Review

Targeting H3N2 Influenza: Advancements in Treatment and Vaccine Strategies

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Abstract: The emergence of the H3N2 influenza virus in 1968 marked a significant event as it crossed the species barrier. Since then, ongoing mutational dynamics have led to the formation of antigenic clusters, prompting the World Health Organization to advocate for regular updates to H3N2 vaccines. Research in the Western Pacific region underscores the necessity for heightened awareness and effective control strategies. Stemming from avian influenza A, the 1968 H3N2 influenza pandemic resulted in the deaths of one million people globally, with its seasonal variants primarily affecting older individuals and causing severe illness due to antigenic drift. To address the challenge of vaccine efficacy against H3N2 mutations, researchers are exploring innovative strategies such as precise antigenic material administration, controlled release patterns, understanding immune system mechanisms, and glycan engineering. This review comprehensively examines various aspects of the Influenza A (H3N2) virus, encompassing its virological characteristics, evolutionary trends, global epidemiology, vaccination strategies, antiviral interventions, and emerging diagnostic approaches. It underscores the impact of antigenic variation on vaccine design and effectiveness, seasonal outbreak patterns, pandemic potential, and the interplay between viral factors and host immune responses. Moreover, the review evaluates antiviral therapies and the issue of drug resistance, emphasising the necessity for multidisciplinary approaches involving researchers, healthcare professionals, and policymakers to comprehend H3N2 and enhance public health interventions.

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Keywords: H3N2 influenza; antigenic variation; vaccine efficacy; pandemic potential; multidisciplinary approach

1. Introduction

Zoonotic influenza remains a significant global public health threat [1–3]. Specially avian influenza, in different forms, represents a menace for a future pandemic [4]. In the case of H3N2, this is one of the emerging forms currently concerning in some areas of the world [5]. For some experts, the risk of spillover from this and other forms seems imminent for multiple epidemics [6,7]. This review examines the host's humoral immune response, primarily targeting primary epitopes carried by the hemagglutinin (HA) surface glycoprotein in influenza A viruses. The swift mutational evolution of HA leads to "antigenic drift," allowing viruses to evade the host's adaptive immune defence [8,9]. Notably, the H3N2 influenza virus crossed the species barrier, initiating human infection in 1968, and recent antigenic modifications have led to the emergence of antigenic clusters [9]. Influenza A virus causes acute respiratory illness, contributing to a global annual mortality estimate of 250,000 to 500,000 individuals, with notable pandemics including the 1918 H1N1, 1957 H2N2, 1968 H3N2, and 2009 H1N1 outbreaks [10–12]. Influenza A viruses are distinguished from types B and C by their nucleoprotein (NP) and matrix (MI) proteins belonging to the Orthomyxoviridae family. The influenza A virus genome comprises 11 protein segments on a single-stranded negative-sense RNA sequence [13]. There are 144 different HA-NA combinations in the influenza A virus family, with 16 HA and 9 NA subtypes identified by neuraminidase (NA or N) and hemagglutinin (HA or H) proteins on the virus surface [14–16].

The World Health Organization (WHO) recommends regular updates to the H3N2 component in influenza vaccines due to ongoing mutational dynamics within H3N2 viruses [17]. Amino acid alterations at residues 222 and 225 in the hemagglutinin chain have been identified, affecting receptor-binding characteristics [18,19]. Research in the Western Pacific region aims to assess the impact of H3N2 viruses on public health and mortality rates, emphasising the need for control strategies [20]. Predicting antigenic characteristics of A (H3N2) viruses outside E-SE Asia could improve vaccine strain selection and reduce morbidity and mortality [21]. H1N1 and H3N2 subtypes have circulated in humans since 1977, with age-specific variations likely due to childhood imprinting [22]. Fever is the most common symptom for both influenza A subtypes, with higher body temperatures during the A/H3N2 season, while myalgia, coughing, and sore throats were more common during the A/H1N1 season [23]. Antigenicity characterisation poses challenges for HA inhibitory antibodies, with differences in neutralising antibody titers between children and adults, suggesting the NI assay's potential in H3N2 infection testing and vaccine selection [24]. Figure 1 provides a visual overview of the Influenza A (H3N2) virus, emphasising recent occurrences and research methodologies for analysis.

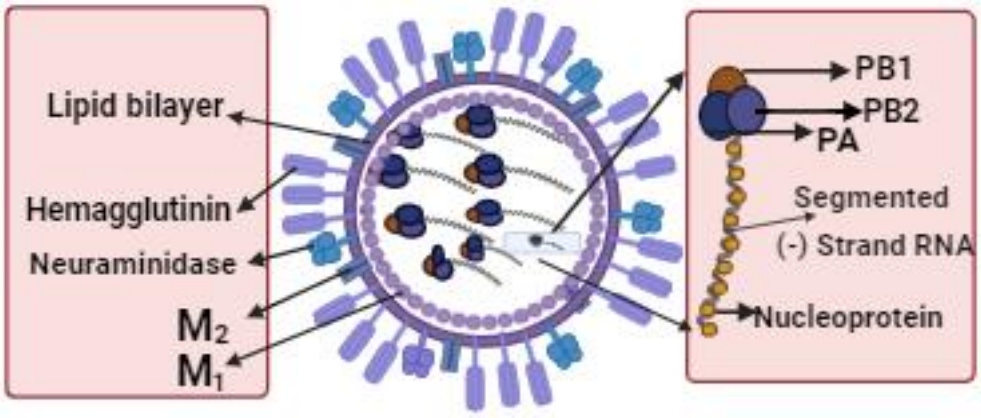

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Figure 1. The figure depicts an enveloped influenza A virus, with each viral protein encoded by eight separate segments of single-stranded RNA. The viral RNA segments and polymerase produce viral ribonucleoprotein by binding to nucleoproteins (vRNP). The viral RNA-dependent RNA polymerases, including polymerase acid (PA), polymerase basic 1 (PB1), and polymerase basic 2 (PB2), are responsible for replication. Two glycoproteins, hemagglutinin (HA) and neuraminidase (NA) bind to the viral surface. Neuraminidase facilitates the virus's escape from infected cells, while hemagglutinin mediates viral entry into host cells. The matrix protein (M1) forms a coat inside the virus envelope, providing structural stability and organisation to the viral components. Additionally, the membrane protein (M2) serves as a proton ion channel, contributing to the acidification of the virus inside the host cell endosome.

2. Global Impact and Evolution of H3N2 Influenza: A Historical Perspective

The H3N2 influenza virus originated from avian influenza A and coupled the N2 neuraminidase from the 1957 H2N2 virus with the distinct H3 hemagglutinin to generate the 1968 pandemic. When this virus was first identified in September 1968, it was thought to have killed one million people worldwide, of whom 100,000 died in the United States of America (USA) [25]. The H3N2 seasonal influenza A virus continues its worldwide circulation. These seasonal H3N2 variants frequently undergo antigenic drift, mainly affecting older individuals and leading to severe illness [26]. Since 1977, human-adapted subtypes of influenza A viruses (H1N1, H1N2, H3N2) have posed a simultaneous infection risk. While these viruses infect various animals, their primary hosts are wild birds. Influenza outbreaks affect 10–30% of the world's population and result in 290,000–650,000 deaths yearly, mostly in adults 65 years of age and older. Sub-Saharan Africa and Southeast Asia have the highest death rates from influenza epidemics [20]. In 2011, 12 cases of human infection with the new A(H3N2) virus were identified in the US, with potential person-to-person transmission [27]. Between 2011 and 2018, A(H3N2) was the cause of 39.2% of confirmed influenza infections in 27 Asian countries. Epizootiology research confirmed the H3N2 pandemic strain resulted from avian-human virus reassortment. Various H3N2 variants have amino acid changes at five antigenic sites on the H3 head, and single amino acid substitutions can alter the virus. A 2021-2022 study investigated 161 A(H3N2) virus strains, showing high genetic linkage [28]. Table 1 simplifies the origin and impact of the 1968 H3N2 influenza pandemic by representing the year-wise occurrences of H3N2 human infections, aiding in a clearer understanding of its global impact.

Table 1. Tracking the Impact: Yearly Incidences of H3N2 Human Infections Worldwide.

#	Year	Event Description	Impact	Ref.
1	1968-1970	The first influenza A/H3N2 pandemic season (1968/1969) it led to significant US mortality, while the second (1969/1970) caused most deaths in England. This reveals a global mortality pattern.	Mortality patterns in Europe and Asia were delayed until the second pandemic season due to higher neuraminidase immunity and a drift in the antigen during 1969/1970.	[29]
2	1970-2006	It has been discovered that pandemic influenza viruses persist in Chinese pigs as intermediate hosts, with triple-, double-, and entirely human-like H3N2 viruses coexisting.	This study analyses eight H3N2 virus genes from 1970 to 2006, revealing that pigs serve as mixing vessels for the virus generation. The coexistence of these viruses underscores the importance of reinforcing swine influenza virus surveillance in China.	[30]
3	1976	Eight segments of the Singapore (H2N2) strain's RNA have been tagged, divided, and associated with proteins and gene functions. The base sequence homology between the H2N2 virus and several influenza	The Singapore strain exhibits a base sequence homology of about 160% compared to the FM1 strain (HlNl). Still, the H3N2 strain is likely descended from an H2N2 subtype, as evidenced by its	[31]

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		A strains was discovered using molecular hybridisation.	retention of four segments and the HA gene from a different strain.	
4	2000-2010	This work recreated the ecological and evolutionary dynamics of influenza using a host metapopulation representative of the tropical, temperate, and southern regions.	Results showed that a region's primary reproductive number significantly impacts the antigenic evolution of its viral population and the probability of its strains spreading globally. Seasonality increases the probability of tropical populations exporting evolutionarily successful strains but doesn't predict their antigenically advanced status alone.	[32]
5	2009-2011	Concerns have been raised about the possibility of a pandemic brought on by four A(H3N2) v influenza viruses that were isolated from US people and examined in a study. It was discovered that the viruses may effectively propagate among ferrets living together and infect newly acquired ferrets via respiratory droplets.	The study found that A(H3N2) v viruses replicated in Calu-3 cells at considerably higher levels than the usual seasonal H3N2 influenza viruses, highlighting the significance of continuous public health surveillance.	[33]
6	2009-2011	During a phylogenetic analysis of influenza viruses from swine and humans in North America, thirty-four rH3N2p viruses with identical H3, N2, and pM segments to the human-identified H3N2v viruses were found.	Combination events between H3N2 viruses and the pM segment have produced these viruses about four to ten times since 2009. All H3N2v viruses recovered from humans have an N2 segment originating from a genetically unique N2 lineage, which may affect the development of influenza vaccines and the possibility of pandemics.	[34]
7	2010-2012	Recent US cases of H3N2v influenza infection, primarily among children, are being studied to determine cross-reactive antibody levels and whether seasonal TIV may increase seroprotection.	While teenagers and young adults have cross-reactive H3N2v antibodies, children and older individuals are susceptible. The lack of seroprotection in recent TIV formulations makes a particular vaccine necessary to spread the epidemic.	[35]

3. Pathophysiology of H3N2 Influenza: Complex Interactions and Inflammatory Response Mechanisms

The H3N2 influenza virus invades the upper respiratory tract, developing within respiratory epithelial cells and triggering an inflammatory response. This response, orchestrated by viral infection, produces inflammatory mediators like chemokines and cytokines. Cytokines recruit leukocytes and activate immune reactions, while chemokines attract leukocytes to infection sites, thus contributing to inflammation. However, this intricate cascade can lead to excessive inflammation, resulting in tissue damage and organ dysfunction in critical illnesses related to the virus [36]. This is comparable to the complex pathophysiology of the H3N2 influenza virus, which starts in the upper respiratory system and progresses to the lower tract. Influenza virus H3N2 damages respiratory epithelium, causing inflammation and airway obstruction [37]. Inside the body, the H3N2 influenza virus attaches to cell receptors, infiltrates host cells, and initiates replication, prompting immune responses that restrict viral replication via antibodies and cytokines.

The virus, however, can also directly damage tissues, leading to complications like pneumonia [38]. Influenza transmission studies using ferrets reveal a significant route through the soft palate, favoured by human influenza viruses' hemagglutinin proteins and similar receptors in humans

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[39,40]. Influenza's primary pathophysiology involves lung inflammation and dysfunction due to viral infection of the respiratory epithelium, compounded by immune responses. Chronic inflammation can lead to multiorgan failure, with predominant effects on the lungs, causing severe respiratory distress [41]. The influenza virus directly affects the respiratory tract or weakens the immune system. Lung obstruction, alveolar structural loss, and extracellular matrix deterioration are possible outcomes. Acute pneumonia is identified in 30–40% of hospitalised patients with laboratory-confirmed influenza; older patients (more than 65), Caucasians, younger than five, and inhabitants of nursing homes are at higher risk of developing pneumonia. Moreover, influenza can result in secondary bacterial infections and severe pneumonia, which increase the risk of bacterial sepsis and ARDS. Influenza A is the most frequent virus that causes adult acute respiratory distress syndrome (ARDS) [36]. H3N2 Influenza Viruses (IAVs) have evolved rapidly since 1968, incorporating N-linked glycans, increased HA molecule net charge, and altered receptor binding preferences. Researchers have modified antigenic characterisation assays to adapt to these changes. The HAI assay's use of guinea pig red blood cells and 20nM oseltamivir carboxylate enables a more precise evaluation of contemporary IAVs (Figure 2) [42].

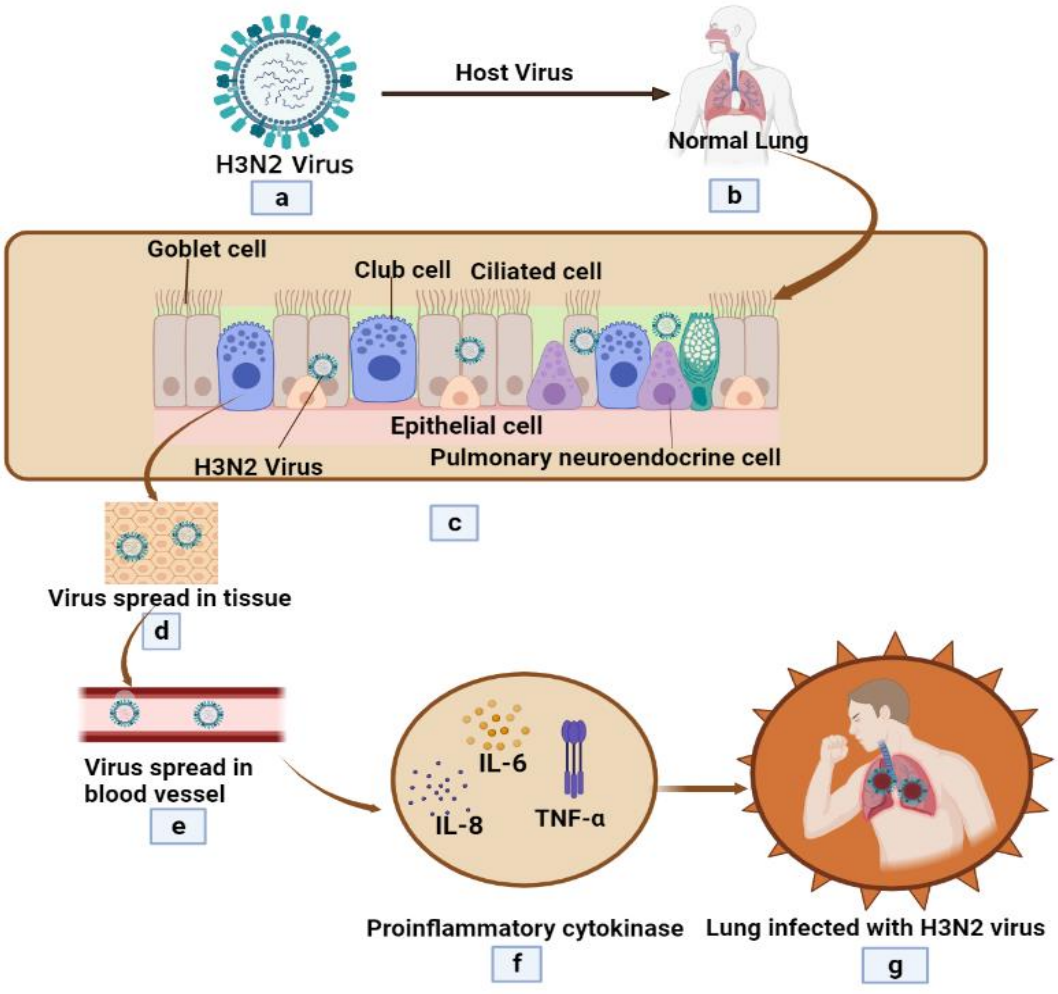

Figure 2. Pathophysiological progression of H3N2 Influenza virus infection. **(a)** Initiation of H3N2 Influenza virus infection, **(b)** Penetration of H3N2 virus into human lung, **(c)** Intracellular replication of the virus within respiratory epithelial cells, **(d)** Dissemination of H3N2 variant throughout the tissue, **(e)** Vascular impairment due to virus-induced damage, **(f)** Induction of TNF-alpha and pro-inflammatory cytokines, such as Interleukin-6 and Interleukin-8, and **(g)** Lung infection with H3N2 virus.

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4. Evolving Vaccination Strategies

The primary method used to create inactivated influenza vaccines is to develop viruses in chicken eggs, which have been regulated for over 70 years. The FDA distributes, tests, and adapts vaccine viruses following the WHO's strain selection process. The global manufacturing infrastructure can produce 1.5 billion doses annually. Influenza vaccination efficacy is assessed through randomised clinical trials and observational studies. Since the mid-2000s, however, nothing has been known about the effectiveness of vaccines targeted to a particular strain. A meta-analysis of influenza vaccine clinical trials conducted between 1967 and 2011 identified eight placebo-controlled trials, most showing no particular benefit against H3N2. With the development of molecular diagnostic tests, influenza infections may now be detected with high sensitivity and specificity. An inventive 'test-negative' observational study design was employed in Canada in 2005 to quantify vaccine effectiveness for the first time using RT-PCR. This methodology is used in annual VE surveys conducted in the US, Canada, Europe, and Australia [43]. The primary public health approach to curbing influenza, including the H3N2 influenza virus, revolves around vaccination. Annual vaccines are advised for individuals who are deemed to be at risk, such as the elderly and those with high rates of morbidity and death, according to the WHO [44]. Seasonal influenza, primarily H3N2, is a significant cause of respiratory disease and mortality, with increased rates of hospitalisation and excess mortality among the elderly [43].

Nevertheless, compared to other subtypes, the efficiency of the existing influenza vaccinations against H3N2 viruses is lacking [20,45]. This discrepancy is partially attributed to the H3N2 virus's rapid and unpredictable evolutionary pace in contrast to other seasonal flu viruses [46]. Further contributing to the reduced vaccine effectiveness is the composition of trivalent inactivated vaccine (TIV) influenza vaccines, which incorporate antigens from two A subtypes (A H3N2 and A H1N1) and only one B lineage. This setup frequently leads to mismatches between circulating and vaccine B strains. Consequently, the Quadrivalent influenza vaccine (QIV) has demonstrated enhanced immunogenicity compared to TIV across various age groups [44]. Furthermore, a prime-boost vaccination strategy involving the Ad5-HA + Ad5-NP vaccine followed by an inactivated H3N2 vaccine has proven effective in inducing cross-reactive immunity against H3N2 viruses in swine[47]. To improve vaccinations, yearly assessments of vaccine efficacy and genetic analysis of circulating influenza viruses are essential. The evidence base for choosing influenza vaccine viruses may be strengthened by combining clinical protection with virologic data [48]. The efficacy of influenza vaccines against influenza A(H3N2) viruses was lower than that of influenza B viruses, necessitating improved effectiveness. However, the 2016–2017 influenza vaccination trials demonstrated modest protection against outpatient influenza [49]. The antigenic distance hypothesis states that harmful interference from the previous season's immunisation may have an adverse effect on this season's protection from influenza. During three outbreaks (2010-2011, 2012-2013, 2014-2015), a study conducted in Canada assessed the effectiveness of vaccines against influenza A(H3N2) disease that required medical attention and laboratory confirmation. Consistent with the ADH, the results revealed considerable variations in the preceding vaccination effects by season. In 2014–2015, adverse effects were evident and statistically significant, indicating that low vaccine effectiveness in subsequent epidemics since 2010 could be attributed to influenza vaccinations administered more than once [50]. The discussion on H3N2 vaccine effectiveness involves summarising and presenting data from studies or clinical trials, providing a general outline to structure the discussion in Table 2. Vaccination remains the chief strategy to contain the H3N2 influenza virus. However, formulating effective vaccines encounters challenges due to the virus's rapid evolutionary rate.

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Table 2. The effectiveness of the H3N2 influenza vaccine, incorporating relevant data for analysis and comparison.

#	Population	Study Design	Vaccine formulation	Vaccine effectiveness %	Key finding	Ref.
1	Paediatric (2-17 Years)	Observational studies	Trivalent inactivated vaccine	Efficacy of vaccination, 5%; 95% confidence interval, -47 to 39	Between 2015 and 2016, influenza vaccinations dramatically decreased the likelihood of contracting the illness.	[49]
2	Adults in the 20–364 age range	Meta-analysis of TND studies	Trivalent influenza	VE of 65%	According to the ADH, the impact of recurrent influenza vaccination may have contributed to the low VE in recent A(H3N2) epidemics in Canada since 2010.	[50]
3	General Population	test-negative design and observational studies	trivalent vaccine	VE was 59%	According to the study, the recently developed A(H1N1)pdm09 vaccine offered reasonable defence against circulating strains. However, VE against A(H3N2) was less than 35% in 2016–17 and 2017–18, presumably due to the antigenic mismatch obtained from egg multiplication.	[51]
4	Aged one year and above.	Test-negative design	vaccine strain uses egg-adaptation mutations	In 2016–17 and even lower in 2017–18, VE against A(H3N2) was below 40%.	The study suggests that VE, influenced by phylogenetic sub-clusters and vaccination history, exhibits informative heterogeneity. However, it requires larger sample sizes and may be linked to pivotal mutations.	[52]
5	Age group is greater than 65-79 years.	test-negative design	trivalent vaccine	Influenza A(H3N2) IVE was 24%, while B IVE was 30%, 37%, and 19%.	IVE against influenza B in hospitalised older adults is similar to A(H3N2), highlighting the importance of influenza vaccination.	[53]
6	Aged 65 and above.	test-negative case-control design	trivalent influenza vaccine	The adjusted VE for inpatients was 7.4%, while outpatients had 19.3%.	Denmark experienced multiple genetically drifted H3N2 viruses during the 2016-17 influenza season, with low estimated VE and varying VEs across four main virus clusters.	[54]
7	Aged one year and older.	test-negative design	-	The vector error (VE) for Canada's influenza A(H3N2) outbreak in 2016–17 is over forty per cent higher than in 2014–15.	The intermediate vector error (VE) is approximately 40% higher in Canada's 2016–17 influenza A(H3N2) epidemic than in the 2014–15 pandemic. To reduce morbidity and death, particularly in high-risk individuals, further steps are required.	[55]

4.1. Current Influenza Vaccines

The influenza vaccination now in use, which targets the H3N2 strain, has been updated for the flu season of 2022–2023 [56,57]. A component of the virus similar to A/Darwin/9/2021 (H3N2) is included in this vaccination [56,57]. However, it's crucial to understand that the flu vaccine's ability to fend off H3N2 viruses changes according to the season. Vaccinations against influenza A(H3N2) are often less effective against influenza B viruses and more effective against influenza A(H1N1) viruses [58]. The H3N2 strain in the flu season of 2021–2022 showed antigenic dissimilarity from the vaccine virus, which led to a decrease in the efficacy of the vaccine against H3N2 viruses [59]. The World Health Organization (WHO) has recommended the A/Darwin/6/2023 (H3N2) component of influenza vaccinations for the 2023–2024 influenza season in the northern hemisphere [60]. Maintaining a yearly vaccination regimen is pivotal in guarding against the flu, given the ongoing variations in the viruses [56].

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4.2. Effectiveness and Limitations of Vaccines

H3N2 vaccines' effectiveness varies annually due to the virus's continuous evolution and adaptation. The potential deterioration in the effectiveness of the H3N2 vaccination can be attributed to several factors, including antigenic mismatch, vaccine component egg-adaptive alterations, and age-related effects, which allow older individuals to be less protected against A(H3N2) viruses due to previous exposure to non-A(H3N2) influenza viruses. According to recent studies, immunisation lowers the risk of influenza hospitalisation in younger, immunocompetent adults during seasons when the vaccine virus and influenza A(H3N2) are antigenically different. In 2020–2021, the COVID-19 pandemic saw a low level of influenza circulation; in 2021–2022, the circulation level increased. Immunisations decreased the likelihood of hospitalisation for younger persons but not for those over 65. Antivirals, vaccinations, and preventative measures all require advancements [59]. Among participants <50 years old, influenza vaccines displayed a 36% effectiveness against A(H3N2)-related illnesses [61].

Studies show that repeated annual influenza vaccine shots are less effective against influenza. In a trial, ferrets were given a prime-boost vaccination regimen twice and once, and they were then challenged with A/Hong Kong/4801/2014 (H3N2). The RV group lost weight more slowly and shed more virus, indicating that variations in the quality of the immune response could influence protection following recurrent immunisation [62]. Despite the continued widespread use of egg-based influenza vaccinations, emerging vaccine platforms promise to resolve drawbacks [20]. The investigation compared the H3N2-specific antibody responses of mice immunised with mRNA-LNP vaccines encoding wild-type and egg-adapted H3 antigens. The results showed that mRNA-LNP encoding wild-type H3 was superior to egg-adapted H3 or the egg-based Fluzone vaccination in neutralising the wild-type 3c.2A H3N2 virus. Both mRNA-LNP vaccinations produced significant levels of group 2 HA stalk-reactive antibodies, suggesting that mRNA-LNP-based vaccines modified with nucleosides can avoid problems associated with egg adaptation in the most recent 3c.2A H3N2 viruses. Because of their distinct glycosylation site, 3c.2A H3N2 viruses, a distinct offshoot of the 1968 H3N2 strain, came to light during the 2014–2015 influenza season and are still circulating worldwide [63,64]. Those with moderate diseases, such as mild upper respiratory tract infections, fever, or diarrhoea, can benefit from the nasal spray flu vaccine. Even if the vaccine does not precisely match the strain that is now circulating, immunisation remains the most effective means of protection against the flu overall [65].

4.3. Novel Approaches to Vaccine Design

Influenza H3N2 undergoes continuous mutations, presenting a challenge for vaccine creation. Nonetheless, researchers have explored innovative avenues to enhance vaccine efficacy. These strategies include the accurate administration of antigenic material, control over release patterns, and well-informed design based on a more profound comprehension of immune system mechanisms and pathogen-host interactions [66]. One study employed an H3N2 microneedle vaccine, which produced a cross-protective immune response against many H3N2 antigenic variants [67]. Alternate approaches encompass employing conserved antigens like HA, NA, matrix, and internal proteins, coupled with diverse vaccine platforms such as recombinant antigen/protein-based, virus-vectored, nanoparticle-based, DNA/RNA-based, virus-like particle (VLP), and multiplex vaccines [68–70]. How to expose highly cross-protective epitopes to the immune system has been investigated using glycan engineering of HA and NA proteins [68]. Researchers have also devised chimeric HAs by transferring unique HA globular head domains from exotic novel strains to the HA stalk domains of presently circulating human influenza viruses [71]. Moreover, adenoviruses have been transformed into vaccine vectors by disabling genes responsible for their replication, exhibiting the potential for developing a "universal" flu vaccine. Overall, pandemic-focused vaccine development aims to mitigate public health repercussions and societal disruption [72].

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5. Antiviral Therapies of H3N2 Influenza Virus

Several antiviral medications are accessible for treating influenza, including H3N2. The four antiviral medications recommended for influenza treatment are oseltamivir, peramivir, zanamivir, and baloxavir [73,74]. These medications function by stopping the surfaces of infected host cells from releasing influenza virions [75]. Antiviral therapy should be started as soon as possible for hospitalised patients with suspected or confirmed influenza who have severe, complicated, or worsening symptoms or are at higher risk of developing influenza-related complications. It is not necessary to wait for laboratory proof of influenza virus infection before starting antiviral therapy in suspected influenza cases [73]. Vaccination is the most reliable method of preventing H3N2 influenza; it is recommended for all individuals six months of age and older and is typically available in the fall. Keeping up with proper sanitary practices is also crucial. These include avoiding direct contact with sick people, frequently washing hands with soap and water, and covering the mouth and nose when coughing or sneezing [75].

5.1. Neuraminidase Inhibitors (Oseltamivir, Zanamivir)

Neuraminidase inhibitors, such as oseltamivir and zanamivir, belong to a drug class that obstructs the neuraminidase enzyme, a vital component for influenza replication. The FDA has approved using oseltamivir, with a twice-daily dosing schedule, for treating acute, uncomplicated influenza within two days of the onset of illness. These antiviral drugs are commonly used to battle influenza viruses A and B [73]. Even in severe influenza, where rhabdomyolysis is present, zanamivir has proven beneficial when treatment is initiated more than 48 hours after the onset of symptoms. When used early in influenza treatment, Neuraminidase inhibitors effectively lower the incidence of severe cases and fatality [76]. However, individuals with severe immunosuppression face the highest risk of developing oseltamivir- and peramivir-resistant influenza virus infections during or after treatment with these drugs [73]. Ongoing observation of oseltamivir resistance is necessary to further protect public health due to the evolution of antiviral medication resistance among influenza viruses [77]. Public health organisations advise using neuroliminase inhibitors to treat and prevent seasonal and pandemic influenza infections (Figure 3) [78]. Sialic acid receptors are crucial for virus attachment and entry into host cells. Blocking these receptors and inhibiting virus-host cell interactions is the best way to control and prevent infection. Neuraminidase inhibitors, including oseltamivir and zanamivir, are the most efficient drugs for treating influenza A and B virus infections [79].

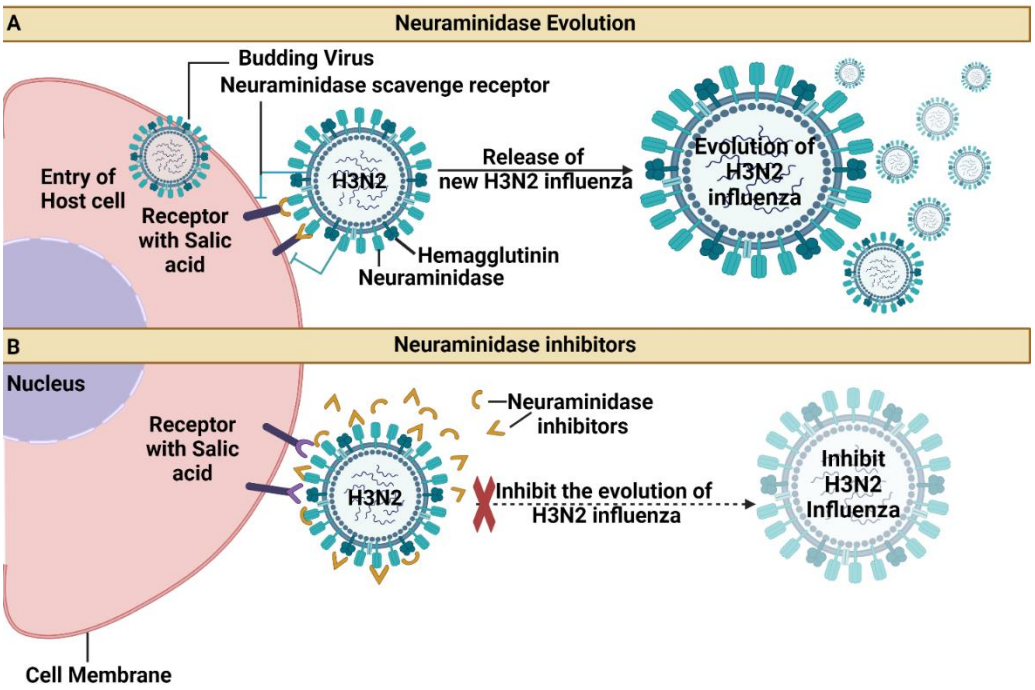

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Figure 3. Mechanism of virus entry **(A)** Neuraminidase Evolution and **(B)** Neuraminidase inhibitors.

5.2. Polymerase Inhibitors (Baloxavir Marboxil)

Baloxavir marboxil, a polymerase inhibitor, serves as a treatment for uncomplicated influenza [80,81]. It prevents the viral polymerase complex's cap-dependent endonuclease activity, which is essential for viral replication [82]. About its use against H3N2 influenza, here are key insights: Clinical trials have established the efficacy of baloxavir marboxil against H3N2 influenza [81]. However, because to changes in the polymerase acidic protein, certain H3N2 viruses have shown reduced susceptibility to baloxavir marboxil [80,83]. Unlike neuraminidase inhibitors (NAIs) like zanamivir and oseltamivir, which are usually advised twice daily for five days, baloxavir marboxil is only given orally once [81]. In vitro, investigations have revealed that baloxavir acid, the active metabolite of baloxavir marboxil, can be combined with other inhibitors, such as NAIs and favipiravir, to augment antiviral efficacy against seasonal influenza A viruses [84]. Baloxavir marboxil exhibits potential as a treatment choice for uncomplicated influenza, including H3N2 strains. Nevertheless, the emergence of resistant strains underscores the ongoing necessity for research and the development of novel antiviral therapies.

6. Advancements in Molecular Detection Methods

6.1. Rapid Diagnostic Methods

Serological testing, antigen detection tests, and molecular assays are quick diagnostic techniques for H3N2 influenza. In ten to fifteen minutes, antigen detection tests known as Rapid Influenza Diagnostic Tests (RIDTs) can identify influenza viral antigens in respiratory specimens with low sensitivity. The antigen-detection assays known as RIDTs have moderate sensitivity and may detect influenza virus antigens in respiratory specimens in 10 to 15 minutes. These immunoassays detect influenza A and B viral nucleoprotein antigens in respiratory samples and yield a qualitative result (positive vs. negative). RIDTs have excellent specificity; however, they only offer modest sensitivity. On the other hand, outstanding sensitivity and specificity are offered by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and other molecular techniques for identifying influenza virus RNA or nucleic acids in respiratory materials [85,86]. Among the techniques used in serological research are the Hemagglutination Inhibition Assay (HAI), Single Radial Hemolysis (SRH), Virus Neutralization Assay (VN) or Microneutralization, and Enzyme-Linked Immunosorbent Assay (ELISA). These tests detect influenza virus antibodies in serum or other bodily fluids. However, their applicability for early influenza diagnosis is limited due to the need for paired serum samples collected at least 2 weeks apart. For H3N2 influenza, molecular assays and antigen detection tests stand out as the most common quick diagnostic techniques [87].

6.2. Molecular Detection Techniques

There are numerous molecular detection methods available for identifying the H3N2 influenza virus. Reverse transcription-polymerase chain reaction (RT-PCR) and other nucleic acid amplification tests are the most widely used methods for detecting influenza virus RNA or nucleic acids in respiratory samples because of their exceptional sensitivity and specificity. Several molecular techniques can distinguish between influenza A and B infections as well as identify seasonal influenza A virus subtypes, such as A(H1N1) pdm09 or A (H3N2) [87–90]. Loop-mediated isothermal amplification (LAMP), an alternative molecular-based influenza diagnostic, provides a rapid, accurate, and dependable molecular detection technique. This technique provides a stable foundation for influenza and COVID-19 testing [91–93]. The Multiplex One-Step Real-Time RT-PCR technology increases efficiency and simultaneously identifies the human H3N2 virus, the pandemic (H1N1) 2009 virus, and the reassortant avian H7N9 virus [94]. Recommended by the WHO, these molecular assays offer high accuracy and efficacy for diagnosing the H3N2 influenza virus [87–90].

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6.3. Point-of-Care Testing Advances

Point-of-care testing (POCT) for influenza is experiencing rapid growth, and multiple studies have demonstrated its benefits in diagnosing influenza among patients with acute respiratory tract infections [95]. POCTs represent swift diagnostic assessments performed at the point of care, whether in a doctor's office or an emergency department, delivering results within minutes [95,96]. Initially criticised for limited sensitivity and result variability, recent research has highlighted the high specificity and sensitivity of POCTs, establishing them as reliable and practical tools for early influenza identification [96–98]. POCTs have the potential to enhance patient flow, reduce hospitalisations, and facilitate targeted treatments [99,100]. Predominant POCT options for influenza encompass antigen detection tests and molecular assays like RT-PCR [95,96,98]. Additionally, the molecular-based Loop-mediated isothermal amplification (LAMP) assay introduces an innovative avenue for molecular diagnosis [99,101]. The introduction of POCTs offers fresh prospects for managing healthcare patients, as illustrated in Table 3.

Table 3. The diagnostic methods of the H3N2 influenza virus, highlighting their descriptions.

#	Method	Description	Ref.
1.	Biosensors	Advanced methods to detect H3N2 influenza viruses based on different parameters, aiming to improve specificity and sensitivity. The human influenza virus binds to α, 2–6 glycosidic bonds, while the avian influenza virus binds to α, 2-3 glycosidic bonds. Viruses detect distinct receptors on host cells. Pigs show both genetic re-assortment and antigenic shift since they have both types.	[87,102]
2.	RT-LAMP stands for Reverse Transcription Loop-Mediated Isothermal Amplification.	Molecular diagnostic tool for influenza A viruses, including H3N2. It is quick, easy, cost-effective, sensitive, and specific, suitable for point-of-care testing during outbreaks. The process involves amplifying nucleic acid using reverse transcriptase, DNA polymerase, and oligos, resulting in double-stranded looped DNA structures that can be detected using pH sensitivity, fluorescent response, and turbidity.	[103,104]
3.	Multiplex PCR (Polymerase Chain Reaction).	The diagnostic tool uses several primer pairs in the same reaction, amplifying different specific amplicons for various targets. Increasingly used for the diagnosis of infectious diseases, including RNA-containing viruses like H3N2 influenza. Using this technique, different influenza viruses' HA, NA, and M gene segments can be amplified simultaneously from clinical specimens or isolates to be sequenced.	[105,106]
4.	Rapid Influenza Diagnostic Tests, or RIDTs	Rapid influenza tests can detect viral nucleoprotein antigens in respiratory specimens in less than 15 minutes; commercial and laboratory-developed RT-PCR assays are suitable reference tests.	[85,107]
5.	Neuraminidase Activity-Based Assay	A chemiluminescent assay for detecting influenza viral neuraminidase (NA) activity utilises a unique substrate related to NA, a target for newer-generation influenza therapeutic drugs. The main goal of mechanism-based drug design is to locate and create target enzyme competitive inhibitors.	[108,109]
6.	one- step RT-PCR	The one-step RT-PCR assay proved quicker and easier than virus isolation and serological methods.	[110]

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7. Future Directions

In moving forward, this review has illuminated crucial paths for future research and development in effectively combatting the challenges of Influenza A (H3N2). These essential directions encompass strengthening global surveillance through advanced sequencing technologies and real-time data sharing, enabling rapid detection of new strains and swift public health responses. Predictive modelling holds promise in guiding the formulation of more effective vaccines by anticipating antigenic shifts. Universal vaccines targeting conserved viral regions could offer enduring protection against diverse strains. Investigating host immune responses can provide insights into disease severity and susceptibility, informing targeted interventions. Exploring combination antiviral therapies and integrating advanced diagnostic tools into public health strategies can enhance treatment and outbreak management. For comprehensive solutions, promoting multidisciplinary collaboration among professionals is essential. Investment in worldwide education and pandemic preparedness will improve the ability to control possible epidemics. Influenza A (H3N2) 's dynamic complexity must be anticipated, understood, and efficiently managed. Proactive collaboration, cutting-edge technology, and a multidisciplinary approach are essential.

8. Conclusion

This review delves into the humoral immune response to influenza A viruses, mainly focusing on recognising hemagglutinin surface glycoprotein epitopes. It emphasises the significant impact of the H3N2 virus, which breached the species barrier in 1968, leading to acute respiratory illness and global mortality. The WHO advocates for regular updates to H3N2 vaccines due to mutational dynamics, with research in the Western Pacific region underscoring the importance of awareness and control strategies. The devastating 1968 H3N2 influenza pandemic claimed one million lives globally, with seasonal variants continuing to cause severe illness. Since 1977, human-adapted subtypes have posed a significant infection risk, affecting a substantial portion of the world's population annually. Influenza A (H3N2) spreads through various means and exhibits mutability, causing sporadic outbreaks and pandemics. Notably, H3N2 has caused fatal outcomes in India, and its molecular epidemiology indicates the possibility of reinfection by the same subtype in a short time frame. The review highlights the challenge posed by H1N1 and H3N2 mutations, affecting vaccination effectiveness. The WHO strongly recommends yearly influenza vaccination, particularly for the 2023-2024 flu season. However, H3N2 mutations present obstacles to vaccine development, prompting exploration into innovative strategies such as alternative antigen formulations and pandemic-focused vaccine development. Despite longstanding regulation of inactivated influenza vaccines, their effectiveness against H3N2 viruses has been limited due to the virus's rapid evolutionary pace. The review comprehensively explores the evolution, epidemiology, clinical manifestations, vaccination strategies, and Influenza A (H3N2) antiviral interventions, underscoring the importance of diagnostic advancements and multidisciplinary collaboration for pandemic preparedness. It is a valuable resource for healthcare professionals and policymakers combating H3N2.

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Abbreviations

H3N2	Hemagglutinin 3 and Neuraminidase 2
CDC	Centres for Disease Control and Prevention
WHO	World Health Organization
HA	Hemagglutinin
RNA	Ribonucleic Acid
IBVs	Influenza B Viruses
A(H3N2)	Influenza A Subtype H3N2
rRT-PCR	Real-Time Reverse Transcription Polymerase Chain Reaction
M	Matrix
ILI	influenza-like illness

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PA	Polymerase acid
PB1	polymerase basic 1
PB2	polymerase basic 2
TIV	trivalent inactivated vaccine
QIV	Quadrivalent influenza vaccine
RIDTs	Rapid Influenza Diagnostic Tests
HAI	Hemagglutination Inhibition Assay
VN	Virus Neutralization Assay
SRH	Single Radial Homolysis
ELISA	Enzyme-Linked Immunosorbent Assay
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
LAMP	Loop-Mediated Isothermal Amplification
POCT	Point-of-care testing
VE	Vaccine Effectiveness
H3N2	Hemagglutinin 3 and Neuraminidase 2
H3N2v	H3N2 variant

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