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Influenza A virus H5N1: Histopathology, Mortality, Preventive, Therapeutic Approaches and Future Prospects

(Virus Influenza A H5N1: Histopatologi, Kematian, Pencegahan, Pendekatan Terapeutik dan Prospek Masa Depan)

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Abstract

Influenza A virus H5N1 remains a significant global health concern due to its high pathogenicity and potential for zoonotic transmission. This review explores the pathological impact of H5N1 infection, emphasizing its severe effects on the lower respiratory tract, including alveolar epithelial apoptosis, diffuse alveolar damage (DAD), and acute respiratory distress syndrome (ARDS). Upon infection, the virus induces a hyperinflammatory response, characterized by excessive cytokine production, leading to multi-organ failure and an alarmingly high human mortality rate of approximately 54%. Key histopathological findings, such as epithelial necrosis, vascular damage, and neurological involvement, underscore the severity of infection. Current treatment options, including neuraminidase inhibitors, remain limited, necessitating novel therapeutic strategies such as monoclonal antibody therapy and immune modulation. Additionally, the emergence of antiviral-resistant strains underscores the importance of continuous disease surveillance and vaccine development. This review highlights the urgent need for global preparedness and further research into H5N1 pathogenesis and countermeasures.

Keywords: H5N1, zoonotic transmission, lower respiratory tract, ARDS

Abstrak

Virus Influenza A H5N1 kekal sebagai kebimbangan kesihatan global yang signifikan disebabkan oleh patogenisiti yang tinggi dan potensinya untuk perebakan zoonotik. Ulasan ini meneroka kesan patologi jangkitan H5N1, dengan penekanan kepada kesan yang teruk pada saluran pernafasan bawah, termasuk apoptosis epitelium alveolar, kerosakan alveolar meresap (DAD), dan sindrom gangguan pernafasan akut (ARDS). Semasa jangkitan, virus ini mencetuskan tindak balas hiper-radang, yang dicirikan oleh pengeluaran sitokin yang berlebihan sehingga membawa kepada kegagalan pelbagai organ dan kadar kematian manusia yang sangat tinggi, iaitu kira-kira 54%. Penemuan histopatologi utama, seperti nekrosis epitelium, kerosakan vaskular, dan penglibatan neurologi, membuktikan keterukan jangkitan. Pilihan rawatan semasa termasuk perencat neuraminidase masih terhad, maka memerlukan strategi terapeutik baharu seperti terapi antibodi monoklonal dan modulasi imun. Di samping itu, kemunculan strain rintang antiviral menekankan kepentingan pengawasan penyakit secara berterusan dan pembangunan vaksin. Ulasan ini menekankan keperluan mendesak untuk kesiapsiagaan global dan penyelidikan lanjut mengenai patogenesis H5N1 dan langkah-langkah pencegahan.

Kata kunci: H5N1, penularan zoonotik, saluran pernafasan bawah, ARDS

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INTRODUCTION

Infectious diseases of zoonotic origin have increasingly captured global attention due to their potential to cause widespread outbreaks and significant threats to public health. Among these, influenza viruses are particularly concerning because of their ability to cross species barriers and cause adverse disease outcomes in hosts. Avian influenza virus, in particular, have emerged as a critical pathogen due to its capacity to mutate and infect avian and mammalian hosts, including humans. One of the subtypes of avian influenza A virus, H5N1 has become a focal point of concern due to its ability to form highly pathogenic strain that can result in global flu pandemic (Charostad et al. 2023).

Influenza A viruses, which belong to the *Orthomyxoviridae* family, are enveloped RNA viruses carrying single-stranded, negative-sense, eight-segmented genomes (Li et al. 2021). They evolve rapidly due to their unique structural and genetic features. The lack of proofreading activity in the viral polymerase results in a high mutation rate, with approximately one error per replicated genome, leading to the production of around 10,000 new viral mutants per infected cell (Nistal & Garcia 2009). This high mutation rate drives antigenic drift, where continual changes are found in the viral glycoprotein sequences, particularly hemagglutinin (HA), enabling the virus to evade host immune responses, thereby necessitating frequent updates to seasonal vaccines (Nistal & Garcia 2009). Additionally, the segmented nature of the influenza genome facilitates genome reassortment, a process in which genetic segments from different co-infecting strains are packaged into new viral particles. The reassortment adapt rapidly to new hosts, enhance transmissibility, and potentially increase virulence. Together, these mechanisms contribute to the virus's capacity for continual evolution, posing persistent challenges for disease control (Boivin et al. 2010).

The United States Department of Agriculture (USDA) (2015) categorizes avian influenza A H5N1, based on its pathogenicity in poultry into highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). LPAI virus typically causes mild symptoms or asymptomatic infections in poultry mutate into HPAI strains, particularly in a densely populated poultry environment. In contrast, HPAI virus causes severe diseases and high mortality in poultry, leading to significant economic losses due to mass culling and trade restrictions (USDA 2015).

The adaptation of H5N1 in human hosts, coupled with its ability to exchange genetic materials with the human influenza A virus has raised significant concerns about its potential to trigger an upcoming human influenza pandemic (AbuBakar et al. 2023). The first recorded outbreak of H5N1 occurred in domestic geese in Guangdong, China, in 1996 (Wan 2012). Since then, the virus has spread globally, infecting a diverse range of hosts, including wild birds, poultry, and domestic animals. Outbreaks have been documented across continents, including Europe, Africa, the Americas, and even Antarctica. Notably, in recent years, extensive outbreaks of HPAI H5N1 have been observed in mammalian species such as farmed mink, foxes, raccoon dogs, sea lions, and elephant seals (CDC 2024). This epidemiological shift is particularly concerning as sustained mammal-to-mammal transmission increases the likelihood of zoonotic spillover events to humans (CDC 2024).

Over the years, the epidemiology of H5N1 has evolved rapidly, with over 954 confirmed human infections and more than 460 associated deaths globally since 2003, yielding a case fatality rate exceeding 54% (WHO 2024). Most cases are linked to direct exposure to infected birds or contaminated environments. The persistent circulation of avian influenza virus in wild and domestic animal reservoirs presents a significant public health risk, particularly in regions where close human-animal interactions are regularly reported but with very limited surveillance infrastructure (Charostad et al. 2023).

In humans, H5N1 infection primarily affects the lower respiratory tract which then rapidly manifests as viral pneumonia accompanied by leukopenia, and elevated serum cytokine levels. In severe cases, the infection leads to acute lung injury, diffuse alveolar damage, acute respiratory distress syndrome (ARDS), and multiorgan failure, contributing to an alarming mortality rate of approximately 54% (Lam et al. 2010). The high pathogenicity of H5N1 is largely driven by an excessive immune response that is often regarded as "cytokine storm" due to by the overproduction of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interferon-gamma (IFN-γ) (Li et al. 2024). This hyperinflammatory state promotes a massive influx of immune cells, including macrophages and neutrophils, which exacerbate lung injury by increasing vascular permeability and subsequently lead to pulmonary edema and alveolar hemorrhage (WHO 2024). These severe clinical outcomes underscore the devastating consequences of potential human-to-human transmission of H5N1.

Despite the advances in understanding H5N1 pathogenesis, the precise underlying mechanisms of H5N1-induced lung injury remains incompletely elucidated. It is strongly believed that the combination of direct viral cytotoxicity, and immune-mediated

damage is highly possible to contribute to the fatal disease course (Xu et al. 2024). The current antiviral treatments, such as neuraminidase inhibitors. i.e. oseltamivir and zanamivir, have limited efficacy in severe cases, highlighting the need for targeted therapies that can modulate the exaggerated immune response while controlling the viral replication (Xu et al. 2024).

Therefore, this review aims to provide a more comprehensive understanding on the pathophysiological changes induced by H5N1 in the lower respiratory tract, its risk factors and the potential therapeutic strategies. By providing an organized and detailed overview of the available evidence, this review aims to support researchers, clinicians, and policymakers in addressing the public health challenges posed by H5N1 and preparing the nation and community for potential future outbreaks.

DISEASE PROGRESSION OF H5N1

H5N1 primarily infects the respiratory system, with a marked predilection for the lower respiratory tract, where the virus replicates within the epithelial cells of the trachea, bronchi, and alveoli (Oshansky et al. 2011). The molecular basis of the tissue tropism lies in the specific binding of the viral HA with the α2,3-linked sialic acid receptor on the lower respiratory epithelia, predominantly expressed in bronchiolar and alveolar epithelial cells (Kumlin et al. 2008). Upon entry, H5N1 undergoes rapid replication within the alveolar and bronchial epithelia, leading to extensive tissue damage and loss of epithelial integrity (Ruan et al.2022). Concomitantly, this also triggers a strong local inflammatory response, signified by the excessive release of pro-inflammatory cytokines (Gu et al. 2021). Altogether, it promotes the development of ARDS, respiratory failure, and multi-organ dysfunction in severe cases (Charostad et al. 2023).

H5N1 infection in the lower respiratory tract is exacerbated by the absence of efficient clearance mechanism. Unlike the upper respiratory tract with a robust mucociliary clearance system, alveoli and bronchioles lack effective mucus secretion and ciliary movement, thereby allowing deeper intrusion of H5N1 into susceptible host cells and inducing progressive lung damage (Button et al. 2012). Generally, the early signs and symptoms of H5N1 are similar to those observed in common cold or influenza. During the disease progression, myalgia and arthralgia, and conjunctivitis might be reported, reflecting the systemic nature of the virus infection (CDC 2024; Charostad et al. 2023). Moderate to severe illness are marked by respiratory distress, including shortness of breath or difficult breathing (Rajabali et al. 2015). In more severe cases, patients may experience altered mental status, confusion, or even seizures (CDC 2024). H5N1-induced pneumonia is characterized by lung consolidation, severe hypoxemia, and the infiltration of inflammatory cells into the lower respiratory tract (Rajabali et al. 2015). These pathological changes closely resemble the clinical features of ARDS (Matthay et al. 2019). In ARDS, the compromised integrity of the alveolar-capillary barrier permits fluid accumulation within the alveolar spaces, impairing pulmonary gas exchange and resulting in profound hypoxemia. If left untreated, the progressive decline in respiratory function culminates in respiratory failure, necessitating mechanical ventilation and intensive supportive care (Powers & Dhamoon 2019).

ARDS can affect individuals of all ages, but certain age groups are more susceptible to severe outcomes due to physiological and immune-related differences. Bellani et al. (2016) found that ARDS incidence and its related mortality increased with age, with the highest incidence rate reported among older patients (≥65 years). The ARDS-related mortality in patients aged >85 years was 70% compared to 34% in those aged <60 years (Kawachi et al. 2009).

HISTOPATHOLOGICAL FINDINGS OF H5N1 INFECTION

Histopathological examination of biopsies from patients infected with H5N1 influenza revealed a broad spectrum of pathological changes, highlighting the involvement of respiratory epithelial injury and systemic organ failure.

The key histopathological features associated with H5N1 infection include necrosis of infected respiratory epithelial cells, leading to the sloughing of damaged cells from the airway surface. This process is particularly evident in the bronchioles and alveolar regions, where the virus induces extensive cell death and tissue breakdown (Uiprasertkul et al. 2007). Beyond the respiratory tract, an experimental study on mute and whooper swans revealed characteristic lesions, including multifocal haemorrhagic necrosis in the pancreas, pulmonary congestion and edema, and subepicardial haemorrhages (Teifke et al. 2007). These findings underscore the ability of the virus to cause severe necrotic damage across multiple tissues, contributing to the high mortality observed in infected avian species.

The damage to the respiratory epithelia often triggers a robust inflammatory response, characterized by the infiltration of various immune cells, including neutrophils, macrophages, and lymphocytes. Initially, the anti-H5N1 immunity is

triggered to clear the virus infection. To evade the immune response, H5N1 expresses and utilizes non-structural protein 1 (NS1) to inhibit key antiviral signalling mechanisms, particularly the host's interferon response. As a result, the virus propagation continues unchecked, leading to widespread infection and systemic inflammation (Bouvier & Palese 2008). The inflammation is most prominent in the alveolar spaces, contributing to the formation of exudates and the development of pulmonary edema (Wonderlich et al. 2017). This intensified inflammatory response is often accompanied by a cytokine storm, marked by a drastic increase of pro-inflammatory cytokines such as TNF-α, IL-6, and IFN-γ (Chan et al. 2005). Clinically, the situation manifests as ARDS, characterized by severe hypoxemia, diffuse pulmonary infiltrates, and reduced lung compliance (Matthay et al. 2019). In terms of pathological observations, conditions such as diffuse alveolar damage, hyaline membranes, fibrin exudates, and fibrotic healing are frequently documented (CDC 2024).

In more severe cases, lung hemorrhage can occur, as a consequence of vascular endothelial damage, disruption of the alveolar-capillary barrier and microvascular thrombosis (Vassiliou et al. 2020). It is speculated that the mortality rate of patients with severe ARDS exhibiting pulmonary haemorrhage and thrombotic complications, can exceed 50-70% (Carsana et al. 2020; Gu et al.2007). Moreover, a recent study found that patients suffering from ARDS experienced remarkably high mortality rates, with 28-day and in-hospital mortality rates of 34.8% and an 40.0% respectively (Xie et al. 2025).

In addition to the pulmonary pathological damages, the manifestation of H5N1 symptoms in the central nervous system (CNS) is also an alarming scenario in patients, especially those showing meningoencephalitis (CDC 2024). This phenomenon was supported by an *in vivo* study in ferrets in which the spread of H5N1 into the CNS was shown to be via the olfactory and trigeminal nerves (Siegers et al. 2023). In the CNS, the virus infects the olfactory nerve, which connects the nasal cavity directly to the olfactory bulb in the brain. Once inside, it travels retrogradely along the axons into the olfactory bulb, bypassing the blood-brain barrier (Charostad et al. 2023).

Alternatively, the trigeminal nerve provides another entry portal for the virus to reach the brainstem and deeper brain structures. When H5N1 virus successfully spreads in the CNS, it causes widespread neuronal loss and extensive neuroinflammation, including microglial activation (Jang et al. 2012). Neuroinflammation disrupts the intactness of the blood-brain barrier (BBB), enabling more virions and immune cells to enter the brain, and exacerbating neurological injury (Qin et al. 2023). Neuronal injury often manifests as neuron degeneration, swelling, and vacuolation (Sian-Hulsmann & Riederer 2024). Eventually, affected individuals exhibit CNS symptoms such as altered mental status, seizures, and even coma in the most critical cases, complicating both disease prognosis and clinical diagnosis (Siegers et al. 2023).

Beside the BBB, the meninges and brain parenchyma are also adversely affected during H5N1-infection, which in turn results in meningoencephalitis (Swanson 2015). Meningoencephalitis is typically observed during the acute phase of the illness, often within the first week after symptom onset. The severity of the symptom varies, ranging from mild neurological disturbance to severe complications such as seizures, altered mental status, and, in some cases, death (Steiner et al. 2010). In a paediatric H5N1 case, the patient experienced minimal respiratory symptoms such as obstructive hydrocephalus with the viral RNA detected in the cerebrospinal fluid (Jang et al. 2009). Owing to the intensified systemic inflammation. The other vital organs such kidneys and liver can suffer from critical injury in severe H5N1 disease. When the kidneys are affected, the disease manifests as acute tubular necrosis (ATN), particularly seen in critically ill patients (Wiwanitkit 2006). ATN is characterized by the degeneration and death of renal tubular epithelial cells, hence impair kidney function to filter waste products from the bloodstream, in the worst-case scenario, kidney dysfunction (Gaut & Liapis 2020; Li et al. 2024). These histopathological findings align with that observed in ischemic or toxic ATN, in which the renal function is impaired and multi-organ failure is described (Hanif et al. 2023).

The hepatic involvement, although less frequent, has been reported in patients suffering from severe H5N1 infection (Esper et al. 2010). Liver injury is defined by the abnormally high transaminase levels in those patients. While the exact mechanism behind the liver injury remains unclear, the cellular damage is most likely attributable to either a direct result of viral replication in hepatic cells or secondary to the systemic inflammatory response. In worst-case scenario, patients experience extensive liver dysfunction, which further complicates the disease management (Liu et al. 2013).

In view of the ability of H5N1 to cause multiple organ injury and systemic inflammation, WHO/CDC has urged the health authorities worldwide to consider H5N1 as a health risk in causing respiratory, hepatic, renal and cardiovascular failure (CDC 2024). Besides ARDS, the disease progression may be accompanied by severe complications such as pulmonary haemorrhage, pneumothorax, and pancytopenia (Gruber et al. 2006).

The pathogenesis of multi-organ failure in H5N1

infection is multifactorial, involving both the virus replication and the uncontrollable inflammatory response (Xu et al. 2024). These events contribute remarkably to the dysfunction of multiple vital organs, culminating in shock and organ failure. Additionally, lymphopenia, is also commonly observed in severely ill patients (Korteweg & Gu 2008). This transient immunodeficiency is believed to impair the host's ability tomount a protective antiviral defence against the virus and further increases susceptibility to secondary infections (Korteweg & Gu 2008). Several lines of clinical evidence provided compelling information about the proposed pathological mechanisms. A retrospective analysis of 26 confirmed human H5N1 cases in China demonstrated that multi-organ failure was present in all 17 fatal cases, with respiratory failure being the major cause of death followed by cardiac failure (71%) and renal failure (27%) (Yu et al. 2008). Notably, pathological biomarkers including decreased platelet counts and elevated lactic dehydrogenase (LDH) levels, were strongly associated with the fatal outcomes (Yu et al. 2008). Similarly, the histopathological analysis of five fatal H5N1 cases in Vietnam confirmed the presence of H5N1 in the lungs accompanied by distinctively high levels of proinflammatory cytokines and chemokines (Nakajima et al. 2023). They were believed to be the driving factors causing immune-mediated lung damage and exacerbation of ARDS (Matthay et al. 2019).

Collectively, these findings imply the critical roles of virus-induced cytopathology and immune dysregulation in the progression of multi-organ failure, reinforcing the need for early intervention and targeted therapeutic strategies to help improve the complications caused by H5N1.

FACTORS INFLUENCING THE MORTALITY OF H5N1

Delayed or Inadequate Antiviral Treatment

A critical factor influencing the high mortality of H5N1 is the delayed or inadequate administration of antiviral treatments (Zheng et al. 2008). Early treatment with antiviral drugs, such as oseltamivir (Tamiflu), can reduce the severity of symptoms and shorten recovery time (CDC 2024). However, due to the rapid replication of H5N1, it is probable to limit the effectiveness of antiviral treatment once the disease progresses to a more severe stage. In some cases, patients may not receive antiviral drugs until after hospitalization, by which time significant damage has already occurred (Charostad et al. 2023). Additionally, limited access to healthcare facilities, especially in regions where H5N1 outbreaks are most prevalent, can delay diagnosis and treatment. This delay can lead to a higher fatality rate, as those infected may not receive appropriate care in time (Wong 2015).

Adisasmito et al. (2010) found that patients receiving oseltamivir early had a better survival rate of 60%, compared to 24% among those who did not receive the antiviral therapy. The study also emphasized the benefit of oseltamivir treatment when treatment was initiated within 6–8 days after the symptom onset. Furthermore, World Health Network (2024) pointed out that limited access to healthcare infrastructures in affected areas exacerbates H5N1 dieseases, leading to delays in the disease diagnosis and treatment, contributing to higher fatality rates.

Pre-existing Health Conditions and Age Groups at Higher Risk

Certain individuals are more vulnerable to severe outcomes after succumbing to H5N1 infection, further contributing to the high mortality rate. Immunocompromised individuals, such as those with underlying conditions like HIV/AIDS, cancers, or diabetes, are at greater risk of developing severe complications. They have weakened immunity which limits defence against the virus infection, hence increased viral replication and extensive tissue damage (CDC 2024). Immunocompromised patients who were hospitalized due to influenza-related pneumonia reportedly experienced greater disease severity and mortality than their counterparts. The study highlighted that immunocompromise was an independent risk factor for a 30-day mortality, underscoring the high vulnerability of immunocompromise patients to severe influenza outcomes (Chen et al.2021). Age is a significant determinant of H5N1 mortality, with epidemiological patterns differing from those observed in seasonal influenza (CDC 2024). Unlike seasonal flu that primarily affects the elderly, H5N1 has historically been more prevalent among younger populations, including children and young adults (CDC 2024). This trend is partly attributed to the heightened immune response in younger individuals, which then leads to severe cytokine storm and increased disease severity (CDC 2024). However, the elderly remains vulnerable to H5N1, particularly due to pre-existing health conditions that may exacerbate infection outcomes (Smallman-Raynor & Cliff 2007). In addition, pregnant women are also at an elevated risk of severe complications and higher mortality, likely due to immunological changes during pregnancy (Oseghale et al. 2022; Purcell et al. 2025).

Early epidemiological reports indicated a higher incidence of H5N1 infection among children

and young adults, with relatively fewer cases among older populations. By July 2006, most of the confirmed cases were reported in individuals below 30 years old (Smallman-Raynor & Cliff, 2007). However, more recent data suggested a shift in the prevalence pattern (Qu et al. 2024). In 2024 the median age of vulnerable individuals was reported to be 38.5 years old, with an interquartile range of 20 - 49.3 years old, indicating that the virus has increasingly affected a broader age demographic, including elderly (Qu et al. 2024). Despite the shift, WHO (2024) still found high H5N1 incidences among children under five years old, followed by a secondary peak in the 25–35 age group. The findings underscore the importance of continuous H5N1 surveillance and research.

PREVENTIVE STRATEGIES AND THERAPEUTIC APPROACHES IN HANDLING H5N1 INFECTIONS

In recent years, implementing preventive measures against H5N1 has become increasingly significant globally to manage the ongoing H5N1 spillover and prevent future outbreaks (CDC 2024). H5N1 virions are shed through contaminated saliva, mucus, and feces, therefore, strategies minimizing human exposure to those means remain effective (WHO 2024). In addition to vaccination, the general public can resort to simple yet crucial steps to prevent the spread of avian influenza. These measures include reporting any sick or dead birds to local authorities, avoiding direct contact with wild or domestic birds, wearing personal protective equipment (PPE) such as gloves and masks if contact is necessary, practicing regular hand hygiene, and self-isolating for symptom monitoring if exposure is suspected (Saenz & Liliana 2024).

Antiviral Treatment

Initiating, antiviral therapy as early as possible is crucial in reducing the H5N1 mortality rate. The presently available antiviral agents aim at various stages of influenza viral replication, for instance adamantanes (amantadine, rimantadine), neuraminidase inhibitors (NAIs) (oseltamivir, zanamivir, peramivir), RNA polymerase inhibitors (favipiravir), and polymerase acidic endonuclease inhibitors (baloxavir marboxil) (Jones et al. 2023). While some antivirals have demonstrated high efficacy, concerns regarding the emergence of resistant H5N1 strains and drug limitations necessitate continuous research and development of novel therapies to fight H5N1 infection (WHO 2024).

Adamantanes, including amantadine and rimantadine, were among the earliest antiviral drugs used against influenza viruses (Bright et al. 2006). They inhibit the M2 ion channel, preventing viral uncoating and replication. However, their widespread use has led to a significant rise of antiviral resistant influenza A strain (CDC 2024). Resistance to adamantanes is primarily due to mutations in the M2 protein, notably the S31N mutation. The S31N mutation prevents the binding of adamantanes to the M2 ion channel, thereby allowing the virus replication to occur (Barr et al. 2007). In particular, H5N1 isolates from multiple outbreaks were found to develop resistance against adamantanes (Bright et al. 2006). In Southeast Asia, the mutation conferring resistance has been detected in A/H5N1 isolates since late 2003 (Wong 2015). Consequently, adamantanes are no longer recommended for H5N1 treatment. Favipiravir (T-705) is an RNA polymerase inhibitor that targets the viral RNA-dependent RNA polymerase (RdRp) (Furuta et al. 2017). It is effective against multiple influenza strains, including H5N1 and H7N9 (Wang et al. 2021). Despite its effectiveness, favipiravir has several limitations. Firstly, its poor solubility. The solubility of antivirals is pH-dependent, with an optimal solubility at pH 6.8 (Timur et al. 2021). It is poorly soluble in neutral or acidic conditions, therefore, affecting its bioavailability in the gastrointestinal tract and requiring higher doses for optimum therapeutic efficacy (Timur et al. 2021). Additionally, favipiravir also exhibits embryotoxicity and teratogenicity, restricting its use in pregnant women. In long-term safety, it potentially causes mitochondrial toxicity (Furuta et al. 2013). Nonetheless, the use of favipiravir does not cause the appearance of resistant viral strains, making it a valuable alternative to NAIs.

Baloxavir marboxil, approved in 2018, offers a valuable alternative for patients unresponsive to NAIs (Ison et al. 2020). A single dose of the antiviral significantly reduces the viral load within 24 hours, hastening the recovery rate (O'Hanlon & Shaw 2019). In a mouse model, baloxavir reduced H5N1 titters in the lungs, brain, and kidneys and prevented acute lung inflammation (Noshi et al. 2018). While baloxavir is highly effective, resistance mutations such as I38T/M in the PA genewere described thereby requiring ongoing surveillance to monitor the antiviral resistance trend (Takashita et al. 2019).

Given the rise of antiviral resistant strains and to better prepare the global health authorities in facing the upcoming H5N1 outbreaks, new drug candidates are being explored. Taurolidine (TRD) exhibits both antiviral and anti-inflammatory properties, making it a promising candidate for H5N1 treatment (Lv et al. 2023). Preclinically, TRD was found to reduce viral replication and suppress excessive inflammation,

potentially mitigating severe disease outcomes (Sato et al. 2020). Additionally, combined antiviral therapies have been proposed to enhance antiviral effects. For example, MEK inhibitors (e.g. ATR-002), when used alongside baloxavir, can supress viral replication and help minimize the occurrence of cytokine storm in severe influenza cases (Schreiber et al. 2021). While continuing surveillance efforts to monitor the antiviral resistance trend, WHO also evaluate new broad-spectrum influenza antivirals such as ENSO-002 and EDP-938. These compounds target different stages of the viral life cycle and could improve treatment outcomes in severe influenza cases (WHO 2024).

Chloroquine, traditionally an anti-malarial drug, has shown potential in treating H5N1 infections by inhibiting viral replication (Yan et al. 2012). It disrupts the acidic environment within endosomes, preventing the release of viral RNA genome into the host nucleus (Yan et al. 2012). Additionally, chloroquine has immunomodulatory effects that are believed to control the magnitude of pro-inflammatory response in the host (Yan et al. 2012). However, the use of chloroquine is not without side effects, resistance and drug efficacy issues, therefore, its use for H5N1 treatment remains limited (Savarino et al. 2003).

Avian Influenza Vaccination

CDC (2024) highlighted the emergence of antiviral-resistant influenza strains, such as oseltamivir and zanamivir resistance. Kode et al. (2019) demonstrated that I117T mutation in the viral RNA genome, conferred cross-resistance to oseltamivir and zanamivir. With the rising prevalence of drug-resistant influenza strains, vaccination and passive immunotherapy have emerged as the most effective preventive measures against influenza H5N1 (Haupt et al. 2023). In January 2020, the FDA approved the use of an adjuvanted monovalent influenza A (H5N1) vaccine, Audenz, in individuals aged six months and older (CDC 2024). In a phase 3 placebo-controlled trial involving 3,196 adults aged 18 years and older, a substantial proportion of the vaccine recipients developed hemagglutination-inhibition (HI) antibodies against the A/turkey/Turkey/1/2005 (H5N1) strain, indicating a robust immune response (Downey & Adalja 2020; Versage et al. 2021).

The first H5N1 vaccine, namely Sanofi Pasteur H5N1 vaccine, was approved by FDA in April 2007 (CDC 2024). It is intended for individuals aged 18 to 64 years (Baz et al. 2013). The vaccine was developed in collaboration with the National Institutes of Health (NIH) and has been considered an effective preparedness measure against potential H5N1 influenza pandemics (Haque et al. 2007). Clinical trials demonstrated that the vaccine elicited profound immune response in participants, indicating its potential to provide protection against H5N1 (Clegg et al. 2013). Following that, in May 2008, GlaxoSmithKline (GSK) pre-pandemic vaccine was approved by the European Medicines Agency (EMA) (Levine et al. 2022). This vaccine was intended for use in adults aged 18 to 60 years (Levine et al. 2022). In October 2009, the EMA granted marketing authorization for GSK H5N1 Adjupanrix (Jones 2009). Adjupanrix contains the inactivated split A/VietNam/1194/2004 NIBRG-14 (H5N1) strain and is adjuvanted to enhance the ability of the vaccine to mount the protective immune response (Jones 2009). These initiatives underscore the importance of pre-emptive measures in pandemic preparedness, particularly in targeting the emerging strains including the H5N1 clade 2.3.4.4b (Clegg et al. 2013).

Passive Immunization and Antibody Therapy

Passive immunization represents a promising alternative approach for the prevention and treatment of H5N1 (Eriksson et al. 2024). Antibody-based therapies, particularly via the use of monoclonal antibodies (mAbs), are potentially used in individuals suffering from severe H5N1 disease (Charostad et al. 2023). Tan et al. (2015) demonstrated the efficacy of humanized H5 antibodies in targeting a wide range of H5N1 strains, whilst Simmons et al. (2007) described the effectiveness of mAbs in preventing and treating H5N1 infection in a mouse model. To enhance the avidity of mAbs in neutralizing H5N1 virions, passive immunization involving a combination of antibodies has been proposed (Eriksson et al. 2024). The strategy enhanced the antibody specificity, hence comprehensive protection. Alternatively, employing polyclonal antibodies to recognize multiple antigenic epitopes also provides enhanced protection and is more cost-effective than monoclonal antibodies (Dixit et al.2016).

FUTURE DIRECTIONS

Despite significant advancements, several knowledge gaps remain unresolved in understanding H5N1 pathogenesis, transmission, and control. Future research should focus on elucidating the molecular mechanisms by which H5N1 suppresses the host immune response and facilitates systemic dissemination (AbuBakar et al. 2023). Investigating host genetic factors that influence infection severity, as well as receptor-binding adaptations that enhance human-to-human transmission, is crucial for assessing pandemic risk (AbuBakar et al. 2023). Additionally, exploring the impact of environmental

factors, such as climate change, on the cross-species transmission of H5N1should be given equal concentration (WHO 2024). Furthermore, co-infections with other respiratory pathogens can exacerbate the adversity of H5N1 disease, prolong viral shedding, and complicated treatment strategies, highlighting the need for further research on their clinical and immunological implications (WHO 2024).

From the One Health perspective, a multidisciplinary approach integrating human, animal, and environmental health is essential for understanding the ecological and epidemiological dynamics of H5N1 (CDC 2024). Strengthening ongoing surveillance in avian and mammalian populations, particularly in high-risk regions is critical for monitoring and detecting the emerging strains with pandemic potential (CDC 2024). In this context, data-driven machine learning models have shed light on improving risk assessment and biosecurity planning by identifying pre-emptive zones based on the farm-level risk scores. These models enhance the precision of preventive measures, minimize unnecessary depopulation of poultry, and represent a valuable addition to traditional surveillance methods (Jeon 2023). Expanding genomic surveillance, real-time monitoring of viral evolution, and international data-sharing mechanisms are among the global efforts to improve early warning systems and outbreak response in the context of H5N1 infection (WHO 2024).

Overall, preparedness strategies should prioritize the development of broad-spectrum antivirals, next-generation vaccines, and immunomodulatory therapies to mitigate severe H5N1 disease (WHO 2024). However, the development of effective therapeutics and vaccines for H5N1 remains challenging. One significant hurdle is developing broadly effective vaccines as influenza viruses are known for their rapid evolution and frequent genetic changes, especially in the hemagglutinin protein (Chan et al.2021). Currently used antivirals, such as oseltamivir and zanamivir, have shown limited efficacy in severe cases, especially in those receiving late treatments (Gao et al.2024). Recently, universal influenza vaccines capable of providing long-lasting protection against multiple strains of H5N1, including newly emergent ones, have gained substantial focus (Webster & Govorkova 2014). Alongside immune-boosting strategies, immunity raised by universal influenza vaccines can potentially outdo virus evolution and reduce the excessive inflammatory response seen in severe H5N1.

In a nutshell, investing in scalable vaccine platforms and improving biosecurity measures in live animal markets, poultry farms, and wildlife habitats can reduce zoonotic transmission risks (WHO 2024). Strengthening global pandemic preparedness through coordinated collaborative efforts, robust healthcare infrastructure, and rapid response frameworks can help address future H5N1 outbreaks better (CDC 2024). Enhancing public health communication and community engagement should not be neglected in controlling the spread of H5N1 and minimizing its socioeconomic impact (CDC 2024).

CONCLUSION

H5N1 infection severely affects the lower respiratory tract, leading to progressive disease that can ultimately result in death in humans. The virus triggers dysregulated immune response, marked by the excessive release of pro-inflammatory which not only exacerbates pulmonary injury but also promote systemic inflammation and multi-organ dysfunction. These pathological events underscore the complexity and severity of H5N1 infection, necessitating a deeper understanding of the underlying molecular mechanisms to inform effective interventions. Given its potential for re-emergence in a pandemic form, advancing H5N1 research is paramount to bridging critical knowledge gaps in the viral pathogenesis, transmission dynamics, and host immune modulation. Therefore, ongoing research and innovation are fundamental in minimizing its impact on the global health and enhancing global vigilance to future outbreaks, thus safeguarding the animal, human and ecosystem from its detrimental impact.

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