/H1N1-Hsw1N1	No	Related to A/duck/Tashkent/78 (H3N1) by NA
A/Almaty/1417/84	Male, 26, nasal wash	Non-fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized; available sequence data in (3)
A/Almaty/1415/84	14 mo child, nasal wash	Non-fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/1414/84	Female, 13, nasal wash	Non-fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/1382/84	Female, 32, blood (2x)	Non-fatal	A/H1N1-Hsw1N1	Unknown	Seropositive to Hsw1N1, seronegative later despite the virus presence
A/Almaty/247/84	Female, 18, blood (2x)	Non-fatal	A/H1N1-Hsw1N1	Unknown	Seropositive to Hsw1N1 and A/Almaty/1417/84; no HAI to Hsw1
A/Almaty/1395/84	Male, 46, nasal wash	Fatal	Hsw1N1	No	Insufficiently characterized
A/Almaty/1367/84	Female, 47, trachea, lung	Fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/3/85	1 day old newborn, CSF	Congenital encephalopathy	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/5/98	Female, 23, ???	Repeated spontaneous abortions	Hsw1N1	No	Insufficiently characterized; sequence data unpublished
A/Almaty/32/98	Female, 25, blood	Prem. birth, spontaneous abortions	A/H1N1-Hsw1N1	No	Insufficiently characterized; sequence data unpublished
A/Almaty/84/98	Female, 31, blood (2x)	Multiple sclerosis, immunodeficit	A/H1N1-Hsw1N1	No	Insufficiently characterized

Beklemishev, et al.(1993) Mol Gen Mikrobiol Virusol 1, 24-27

Phylogenetic analysis of the HA gene of one swine-like virus isolate

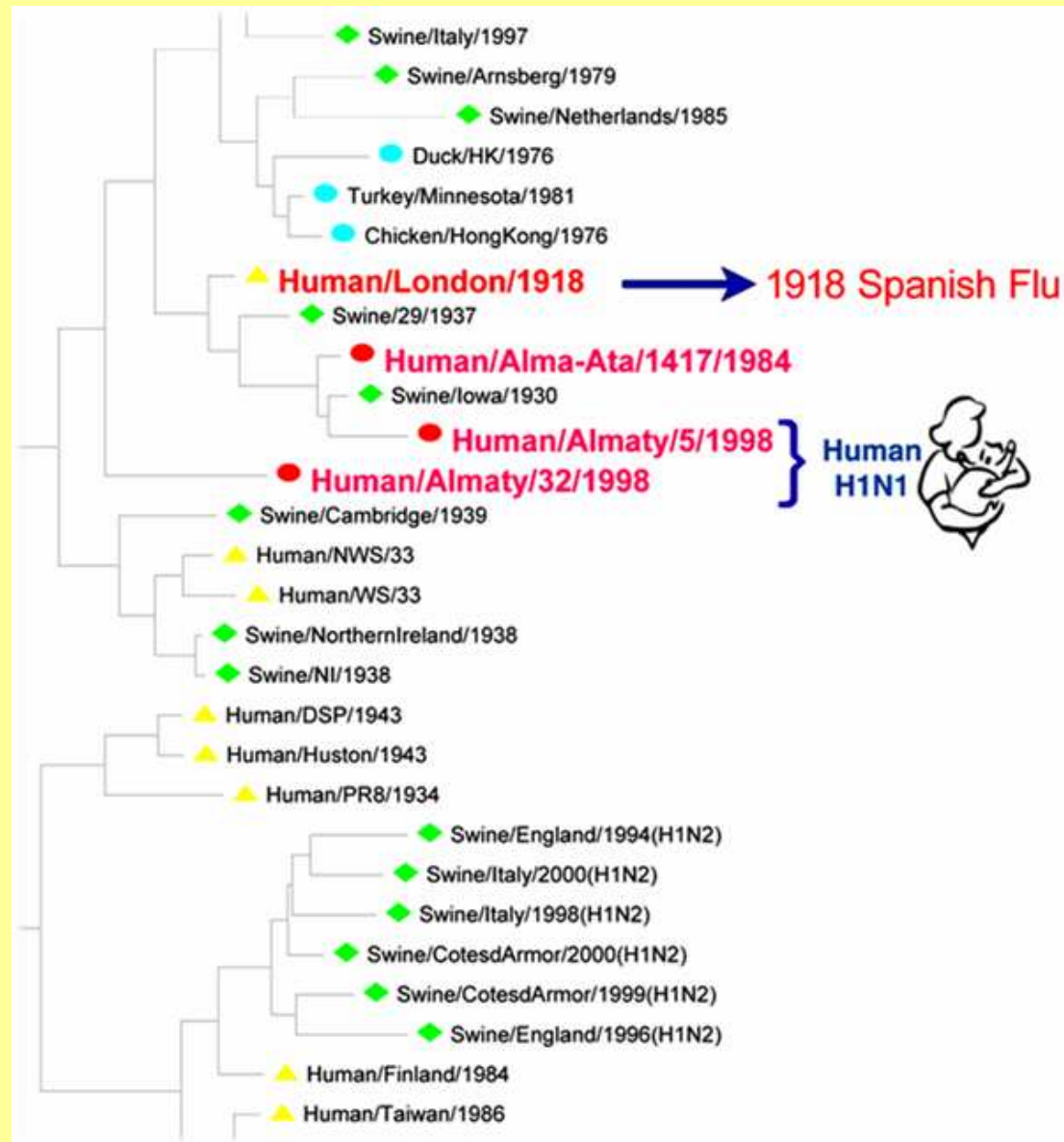


Adapted from: Taubenberger, et al. (1997) *Science* **275**(5307), 1793-1796

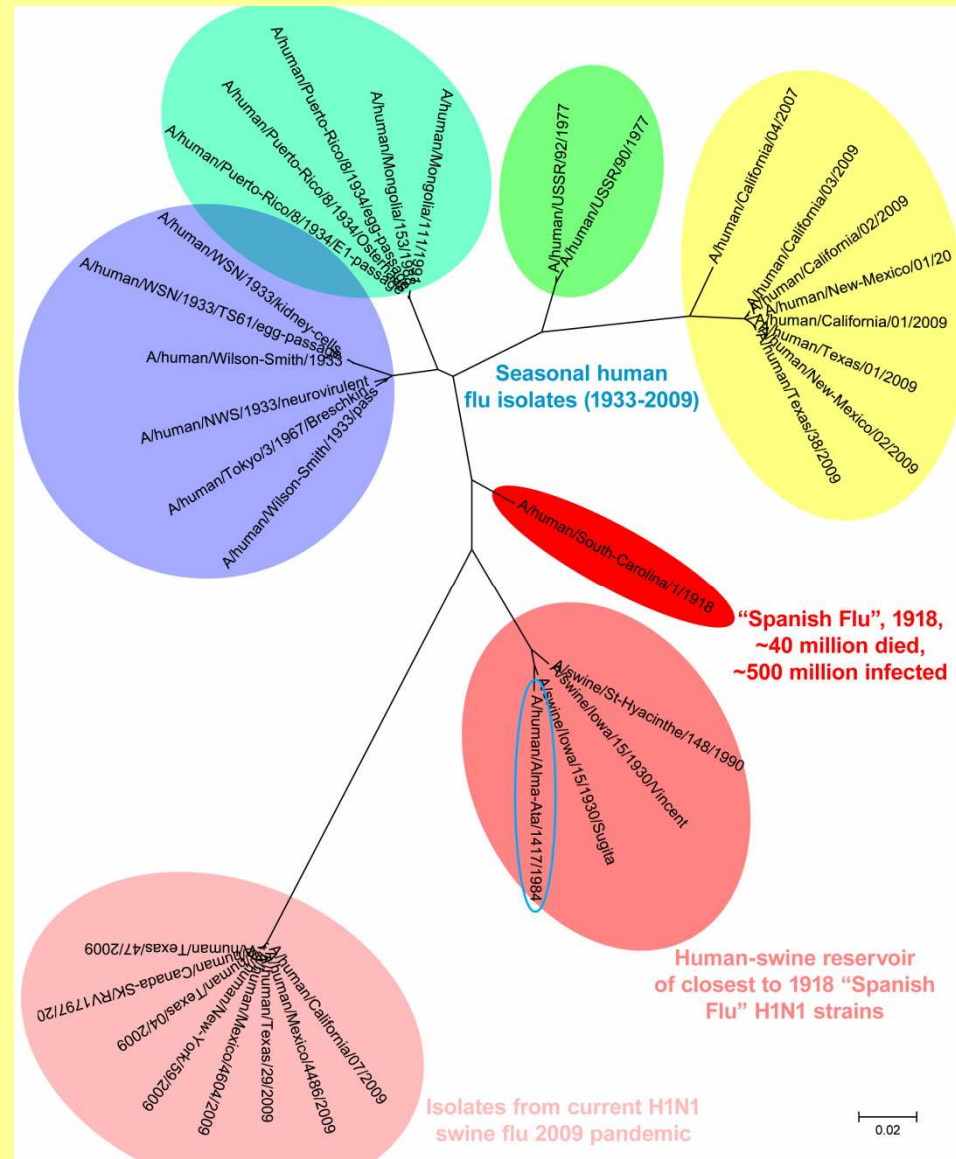
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A/Almaty/1135/83	Female, 10, nasal wash	Non-fatal	A/H1N1-Hsw1N1	No	Related to A/duck/Tashkent/78 (H3N1) by NA
A/Almaty/1417/84	Male, 26, nasal wash	Non-fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized; available sequence data in (3)
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A/Almaty/1414/84	Female, 13, nasal wash	Non-fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/1382/84	Female, 32, blood (2x)	Non-fatal	A/H1N1-Hsw1N1	Unknown	Seropositive to Hsw1N1, seronegative later despite the virus presence
A/Almaty/247/84	Female, 18, blood (2x)	Non-fatal	A/H1N1-Hsw1N1	Unknown	Seropositive to Hsw1N1 and A/Almaty/1417/84; no HAI to Hsw1
A/Almaty/1395/84	Male, 46, nasal wash	Fatal	Hsw1N1	No	Insufficiently characterized
A/Almaty/1367/84	Female, 47, trachea, lung	Fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/3/85	1 day old newborn, CSF	Congenital encephalopathy	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/5/98	Female, 23, ???	Repeated spontaneous abortions	Hsw1N1	No	Insufficiently characterized; sequence data unpublished
A/Almaty/32/98	Female, 25, blood	Prem. birth, spontaneous abortions	A/H1N1-Hsw1N1	No	Insufficiently characterized; sequence data unpublished
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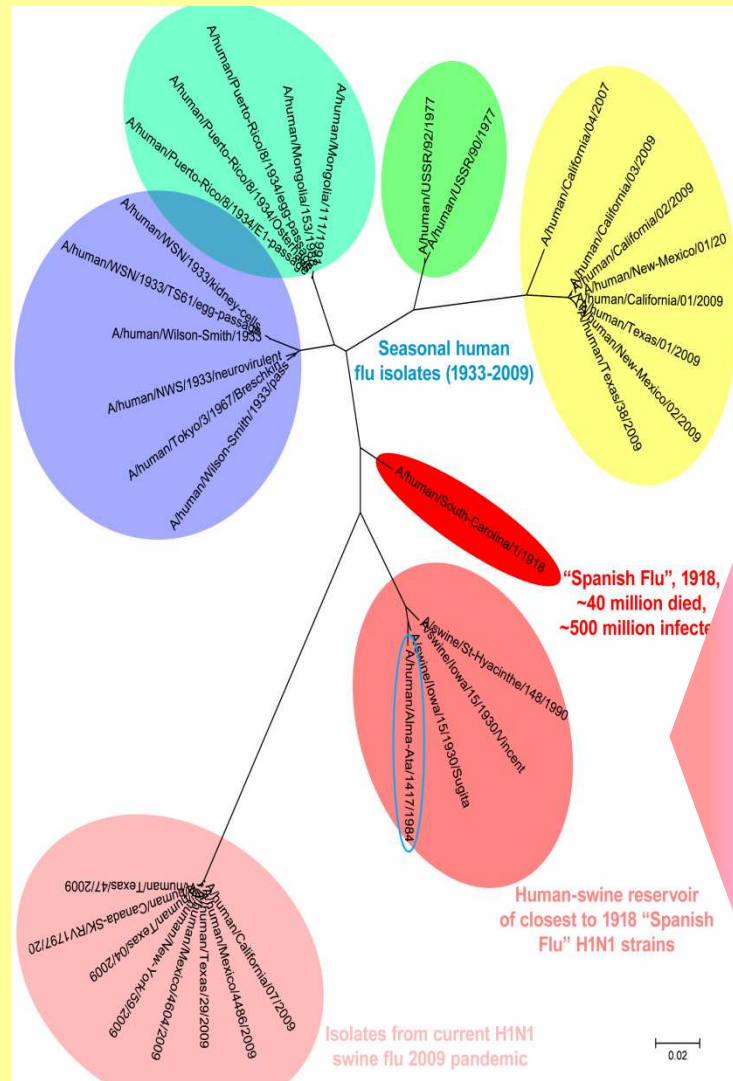
Phylogenetic analysis of the HA gene of additional swine-like virus isolates



Phylogenetic analysis of the HA gene of H1N1 viruses that have been circulating in human population over decades (1933-2009)

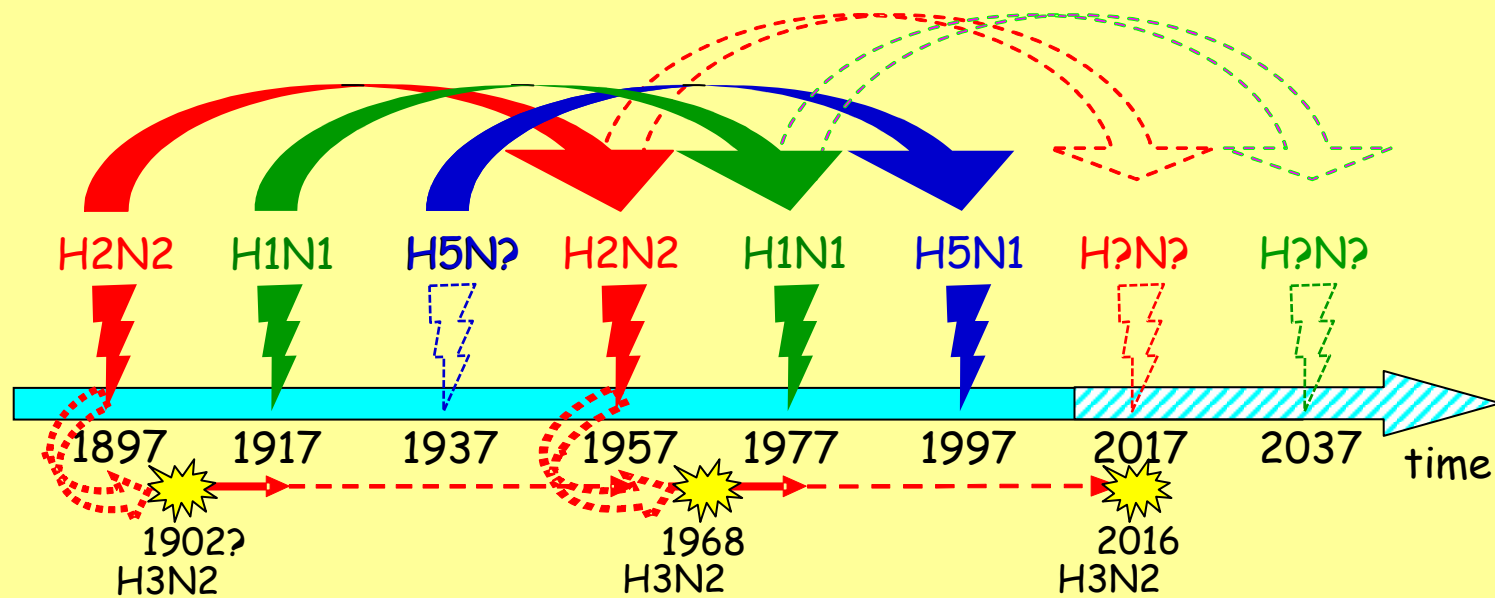


A human-swine circulation reservoir of swine-like influenza A?



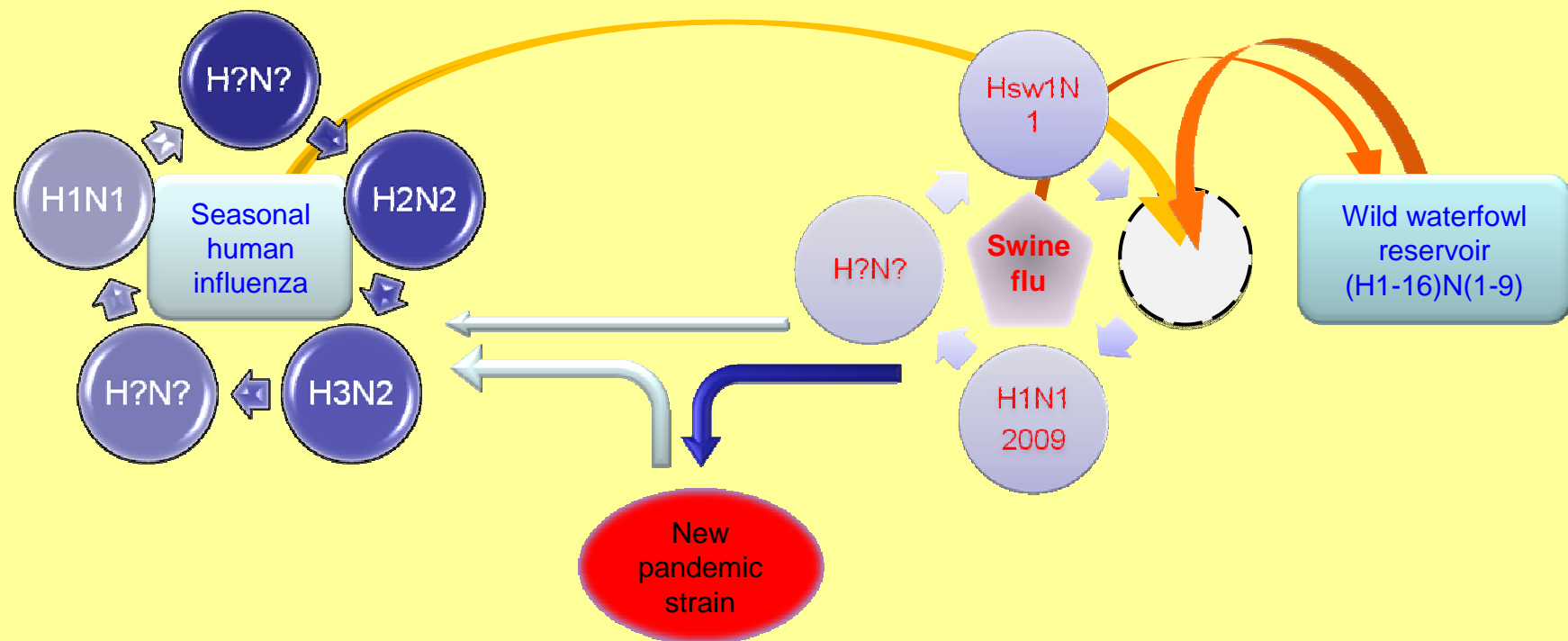
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A/Almaty/1414/84	Female, 13, nasal wash	Non-fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/1382/84	Female, 32, blood (2x)	Non-fatal	A/H1N1-Hsw1N1	Unknown	Seropositive to Hsw1N1, seronegative later despite the virus presence
A/Almaty/247/84	Female, 18, blood (2x)	Non-fatal	A/H1N1-Hsw1N1	Unknown	Seropositive to Hsw1N1 and A/Almaty/1417/84; no HAI to Hsw1
A/Almaty/1395/84	Male, 46, nasal wash	Fatal	Hsw1N1	No	Insufficiently characterized
A/Almaty/1367/84	Female, 47, trachea, lung	Fatal	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/3/85	1 day old newborn, CSF	Congenital encephalopathy	A/H1N1-Hsw1N1	No	Insufficiently characterized
A/Almaty/5/98	Female, 23, ???	Repeated spontaneous abortions	Hsw1N1	No	Insufficiently characterized; sequence data unpublished
A/Almaty/32/98	Female, 25, blood	Prem. birth, spontaneous abortions	A/H1N1-Hsw1N1	No	Insufficiently characterized; sequence data unpublished
A/Almaty/84/98	Female, 31, blood (2x)	Multiple sclerosis, immunodeficient	A/H1N1-Hsw1N1	No	Insufficiently characterized

Host-of-origin cycles of influenza A emergence



Influenza B
Influenza C

Hypothetical model of emergence of new highly pathogenic pandemic strains



What does need to be done urgently?

1. Systematic collection of samples in the southern part of the Novosibirsk oblast bordering the north-eastern part of Kazakhstan, in the Almaty city area, in the Almaty region, and in eastern Kazakhstan along routes of bird migration from China to Eurasia through the Jungar gates involving lakes Alakol, Sasykkol, along the Black Irtysh river leading to lakes Zaisan, Markakkol, along the Ili river, and lakes Kapchagai and Balkhash
2. Detection and amplification of influenza virus isolates in chick embryos, primary chick fibroblasts or in MDCK cell lines
3. Characterization and serotyping of virus isolates using hemagglutination inhibition, ELISA and neutralization assays with type-specific control reference sera
4. Characterization of virus isolates by sequencing of genome segments amplified by RT-PCR to identify new reassortants and new genetic variants with subsequent phylogenetic analysis to detect and predicts increase in mammalian virulence and transmissibility
5. Characterization of virulence and transmission of selected influenza virus isolates to verify predictive algorithms of increased virulence and transmissibility in the ferret model of influenza developed at the Southern Research Institute (Birmingham, AL, USA) under contract with NIAID
6. Development of predictive algorithms for timely design of influenza vaccines effective against emerging viruses with epidemic and pandemic potential
7. Preparation of vaccines using reverse genetics and their testing in ferrets