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Title

Likelihood of prior exposure to circulating influenza viruses resulting in cross protection by CD8+ T cells against emergent H3N2v swine viruses infecting humans

Running Title: H3N2v immunogenic peptides

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Abstract

Outbreaks of influenza in swine can result in potential threats to human public health. A notable occurrence was the emergence of swine-origin H1N1 influenza viruses in 2009. Since then, there have been several documented outbreaks of swine origin influenza infecting humans in several countries. Sustained events

have occurred when H1N1v, H1N2v and H3N2v swine origin viruses have infected humans visiting agricultural shows in the US. The predominant H3N2v viruses gained the matrix protein from the A(H1N1)pdm09 viruses, with reported human-to-human transmission raising fears of another pandemic. Current vaccines do not induce secondary cell-mediated immune responses, which may provide cross-protection against novel influenza A subtypes, however population susceptibility to infection with seasonal influenza is likely to be influenced by cross-reactive CD8+T-cells directed towards immunogenic peptides derived from viral proteins. This study involved a retrospective review of historical influenza viruses circulating in human populations from 1918 to 2020 to identify evidence of prior circulation of H3N3v immunogenic CD8+T-cells peptides found in the NP and M1 proteins. We found evidence of prior circulation of H3N2v NP and M1 immunogenic peptides in historical influenza viruses. This provides insight into the population context in which influenza viruses emerge and may help inform immunogenic peptide selection for Cytotoxic T-cell Lymphocytes (CTL)-inducing influenza vaccines. Next generation vaccines capable of eliciting CD8+T-cell mediated cross-protective immunity may offer a long-term alternative strategy for influenza vaccines.

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Introduction

Respiratory viruses are responsible for a number of diseases in humans, with novel respiratory viruses having the potential to cause pandemics. The

emergence of the novel coronavirus, SARS-CoV-2 and the subsequent morbidity and mortality associated with the COVID-19 pandemic have been a stark reminder of the potential impact on the human population. Of the known respiratory pathogens, influenza A viruses in particular have significantly affected human health over the course of recorded history, causing the deadly pandemic in 1918, as well as inflicting an annual seasonal burden (1). Whilst influenza viruses are of avian origin, swine populations are also susceptible to type A influenza infection. Outbreaks in swine herds are an economic burden to the agricultural industry and pose a potential threat to public health through spill-over infections to humans (2).

There have been numerous introductions of influenza viruses from swine to humans. In 1976, a H1N1 outbreak at Fort Dix (United States) involved 230 human cases (3). In 2009, transmission of influenza from swine to humans resulted in a global pandemic (4). In this case, human viruses that had been transmitted to animal reservoirs decades earlier evolved over time and were then reintroduced into the human population as the 2009 pandemic H1N1 reassortant virus. In humans, influenza re-infections occur over a lifetime allowing a robust immune response to exert pressure on the virus leading to genetic changes and subsequent immune escape. In contrast, the short lifespan of swine raised for meat leaves little opportunity for re-infection or exertion of immune pressure on the virus within swineherds (5). Because the 2009 novel H1N1 virus was antigenically distinct from recently circulating human influenza viruses, when it emerged most of the human, population under 65years of age would likely have been susceptible.

The risk of viruses of pandemic potential being transmitted from swine to humans has also been demonstrated by a number of USA reports between 2010 and 2012 of novel H1N1, H1N2 and H3N2 human infections associated with close contact with pigs at agricultural shows (5, 6). Whilst the H1N1 variant (v) viruses and H1N2v viruses were sporadic, infecting only small numbers of humans, the H3N2v were much more prolific with 432 cases isolated from humans between 2010 to 2020 (7). The true number of infections however, was likely to have been much higher due to under reporting, with some estimates suggesting thousands of human infections (8). Young people in the USA are encouraged to participate in school-run agricultural programs such as rearing and exhibiting swine at agricultural fairs. Youth exhibiting swine at agricultural fairs are usually in close proximity to their swine for extended periods, providing an ideal opportunity for transmission of influenza from swine to humans. As millions attend these fairs, the opportunity for further transmission remains significant (9). Infection of swineherds in the US with human H3N2 viruses occurred during 1995, becoming endemic in swine by 1998 (10). The H3N2v swine origin influenza virus which emerged in 2010 was a novel reassortant that had gained the matrix gene of the A(H1N1)pdm09 virus circulating in humans (11). This event raised fears of increased transmissibility in humans, particularly as there were reports of human-to-human transmission(12).

Both natural infection and influenza vaccines provide immunity against influenza (13). Whereas vaccination induces antibodies against the haemagglutinin (HA) and neuraminidase (NA) antigens, natural infection triggers both the production of antibodies as well as cell-mediated immunity. Cell mediated immunity

produces long-term memory CD8+T-cells (14, 15), that may provide cross-protection to other influenza A subtypes via CD8+T-cells responses directed against immunogenic peptides in the highly conserved internal influenza viral proteins, in particular the nucleoprotein (NP) and matrix (M). Therefore, whereas seasonal vaccines, are not anticipated to provide protection against influenza viruses which have undergone major genetic changes (16), immunity gained from prior exposure to influenza viruses may provide protection against both known and novel influenza viruses, potentially including those of animal origin associated with spill over infection events (17, 18).

To test the hypothesis that prior exposure to CD8+T-cell immunogenic peptides from past influenza infections might induce cross protection against novel influenza viruses, we examined the CD8+T-cell immunogenic peptides found in the NP and M1 of novel H3N2v viruses and compared them to immunogenic peptides found in human influenza viruses which have circulated from 1918-2020. We further hypothesise that if prior exposure, to CD8+T-cell immunogenic peptides protects against novel influenza viruses that cross the species barrier, this could impact on which population cohorts are vulnerable to infection by novel viruses. Understanding exposure patterns to CD8+ T-cell immunogenic peptides of different age cohorts and potential cross protection provided against novel influenza viruses could help inform development of broadly protective vaccines. 'Universal' vaccine candidates would ideally provide a level of cross protection against multiple influenza A subtypes similar to that afforded by naturally acquired immunity gained through infection.

Methods

This study involved a retrospective review of historical influenza viruses circulating in human populations from 1918 to 2020 to identify evidence of prior circulation of H3N2v immunogenic CD8+T-cell peptides found in the NP and M1 proteins. This study has focused on US outbreaks, as H3N2v viruses have been isolated from humans since the emergence of the H3N2v viruses in 2010.

Reference Peptide selection:- Sequences of swine-origin H3N2v viruses identified from humans were sourced from the Global Initiative on Sharing All Influenza Data (GISAID EpiFlu™) database, (www.gisiad.org) (19). The consensus sequences were determined for each of the proteins of interest using Geneious Prime 2019.0.3 (www.geneious.com). A total of 73 NP and 37 M1 immunogenic peptides were selected from the IEDB (www.iedb.org) by searching for experimentally defined epitopes that had been identified by the use of T-cell assays (20), Supplementary Material Table S1. Diversity amongst the peptides in the NP and M1 proteins of the H3N2v viruses was calculated as the proportion of viruses per peptide that matched the reference peptide consensus. This was plotted using Rv3.6.3 (www.cran-r-project.org) (21).

Data Preparation: Geneious was used to select sequences using the following criteria. Protein sequences were excluded if they were; derived from laboratory adapted or generated viruses; duplicates; did not span the full range of peptides; and were incomplete. Sequences which were derived from clinical specimens, lowest passage history or passaging in cell rather than eggs were preferentially selected from available duplicate sequences to minimize the occurrence of

mutations due to adaptation in the regions of interest. Data were trimmed to begin with the first Methionine of the NP and M1 genes. Peptide sequences for each of the 73 NP and 37 M1 peptides were extracted from the historical data set and prepared for analysis using R. v3.6.3, (www.cran.r-project.org) (21)

Analysis of historical peptide sequences: The H3N2v reference peptide sequences were compared with the corresponding peptide sequences of historical subtypes; i.e. H1N1 (pre-1957 and post-1977), H2N2, H3N2 and A(H1N1)pdm09 viruses. The proportions of peptide sequences identical to the reference peptide sequence for each peptide, each subtype and each year were calculated. For instance, the sequence of reference peptide NP_{17-25} was compared to the NP_{17-25} peptides for each of the four influenza viral subtypes. The proportion of identical sequences were generally aggregated into 5-year blocks from 1918 to 2013. Prior to 1933, there was only one historical H1N1 NP and M1 sequence available for comparison. Peptide diversity per protein per peptide per year per subtype was calculated and plotted using R. v3.6.3 (21). Age cohort ranges were determined from the time of circulation of each novel influenza subtype and the time of emergence of the H3N2v viruses in 2010.

Epidemiology: A small number of H3N2v sequences available on the GISAID database included patient meta-data such as age and gender. Characteristics by age groups (≥18 and <18 years), gender, hospitalisation rates, those who attended agricultural fairs and had direct contact with swine of all cases of H3N2 variant virus infections in the USA were available at https://gis.cdc.gov/grasp/fluview/Novel_Influenza.html

Results

Sequence data: A total 1921 H1N1, 188 H2N2, 77312 H3N2, 47947 A(H1N1)pdm09 NP and M1 protein sequences from the time-period 1918-2020 were identified in GISAID. The number of available sequences for each of the NP and M1 immunogenic peptides per subtype are shown in the supplementary material, 0Table S2.

H3N2v reference data: For the H3N2v reference data, 143 NP and 135 M1 protein sequences were identified. The consensus sequence was used to calculate peptide diversity within the NP and M1 H3N2v peptides with the mean from the consensus sequence for each gene being 98% (minimum 91%, maximum 100%) for the NP peptides and 99% (minimum 98%, maximum 100%) for the M1 peptides.

Epidemiology: Novel H3N2v viruses were isolated from 432 humans in the USA between the years 2010 and 2020. The number of cases peaked in 2012 at 309 with a secondary peak of 61 cases in 2017 (Figure 1). The majority (85%) of cases occurred in Indiana and Ohio with small numbers in a further eight states. Most cases reported direct contact with swine at agricultural shows. Human-to-human transmission was suspected to have occurred in a small number of cases (35, 8%) who reported no contact with swine but whose family members had close contact with swine, displayed symptoms and were not tested or were laboratory confirmed cases (22). The median age of the 432 cases for which data were available was 7 years (3 months to 74 years) with 91% of individuals <18 years of age and 51% were female. Around 7% were hospitalised, one

death was recorded of a 61 year old female who had underlying medical conditions (6, 8, 23).

Comparison of reference H3N2v peptides with historical immunogenic peptide circulating from 1918 to 2013 in human influenza viruses.

NP immunogenic peptides: A comparison of the 73 H3N2v NP peptides with corresponding peptides identified in the four viral subtypes found that six peptides (8%) were absent from all circulating human viruses over the study period, whereas nine (12%) H3N2v peptides were present in 100% of viruses reviewed (Table 1). Furthermore, as shown in Table 2, characterisation of NP H3N2v peptides found additional instances of high conservation (H3N2v peptides present in ≥ 95% of in previously circulating viruses) and near absence (H3N2v peptides detected in ≤ 5% of previously circulating viruses). The H3N2 viruses which emerged in humans in 1968 and are currently circulating in humans had the greatest decrease in the number of viruses with H3N2v peptides with twelve,16% found in ≥ 95% of H3N2 human viruses (Table 2).

The percentage of H3N2v NP peptides found in circulating influenza viruses is shown in Figures 2 and 3(a). The number of circulating H1N1, H2N2 and H3N2 viruses with H3N2v NP peptides decreased over time (Figure 2). In 1918, 68% of H1N1 viruses and in 1968, 46% of H3N2 viruses had H3N2v peptides; by 2010 this had decreased to 47% and 36%, respectively. The number of circulating A(H1N1)pdm09 viruses with the H3N2v peptides were stable, 84% to 83% between 2009 and 2010. Between 2010 and 2020 whilst H3N2v viruses were

being isolated from humans, the number of H3N2 viruses with H3N2v NP peptides were stable, 37% to 36%. The number of A(H1N1)pdm09 with H3N2v NP peptides decreased slightly from 84% to 80% from 2010 to 2020. The potential for exposure to the H3N2v immunogenic peptides varied for different age cohorts. In 1918, of those whose first potential exposure was to H1N1 viruses, the age cohort ≥ 75 years had potential exposure to 68% of H3N2v peptides. In contrast, by the late 1990s, the ≤16 years age cohort whose first potential exposure was to H3N2 influenza viruses saw a decrease in potential exposure to the lowest level of H3N2v peptides, 34% to 37%. During 1990 to 1994, circulation of H1N1 viruses was mostly sporadic in the human population with 3% of influenza viruses isolated in 1994 being H1N1 viruses with H3N2 and type B viruses being the dominant circulating viruses at the time (24, 25).

M1 immunogenic peptides: Of the 37 M1 peptides compared in the four subtypes, seventeen peptides, 46% of the corresponding H3N2v peptides were highly conserved and present in all human influenza viruses and H3N2v viruses (Table 1). There were no peptides that were absent in all four circulating subtypes. Additionally, as shown in Table 2, characterisation of M1 H3N2v peptides found additional instances of high conservation (H3N2v peptides present in ≥ 95% of in previously circulating viruses) and near absence (H3N2v peptides detected in ≤ 5% of previously circulating viruses). The percentage of H3N2v peptides found in circulating human influenza viruses are in Figure 4. The proportion of each of the 37 H3N2v M1 peptides circulating in the H1N1, H2N2, H3N2 and pdmH1N1 human influenza viruses are shown in Figure 3(b).

In 1918, 68% of the H1N1 viruses had H3N2v M1 peptides present, decreasing to 57% found in circulating human H3N2 viruses by 2010. By 2010 the number H3N2v peptides in post 1977 H1N1 viruses was similar to that in 1918 with 71% of H1N1 viruses with H3N2v M1 peptides present. The number of H3N2 viruses with H3N2v M1 peptides remained at 57% from 2010 to 2020, the time-period during which H3N2v viruses were still being isolated from humans. The number of A(H1N1)pdm09 viruses with H3N2v peptides had a marginal decrease from 99% to 97% between 2010 to 2020. Each age cohort whose first potential exposure to the H3N2v peptides was to each of the emergent virus in 1918, 1957 and 1968 was found to have a similar potential for exposure to the H3N2v M1 peptides. However, in 1977 when H3N2 and the re-emergent H1N1 viruses began to co-circulate, those whose first potential exposure was post 1977 were potentially exposed to differing levels of H3N2v peptides dependant on whether they were infected with H3N2 or H1N1 viruses (Figure 4). Exposure to the H3N2v peptides in the post H1N1 viruses was less likely to occur as the virus circulated in very low numbers (26, 27), although there was increase in the numbers of H1N1 viruses in circulation between 2007 and 2009. There was a decrease in the number of H3N2 viruses with H3N2v peptides from 65% to 57% in ≥ 95% of circulating viruses, post 1977. For the age cohort ≤ 1 to 33 years, this period of co-circulation was the first potential infection to either of these viruses, with potential exposure to differing profiles of H3N2v peptides and consequently different levels of potential cross-protection (Figure 4).

Discussion:

In this study, we examined all available complete viral sequences of the NP and M1 proteins circulating in human influenza viruses since 1918 and compared these with H3N2v CD8+T-cell immunogenic peptides. We found evidence of prior circulation of H3N2v NP and M1 immunogenic peptides in influenza viruses circulating in humans, between1918 to 2010. The numbers of H3N2v immunogenic peptides found in the H1N1, H2N2 and H3N2 human viruses decreased over time, suggesting a link between prior exposure to influenza viruses and age-related morbidity patterns noted during the outbreak of the novel H3N3v viruses in humans. We chose to study NP and M1 immunogenic peptides as the NP protein carries the greatest number of immunogenic peptides (20), and both of these peptides have also been extensively studied, this offering a greater understanding of the characteristics of their immunogenic peptides. However, this study did have some unavoidable limitations such as the low number of influenza viral NP and M1 sequences available from the early 20th century leading to a possible underestimation of population level diversity. Furthermore, as not all CD8+T-cell peptides have been fully characterised and as the importance of sequences changes are not well understood, setting the criteria for inclusion at 100% match to the H3N2v peptides may have excluded a number of relevant peptides.

Several highly conserved NP and M1 immunogenic peptides were noted in the four influenza A subtypes viruses circulating in humans since 1918 and the novel H3N2v virus. Presumably, those who had experienced an influenza infection with either H1N1, H2N2, H3N2 or A(H1N1)pdm09 viruses had been exposed to a

number of the conserved immunogenic peptides. The NP and MP genes in the swine origin H3N2v viruses have undergone less change due to immune pressure than in viruses circulating in humans (5). Other studies have also noted the existence of these same conserved peptides in H7N9 studies (20, 28). A further observation is that over time there was a cumulative decrease in the number of NP and M1 H3N2v immunogenic peptides found in viruses circulating in humans. There was also a decrease in the number of H3N2v peptides between the emergence and disappearance of each subtype. Those cohorts whose infection occurred at the time of emergence of each of the influenza subtypes generally had opportunity for exposure to a higher number of H3N2v peptides and therefore a higher level of cross-protection.

A relationship was observed between those whose first exposure was to the H3N2 human viruses emerging in 1968 and age-related morbidity patterns, particularly for those ≤7 years of age, as both the NP and M1 H3N3v peptides in circulating H3N2 viruses decreased to the lowest levels. Those aged 42-50 years, whose potential first exposure was to the H2N2 viruses and those aged 8-17 years, whose potential first exposure was to H3N2 viruses, may have also had an increased vulnerability to infection with H3N2v viruses. For the very young infected with H3N2v virus, it is possible that exposure to this variant was their first exposure to the influenza virus. In contrast, older cohorts previously exposed to higher numbers of H3N2v immunogenic peptides found in the H1N1, H2N2 and the early H3N2 human viruses were likely to have gained some level of pre-existing immunity providing secondary cross protection by the CD8+ T cell immunogenic peptides. Similarly in 2009, studies in older adults at the time were

found to have pre-existing immunity to the novel pdmH1N1 virus as they had most likely been exposed to the 1918-like H1N1 virus (29). Young adults were also disproportionately affected by the pdmH1N1 virus in 2009/2010 (30) and this study also found those who had been infected with the pdmH1N1 virus had been exposed to high numbers of immunogenic peptides found in the H3N2v viruses post 2010.

Influenza A HA subtypes H1, H2 and H3, are known through sero-archeological studies to have circulated in humans from around 1850. It has been suggested that these HA subtypes are recycled as novel reassortant viruses causing pandemics as happened in 2009 with a H1N1 virus that had a different constellation of genes to the previous H1N1 virus of 1918 (31). In 2006, a novel H2N3 virus was isolated from swine in North America raising concern of a H2 virus with a novel constellation of genes emerging in humans (32). Studies of H2N2 viruses circulating in birds are known to have genetic similarities to the H2N2 viruses previously circulating in humans; coupled with the knowledge that those under 60 years of age lack any immunity to the H2N2 viruses continues to cause concern regarding a re-emergence of H2N2 viruses in humans (33). Currently, influenza surveillance on swine and birds is not comprehensively undertaken globally, leaving important knowledge gaps in this zoonotic infection.

In order to provide better, more durable protection against emerging influenza strains, better understanding is needed of the conservation of immunogenic peptides found in non-human influenza viruses, and whether they have previously circulated in human viruses (20, 34). CD8+ T cell responses, which is not generated by inactivated vaccines but is generated by infection to seasonal

influenza viruses, have been found to provide a level of cross-protection against natural influenza infection in humans (35, 36). This suggests that the challenging goal of developing influenza vaccines capable of eliciting a broadly cross-protective immunity against a number of influenza A subtypes should be pursued. The ability of a cell mediated T-cell response in the recognition of conserved internal epitopes resulting in the broad protection against other influenza strains has been raised in other studies (37, 38). This study provides further insight into the role of CD8+T-cell immunogenic peptides with prior circulation in humans and the provision of some cross-protection against novel yet related zoonotic influenza viruses.

Word count: 3337

References

1.   Lee VJ, Ho ZJM, Goh EH, Campbell H, Cohen CASA, Cozza V, et al. Advances in measuring influenza burden of disease. Influenza Other Respir Viruses. 2018;12(1):3-9.

2.   Rajao DS, Vincent AL, Perez DR. Adaptation of Human Influenza Viruses to Swine. Frontiers in Veterinary Science. 2019;5:347.

3.   Gaydos JC, Top FH, Hodder RA, Russell PK. Swine influenza a outbreak, Fort Dix, New Jersey, 1976. Emerging infectious diseases. 2006;12(1):23.

4.   Gabriele N, Takeshi N, Yoshihiro K. Emergence and pandemic potential of swine-origin H1N1 influenza virus. Nature. 2009;459(7249):931.

5.   Komadina N, McVernon J, Hall R, Leder K. A historical perspective of influenza A(H1N2) virus. Emerging infectious diseases. 2014;20(1):6-12.

6.   MMWR. MMWR Morbidity and mortality weekly report. 2012;61(39):785-9 were detected than in the summer months of previous years Influenza A report summarizes influenza activity in the United States and worldwide since May.

7.   Houser KV, Katz JM, Tumpey TM. Seasonal trivalent inactivated influenza vaccine does not protect against newly emerging variants of influenza A (H3N2v) virus in ferrets. Journal of virology. 2013;87(2):1261-3.

8.   Biggerstaff M, Reed C, Epperson S, Jhung MA, Gambhir M, Bresee JS, et al. Estimates of the number of human infections with influenza A (H3N2) variant virus, United States, August 2011–April 2012. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2013;57 Suppl 1:S12-5.

9.   Stewart RJ, Rossow J, Conover JT, Lobelo EE, Eckel S, Signs K, et al. Do animal exhibitors support and follow recommendations to prevent transmission of variant influenza at agricultural fairs? A survey of animal exhibitor households after a variant influenza virus outbreak in Michigan. Zoonoses and public health. 2018;65(1):195-201.

10.   Richard JW, Sabrina LS, Scott LK, Philip JG, Sagar MG, Robert GW. Evolution of Swine H3N2 Influenza Viruses in the United States. Journal of virology. 2000;74(18):8243-51.

11.   Chou Y-y, Albrecht RA, Pica N, Lowen AC, Richt JA, Garcia-Sastre A, et al. The M Segment of the 2009 New Pandemic H1N1 Influenza Virus Is Critical for Its High Transmission Efficiency in the Guinea Pig Model. Journal of virology. 2011;85(21):11235.

12.   Lindstrom S, Garten R, Balish A, Shu B, Emery S, Berman L, et al. Human infections with novel reassortant influenza A(H3N2)v viruses, United States, 2011. Emerging infectious diseases.18(5):834-7.

13.   van de Sandt CE, Kreijtz JH, Rimmelzwaan GF. Evasion of influenza A viruses from innate and adaptive immune responses. Viruses. 2012;4(9):1438-76.

14.   Nüssing S, Sant S, Koutsakos M, Subbarao K, Nguyen THO, Kedzierska K. Innate and adaptive T cells in influenza disease. Frontiers of medicine. 2018;12(1):34-47.

15.   Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, et al. Influenza. Nature reviews Disease primers. 2018;4(1):3.

16.   Kelso A. CD4+ T cells limit the damage in influenza. Nature medicine.18(2):200-2.

17.   Hayward AC, Wang L, Goonetilleke N, Fragaszy EB, Bermingham A, Copas A, et al. Natural T Cell-mediated Protection against Seasonal and

Pandemic Influenza. Results of the Flu Watch Cohort Study. American journal of respiratory and critical care medicine. 2015;191(12):1422-31.

18.   Sridhar S, Begom S, Bermingham A, Hoschler K, Adamson W, Carman W, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. Nature medicine. 2013;19(10):1305-12.

19.   Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2017;22(13).

20.   Quinones-Parra S, Grant E, Loh L, Nguyen TH, Campbell KA, Tong SY, et al. Preexisting CD8+ T-cell immunity to the H7N9 influenza A virus varies across ethnicities. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(3):1049-54.

21.   R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria2018. Available from: https://www.R-project.org.

22.   Jhung MA, Epperson S, Biggerstaff M, Allen D, Balish A, Barnes N, et al. Outbreak of Variant Influenza A(H3N2) Virus in the United States. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2013;57(12):1703-12.

23.   Influenza A (H3N2) variant virus-related hospitalizations: Ohio, 2012. MMWR Morbidity and mortality weekly report. 2012;61:764-7.

24.   Centers for Disease C. Update: Influenza Activity – Worldwide and Recommendations for Influenza Vaccine Composition for the 1990–91 Influenza Season. MMWR Morbidity and mortality weekly report. 1990;39(17):293-6.

25.   Brammer TL, Izurieta HS, Fukuda K, Schmeltz LM, Regnery HL, Hall HE, et al. Surveillance for Influenza — United States, 1994–95, 1995–96, and 1996–97 Seasons. MMWR CDC surveillance summaries: Morbidity and mortality weekly report CDC surveillance summaries / Centers for Disease Control. 2000;49(SS-3):13-28.

26.   Influenza Activity—Worldwide and Recommendations for Influenza Vaccine Composition for the 1990-1991 Influenza Season. JAMA: the journal of the American Medical Association. 1990;263(22):3002-6.

27.   Brammer TL, Izurieta HS, Fukuda K, Schmeltz LM, Regnery HL, Hall HE, et al. Surveillance for influenza--United States, 1994-95, 1995-96, and 1996-97 seasons. MMWR CDC surveillance summaries: Morbidity and mortality weekly report CDC surveillance summaries / Centers for Disease Control. 2000;49(3):13-28.

28.   Mao H, Yen HL, Liu Y, Lau YL, Malik Peiris JS, Tu W. Conservation of T cell epitopes between seasonal influenza viruses and the novel influenza A H7N9 virus. Virologica Sinica. 2014;29(3):170-5.

29.   Hancock K, Veguilla V, Lu X, Zhong W, Butler EN, Sun H, et al. Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus. The New England journal of medicine. 2009;361(20):1945-52.

30.　Verma N, Dimitrova M, Carter DM, Crevar CJ, Ross TM, Golding H, et al. Influenza virus H1N1pdm09 infections in the young and old: evidence of greater antibody diversity and affinity for the hemagglutinin globular head domain (HA1 Domain) in the elderly than in young adults and children. Journal of virology. 2012;86(10):5515-22.

31.　Dowdle WR. Influenza A virus recycling revisited. Bulletin of the World Health Organization. 1999;77(10):820-8.

32.　Wenjun M, Amy LV, Marie RG, Christy BB, Kelly ML, Bruce HJ, et al. Identification of H2N3 influenza A viruses from swine in the United States. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(52):20949-54.

33.　Joseph U, Linster M, Suzuki Y, Krauss S, Halpin RA, Vijaykrishna D, et al. Adaptation of pandemic H2N2 influenza A viruses in humans. Journal of virology. 2015;89(4):2442-7.

34.　Komadina N, Quinones-Parra SM, Kedzierska K, McCaw JM, Kelso A, Leder K, et al. High conservation level of CD8 T cell immunogenic regions within an unusual H1N2 human influenza variant. Journal of medical virology. 2016.

35.　Sridhar S, Begom S, Bermingham A, Ziegler T, Roberts KL, Barclay WS, et al. Predominance of heterosubtypic IFN-γ-only-secreting effector memory T cells in pandemic H1N1 naive adults. European journal of immunology. 2012;42(11):2913-24.

36.   Sridhar S. Heterosubtypic T-Cell Immunity to Influenza in Humans: Challenges for Universal T-Cell Influenza Vaccines. Frontiers in immunology. 2016;7:195-.

37.   Rimmelzwaan GF, Kreijtz JH, Bodewes R, Fouchier RA, Osterhaus AD. Influenza virus CTL epitopes, remarkably conserved and remarkably variable. Vaccine. 2009;27(45):6363-5.

38.   Agrati C, Castilletti C, Cimini E, Lapa D, Quartu S, Caglioti C, et al. Cellular and humoral cross-immunity against two H3N2v influenza strains in presumably unexposed healthy and HIV-infected subjects. PloS one. 2014;9(8):e105651-e.

Figures

Figure 1: Reported cases of H3N2v isolated from humans in the USA between 2010 and 2020.
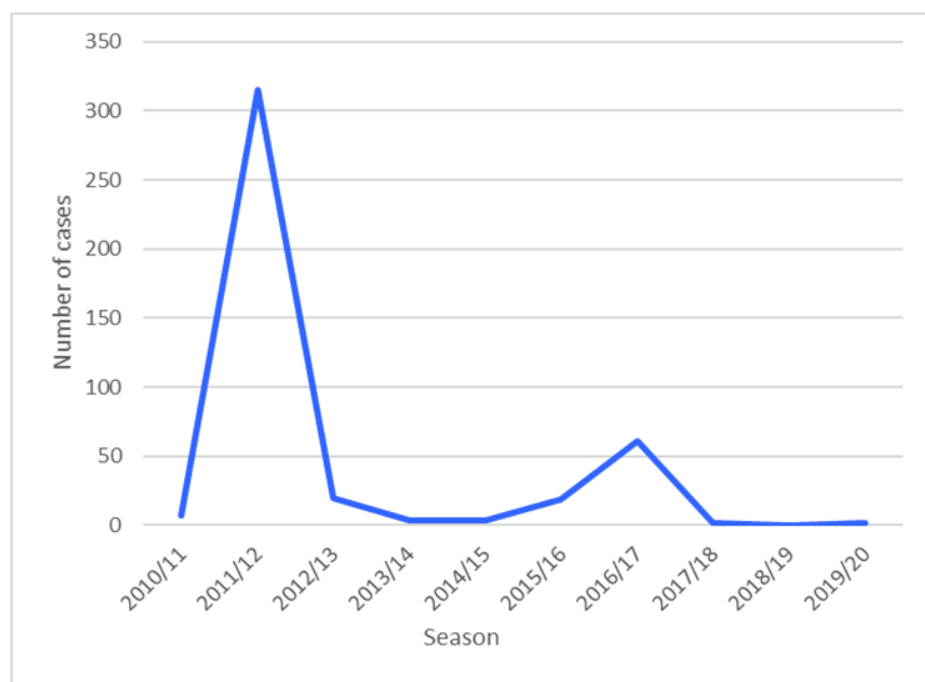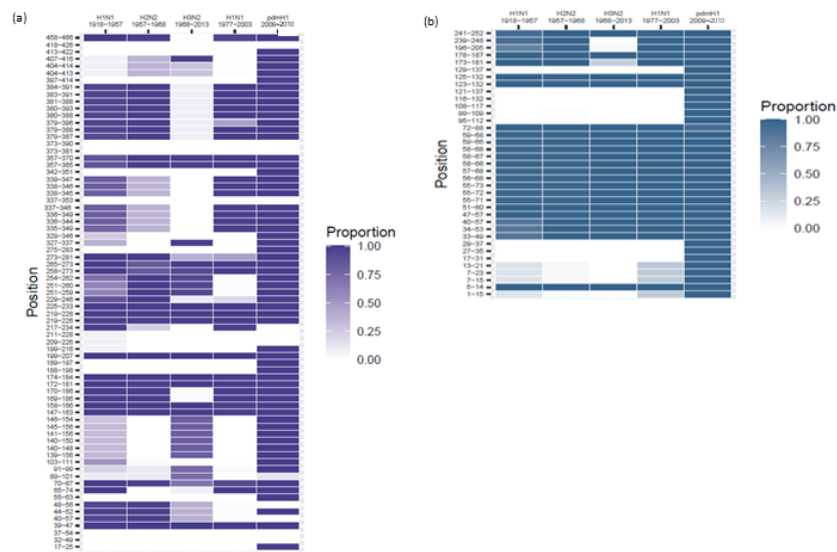
Figure 2. The percentage of all 73 H3N2v peptides in circulating viruses identical to the H3N2v NP peptides per year from 1918 to 2020. No H1N1 NP sequences were available for 1990 and 1992-1994. Ages of each cohort at the time of emergence of the H3N2v virus, 2010, exposed to each of the circulating human influenza virus subtype are shown. Levels of exposure to H3N2v peptides of different age cohorts over time are included. Post 2010 those who infected with swine origin pdmH1N1 virus received exposure to the highest number of H3N2v peptides.
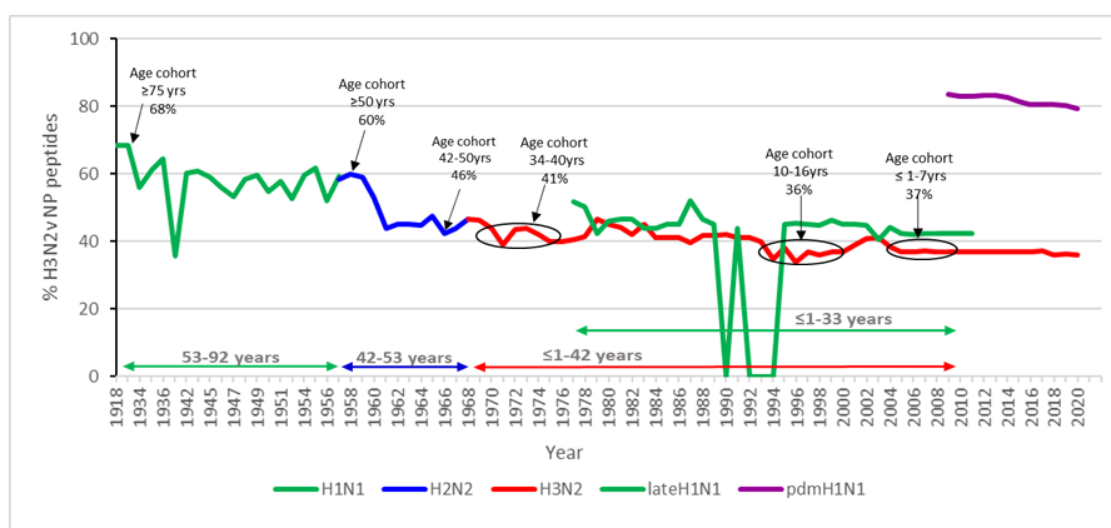
Figure 3:- The proportion of H3N2v influenza virus NP and M1 immunogenic peptides found in the four influenza A subtypes, H1N1, H2N2, H3N2 and pdmH1N109 which circulated in humans from 1918- 2010.

Figure 4:- The percentage of all 37 H3N2v peptides in circulating viruses identical to the H3N2v M1 peptides per year from 1918 to 2020. No H1N1 M1 sequences were available for 1990 and 1992-1994. Ages of each cohort at the time of emergence of the H3N2v virus, 2010, exposed to each of the circulating human influenza virus subtypes are coloured to match the virus subtype. Between 1918 and 1947, the number of H3N2v peptides in ≥ 95% of H1N1

viruses fluctuated between 76% (1936) to 53% (1946). Between 1947 and 1977, the number of H3N2v peptides remained steady at 65% ≥ 95% of H1N1, H2N2 and H3N2 viruses, with the exception of 1961 where 53% of peptides were found in circulating H2N2 viruses. Post 1977, the number of H3N2v peptides in H3N2 viruses decreased whereas those in circulating late H1N1 viruses increased. Post 2010, those infected with swine origin pdmH1N1 virus received exposure to the highest number of H3N2v peptides.
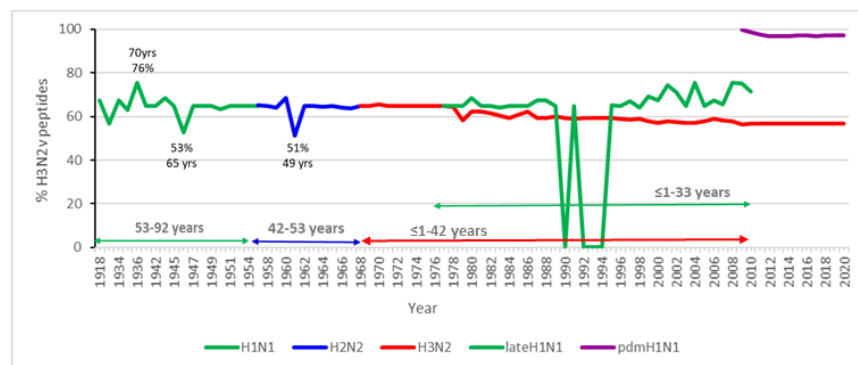
Tables

Table 1:- Peptides with 100% match to the H3N2v immunogenic peptides are highly conserved in both the NP and M1 proteins. These peptides were found in ≥ 99% of all H1N1, H2N2, H3N2 and pdmH1N1 influenza viruses circulating in humans over the study period. H3N2v NP peptides not detected in of previously circulating viruses

NP conserved peptides	M1 conserved Peptides	
39-47 FYIQMCTEL	5-14	TEVETYVLSI
147-163 TYQRTRALVRTGMDPRM	47-57	KTRPILSPLTK
158-166 GMDPRMCSL	51-60	ILSPLTKGIL
172-181 LPRRSGAAGA	55-71	LTKGILGFVFTLTVPSE
174-184 RRSGAAGAAVK	55-72	LTKGILGFVFTLTVPSER
199-207 RGINDRNFW	55-73	LTKGILGFVFTLTVPSERG
219-266 YERMCNIL	56-68	LTKGILGFVFTLTV
219-228 YERMCNILKG	57-68	KGILGFVFTLTV
225-233 ILKGKFQTA	58-66	GILGFVFTL
NP absent peptides	58-67	GILGFVFTLT

32-49	MIGGIGKFYIQMCTELKL	58-68	GILGFVFTLTV
37-54	GKFYIQMCTELKLSDYEG	59-66	ILGFVFTLTVP
373-381	AMDSNTLEL	59-68	ILGFVFTLTV
373-390	AMDSNTLELRSRYWAIRT	72-88	RGLQRRRFVQNALNGNG
418-426	LPFERATVL	123-132	ALASCMGLIY
		125-132	ASCMGLIY

Table 2: The number of highly conserved peptides NP and M1 found in ≥ 95% of circulating viruses and the number of H3N2v peptides found in very low numbers, ≤ 5%, of human influenza viruses circulating from 1918 to 2010.

	Influenza Subtype	Period of Circulation	≥ 95% circulation (n)	≤ 5% Circulation (n)	Age Cohorts (yrs)
NP peptides	H1N1	1918-1957	53%(39)	14%(19)	53-92
	H2N2	1957-1968	42%(31)	33%(24)	42-53
	H3N2	1968-2010	16%(12)	38%(28)	1-42
	H1H1	1977-2010	47%(36)	32%(23)	1-33
M1 Peptides	H1N1	1918-1957	57%(21)	22%(8)	53-92
	H2N2	1957-1968	65%(24)	24%(9)	42-53
	H3N2	1968-2010	57%(21)	32%(12)	1-42
	H1N1	1977-2010	65%(24)	24%(9)	1-33