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Review

Seven decades after the Asian influenza pandemic: A historical review about immunity and vaccines against H2N2

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ABSTRACT

In 1957, a reassortant influenza A virus (IAV) H2N2 subtype emerged in humans and encountered a population that was antigenically naïve to this subtype. The lack of pre-existing immunity to the H2 hemagglutinin (HA) facilitated efficient human-to-human transmission, and by the end of the Summer of 1957, most countries around the world reported increasing influenza cases caused by the new influenza virus subtype. The pandemic lasted until 1958, resulting in millions of infections globally, with 1–4 million estimated deaths. The first vaccines targeting specifically the H2N2 subtype were available in autumn 1957, but their limited immunogenicity hampered a successful fight against the "Asian influenza pandemic". After the pandemic, H2N2 became seasonal in the following years. Most individuals developed immunity against both the H2 HA and N2 neuraminidase (NA) proteins of the virus, and vaccines administered in the early 1960s successfully boosted this immunity. In 1968, the circulating H2N2 was replaced by the H3N2 subtype, and individuals with pre-existing N2 immunity were partially cross-protected against severe H3N2 infection, as the two N2 NAs were antigenically similar. Since the disappearance of H2N2 from the human population in 1968, global H2 immunity has been decreasing. This raises concerns about a possible re-emergence of the H2 subtype from animal reservoirs, where the virus has circulated for decades, into the human population. As preparedness for future pandemics, research on H2-specific vaccines is currently ongoing, with several candidates being tested in preclinical studies and early-phase clinical trials. In contrast to 1957, vaccine technology platforms, but also the assays used to assess vaccine immunogenicity, and efficacy, have significantly improved. This review aims to summarize the key historical milestones of the Asian influenza pandemic, the impact of H2N2 immunity during and after the 1957 pandemic, the immunogenicity of H2N2-specific vaccines in both a pandemic and pre-pandemic situation, and H2N2-specific antiviral treatment.

1. Introduction

The H2N2 pandemic, originating in Asia, erupted in 1957 and was caused by a reassortant human influenza A (IAV) H2N2 subtype (Fig. 1A). The reassortment occurred between a contemporary IAV H1N1 subtype, circulating in the human population since 1918, and an avian IAV H2N2 subtype, circulating in ducks. Sequence analysis of the H2N2 virus revealed a replacement of the two surface proteins, hemagglutinin (HA) and neuraminidase (NA), of the H1N1 subtype by the avian-like H2 and N2 subtypes. In addition, the gene segment encoding the polymerase basic (PB) protein 1 (part of the trimeric polymerase complex), also originated from an avian species (wild waterfowl) [1,2]. Interestingly, it is unknown, in which host species this reassortment occurred [3] and how long it took for the virus to evolve to adapt to humans. However, there is speculation that the three gene segments may already have been introduced into the human population two to six years before the pandemic [4,5].

Researchers in the 1950s faced several obstacles in fighting against the newly emerged virus. This included the lack of pre-existing immunity against H2 and N2, which hampered the induction of robust immune responses to vaccines administered early during the pandemic. Furthermore, vaccine technology platforms were less advanced in the 1950s, and standardized assays to assess the antigen content of vaccines, as well as to detect antibodies in the sera of convalescent patients, were

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less developed. Lastly, in the 1950s, global surveillance systems to monitor influenza virus infections and the circulation of strains in both humans and the animal reservoir were lacking.

In 1968, H2N2 was replaced by H3N2, the causative agent of the Hong Kong influenza pandemic [9], and has not been detected in humans since then. However, the H2 subtype continues to circulate in animal reservoirs (e.g., wild birds, pigs), raising concerns about a potential re-introduction of the virus into the human population [10]. Therefore, in terms of preparedness for a future H2 influenza pandemic, vaccine development targeting this subtype is urgently needed. In this review, we aim to summarize the historical milestones of the Asian influenza pandemic, the impact of H2N2 immunity during, and after the pandemic, the vaccines developed in the 1950s, the current status of the most advanced H2 subtype-specific vaccines in clinical trials, and antiviral treatment of H2N2.

2. Historical milestones of the pandemic

In February 1957, the first Asian influenza cases were reported in Guizhou province in China (formerly Kweichow), followed by Yunnan province in March 1957 (Fig. 1B) [11]. A few weeks later, in April 1957, numerous regions across China, Taiwan, Hong Kong, Indonesia (Borneo), and Singapore reported influenza virus infections [6,12]. The virus was first isolated in Changchun and Beijing, and later characterized [11]. In May 1957, the virus reached Japan [12], the Philippines, Guam, and Australia [11]. During the same month, the virus was also isolated in Singapore and Japan and identified as a new IAV subtype at the Walter Reed Army Institute of Research in Washington, the World Influenza

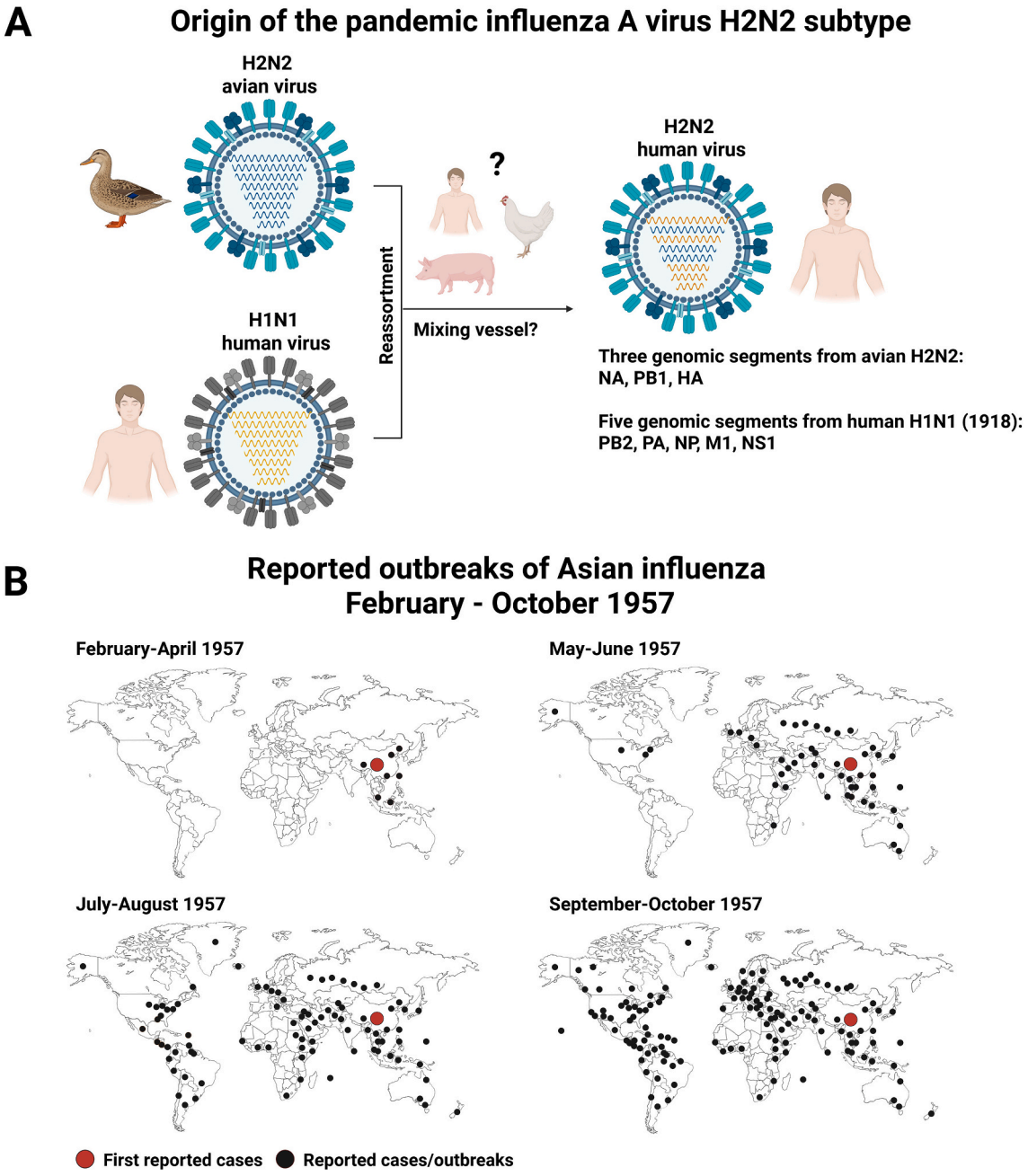

Fig. 1. Origin of the Asian influenza virus pandemic strain. (A) The pandemic H2N2 strain emerged from a reassortment between a contemporary human H1N1 virus and an avian H2N2 virus in an unknown intermediate host. (B) Global spread of the H2N2 virus during the first year of the H2N2 pandemic. Based on [6–8]. Created in BioRender. Tscherne, A. (2026) https://BioRender.com/yv0kcpk.

Centre in London, and the Walter and Eliza Hall Institute in Melbourne [11,13]. In summer 1957, travelers introduced the virus to areas along the Atlantic coast of the U.S., from where it spread across the country [14].

In June 1957, the first influenza case caused by the novel H2N2 virus was reported in Europe. A passenger with febrile respiratory illness had travelled from Djakarta to the Netherlands by plane [11]. By the end of August 1957, Asian countries and Australia reached the peak of the pandemic and reported a decline in influenza cases. In contrast, the outbreak was on the upswing in other parts of the world, including Europe, Africa, Oceania, and Central and South America, and by mid-September 1957, additional European countries reported an increase in H2N2 influenza cases [11]. This was related to school openings, where the first influenza cases were reported, followed by spreading of the virus via siblings and parents to factories, schools, and subsequently to the general population [11,15]. In October 1957, most regions around the world (Europe, Africa, North and South America) reported a decline in influenza cases. Interestingly, among Asian countries, only Japan experienced a second wave with major outbreaks in October 1957 [12]. In addition, the former U.S.S.R. and Czechoslovakia also experienced a second wave [11]. By November/December 1957, all affected countries reported a decline in influenza cases [16].

The clinical symptoms resembled H1N1 infections, including headache, myalgia, scratchy sore throat, chills, muscle pain, anorexia, sudden fever onset, non-productive cough and malaise [17–21]. The attack rate was very high (up to 60%), especially among younger age groups [7,8,12,16,18]. However, the estimated mortality rate of the 1957 H2N2 pandemic was moderate compared to the 1918 H1N1 pandemic (1–4 million deaths versus ~20–100 million deaths) [14,22–24]. Complications of severe influenza included acute chest infections and acute otitis media [18]. Most deaths were associated with pneumonia and bronchitis [15,17]. Interestingly, there were reports of toxic psychosis after influenza virus infection or during the acute phase. The patients were noisy, resistive, disoriented, or experienced hallucinations [25].

At the beginning of 1958, there were no reports of larger outbreaks, and the pandemic H2N2 virus soon became seasonal, emerging sporadically in the human population in the subsequent years [26,27]. The emergence and circulation of H2N2 in the human population led to the extinction of H1N1, which had been circulating for about 39 years. H1N1 emerged originally in humans in 1918, and was the causative agent of the Spanish influenza pandemic. During 1956 and the early months of 1957, H1N1 was still detectable in humans, but disappeared after the global spread of H2N2 [28].

3. Evolution of H2N2 between 1957 and 1968

Researchers were interested in how the novel H2N2 virus differed from previously circulating viruses and how the virus evolved during the ~11 years of circulation in humans. The global World Health Organization (WHO) influenza centers isolated various influenza virus strains between 1957 and 1960, which were sent to the World Influenza Centre in London for subsequent analysis. The received H2 viruses were antigenically similar and showed comparable *in vitro* characteristics [28]. During seasonal outbreaks in the Winter of 1961/1962 and 1962/1963 [29], influenza virus strains from geographically separated locations (e.g., New Zealand, the U.S., Europe, Far East) were recovered in laboratories, and human sera were analyzed for the presence of H2-specific antibody responses. The responses in human sera against the strains circulating from 1961 to 1963 were lower than against the prototype 1957 strain, suggesting antigenic drift.

Sequence analyses revealed that during the circulation in the human population, the virus evolved antigenically more slowly compared to other pandemic viruses, such as the later H3N2 virus [30]. The evolution affected both the antigenicity and transmissibility of the virus [31–33]. Strains isolated in 1957 and 1968 showed at least 25 amino acid changes within the HA. Of these, 12 amino acid changes (T126E, T128D, R132K, N139K, E151K, S154P, N181K, T184A, T188A, N192K, T214A, K217E/K) within the HA1 domain are considered to play a key role for antigenicity [30,34–36].

One key factor that restricts the transmission and host range of IAV is the receptor specificity of HA, which differs between avian and human IAV. HAs from avian IAV bind to glycan receptors on epithelial cells in the intestine, which contain terminal sialic acids with an α2,3-linkage to the vicinal galactose residue. In contrast, HAs from human IAV bind to glycan receptors on epithelial cells in the upper respiratory tract and lungs that contain sialic acids with an α2,6-linkage [37,38]. Thus, switching the specificity of avian IAV from α2,3- to α2,6-linked receptors promotes efficient human-to-human transmission. *In vivo* transmission studies in ferrets and *in vitro* studies using glycan array-binding assays indicate that the initial 1957 H2N2 strain had a dual receptor binding specificity, with a stronger affinity for α2,3 glycan receptors. The initial circulation and replication of H2N2 in humans might have led to amino acid substitutions within the HA gene - particularly in the receptor binding site - which shifted the specificity to α2,6 glycan receptors [32,33], facilitating the rapid spread of the virus. Amino acid substitutions at positions Q226, G228, R137, N144E and A193 within HA [38,39] were found to correlate with changes in receptor binding specificity. Avian-like H2N2 viruses containing Q226 and G228 showed preferred binding to α2,3 glycan receptors, whereas human H2N2 viruses with either Q226L or both Q226L and G228S, showed binding to both α2,3 and α2,6 glycan receptors [32,40]. In addition, R137K/M, N144E/S, and A193T substitutions were found to change the receptor specificity from an initial dual receptor binding of pandemic 1957 H2N2 to a preferred α2,6 glycan receptor binding later on [33,41].

Besides the switch from avian- to human-type receptor specificity, adaption and increased replication/infectivity of avian-like H2N2 viruses are crucial factors to cross the species barrier and to infect mammals and humans. It has been shown that a E627K substitution within PB2 of recently isolated H2Ny viruses promoted the replication capability *in vitro* and increased the virulence in mice, ferrets and guinea pigs [33]. In *in vivo* experiments, the PB2-E627K mutation had emerged already at day 5 during the first lung-to-lung passage of a low pathogenic avian H2N2 virus in mice, demonstrating a rapid adaption of the virus to mammals. After a few serial lung-to-lung passages, the virus caused 100% lethality in mice [33]. The PB2-E627K substitution was found in different subtypes (e.g., H5 or H7) [42] to promote enhanced activity of the viral polymerase in mammalian cells, and allowed the viral polymerase to interact with human acidic nuclear phosphoprotein 32 (ANP32) for efficient viral replication in human cells [43–45]. Increased infectivity in mammals was also associated with a G183S substitution within the non-structural protein (NS1) of recently isolated avian H2N2 viruses, most likely due to an evasion of the host immune response [33].

The N2 NA gene also evolved during circulation of H2N2 in humans between 1957 and 1968, with at least 21 conserved amino acid changes occurring [36]. The affected regions included the enzymatically active globular head region. As mentioned before, the H2 HA acquired mutations at the receptor binding domain, switching the specificity from avian- to human-type receptor binding. A gradual shift for preferred cleavage of α2,6 human-type receptor has been observed for the N2 NA in H2N2 and H3N2 viruses isolated between 1957 and 1987. Thus, both H2/ H3 HA and N2 NA demonstrated co-evolution to maintain HA and NA balance [46]. Furthermore, sequence analyses of avian N2 and human N2 NAs, isolated since 1957, indicated independent evolution. Human N2s, but not avian N2s, antigenically developed in a ladder-like pattern, presumably due to an immune driven evolution [47]. From 1957 to early 1970, human and avian N2s shared approximately 90% sequence identity. However, the human N2 NAs diverged, and analysis of viruses circulating in the past 20 years showed only 75–80% similarity to the ancestral avian N2 NAs. These findings were further supported by analyzing the sera of migrating birds for the presence of N2-specific antibodies [48]. In 1972, antibodies to the 1957 H2N2 strain were detected in sera of various migrating bird species (e.g., short-tailed

shearwaters, wedge-tailed shearwaters, noddy terns) around Australia. These sera also inhibited NA from A/Hong Kong/68 (H3N2), although the neuraminidase inhibiting (NI) titers were lower compared to the 1957 H2N2 strain. No cross-reactive NA-specific responses were observed with earlier circulating strains, such as A/BEL/42 (H1N1) or A/FM/1/47 (H1N1).

4. Immunity against H2N2

Although there have been speculations about the circulation of an H2 subtype in the human population in the late 19th century, only inconsistent reports are found in the literature, and robust data to confirm these speculations are lacking [49–52]. At the beginning of the H2N2 pandemic in 1957, most of the population was antigenically naïve to the newly emerged subtype (Fig. 2). However, this changed rapidly, as H2N2 spread globally within a few months among the naïve population. When the virus disappeared in 1968, most of the population had acquired immunity against both H2 and N2, either by infection and/or vaccination. The H2 subtype persists in domestic and wild birds (H2Ny) [53,54] and has also been found in pigs (H2N3) [55] and muskrats (H2N2) [56]. This raises concerns about a possible re-introduction into the human population. Seroprevalence studies showed that individuals born after 1968 are antigenically naïve to H2N2 [57] and lack protective immunity. In contrast, individuals born before 1968 have immunity due to earlier exposure to H2N2, which has been shown to be long-lasting [58–61].

However, global H2 immunity would be insufficient to prevent the rapid spread of the H2 subtype if it re-emerges in the human population [33]. The proportion of seropositive individuals is decreasing over time due to the declining number of individuals born before 1968 and the

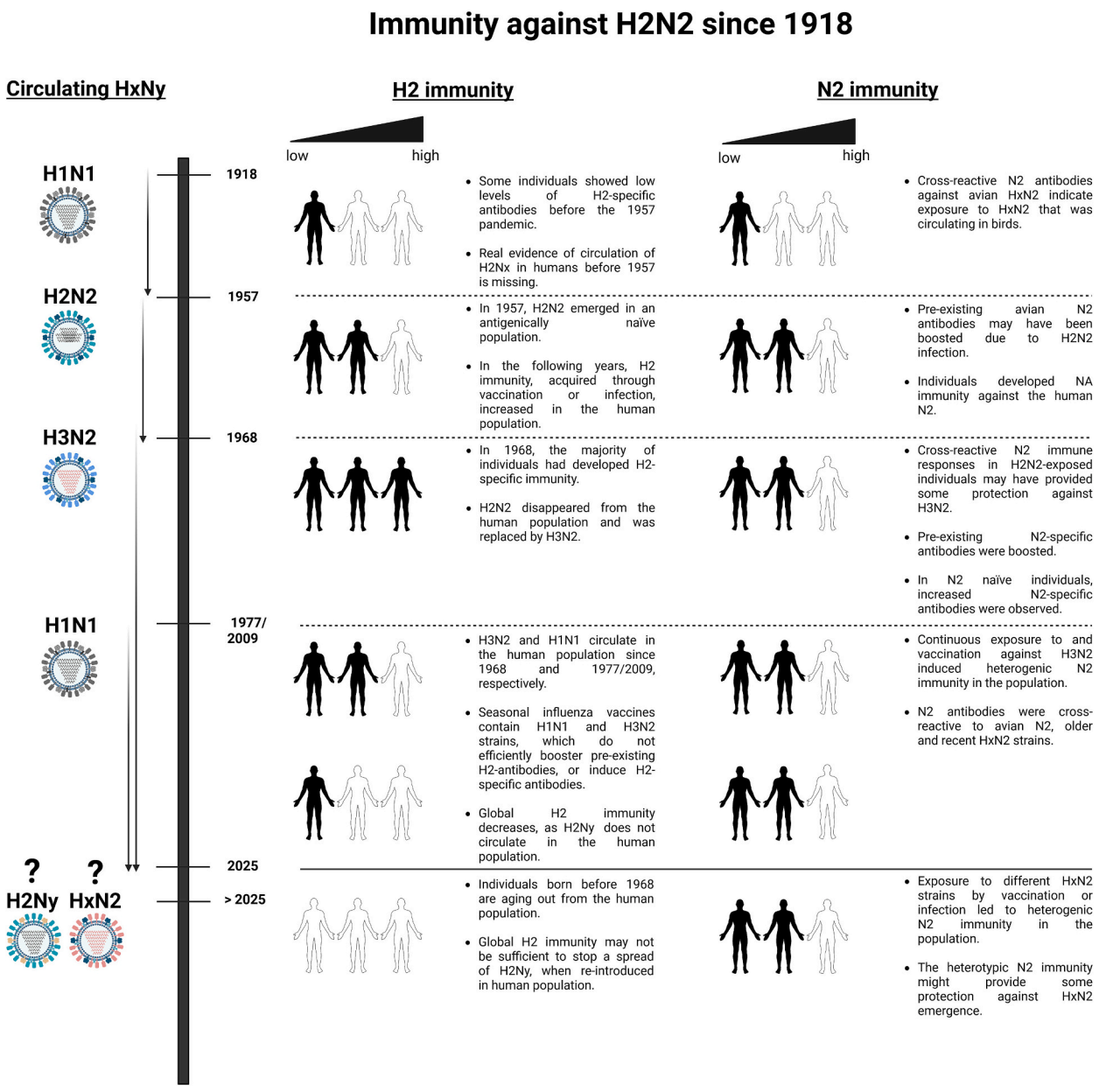

Fig. 2. Pre-existing immunity against H2 hemagglutinin (HA) and N2 neuraminidase (NA) in the human population since the 19th century. Before the circulation of H2N2 in the human population, the immunity against both H2 HA and N2 NA was low-to-negligible, but increased rapidly during 1957 and1968. After H2N2 disappeared from the human population, vaccinations against this subtype stopped and immunity against the H2 subtype has since been waning. In contrast, individuals are constantly exposed to the N2 subtype, either through natural infection or through seasonal vaccines. Created in BioRender. Tscherne, A. (2026) https://BioRender.com/7ty62fkx.

increasing number of naïve individuals, those neither vaccinated nor exposed to H2N2 (Fig. 3). The current seasonal influenza virus vaccines do not include the H2 antigen, and only a low-to-negligible booster effect of H2-specific antibody responses after vaccination [62] has been observed.

Most seroprevalence studies focused on the presence of H2 immunity in the population, while responses to N2 were largely neglected. One reason was the lack of suitable assays to measure N2-specific immune responses at that time. Over the past decades, researchers have become interested in NA as a potential target for vaccine development [64,65], as NA-antibodies are often cross-reactive, can reduce disease severity after infection, and can provide protection independently of HA-antibodies [47,66–70]. Unlike pre-existing HA antibodies, which are acquired via infection and vaccination, NA-specific antibodies are mostly obtained after infection, as the NA responses after vaccination are significantly lower [71].

When N2 emerged in the human population during the 1957 pandemic, individuals developed human N2-specific antibodies following natural infection. Serological studies revealed that before the H3N2 pandemic, N2-specific antibodies at low levels were present in 42% of individuals [72]. It was believed that those N2-specific antibodies may have provided some degree of protection against the H3N2 strain causing the Hong Kong influenza pandemic in 1968, since these two viruses share a similar NA [73]. Individuals infected with H2N2 in 1967/68 were better protected against infection with H3N2 early during the 1968 Hong Kong influenza pandemic than uninfected individuals [72]. Those with pre-existing N2 antibodies were more likely to develop asymptomatic influenza compared to individuals lacking such antibodies [72]. NA antibodies can exhibit high cross-reactivity that is not limited to human N2, as cross-reactivity against avian N2 has been observed in adults and the elderly, suggesting the presence of conserved epitopes [47,67]. The human population is constantly exposed to human N2 NAs through seasonal influenza virus circulation and vaccination-inducing a heterologous N2 immunity [71] that may offer some protection against H2N2 re-emergence.

While humoral immunity against both H2 HA and N2 NA has been studied extensively over the last decades, only limited information is available on the potential protective effect of cellular immunity against H2N2 in humans. Studies have been carried out over the last years to assess the level of cellular immunity against H2N2 in the human population. Low levels of cross-reactive cellular immunity directed against the nucleoprotein (NP), matrix protein (M) 1, PB1, polymerase acidic (PA) protein, PB2, NS1, HA, or NA of pandemic 1957 H2N2 could be observed in individuals born after 1968, most likely induced by vaccination against or infection with circulating seasonal influenza A viruses [74–76]. In addition, low levels of H2 HA-specific cellular immune responses could be found in individuals born before 1968 [76]. These studies assessed the cellular immunity in humans based on the identification of immunogenic T cell epitopes within the viral proteins, rather than testing for protective efficacy against H2N2. However, there is evidence that pre-existing IAV-specific $CD4^+$ and $CD8^+$ T cells, acquired through vaccination or infection, provide some degree of cross protection against different IAV subtypes, such as H1N1 or H3N2, in humans [77–79]. Based on these observations, the level of cross-reactive T cell immunity found in the population might provide some degree of protection against H2N2 re-emergence.

5. Vaccination against H2N2

5.1. H2N2 vaccination from 1957 until 1968

The causative agent of the Asian influenza pandemic was rapidly identified and isolated. Nasopharyngeal washings [80] from patients suffering from influenza were collected, filtered through cotton,

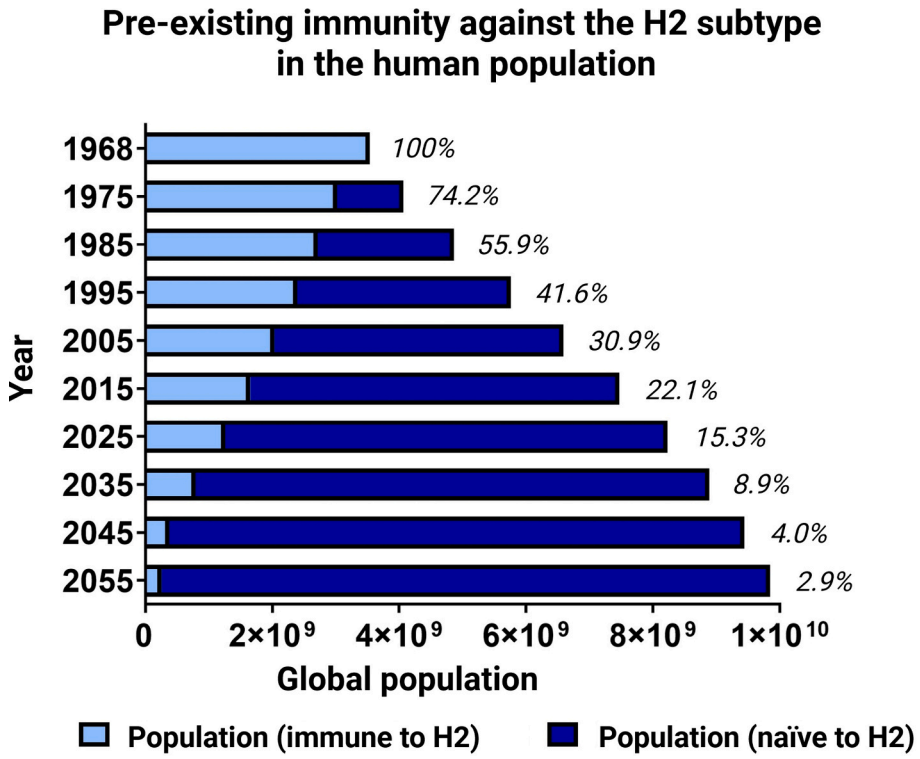

Fig. 3. Distribution of pre-existing immunity against the H2 subtype in the population from 1968 to 2055. Total number and percentage of individuals with pre-existing H2 immunity were analyzed in relation to the total global population. For the displayed years, the number of individuals born before 1968 were used for calculation: 1975 (aged >10 years), 1985 (aged >20 years), 1995 (aged >30 years), 2005 (aged >40 years), 2015 (aged >50 years), 2025 (aged >60 years), 2035 (aged >70 years), 2045 (aged >80 years), 2055 (aged >90 years). The numbers next to the bars indicate the calculated percentage of H2 immune people for each year. Data obtained from [63]. Created with Graphpad Prism 10 and in BioRender. Tscherne, A. (2026) https://BioRender.com/3z2x8kc.

concentrated, and injected into the amniotic cavity of embryonated eggs to amplify the virus. After incubation for a few days, the allantoic fluid was collected and either analyzed or used to reinfect embryonated eggs to obtain higher titers for vaccine development.

In September 1957, several countries, including the U.S., started large scale production of inactivated H2N2 virus vaccines. The vaccines were based on circulating strains (A/Singapore/1/57, or A/Adachi/2/57) in the respective countries. In the U.S., a total of 50 million vaccine doses were produced during the first 7 months after the first reported cases in the country [81]. However, the manufacturers faced various challenges and issues related to the vaccine production [81,82]: 1) large numbers of eggs were needed to generate the vaccine virus; 2) the vaccine preparations suffered from low yields of virus; 3) a lack of suitable assays to determine potency; 4) difficulties in assessing the immunogenicity of the new vaccines in humans; 5) the need to renovate or build new facilities for vaccine manufacturing. The vaccine formulations used during the circulation of H2N2 in humans included both monovalent and polyvalent inactivated vaccine preparations, as well as live attenuated vaccines (Fig. 4). At the beginning of the pandemic, vaccine studies were predominantly conducted in H2-naïve individuals [16,83–88]. After the first wave of influenza, vaccines were also evaluated in individuals with pre-existing H2 immunity, acquired through vaccination or infection [88–92].

While vaccines became quickly available after the outbreak, insufficient supply and uneven distribution resulted in many avoidable deaths. Although public health authorities categorized priority groups (e.g., pregnant women, healthcare and military personnel), evidence showed that vaccine doses were not efficiently distributed to these

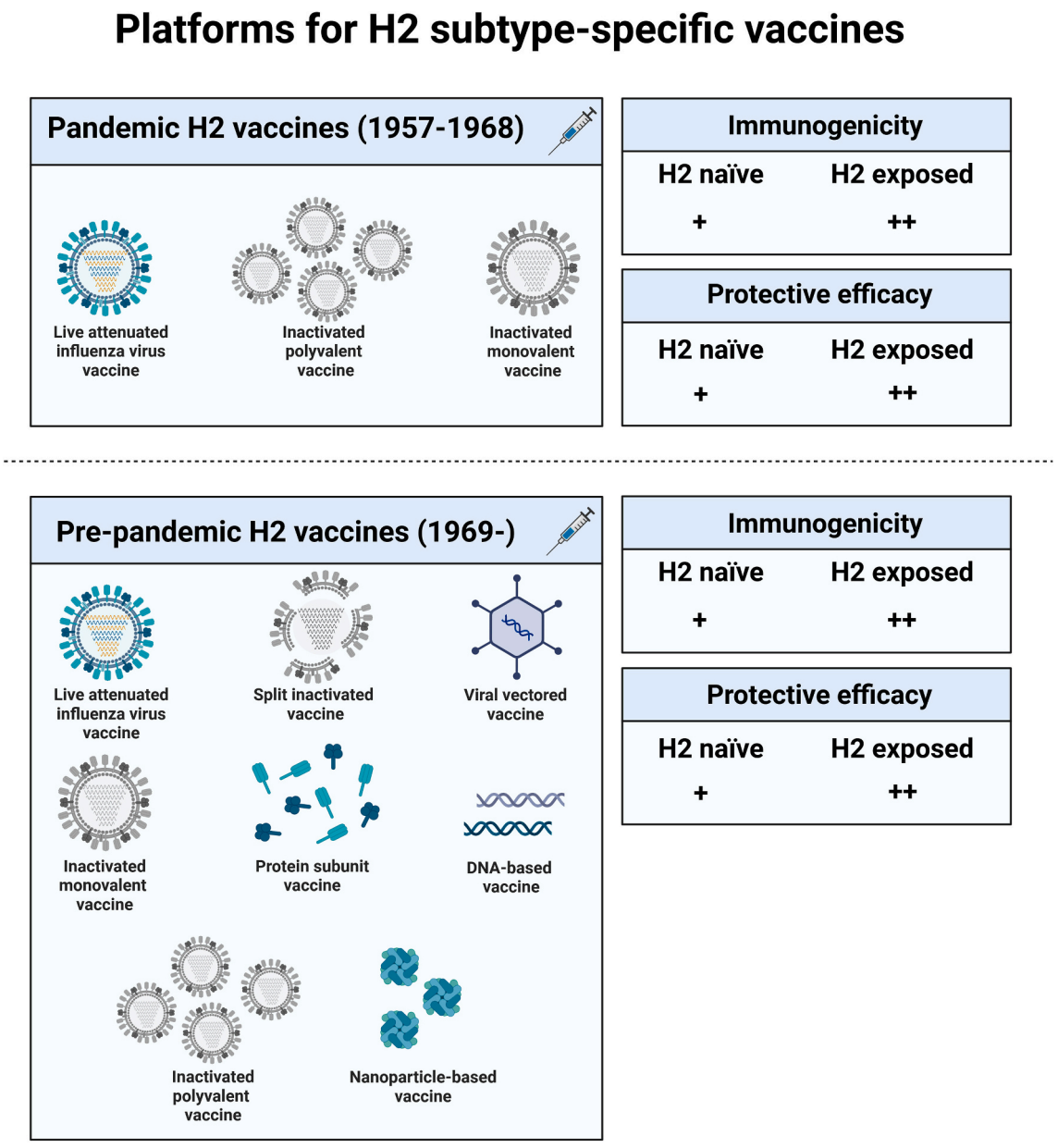

Fig. 4. Platforms for H2 subtype-specific vaccine development. During 1957 to1968, predominantly inactivated (mono-, and polyvalent) and live attenuated virus vaccines were used to combat the H2N2 pandemic. After the pandemic, the vaccine technology platforms improved, and in addition to inactivated and live attenuated virus vaccines, other platforms such as DNA-based, protein subunit-based, and split virus vaccines were used to design vaccines for potential future H2 emergence (**Pre-pandemic H2 vaccines**). The observed immunogenicity and protective efficacy in both pandemic and pre-pandemic vaccines is/was similar. Individuals with a pre-existing H2 immunity respond(ed) better to the vaccines which led to an increased immunogenicity and protective efficacy. Created in BioRender. Tscherne, A. (2026) https://BioRender.com/am6r51w.

groups [93]. After the first wave of the pandemic ended, and in preparation for future epidemics/pandemics, committees were established to inform the public on who should receive an influenza vaccine each year. In 1960, the annual vaccination of individuals at high risk was recommended by the U.S. Surgeon General. He tasked the Centers for Disease Control and Prevention (CDC) with establishing a concept to ensure that recommended individuals receive the vaccine. CDC and other public health agencies launched outreach programs, including songs, radio advertisements, posters, and service announcements [94], and began mass vaccination programs [95].

5.1.1. Inactivated monovalent vaccines

Vaccination campaigns with monovalent vaccine formulations (**Supplementary Table 1**) matching the circulating H2 strains (e.g., A/Adachi/2/57 [88,92], A/Singapore/1/57 [89–91,96,97], A/Japan/305/57 [19,86,87], A/Kumamoto/Y5/57 [88], A/Kumamoto/K9/57 [88]) started in 1957. These campaigns included monitored studies to evaluate the protective efficacy of the vaccines in different age groups, ethnic groups and sexes. The vaccine formulations contained between 20 and 500 [16,19,83–88,92,98,99] chicken erythrocyte agglutination (CCA) units or 15–7000 [90,91,96,100] hemagglutinating units (HU) of H2 strain per dose. Vaccines were administered subcutaneously (SC) [16,83,84,87,92,96–100], intradermally (ID) [16,83–85,87,88] or intramuscularly (IM) [19,86,90,91]. They were given once [86–89,92,96,98–100] or twice over 7- [83,88], 14- [16,85], 21- [88,89,100], or 28- [97] day intervals.

The most frequently reported adverse events after vaccination included swelling, pain and redness at the injection site, along with systemic reactions such as fever, headache or nausea [16,86,97–99]. Vaccinees were predominantly tested for hemagglutination inhibition (HI) antibody titers (the state-of-the-art method to measure immunity at the time), which increased in a dose dependent manner [16,19,83–86,88,90,92]. H2N2 HI antibody levels in sera of immunized individuals peaked a few weeks after immunization and declined to low or baseline levels within a few months [19,88,91]. Interestingly, in H2N2-naïve individuals, two low-dose immunizations at least 7 days apart induced higher antibody responses than a single high-dose immunization (total vaccine quantity was the same for both schedules) [83,88]. Consistent with these findings, vaccine responses during the first year of H2N2 circulation were low [16,84], and higher antigen doses were required to induce a robust immune response in individuals lacking pre-existing H2 immunity [84]. Vaccine studies conducted after the first influenza wave showed higher and more robust responses to vaccination, as individuals already had pre-existing H2 immunity from vaccination or natural infection [89–91,101,102]. Naturally infected individuals also developed H2-specific antibodies which were slightly higher than those induced by vaccination [16,85].

However, the vaccines only provided partial protection against influenza virus infection [19,21,85–88,90,99], with vaccine efficacy against symptomatic, respiratory influenza estimated to range between 38 and 81% [16,19,86,87,100]. Notably, individuals immunized with lower vaccine doses experienced more infections but were equally protected during a second influenza wave compared to those vaccinated with higher doses, indicating a potential immune recall following repeated exposure to H2N2 [19].

5.1.2. Inactivated polyvalent (bi-, tri-, quadrivalent) vaccines

Vaccinations with polyvalent vaccines (**Supplementary Table 1**) were carried out using either vaccine preparations from previous years (without H2 antigen) or adapted formulations including H2 strains. During the 1956/1957 influenza season (before the pandemic began), a study comparing polyvalent and monovalent vaccine formulations was initiated. The initial goal was to evaluate the effectiveness of a monovalent vaccine based on an IAV strain (A/Ann Arbor/56 (H1N1)) isolated early in 1956 [103]. Individuals received a single SC immunization with either monovalent vaccines (A/Ann Arbor/56 or B/Great Lakes/54) or a polyvalent vaccine (A/Puerto Rico/8/34, A/swine/31, A/Puerto Rico/301/54, B/Lee/40, B/Great Lakes/54). Pre-vaccination HI antibodies against heterologous H2 strain A/Denver/57 (H2N2) were mostly absent but could be slightly boosted with both mono- and polyvalent vaccines. During the 1956/1957 Winter season, influenza cases were reported across all groups; however, vaccinated individuals experienced fewer infections. Another trial [96] with a polyvalent vaccine formulation, containing three influenza A/H1 strains (A/swine/31, A/Puerto Rico/8/34, A/FM/1/47), but no H2 strain, was compared to a monovalent H2 vaccine (A/Singapore/1/57 H2N2) in patients with chronic bronchitis. Protective efficacy against infection with H2N2 was confirmed with the monovalent vaccine, but not with the polyvalent one. In addition, a polyvalent formulation (two A/H1 strains, B strain) [84] without H2 strain, tested in a prime only immunization schedule, failed to boost pre-existing H2 HI antibody responses or to induce H2-specific HI antibody responses in H2-naïve individuals.

It soon became clear, that polyvalent vaccines lacking the H2 antigen were ineffective in inducing H2-specific antibody responses and providing protection against H2N2 infection, leading to the adaption of vaccine formulations. The new formulations included H1 strains (e.g., A/Swine/1976/31 [102], A/Puerto Rico/8/34 [20,102], A/Puerto Rico/301/54 [102], Ann Arbor/1/57 [20,102]) and different H2-strains (e.g., A/Adachi/2/57 [88], A/Singapore/1/57 [104,105], A/England/1/61 [104,105], A/Netherlands/67/1963 [106], AA/23 (Asian-1957) [102]) in combination with influenza B virus strains (e.g., B/England/939/59 [104,105], B/Thailand/4/62 [104], B/Johannesburg/33/1958 [27,106], B/Great Lakes/1739/54 [20]). These vaccines were given once [27,88,96,104,105] or twice over 4- [107], 5- [20], 6- [108], 8- [102], or 12- [102] week intervals using ID [84,88,108], IM [102,104,105,108] or SC [27,84,88,96,102,105,107,108] administration routes. A few years before the pandemic, researchers started evaluating adjuvanted polyvalent vaccines compared to vaccines diluted in saline. This comparison continued during the pandemic, and some vaccines were tested in oil-in-water emulsions, which led to increased and long-lasting immune responses compared to unadjuvanted vaccines [102,105].

Like monovalent vaccines, the polyvalent vaccine formulations were tested for the induction/boosting of H2-specific antibody responses and protective efficacy against symptomatic H2N2 infection. The polyvalent vaccines contained less H2 antigen than the monovalent vaccines; thus, the induced levels of H2-specific HI titers were lower in the polyvalent vaccine groups [88,96]. The vaccines elicited HI antibody titers against the vaccine strains [102,104], but also against H2N2 strains, which circulated later in humans, such as A/Netherlands/1/64 [104]. Interestingly, adults developed a stronger immune response against the other vaccine strains (e.g., A/Swine/34, PR8), likely due to pre-existing immunity from earlier exposure to these strains [102]. In children with pre-existing H2 immunity, low doses of the polyvalent vaccine already stimulated H2-specific antibody responses after a prime immunization, which were further boosted after the second shot [102]. In adults with pre-existing H2 immunity, a prime immunization boosted the H2-specific antibody responses, however, the second immunization did not further increase these responses [108]. In terms of protection, polyvalent vaccines provided some protection against symptomatic H2N2 infection [20,27,107]. However, as seen with the induction of HI antibodies, the protection was lower compared to the monovalent vaccines [88,96,108].

5.1.3. Live attenuated vaccines

Another approach involved the use of live attenuated vaccines [7,80,109] (**Supplementary Table 1**), which were capable of replicating in the nasal cavity and the upper respiratory tract. The main advantage of using live virus is its intranasal (IN) application route, which preferably induces a local immune response at the side of viral entry (e.g., secretory IgA antibodies) [110]. Furthermore, a lower amount of virus is needed for immunization, and since this method is

needle-free, it is less painful compared to SC or IM administered vaccines. Live attenuated vaccines were mainly given in the former U.S.S.R [111], where by the end of 1957 more than 3 million people were vaccinated [80]. Zhdanov *et al.* [80] IN inoculated volunteers with allantoic fluid containing live H2N2 virus (Iksha strain) passaged four to six times in embryonated eggs. The vaccine was well tolerated, and the most frequently reported adverse events included fever and hyperemia of the nasal mucosa [80]. The vaccine slightly increased the HI antibody responses against H2 [7,80]. Isaacs *et al.* [109] passaged an H2N2 strain once each in amniotic and allantoic fluids for a first trial, and performed two additional passages at high dilutions in allantoic fluid for a second trial. The volunteers in the study tested negative for pre-existing H2 HI antibody titers. A total of ~1000 EID_{50} (50% egg infectious dose) of the virus was given intranasally and via swabs to the throat. Those who had developed symptoms (e.g., fever, cough), showed an increase in HI antibodies against H2N2. In the second trial, no volunteers developed symptoms, or showed an increase in antibodies against H2N2. Efforts were made to increase the immunogenicity and reduce the virulence of the live attenuated vaccines. Beare *et al.* [112] tested the effect of up to 30 egg passages on the immunogenicity of H2 strain A/Leningrad/4/65 and two influenza B strains (B/England/101/62, B/England/13/65), and found no differences in infectivity or induction of HI antibody responses between early passages and those after 30 passages. After three human passages of B/England/101/62, an increase in virulence was observed, and individuals developed more severe symptoms after immunization. Furthermore, Mills *et al.* [113] demonstrated that a cold-adapted H2N2 vaccine resulted in reduced virulence in humans, as no symptoms developed after immunization and no infectious virus could be isolated following immunization. However, study participants showed increased serum neutralizing antibodies and minor elevations in antibodies in sputum and nasal washings.

In 1968, when the Hong Kong influenza pandemic started, vaccination of the human population against H2N2 ended.

5.2. Vaccination in the post-H2N2 era (1969-)

When the H2N2 subtype was replaced by H3N2 in 1968, work on H2N2 vaccines mostly stopped. However, because there is an increasing potential for a re-emergence of the H2Ny subtype in the human population, the development of H2-specific vaccines should be prioritized again. Seasonal vaccines target only circulating influenza H1N1 and H3N2 viruses, and studies have confirmed the low capability of these vaccines to trigger effective cross-reactive immune responses against H2 viruses [62].

Although additional vaccine platforms have been developed since the 1950s, the current experimental H2N2 vaccines are predominantly based on the same inactivated [114–118], and live attenuated [119,120] virus platforms as were used during the 1957 pandemic (Fig. 4). Aside from these, new approaches involve protein-based [121–123], and viral vectored vaccines [124]. These vaccines were highly immunogenic in mice [114,115,118–121,123,124], and ferrets [119], by inducing systemic or mucosal immune responses against homologous [114,115,118–121,123,124] and heterologous H2 strains [114,115,118–121,123,124]. The vaccines provided complete or partial protection against infection in the respiratory tract with homologous [114,115,118–121,124] or heterologous H2 strains [114,115,118–121,124]. Of those that have been successfully tested in preclinical studies, at least five vaccine candidates have been evaluated in clinical trials over the last 20 years (Table 1).

5.2.1. Inactivated monovalent vaccines

An open, randomized, multi-center, and comparative bioequivalence study [116,117] with 200 participants was conducted in the early 2000s to evaluate the immunogenicity and reactivity of a monovalent H2N2 vaccine (A/Singapore/1/57). Individuals were IM immunized twice over a 21-day interval with 1) 15 μg split vaccine, 2) 7.5 μg whole virus vaccine +0.5 mg aluminum, 3) 3.8 μg whole virus vaccine +0.5 mg aluminum or 4) 1.9 μg whole virus vaccine +0.5 mg aluminum. Only 10/200 participants had pre-existing H2 HI antibody titers. After the first immunization, titers slightly increased in all groups in a dose dependent manner, with an overall seroconversion rate between 32 and 48%. The booster immunization increased H2 HI-specific immune responses, with the split vaccine and the highest doses (7.5 μg, 3.8 μg) of the whole virus vaccine eliciting the strongest responses. The overall seroconversion rate was between 88 and 100% after two immunizations. Almost all individuals (≥ 98%) vaccinated with 15 μg split vaccine or 3.8 μg whole virus vaccine developed HI titers ≥1:40. The lower dose of the whole virus vaccine (1.9 μg) and the split vaccine (15 μg) increased both pre-existing neutralizing antibody titers and NI responses after one immunization, which further increased after the second immunization. The seroconversion rates after the second immunization ranged between 82 and 94% (for neutralizing antibody responses) and 94–98% (for NI responses). No increase in nasal IgA could be detected in any of the vaccine groups.

5.2.2. Live attenuated cold-adapted vaccines

A/Ann Arbor/6/60 (H2N2) is licensed in the U.S. and Europe as the backbone virus for the live attenuated seasonal influenza vaccine. This cold-adapted, temperature-sensitive strain was originally generated in the 1960s [127], and it replicates in cell culture at 25 °C and 33 °C, but not above 39 °C. Furthermore, the replicative capacity *in vivo* is limited to the respiratory tract [128]. Two IN immunizations of BALB/c mice with this vaccine strain led to a ≥ 4-fold increase in serum HI titers and provided complete protection against infection with the homologous A/Ann Arbor/6/60 strain and partial protection against heterologous H2 strains (e.g., A/Japan/305/57) in the respiratory tract. A single IN immunization in ferrets led to a ≥4-fold increase in serum HI titers, provided complete protection in the upper respiratory tract against homologous challenge, and offered partial protection against heterologous H2 strain challenge.

As a follow-up [125], the A/Ann Arbor/6/60 vaccine was evaluated in a phase I clinical trial during the summer of 2008 in Baltimore (U.S.) with 21 participants to evaluate safety, immunogenicity, and infectivity. Individuals were IN vaccinated twice with 10^7 $TCID_{50}$ (50% tissue culture infectious dose) over a 4-week interval. After the first and second immunizations, the vaccine strain was detected in 24% and 17% of individuals, respectively. None showed a ≥4-fold increase in HI antibody responses after one immunization, and only 12% developed a ≥4-fold increase in HI antibody responses after the second immunization. None of the participants developed neutralizing antibody responses after one or two immunizations. After one or two immunizations, only 5/21 participants developed a ≥4-fold increase in serum IgG titers, and 3/21 developed a ≥4-fold increase in serum IgA titers. After two immunizations, only 1/21 participant developed a ≥4-fold increase in nasal IgA titers. Regarding cellular immunity, 2/18 participants had a ≥ 5-fold rise in vaccine-specific IgG-secreting cells, while none showed a ≥ 5-fold rise in vaccine-specific IgA-secreting cells. As expected, most participants had pre-existing seasonal N2 NI antibody titers, with only one participant having pre-existing NI titers against the pandemic N2 NA. Only one participant developed a ≥4-fold increase in NI titers against pandemic N2, and four developed a weak increase in NI titers against seasonal N2.

Isakova-Sivak and colleagues [120] developed live attenuated, cold-adapted vaccines based on the A/Leningrad/134/17/57 (H2N2) strain, with the HA and NA of either A/California/1/66 (17/Cal/395) or A/Tokyo/3/67 (17/Tok/326). Two IN immunizations of CBA mice with 17/Cal/395 induced higher homologous HI antibody titers compared to immunization with 17/Tok/326. In contrast, two IN immunizations with 17/Tok/326 resulted in higher heterologous HI antibody titers compared to immunization with 17/Cal/395. Immunizations with either 17/Cal/395 or 17/Tok/326 provided complete protection against heterologous or homologous challenge infection in the upper and lower

Table 1
Recent H2 subtype-specific vaccine candidates in clinical trials.

Date of last publication	Route	Vaccine platform	Name of vaccine	Vaccine strain	Developer/ manufacturer/ sponsor	Key findings	Phase	Status	Trial registries, public reports	Clinical data
2002, 2004	IM	• Inactivated whole virus vaccine (adjuvanted with aluminum) • Split formulation	Monovalent H2N2	A/Singapore/1/ 57(H2N2)	n/a	• No severe adverse reactions • Most frequently reported adverse events: redness, swelling at injection side • 15 μg of split vaccine induced similar antibody responses compared to 7.5 μg and 3.8 μg aluminum adjuvanted whole virus vaccine • Single immunization did not efficiently booster pre-existing H2 HI antibodies (pre-vac; one dose; two doses): o Split vaccine (15 μg): 1:5; 1:18-1:26; 1:126 o Whole virus (7.5 μg): 1:6; 1:16–34; 1:93 o Whole virus (3.8 μg): 1:6; 1:18–1:39; 1:95 o Whole virus (1.9 μg): 1:6; 1:13–1:25; 1:63 • Pre-existing nAb were boosted after vaccination with 15 μg split vaccine or 1.9 μg whole virus vaccine (pre-vac; one dose; two doses) o Split vaccine (15 μg): 1:74; 1:423–448; 1:1376 o Whole virus (1.9 μg): 1:99; 1:250–1:330; 1:802 • Pre-existing NI antibodies were boosted after vaccination with 15 μg split vaccine or 1.9 μg whole virus vaccine (pre-vac; one dose; two doses) o Split vaccine (15 μg): 1:12; 1:78–86; 1:151 o Whole virus (1.9 μg): 1:9; 1:35–1:56; 1:88	n/a	completed	n/a	[116,117]
2012	IN	LAIV (ca, ts)	H2N2 1960 AA ca	A/Ann Arbor/6/ 60 (H2N2)	MedImmune (U.S.)	• Infectious virus detectable after first (24%) and second dose (17%) • Weak immunogenic after one or two doses: o H2 HI titer: 10% (≥ 4-fold increase) o Serum H2 titer: IgG (24%, (≥ 4-fold increase)), IgA (16%, (≥ 4-fold increase) o Nasal HA-IgA: 5% (≥ 4-fold increase) o No nAb responses • Weak induction of cellular immunity after one or two doses: o IgG secreting cells: 19% (≥ 5-cell rise) o IgA secreting cells: 0% (≥ 5-cell rise) • 1/21 participant had pre-existing NI titers specific to vaccine strain o No boost of vaccine specific NA titers o 1/21 participant developed ≥4-fold increase to vaccine NA • 18/21 participants had pre-existing NI titers to seasonal N2 NA (mean 1:21,6) • 4/21 showed modest increase in titer against seasonal N2	I	Completed	n/a	[125]

(*continued on next page*)

Table 1 (*continued*)

Date of last publication	Route	Vaccine platform	Name of vaccine	Vaccine strain	Developer/ manufacturer/ sponsor	Key findings	Phase	Status	Trial registries, public reports	Clinical data
2015	IN	LAIV (ca)	LAIV-H2N2	A/17/California/ 66/395 (H2N2)	PATH, Research Institute of Influenza (Saint Petersburg, Russia)	• No severe adverse reactions • Vaccine strain was highly infectious (74–78% of individuals shed virus after one or two doses) • Highly immunogenic (humoral immunity): o Cumulative seroconversion: 85% o HI (serum): 60% o MN (serum): 60% o IgA ELISA (serum): 52% o IgG ELISA (serum): 8% o IgA ELISA (nasal wick): 48% o IgA ELISA (saliva): 44% • Moderate immunogenic (cellular immunity): o Cumulative: 56% o CD4+/CD8+ T cells: 22%/22% o CD4+/CD8+ T_{cm} cells: 15%/15% o CD4+/CD8+ T_{em} cells: 15%/45%	I	Completed	NCT01982331	[126]
2022, 2025	IM	Ferritin nanoparticle-based vaccine, H2 DNA vaccine	H2HA-ferritin (VRC-FLUNPF081–00-VP), H2 DNA (VRC-FLUDNA082–00-VP)	A/Singapore/1/ 57 (ectodomain of HA)	National Institute of Allergy and Infectious Diseases (NIAID) (U.S.)	• No severe adverse reactions • H2-exposed individuals: o HI titers against H2N2 A/Singapore/1/ 57 measurable after prime immunization o No increase in antibody responses against HA stem from H1, H2, H5 or H6 subtypes o No increase in nAb responses against H5N1 and H6N1 after prime-boost immunization o Rapid secretion of potent H2-specific antibodies • H2-naïve individuals: o HI titers against H2N2 A/Singapore/1/ 57 measurable after prime-boost immunization o Antibody responses against HA stem from H1, H2, H5 and H6 subtypes measurable after prime immunization and were boosted after the second immunization o Increase in neutralizing antibodies against H5N1 and H6N1 after prime-boost immunization o Secreted H2-specific antibodies were less potent than in H2-exposed individuals	I	Completed	NCT03186781	[58,61,122]
n/a	IM	Mosaic hexavalent vaccine	FluMos-v2	A/Idaho/07/ 2018 A/Singapore/1/ 1957 A/Perth/1008/ 2019 A/Darwin/106/ 2020 B/Colorado/06/ 2017 B/Phuket/3073/ 2013	National Institute of Allergy and Infectious Diseases (NIAID) (U.S.)	n/a	I	Active, not recruiting	NCT05968989	n/a

AA: Ann Arbor; ca: cold-adapted; H/HA: hemagglutinin; HI: hemagglutination inhibition; IN: intranasal; IM: intramuscular; LAIV: live attenuated influenza virus; MN: micro-neutralization; n/a: not applicable; nAb: neutralizing antibody; N/NA: neuraminidase; T_{cm}: central memory T cells; T_{em}: effector memory T cells; ts: temperature-sensitive.

respiratory tract. In ferrets, a single immunization with 17/Cal/395 or 17/Tok/326 induced high homologous and moderate heterologous HI and virus-neutralization (VN) antibody titers. A single immunization with either 17/Cal/395 or 17/Tok/326 provided partial protection in the upper respiratory tract and complete protection in the lower respiratory tract against homologous or heterologous challenge infections.

In a follow-up study, the A/17/California/66/395 vaccine was evaluated in a randomized, double-blind, placebo-controlled phase I clinical trial between 2013 and 2014 in Saint Petersburg (Russia) (NCT01982331) [126]. The study design involved two IN immunizations with the H2N2 vaccine 28-days apart and subsequent analyses of neutralizing antibodies, mucosal antibodies (nasal and salivary IgA), and cellular immune responses. A total of 38 individuals were enrolled, of which 28 were included in the vaccine group. The most common reported reactions included vomiting, sore throat, dry nose, and headache. Shedding of the virus was observed in 78.6% and 74.1% of the individuals after the prime and booster immunizations, respectively. Overall, 85% and 56% of the individuals showed detectable antibody and cellular immune responses, respectively.

5.2.3. Ferritin nanoparticle-based vaccines

A first-in-human, dose-escalation, open-label, randomized, phase I clinical trial (NCT03186781) [122] with 50 individuals with or without pre-existing H2 immunity was conducted between October 2017 and September 2019 in Bethesda, Maryland (U.S.). The study aimed to evaluate the safety, tolerability, and immunogenicity (antibody responses) of a ferritin nanoparticle-based vaccine. This vaccine consisted of the ectodomain of the HA from A/Singapore/1/57 (H2N2) fused to a *Helicobacter pylori*-derived ferritin protein (H2HA-Ferritin). The study design included 5 groups, in which the H2 vaccine was tested over a prime only or homologous or heterologous prime-boost vaccination regimen: 1) H2HA-ferritin (20 μg) as single dose in H2-naïve individuals, 2) H2HA-ferritin (60 μg/dose) in H2-naïve individuals in a homologous prime-boost regimen with a 16-week interval, 3) H2 DNA vaccine (4 mg) and H2HA-ferritin (60 μg/dose) in H2-naïve individuals with a 16-week prime-boost interval, 4) H2 DNA vaccine (4 mg) and H2HA-ferritin (60 μg) in H2-exposed individuals with a 16-week prime-boost interval and 5) H2HA-ferritin (60 μg/dose) in H2-exposed individuals in a homologous prime-boost regimen in a 16-week interval. Individuals with pre-existing H2 immunity showed HI titers against A/Singapore/1/57 (H2N2) after the first immunization, whereas in H2-naïve individuals, HI titers were measurable after two immunizations. The overall seroconversion rate ranged from 31 to 89% after booster immunization. Subsequently, antibody responses against the HA stalk (from subtypes H1, H2, H5, H6, H7) were evaluated using an electrochemiluminescence immunoassay (ECLIA). Interestingly, H2-naïve individuals showed increased responses to H1, H2, H5 and H6 after the H2HA-ferritin prime immunization, which remained stable up to 6 months following the booster vaccination. H2- exposed individuals tended to exhibit higher baseline stalk HA antibody responses, and immunizations did not boost these responses. Neutralizing H2N2-specific antibodies were detectable at baseline in H2-exposed individuals and increased after the homologous (4.4-fold) and heterologous (6.6-fold) prime-boost immunizations. In H2-naïve individuals, no pre-existing H2N2 neutralizing antibodies were detectable at baseline and were induced after the homologous (13-fold) and heterologous (25-fold) prime-boost immunizations. In addition, H2-naïve individuals, but not H2-exposed individuals, exhibited increased neutralizing antibody responses against H5N1 and H6N1 after heterologous and homologous prime-boost vaccinations, suggesting induction of anti-stalk antibodies. Similar findings have been reported following H5N1 vaccination in H5-naïve individuals [129,130]. In H2-exposed individuals [58], a single immunization with H2HA-ferritin induced a strong H2 memory response, whereas in H2-naïve individuals two immunizations were required. Furthermore, in H2-exposed individuals, vaccination with H2HA-ferritin could expand pre-existing H2-specific memory B cells (IgG, IgA) without changing

isotype usage or somatic hypermutation (SHM). In contrast, in H2-naïve individuals, immunization with H2HA-ferritin recruited H2-specific naïve B cells that underwent IgG isotype switching and increased SHM. H2-specific antibodies produced by B cells following immunization were more potent in H2-exposed individuals compared to H2-naïve individuals. Overall, H2-exposed individuals had a more rapid and high-affinity H2 response following immunization than H2-naïve individuals, although their H2N2 exposure during the 1957 pandemic was over 50 years ago.

6. Treatment against H2N2

Due to the rapid spread of H2N2 in 1957 within a short time, it became apparent for health officials that efforts to stop the viral spread would be futile. Thus, neither were decisions made to quarantine infected or contact persons, nor to cancel large gatherings such as sport events, or conferences, nor to restrict travel. The main focus was on providing medical care for sick people and to ensure functionality of health services and the community. The treatment options in the 1950s/1960s were only limited, and were lacking uniformity. To reduce hospital burden, it was recommended that non-severely sick patients stayed at home, rested and drank enough water. Suitable antiviral treatments against IAV were not available in the 1950s [131,132]. In the 1960s, amantadine, an M2 proton channel inhibitor, was the first antiviral drug which received approval by the U.S. Food and Drug Administration (FDA) for treatment and prophylaxis of IAV. A daily dose of amantadine was found to inhibit infection with H2N2 [133]. However, by the time the drug received approval for human use, H2N2 was already a seasonal circulating virus and most individuals had developed immunity.

Although H2N2 stopped circulating in the human population, H2Ny still persists in the animal reservoir, posing a potential risk of spillover infections to humans. Antiviral susceptibility testing of currently circulating avian H2N2 viruses using FDA-approved neuraminidase inhibitors revealed high susceptibility to oseltamivir, zanamivir, and peramivir. Furthermore, genetic analysis of >500 H2N2 isolates of human or avian origin indicated susceptibility to adamantanes, as specific mutations within the M2 protein at positions 26, 27, 30, 31, or 34, which were associated with antiviral resistance [134–136], were absent. In contrast, high percentages of adamantane-resistant variants were observed for H1 (~70%) and H3 (~44%) subtypes [54,135]. Based on these observations, it is most likely that an initial treatment of H2N2 infections in humans with adamantanes, neuraminidase inhibitors or the newly developed cap-snatching inhibitors will be successful. However, as observed for other subtypes, prolonged exposure of H2N2 to antiviral drugs might favor the emergence of resistant variants.

7. Discussion and future perspective

The 1957 H2N2 pandemic provided a unique opportunity to investigate the immune responses following infection with and vaccination against an influenza virus subtype that emerged in a naïve population. Although some believed that the H2-subtype circulated in the human population before 1900, as some individuals had low levels of pre-existing H2 immunity, the majority had no immunity against H2. Vaccination campaigns and studies started in Fall of 1957.

While in the U.S. and Europe preferentially inactivated monovalent or polyvalent vaccine formulations based on the Asian strain were used, the former U.S.S.R. started vaccinating with live attenuated vaccines. It soon became apparent that polyvalent vaccines, which at that time targeted H1 subtypes and influenza B viruses, did not effectively induce H2 immunity. Furthermore, the inactivated vaccines based on the circulating H2 strains were not highly immunogenic, and in H2-naïve individuals, higher antigen doses and two immunizations were needed to induce immunity. This posed a problem, as initial vaccine supplies during the pandemic were limited. In addition, the live attenuated vaccines stimulated only low levels of H2-specific antibodies. When H2N2 became seasonal in subsequent years, most individuals had developed pre-existing H2 immunity, either acquired through natural infection or vaccination. The responses to the vaccines changed, as single immunizations with inactivated mono- and polyvalent vaccines (containing H1 subtype, influenza B viruses, and H2 subtype) were sufficient to boost pre-existing H2 antibodies, and a second immunization did not further increase these responses. However, none of these vaccines provided complete protection against symptomatic influenza virus infection.

Immunogenicity studies published in the 1950s and 1960s relied on HI assays and complement-fixation assays to measure H2 antibody responses in sera of infected and/or vaccinated individuals. Differences between these methodologies led to discrepancies that were observed in the obtained titers from the same set of sera. Furthermore, Holland *et al.* discovered that the level of measured HI titers and the percentage of seropositive individuals varied considerably, depending on the batch of *Vibrio (V.) cholerae* receptor-destroying enzyme used for serum treatment. When a weak batch of *V. cholerae* filtrate was used to treat sera, the number of positive individuals changed from 6 to 25 out of >100 samples [50]. Nowadays, more advanced assays are available, and aside from HI, binding antibody responses, neutralization titers, and detailed analysis of B and T cell activation are used to assess immune responses.

When H2N2 was replaced by H3N2 in 1968, vaccination campaigns and further development of H2 subtype-specific vaccines stopped, and researchers instead focused on the circulating H3N2 strain. Today, the global population faces a similar situation regarding pre-existing H2 immunity as in 1957. A minority of individuals who were born before 1968 still have H2 immunity, but most people alive today are antigenically naïve to H2 since the virus has not circulated for the past 60 years. Continued circulation of H2 in animal reservoirs raises concerns about a possible re-introduction of the virus to a population that lacks H2 immunity.

Surveillance of circulating influenza virus strains, including H2Ny strains, in the animal reservoir is of great importance [137]. The low rate of H2N2 antigenic drift in avian species may indicate that the vaccines licensed in 1957 would still be useful in the case of a future H2 pandemic [117]. This might be one reason why several candidate vaccines under evaluation in preclinical and clinical trials are based on H2 strains (e.g., A/Singapore/1/57, A/Ann Arbor/6/60) which were used during the H2N2 era. In addition, avian H2 (e.g., circulating in waterfowl) could be added to the vaccine formulations [114]. Most H2-based vaccine candidates remain in preclinical testing or early-phase clinical trials, and, as the virus does not circulate in the human population, it will be difficult to assess the protective efficacy in later phase clinical trials. Furthermore, the results obtained from these early-phase clinical trials already indicate that these vaccines are less effective in inducing a robust immune response in individuals without pre-existing H2-immunity, resembling the situation seen in the 1950s or the situation seen with H5N1 and H7N9 vaccines. Nevertheless, vaccine technologies and strategies have improved significantly since the 1950s, including the development of mRNA vaccines, viral vectored vaccines, the use of safe and potent adjuvants, the use of new targets besides the HA (e.g., NA), and new administration routes/methods (e.g., via the gingival sulcus using dental floss) [138]. Additionally, vaccine candidates against other influenza virus subtypes (e.g., H5, H7) with pandemic potential have been designed that induce cross-reactive and broadly protective immune responses, an approach different from past vaccines, which targeted specific subtypes or strains. If an effective candidate is available, ideally inducing cross-reactive immunity against various H2 influenza viruses, one could consider generating stockpile vaccine preparations to be distributed rapidly in the case of an H2Ny outbreak scenario in the human population.

It has been almost 70 years since the H2N2 pandemic and almost 60 years since the virus disappeared from the human population. The virus is still circulating in the avian reservoir, sometimes close to high-density urban areas like New York City, and the risk for another H2 pandemic

has increased, as most of the population is now susceptible to H2. Although vaccine technologies have improved, inducing strong immune responses to influenza viruses to which individuals are naïve remains challenging as seen with H5N1 and H7N9 vaccines. Furthermore, far less effort is being put into preparing for a potential H2 pandemic than into pandemic preparedness for H5N1. However, the H2N2 subtype has already demonstrated its ability to cause a pandemic, while H5N1 – despite very high prevalence in animals globally – has not yet resulted in a pandemic.

CRediT authorship contribution statement

Alina Tscherne: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Florian Krammer:** Writing – review & editing, Investigation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays, NDV-based SARS-CoV-2 vaccines, influenza virus vaccines and influenza virus therapeutics which list FK as co-inventor and FK has received royalty payments from some of these patents. Mount Sinai has spun out a company, Castlevax, to develop SARS-CoV-2 vaccines. FK is co-founder and scientific advisory board member of Castlevax. FK has consulted for Merck, GSK, Gritstone, Sanofi, Curevac, Seqirus and Pfizer and is currently consulting for 3rd Rock Ventures and Avimex. The Krammer laboratory is also collaborating with Dynavax on influenza vaccine development.

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Appendix A. Supplementary data

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Data availability

No data was used for the research described in the article.

References

[1] Scholtissek C, Rohde W, Von Hoyningen V, Rott R. On the origin of the human influenza virus subtypes H2N2 and H3N2. Virology 1978;87:13–20.
[2] Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. J Virol 1989;63:4603–8.
[3] Wallace GD, Kissling RE. Studies in swine of Asian influenza virus. Bull World Health Organ 1959;20:455–63.
[4] Smith GJ, Bahl J, Vijaykrishna D, Zhang J, Poon LL, Chen H, et al. Dating the emergence of pandemic influenza viruses. Proc Natl Acad Sci USA 2009;106:11709–12.
[5] Joseph U, Linster M, Suzuki Y, Krauss S, Halpin RA, Vijaykrishna D, et al. Adaptation of pandemic H2N2 influenza A viruses in humans. J Virol 2015;89:2442–7.
[6] Dunn FL. Pandemic influenza in 1957; review of international spread of new Asian strain. JAMA 1958;166:1140–8.
[7] Zhdanov VM. The 1957 influenza pandemic in the USSR. Bull World Health Organ 1959;20:489–94.
[8] Trotter Jr Y, Dunn FL, Drachman RH, Henderson DA, Pizzi M, Langmuir AD. Asian influenza in the United States, 1957-1958. Am J Hyg 1959;70:34–50.
[9] Jester BJ, Uyeki TM, Jernigan DB. Fifty years of influenza A(H3N2) following the pandemic of 1968. Am J Public Health 2020;110:669–76.
[10] Chung DH, Torchetti MK, Killian ML, Swayne DE, Lee DH. Transmission dynamics of low pathogenicity avian influenza (H2N2) viruses in live bird markets of the Northeast United States of America, 2013-2019. Virus Evol 2022;8:veac009.
[11] Mc DJ. Asian influenza in Great Britain 1957-58. Proc R Soc Med 1958;51:1016–8.
[12] Fukumi H. Summary report on the Asian influenza epidemic in Japan, 1957. Bull World Health Organ 1959;20:187–98.
[13] Stewart WH. Administrative history of the Asian influenza program. Public Health Rep (1896) 1958;73:101–13.
[14] Langmuir AD, Pizzi M, Trotter WY, Dunn FL. Asian influenza surveillance. Public Health Rep (1896) 1958;73:114–20.
[15] Dauer CC. Mortality in the 1957-58 influenza epidemic. Public Health Rep (1896) 1958;73:803–10.
[16] Sigurjonsson J, Sigurdsson B, Grimsson H. Experience with influenza vaccination in Iceland, 1957. Bull World Health Organ 1959;20:401–9.
[17] Chin TD, Foley JF, Doto IL, Gravelle CR, Weston J. Morbidity and mortality characteristics of Asian strain influenza. Public Health Rep (1896) 1960;75:148–58.
[18] Fry J. Influenza A (Asian) 1957; clinical and epidemiological features in a general practice. Br Med J 1958;1:259–61.
[19] Dull HB, Jensen KE, Rakich JH, Cohen A, Henderson DA, Pirkle CI. Monovalent Asian influenza vaccine: evaluation of its use during two waves of epidemic Asian influenza in partly immunized penitentiary population. JAMA 1960;172:1223–9.
[20] Krag CL. Influenza virus vaccine. The effect upon an outbreak of the disease among a geriatric population. Calif Med 1963;99:310–1.
[21] Blumenfeld HL, Kilbourne ED, Louria DB, Rogers DE. Studies on influenza in the pandemic of 1957-1958. I. An epidemiologic, clinical and serologic investigation of an intrahospital epidemic, with a note on vaccination efficacy. J Clin Invest 1959;38:199–212.
[22] Viboud C, Simonsen L, Fuentes R, Flores J, Miller MA, Chowell G. Global mortality impact of the 1957-1959 influenza pandemic. J Infect Dis 2016;213:738–45.
[23] Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918-1920 "Spanish" influenza pandemic. Bull Hist Med 2002;76:105–15.
[24] Patterson KD, Pyle GF. The geography and mortality of the 1918 influenza pandemic. Bull Hist Med 1991;65:4–21.
[25] Still RM. Psychosis following Asian influenza in Barbados. Lancet 1958;2:20–1.
[26] Eickhoff TC, Robinson RQ. Influenza surveillance, United States, 1960. Public Health Reports (1896-1970) 1961;76:1099–106.
[27] Fortuin GJ, Soeters GC. Inactivated influenza vaccine in an industrial undertaking. Br Med J 1962;1:1042–5.
[28] Isaacs A, Hart RJ, Law VG. Influenza viruses, 1957-60. Bull World Health Organ 1962;26:253–9.
[29] Morris JA, Hatano M, Robinson RQ, Aulisio CG, Smadel JE. Antigenic relationship of 1961-1963 A2 influenza viruses to prototype A2 1957 strain. Proc Soc Exp Biol Med 1963;114:406–13.
[30] Linster M, Schrauwen EJA, van der Vliet S, Burke DF, Lexmond P, Bestebroer TM, et al. The molecular basis for antigenic drift of human a/H2N2 influenza viruses. J Virol 2019;93.
[31] Kutter JS, Linster M, de Meulder D, Bestebroer TM, Lexmond P, Rosu ME, et al. Continued adaptation of A/H2N2 viruses during pandemic circulation in humans. J Gen Virol 2023;104.
[32] Pappas C, Viswanathan K, Chandrasekaran A, Raman R, Katz JM, Sasisekharan R, et al. Receptor specificity and transmission of H2N2 subtype viruses isolated from the pandemic of 1957. PLoS One 2010;5:e11158.
[33] Sun J, Zheng T, Jia M, Wang Y, Yang J, Liu Y, et al. Dual receptor-binding, infectivity, and transmissibility of an emerging H2N2 low pathogenicity avian influenza virus. Nat Commun 2024;15:10012.
[34] Matsuzawa Y, Iwatsuki-Horimoto K, Nishimoto Y, Abe Y, Fukuyama S, Hamabata T, et al. Antigenic change in human influenza a(H2N2) viruses detected by using human plasma from aged and younger adult individuals. Viruses 2019;11.
[35] Wiley DC, Wilson IA, Skehel JJ. Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation. Nature 1981;289:373–8.
[36] Lindstrom SE, Cox NJ, Klimov A. Genetic analysis of human H2N2 and early H3N2 influenza viruses, 1957-1972: evidence for genetic divergence and multiple reassortment events. Virology 2004;328:101–19.
[37] Stevens J, Blixt O, Glaser L, Taubenberger JK, Palese P, Paulson JC, et al. Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. J Mol Biol 2006;355:1143–55.
[38] Connor RJ, Kawaoka Y, Webster RG, Paulson JC. Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. Virology 1994;205:17–23.
[39] Matrosovich M, Tuzikov A, Bovin N, Gambaryan A, Klimov A, Castrucci MR, et al. Early alterations of the receptor-binding properties of H1, H2, and H3 avian

influenza virus hemagglutinins after their introduction into mammals. J Virol 2000;74:8502–12.
[40] Xu R, McBride R, Paulson JC, Basler CF, Wilson IA. Structure, receptor binding, and antigenicity of influenza virus hemagglutinins from the 1957 H2N2 pandemic. J Virol 2010;84:1715–21.
[41] Viswanathan K, Koh X, Chandrasekaran A, Pappas C, Raman R, Srinivasan A, et al. Determinants of glycan receptor specificity of H2N2 influenza A virus hemagglutinin. PLoS One 2010;5:e13768.
[42] MacCosham A, Vasiliu AG, Atchessi N. A rapid review of the avian influenza PB2 E627K mutation in human infection studies. Can Commun Dis Rep 2025;51:137–44.
[43] Wang J, Sun Y, Xu Q, Tan Y, Pu J, Yang H, et al. Mouse-adapted H9N2 influenza A virus PB2 protein M147L and E627K mutations are critical for high virulence. PLoS One 2012;7:e40752.
[44] Cheng K, Yu Z, Chai H, Sun W, Xin Y, Zhang Q, et al. PB2-E627K and PA-T97I substitutions enhance polymerase activity and confer a virulent phenotype to an H6N1 avian influenza virus in mice. Virology 2014;468-470:207–13.
[45] Long JS, Giotis ES, Moncorge O, Frise R, Mistry B, James J, et al. Species difference in ANP32A underlies influenza A virus polymerase host restriction. Nature 2016;529:101–4.
[46] Baum LG, Paulson JC. The N2 neuraminidase of human influenza virus has acquired a substrate specificity complementary to the hemagglutinin receptor specificity. Virology 1991;180:10–5.
[47] Liang Z, Lin X, Sun L, Edwards KM, Song W, Sun H, et al. A(H2N2) and a(H3N2) influenza pandemics elicited durable cross-reactive and protective antibodies against avian N2 neuraminidases. Nat Commun 2024;15:5593.
[48] Laver WG, Webster RG. Antibodies to human influenzavirus neuraminidase (the A-Asian-57 H2N2 strain) in sera from Australian pelagic birds. Bull World Health Organ 1972;47:535–41.
[49] Dowdle WR. Influenza A virus recycling revisited. Bull World Health Organ 1999;77:820–8.
[50] Clarke SK, Heath RB, Sutton RN, Stuart-Harris CH. Serological studies with Asian strain of influenza A. Lancet 1958;1:814–8.
[51] Masurel N. Serological characteristics of a “new” serotype of influenza A virus: the Hong Kong strain. Bull World Health Organ 1969;41:461–8.
[52] Mulder J, Masurel N. Pre-epidemic antibody against 1957 strain of Asiatic influenza in serum of older people living in the Netherlands. Lancet 1958;1:810–4.
[53] Webster RG, Laver WG, Tumova B. Studies on the origin of pandemic influenza viruses V. Persistence of Asian influenza virus hemagglutinin (H2) antigen in nature? Virology 1975;67:534–43.
[54] Jones JC, Baranovich T, Marathe BM, Danner AF, Seiler JP, Franks J, et al. Risk assessment of H2N2 influenza viruses from the avian reservoir. J Virol 2014;88:1175–88.
[55] Ma W, Vincent AL, Gramer MR, Brockwell CB, Lager KM, Janke BH, et al. Identification of H2N3 influenza a viruses from swine in the United States. Proc Natl Acad Sci USA 2007;104:20949–54.
[56] Gulyaeva M, Sharshov K, Suzuki M, Sobolev I, Sakoda Y, Alekseev A, et al. Genetic characterization of an H2N2 influenza virus isolated from a muskrat in Western Siberia. J Vet Med Sci 2017;79:1461–5.
[57] Babu TM, Perera R, Wu JT, Fitzgerald T, Nolan C, Cowling BJ, et al. Population serologic immunity to human and avian H2N2 viruses in the United States and Hong Kong for pandemic risk assessment. J Infect Dis 2018;218:1054–60.
[58] Spangler A, Shimberg GD, Mantus GE, Malek R, Cominsky LY, Tsybovsky Y, et al. Early influenza virus exposure shapes the B cell response to influenza vaccination in individuals 50 years later. Immunity 2025;58. 728–44 e9.
[59] Krause JC, Tsibane T, Tumpey TM, Huffman CJ, Albrecht R, Blum DL, et al. Human monoclonal antibodies to pandemic 1957 H2N2 and pandemic 1968 H3N2 influenza viruses. J Virol 2012;86:6334–40.
[60] Wang W, Alvarado-Facundo E, Chen Q, Anderson CM, Scott D, Vassell R, et al. Serum samples from middle-aged adults vaccinated annually with seasonal influenza vaccines Cross-neutralize some potential pandemic influenza viruses. J Infect Dis 2016;213:403–6.
[61] Andrews SF, Raab JE, Gorman J, Gillespie RA, Cheung CSF, Rawi R, et al. A single residue in influenza virus H2 hemagglutinin enhances the breadth of the B cell response elicited by H2 vaccination. Nat Med 2022;28:373–82.
[62] Beau Reneer Z, Abreu RB, Jamal US, Corn MR, Paugh JL, Ross TM. Seasonal influenza vaccination does not effectively expand H2 cross-reactive antibodies in humans. Vaccine 2021;39:4173–83.
[63] PopulationPyramid.net. Population Pyramids of the World from 1950 to 2100. Accessed October 26, 2025. https://www.populationpyramid.net/world/2025/.
[64] Krammer F, Fouchier RAM, Eichelberger MC, Webby RJ, Shaw-Saliba K, Wan H, et al. NAction! How can neuraminidase-based immunity contribute to better influenza virus vaccines? mBio 2018;9.
[65] Wohlbold TJ, Krammer F. In the shadow of hemagglutinin: a growing interest in influenza viral neuraminidase and its role as a vaccine antigen. Viruses 2014;6:2465–94.
[66] Monto AS, Petrie JG, Cross RT, Johnson E, Liu M, Zhong W, et al. Antibody to influenza virus neuraminidase: an independent correlate of protection. J Infect Dis 2015;212:1191–9.
[67] Rajendran M, Nachbagauer R, Ermler ME, Bunduc P, Amanat F, Izikson R, et al. Analysis of anti-influenza virus neuraminidase antibodies in children, adults, and the elderly by ELISA and enzyme inhibition: evidence for original antigenic sin. mBio 2017;8.
[68] Wang X, Kong H, Chu B, Yang Q, Lin C, Liu R, et al. Identification of a broad-inhibition influenza neuraminidase antibody from pre-existing memory B cells. Cell Host Microbe 2025;33:151–66 e8.
[69] Laguio-Vila MR, Thompson MG, Reynolds S, Spencer SM, Gaglani M, Naleway A, et al. Comparison of serum hemagglutinin and neuraminidase inhibition antibodies after 2010-2011 trivalent inactivated influenza vaccination in healthcare personnel. Open Forum Infect Dis 2015;2:ofu115.
[70] Lei R, Kim W, Lv H, Mou Z, Scherm MJ, Schmitz AJ, et al. Leveraging vaccination-induced protective antibodies to define conserved epitopes on influenza N2 neuraminidase. Immunity 2023;56. 2621–34 e6.
[71] Chen YQ, Wohlbold TJ, Zheng NY, Huang M, Huang Y, Neu KE, et al. Influenza infection in humans induces broadly cross-reactive and protective neuraminidase-reactive antibodies. Cell 2018;173:417–29 e10.
[72] Monto AS, Kendal AP. Effect of neuraminidase antibody on Hong Kong influenza. Lancet 1973;1:623–5.
[73] Viboud C, Grais RF, Lafont BA, Miller MA, Simonsen L, Multinational Influenza Seasonal Mortality Study G. Multinational impact of the 1968 Hong Kong influenza pandemic: evidence for a smoldering pandemic. J Infect Dis 2005;192:233–48.
[74] van de Ven K, de Heij F, van Dijken H, Ferreira JA, de Jonge J. Systemic and respiratory T-cells induced by seasonal H1N1 influenza protect against pandemic H2N2 in ferrets. Commun Biol 2020;3:564.
[75] Jameson J, Cruz J, Ennis FA. Human cytotoxic T-lymphocyte repertoire to influenza A viruses. J Virol 1998;72:8682–9.
[76] Babon JA, Cruz J, Ennis FA, Yin L, Terajima M. A human CD4+ T cell epitope in the influenza hemagglutinin is cross-reactive to influenza A virus subtypes and to influenza B virus. J Virol 2012;86:9233–43.
[77] Tsang TK, Lam KT, Liu Y, Fang VJ, Mu X, Leung NHL, et al. Investigation of CD4 and CD8 T cell-mediated protection against influenza a virus in a cohort study. BMC Med 2022;20:230.
[78] Dong W, Bhide Y, Sicca F, Meijerhof T, Guilfoyle K, Engelhardt OG, et al. Cross-protective immune responses induced by sequential influenza virus infection and by sequential vaccination with inactivated influenza vaccines. Front Immunol 2018;9:2312.
[79] Scheible K, Zhang G, Baer J, Azadniv M, Lambert K, Pryhuber G, et al. CD8+ T cell immunity to 2009 pandemic and seasonal H1N1 influenza viruses. Vaccine 2011;29:2159–68.
[80] Zhdanov VM, Ritova VV, Orlova AV, Sokolova NN. The characteristics of influenza-virus strains isolated in 1957. Lancet 1957;273:735–6.
[81] Smadel JE. Influenza vaccine. Public Health Reports (1896-1970) 1958;73:129–32.
[82] Andrews JM. Research programs on Asian influenza. Public Health Reports (1896-1970) 1958;73:159–63.
[83] Mc CJ, Kilbourne ED. Immunization with Asian-strain influenza vaccine: equivalence of the subcutaneous and intradermal routes. N Engl J Med 1958;259:618–21.
[84] Boger WP, Liu OC. Subcutaneous and intradermal vaccination with Asian influenza vaccine. JAMA 1957;165:1687–9.
[85] Buebendorf DP, Carson TE, Cooper NR, Moynihan MJ, Yankee RA. Asian influenza: a study of 150 persons during the 1957 pandemic. Yale J Biol Med 1958;31:14–28.
[86] Bell JA, Ward TG, Kapikian AZ, Shelokov A, Reichelderfer TE, Huebner RJ. Artificially induced Asian influenza in vaccinated and unvaccinated volunteers. JAMA 1957;165:1366–73.
[87] Stille WT, Schultz I, Gundelfinger BF, Miller LF. Combinations of Asian influenza virus and adenovirus vaccine in the prophylaxis of respiratory illness of navy recruits. II. Am J Public Health 1962;52:275–89.
[88] Fukumi H. Human vaccination experiments with Asian influenza vaccine in Japan. Bull World Health Organ 1959;20:355–76.
[89] Holland WW, Isaacs A, Clarke SR, Heath RB. A serological trial of ASIAN-influenza vaccine after the AUTUMN epidemic. Lancet 1958;271:820–2.
[90] Ujhazy V. Clinical trials of oil-adjuvant influenza vaccines, 1960-3. Report to the Medical Research Council by its Committee on influenza and other respiratory virus vaccines. Br Med J 1964;2:267–71.
[91] Hobson D, Lane CA, Beare AS, Chivers CP. Serological studies on adult volunteers inoculated with oil-adjuvant ASIAN influenza vaccine. Report to the M.R.C. Committee on influenza and other respiratory virus vaccines. Br Med J 1964;2:271–4.
[92] Morita K. Serologic response of atomic bomb survivors following Asian influenza vaccination. Sapporo Igaku Zasshi 1966;29:63–74.
[93] David J. Sencer CDC Museum. The First U.S. National Vaccination Campaign Launches. Accessed October 26, 2025. https://cdcmuseum.org/exhibits/show/influenza/1957-pandemic/vaccine-rollout.
[94] David J. Sencer CDC Museum. Reviewing the 1957 Influenza Pandemic. Accessed October 26, 2025. https://cdcmuseum.org/exhibits/show/influenza/1957-pandemic/reviewing-1957-pandemic.
[95] Hirota Y, Kaji M. History of influenza vaccination programs in Japan. Vaccine 2008;26:6451–4.
[96] Field trial of influenza virus vaccine in patients with chronic bronchitis during the winter 1957-8: report by the joint working Party of the Medical Research Council's Committee on influenza and other respiratory virus vaccines and of the research Committee of the British Tuberculosis Association. Br Med J 1959;2:905–8.
[97] Jhaveri SS. Influenza Vaccination. Br Med J 1958;1:459.
[98] Sadusk Jr JF, Nesche G. Influenza vaccine, Asian strain; reactions following its use in adults. Calif Med 1957;87:301–6.

[99] Nelson AJ. Evaluation of Asian influenza vaccine in an industrial population. Can Med Assoc J 1958;79:888–91.
[100] Heller L, Korlof B, Morner J, Zetterberg B. Serological and prophylactic trials of Asian influenza vaccines in Sweden, 1957. Bull World Health Organ 1959;20:377–400.
[101] Forsyth JR, Russell EE. An assessment of oil adjuvant and aqueous influenza vaccines. II. Antibody responses to the vaccines. J Hyg (Lond) 1967;65:497–504.
[102] Hennessy AV, Davenport FM. Relative merits of aqueous and adjuvant influenza vaccines when used in a two-dose schedule. Public Health Rep (1896) 1961;76:411–9.
[103] Meiklejohn G. Effectiveness of monovalent influenza A-prime vaccine during 1957 influenza A-prime epidemic. Am J Hyg 1958;67:237–49.
[104] Blow RJ. Polyvalent Influenza Vaccine in General Practice. Br Med J 1964;2:943.
[105] Herbert WJ, Selwyn S, Philp JR. Field trials of adjuvant and saline influenza vaccines. Br J Prev Soc Med 1965;19:97–100.
[106] Newman J. Inactivated polyvalent influenza virus vaccine–a controlled study of its efficacy in the prevention of respiratory illness in industrial workers. S Afr Med J 1967;41:856–8.
[107] Hulka JF. Effectiveness of polyvalent influenza vaccine in pregnancy. Report of a controlled study during an outbreak of Asian influenza. Obstet Gynecol 1964;23:830–7.
[108] Clark ML, Reinhardt H, Miller III MC, Wilson R. Polyvalent influenza vaccine: comparison of jet injection with intradermal and subcutaneous syringe methods of administration. J Lab Clin Med 1965;66:34–41.
[109] Isaacs A, Negroni G, Tyrrell DA. Infection of volunteers with Asian influenza virus. Lancet 1957;273:886–7.
[110] Tyrrell DA, Beare AS. Some studies on the selection and efficiency of live influenza vaccine viruses. Bull World Health Organ 1969;41:581–4.
[111] Zhdanov VM. Results of further research on influenza in the USSR with special reference to the 1957 pandemic. Bull World Health Organ 1959;20:261–96.
[112] Beare AS, Bynoe ML, Tyrrell DA. Investigetion into the attenuation of influenza viruses by serial passage. Br Med J 1968;4:482–4.
[113] Mills J, Van Kirk J, Hill DA, Chanock RM. Evaluation of influenza virus mutants for possible use in a live virus vaccine. Bull World Health Organ 1969;41:599–606.
[114] Kaverin NV, Smirnov YA, Govorkova EA, Rudneva IA, Gitelman AK, Lipatov AS, et al. Cross-protection and reassortment studies with avian H2 influenza viruses. Arch Virol 2000;145:1059–66.
[115] Suzuki M, Okamatsu M, Hiono T, Matsuno K, Sakoda Y. Potency of an inactivated influenza vaccine prepared from A/duck/Hokkaido/162/2013 (H2N1) against a challenge with A/swine/Missouri/2124514/2006 (H2N3) in mice. J Vet Med Sci 2017;79:1815–21.
[116] Hehme N, Engelmann H, Kuenzel W, Neumeier E, Saenger R. Immunogenicity of a monovalent, aluminum-adjuvanted influenza whole virus vaccine for pandemic use. Virus Res 2004;103:163–71. https://doi.org/10.1016/j.virusres.2004.02.029. PMID: 15163505.
[117] Hehme N, Engelmann H, Kunzel W, Neumeier E, Sanger R. Pandemic preparedness: lessons learnt from H2N2 and H9N2 candidate vaccines. Med Microbiol Immunol 2002;191:203–8.
[118] Lenny BJ, Sonnberg S, Danner AF, Friedman K, Webby RJ, Webster RG, et al. Evaluation of multivalent H2 influenza pandemic vaccines in mice. Vaccine 2017;35:1455–63.
[119] Chen GL, Lamirande EW, Cheng X, Torres-Velez F, Orandle M, Jin H, et al. Evaluation of three live attenuated H2 pandemic influenza vaccine candidates in mice and ferrets. J Virol 2014;88:2867–76.
[120] Isakova-Sivak I, de Jonge J, Smolonogina T, Rekstin A, van Amerongen G, van Dijken H, et al. Development and pre-clinical evaluation of two LAIV strains against potentially pandemic H2N2 influenza virus. PLoS One 2014;9:e102339.
[121] Reneer ZB, Jamieson PJ, Skarlupka AL, Huang Y, Ross TM. Computationally optimized broadly reactive H2 HA influenza vaccines elicited broadly Cross-reactive antibodies and protected mice from viral challenges. J Virol 2020;95.
[122] Houser KV, Chen GL, Carter C, Crank MC, Nguyen TA, Burgos Florez MC, et al. Safety and immunogenicity of a ferritin nanoparticle H2 influenza vaccine in healthy adults: a phase 1 trial. Nat Med 2022;28:383–91.
[123] Xu D, Carter JJ, Li C, Utz A, Weidenbacher PAB, Tang S, et al. Vaccine design via antigen reorientation. Nat Chem Biol 2024;20:1012–21.
[124] Petro-Turnquist EM, Bullard BL, Pekarek MJ, Weaver EA. Adenoviral-vectored centralized consensus hemagglutinin vaccine provides broad protection against H2 influenza a virus. Vaccines (Basel) 2022;10.
[125] Talaat KR, Karron RA, Liang PH, McMahon BA, Luke CJ, Thumar B, et al. An open-label phase I trial of a live attenuated H2N2 influenza virus vaccine in healthy adults. Influenza Other Respir Viruses 2013;7:66–73.
[126] Isakova-Sivak I, Stukova M, Erofeeva M, Naykhin A, Donina S, Petukhova G, et al. H2N2 live attenuated influenza vaccine is safe and immunogenic for healthy adult volunteers. Hum Vaccin Immunother 2015;11:970–82.
[127] Maassab HF. Adaptation and growth characteristics of influenza virus at 25 degrees c. Nature 1967;213:612–4.
[128] Chen GL, Lamirande EW, Jin H, Kemble G, Subbarao K. Safety, immunogencity, and efficacy of a cold-adapted A/Ann Arbor/6/60 (H2N2) vaccine in mice and ferrets. Virology 2010;398:109–14.
[129] Ellebedy AH, Krammer F, Li GM, Miller MS, Chiu C, Wrammert J, et al. Induction of broadly cross-reactive antibody responses to the influenza HA stem region following H5N1 vaccination in humans. Proc Natl Acad Sci USA 2014;111:13133–8.
[130] Nachbagauer R, Wohlbold TJ, Hirsh A, Hai R, Sjursen H, Palese P, et al. Induction of broadly reactive anti-hemagglutinin stalk antibodies by an H5N1 vaccine in humans. J Virol 2014;88:13260–8.
[131] Jackson C. History lessons: the Asian flu pandemic. Br J Gen Pract 2009;59:622–3.
[132] Henderson DA, Courtney B, Inglesby TV, Toner E, Nuzzo JB. Public health and medical responses to the 1957-58 influenza pandemic. Biosecur Bioterror 2009;7:265–73.
[133] Maugh 2nd TH. Panel urges wide use of antiviral drug. Science 1979;206:1058–60.
[134] Pielak RM, Chou JJ. Flu channel drug resistance: a tale of two sites. Protein Cell 2010;1:246–58.
[135] Dong G, Peng C, Luo J, Wang C, Han L, Wu B, et al. Adamantane-resistant influenza a viruses in the world (1902-2013): frequency and distribution of M2 gene mutations. PLoS One 2015;10:e0119115.
[136] Bright RA, Medina MJ, Xu X, Perez-Oronoz G, Wallis TR, Davis XM, et al. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. Lancet 2005;366:1175–81.
[137] Fouchier RA, Osterhaus AD, Brown IH. Animal influenza virus surveillance. Vaccine 2003;21:1754–7.
[138] RSJ Ingrole, Shakya AK, Joshi G, Lee CH, Nesovic LD, Compans RW, et al. Floss-based vaccination targets the gingival sulcus for mucosal and systemic immunization. Nat Biomed Eng 2025.