Review Article

Equine Influenza Disease and Current Global Situation

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Abstract | Equine influenza (EI) is a highly contagious viral respiratory disease that poses significant threats to equine health, with potential socioeconomic impacts on the equine industry globally. This study provides an overview of equine influenza, focusing on its epidemiology, including clinical presentation, transmission dynamics, laboratory diagnosis, and prevention strategies of the disease. Additionally, the current situation of equine influenza in Nigeria was examined, highlighting gaps in the disease surveillance. Despite the presence of a substantial number of equine population in the country, data on equine influenza remains limited, with dearth of information indicating underreporting and underestimation of the disease in Nigeria. Challenges such as unavailability of vaccination program, poor biosecurity, limited diagnostic capacity and low awareness among stake holders hinder effective management of the disease in the country. This review highlights the epidemiology of equine influenza and underscores the urgent need for comprehensive surveillance programs, improve diagnostic infrastructure and awareness campaigns to enhance control and prevention of the disease, globally especially in Nigeria. Addressing these challenges will not only safeguard equine health but also protect livelihoods dependent on equine-related activities.

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Introduction

Equine influenza disease (EID) is a highly contagious viral respiratory disease that poses significant health and economic risks to the global equine industry. The disease is caused by two subtypes of influenza A viruses, the H7N7 and H3N8 of the family *Orthomyxoviridae* (Dally *et al*., 2011). EID primarily affects horses, donkeys, and mules, leading to outbreaks that can result in severe economic losses due to restrictions on equine movement, reduced performance, and veterinary costs (Cullinane, 2014). The virus, particularly the H3N8 subtype has been reported to be responsible for major outbreaks worldwide, with sporadic reports of infection in new regions, raising concerns about its evolving epidemiology (Diallo *et al*., 2021).

Historically, Equine influenza virus (EIV) was initially discovered in horses in Prague in the year 1956, however, its presence was suggested as far back as 433 AD by the Greek veterinarian Absyrtus (Chambers, 2022). Since the 13th century, respiratory illnesses that are strikingly similar to EIV have been

documented (Sack *et al*., 2019). A review of records from 1688–1888 revealed documented outbreaks of human and equine influenza-like diseases in the Western Hemisphere, with outbreaks in human occurring mainly in the spring or autumn, while in horse infections were more frequent in the winter (Morens and Taubenberger, 2010). In 1872, there was massive outbreak of EID which affected considerable number of the horse population in North America, the morbidity of the epizootic was high, which cripped transportation resulting in significant economic loss associated with the mortality rate of 2 to 4% (Chambers, 2022). The outbreak started in Toronto in late September 1872, and spread throughout the North America along shipping routes across the United States to Central America and the Caribbean eventually stopping in Panama, which had no equine population to support the spread of the EIV (Cullinane and Newton, 2013). This resulted to the complete shutdown of all essential services including road and see transportations as illness rates approached approximately 100% (Chambers, 2022). EIV-like sickness was known to occur concurrently with human influenza disease, although historical records showed that equine and human influenza may be related though the lack of diagnostic testing at the time limited the reliability of this link (Chambers, 2020). This claim was also observed after the 1872 EIV epidemic which was followed be a mild human influenza reported in people working with horses during the 1872 outbreak, unfortunately it cannot be concluded whether this occurrence was a result of an EIV infection or human virus infection (Sack *et al*., 2019). Subsequent major outbreaks of EIV epidemic occurred in South Africa (1986), India (1987), Hong Kong (1992), Dubai (1995) and Australia (2007) affecting approximately 10,000 – 100,000 equines at each occurrence despite improved biosafety measures (Whitlock *et al*., 2022).

Surveillance and control of equine influenza are essential due to its rapid spread, antigenic drift, and potential for cross-species transmission. Vaccination remains a critical preventive measure; however, variations in vaccine efficacy due to viral mutations highlight the need for continuous monitoring and research. In regions like Nigeria, where equine populations are integral to cultural and economic activities, understanding the prevalence, risk factors, and management strategies for equine influenza is crucial for effective disease control. This literature review provides an overview of equine influenza, including its virology, epidemiology, clinical manifestations, diagnostic techniques, and control measures. Additionally, it examines the current status of equine influenza in Nigeria, identifying gaps in research and surveillance efforts. By synthesizing existing knowledge, this review aims to contribute to a better understanding of equine influenza and inform future strategies for its prevention and management.

The Equine Influenza Virus

Equine influenza virus (EIV) belongs to the family of the *Orthomyxoviridae,* genus *influenzavarius*, type A (Laabassi, 2016). The size of EIV ranges from 80-120 nm in diameter while the genome is composed of eight negative-sense RNA segments, which are encapsulated by a nucleoprotein, giving them helical symmetry (Krumbholz *et al*., 2011). Each of these RNA segments encodes up to 10 structural and non-structural proteins. The seventh segment is responsible for the form of the EIV because it encodes the membrane protein and the segmented genome of EIV encodes for at least 10 classical proteins (Elton *et al*., 2013). The segmented genome encodes the following proteins: three polymerase proteins (PB1, PB2, and PA), one nuclear export protein (NEP), one non-structural protein called NS1, matrix proteins M1 and M2, structural proteins called HA, NA, and nucleoprotein (NP). As a result of complementary sequences and frame shift other smaller but no less significant proteins are also expressed (Krumbholz *et al*., 2011). HA and NA glycoproteins are referred to as spikes as these projects outside the envelope and they are necessary for viral entry and release. NS1 and PB1-F2 are proteins with an active role in viral replication but are not incorporated into the viral structure while, PB1-F2 is a derivation of PB1 and is a smaller protein encoded by the open reading frame observed in some strains. NS1 is considered the most antagonistic protein in the immunological reaction of target cells, interfering with type 1 interferons (INF) and thus reducing the production of IFN-β. NS1 is made up of 230 amino acids, however, it presents a different size when compared to that present in other species, especially with that observed in humans and swine. It also performs an RNA binding function and effector function (Singh *et al*., 2018).

Complete transcription of segment 8 leads to

expression of NS1 while pre-mature splicing leads to expression of Nuclear Export Protein (NEP). Previously, NEP was thought to be a non-structural protein and termed as NS2 but later studies revealed that this protein was found within the virion interacting with M protein. NEP is important for the release of viral ribonucleoprotein from the host nucleus. Viral RNA segments 2 and 3 codes for PB1 and PA which are the major virulence factors. Furthermore, PB1 subunit can give rise to three proteins namely, PB1, PB1-F2, and PB1-N40. Notably +1 reading frame of PB1 segment codes for the PB1-F2 (on average 90 amino acid length) which has apoptosis induction function (Krumbholz *et al.*, 2011; Paterson and Fodor, 2012). N40 is another version of PB1 where there is truncation in the N terminal region of PB1 while, PA-X is a protein which is the outcome of ribosomal frame shifting of segment 3 mRNA during translation.

It is noteworthy that the PA-X protein of the virus causes suppression of host gene expression (Oishi *et al.*, 2018). NS1, a homodimer protein (215–237 amino acids), is an important virulence factor of influenza virus as it modulates several viral and host cellular mechanisms during influenza replication cycle. There are two functional domains in case of NS1 protein named as RNA binding domain (N-terminal end) and effector domain (C-terminal end). NS1 possess different epitopes hence having multifunctional activities. NS1 protein plays a crucial role in influenza infection by antagonizing type I interferon of host and reducing IFN β production. HA and NA proteins are the important surface antigens in equine influenza virus and antibodies generated against them provide resistance to infection (Landolt, 2014). Neutralizing antibodies formed against HA block virus entry and antigenic drift at this molecule leading to vaccine failure. Similarly, protective antibodies against NA aggregates the virus on host cell surface and hinders the virus release from the cells. Heterotypic immunity is provided at minimum level by humoral responses; whereas cross-reactive response (mediated by cytotoxic T lymphocytes) is observed between the viral subtypes, true for all the subtypes of type A virus (Hemann *et al.*, 2013; Landolt, 2014). A detailed investigation of 1989 UK outbreak using reverse genetics and site-directed mutagenesis determined the role of amino acid substitutions within HA glycoprotein and mutations at positions 159, 189, and 227 were found to be associated with altered antigenicity, as revealed by HI assays (Woodward *et al.*, 2015).

H7N7 (subtype 1) and H3N8 (subtype 2) initially isolated in Prague 1956 and Miami 1963, respectively were the two subtypes responsible for the outbreak of equine influenza (Laabassi, 2016). It is reported that since 1979, the H7N7 subtype has not been isolated from any equid (Dionísio *et al.*, 2021). The non-incriminating effect of H7N7 subtype is a consequence of the strong bonds of the codon of the EIV virus made by a sequence of three nitrogenous bases of messenger RNA that encode an amino acid or alterations based on the mutation or nucleotide composition, while it is believed that induce protection against this virus and the widespread vaccination program contributed to the disappearance of this virus (Dionísio *et al.*, 2021).

H3N8 subtype viruses was previously proposed to exist as a single lineage but further sequence analysis of the HA gene of the EIV H3N8 shows two genetic and antigenic variants evolving after 1980s namely Eurasian and American lineages (Daly *et al.*, 2011). Subsequently, American lineage evolved into three sub-lineages namely Argentinian, Kentucky, and Florida and further evolution of the Florida sub-lineage has resulted in the emergence of two groups of viruses with divergent HA sequences which are provisionally referred to as Florida sub-lineage clades 1 and 2 viruses. Currently, Clade 1 and Clade 2 lineage viruses have been circulating across the globe and leading to outbreaks. Clade 1 viruses have been circulating more in American continent while Clade 2 viruses have been incriminated for most of the outbreaks in Europe and Asia. Both clades have been reported in massive outbreaks throughout the world (Sack *et al.*, 2019). The emergence of new strains is due to antigenic drift, which consists of the accumulation of mutant spots in the gene that encodes the surface of the HA and NA protein. Antigenic shift is a remarkable event in the viral genome occurring through rearrangement of the genes at the level of NA, HA, or both, which may result from cross-infection or co-infection with another strain. The antigenic site B was suggested to be the major antigenic site for antigenic drift. The possession of K189 in the antigenic site B is important for the differentiation in Eurasian sub-lineage of EIV H3N8 because K189 residue essential for antigenicity and switching between uncharged, acidic and basic amino acids responsible for antigenic properties (Virmani *et al.*, 2020).

Epidemiology of equine influenza

Equine influenza viral disease is a prime example of re-emerging and transboundary disease with widespread outbreak in multiple countries from new strains occurring simultaneously around the world (Khan *et al*., 2021). Outbreaks of EIV H3N8 subtype of different strains were isolated and characterized in several countries such as Argentina, Germany, Chile, China, United States, France, Holland, Ireland, Nigeria, Sweden, United Kingdom, and Uruguay (Dionísio *et al*., 2021). Since EIV H7N7 subtype was last isolated in 1979, only H3N8 has been circulating all around the world (Xia *et al*., 2016). EIV has not undergone antigenic shift to lose its surface antigen to form a new subtype but overtime, the H3N8 has gone through tremendous antigenic drift to develop mutants of different clades (Oladunni *et al*., 2021). The avian influenza virus is the ancestral lineage of the EIV H3N8 subtype therefore, phylogenetic studies have shown that H3N8 virus evolved in the late 1980s, into the American and the Eurasian lineages while, the American lineage strains had evolved into South America, Kentucky, and Florida lineages with the Florida lineage further evolved into two antigenically distinct clades: Florida clade (FC)-1 and FC-2 (Oladunni *et al*., 2021). Florida Clade 1 viruses was dominated on the American continent, nevertheless, this clade has been incriminated to be the cause of widespread outbreaks in Africa, Asia, Australia, Europe and South America, similarly, Clade 2 viruses was dominated in Europe but also have been isolated in Asia, North Africa (Cullinane *et al*., 2010). The main cause of outbreak is due to the transboundary movement of horse movements between North America and Europe allowing the American lineage to spread to Europe while the Eurasian lineage was only detected in Canada, North America in 1990 (Oladunni *et al*., 2021). According to OIE Expert Surveillance Panel on Equine Influenza Vaccination Composition 2020, outbreak of EIV have been reported in Asia, Africa, North America and Europe while Bangladesh, Belarus, Bolivia, Bulgaria, Ethiopia, Georgia, Greenland, French Guiana, Honduras, Iran, Iceland, Laos, Latvia, Lithuania, Malawi, Madagascar, Myanmar, New Zealand, Central African Republic, Sri Lanka, Swaziland, Sudan, Togo, Thailand, Chinese Taipei, and Zimbabwe are countries that have never reported the outbreak of equine influenza virus (Miño *et al*., 2019; Dionísio *et al*., 2021; Kareche *et al*., 2022).

Transmission of equine influenza virus

Equine influenza is a seasonal disease usually occurring in epidemic form spreading through direct contacts and indirect contacts such as fomites, and it can be particularly contagious when it infects a naïve population of equines, with a 100% morbidity rate (Laabassi, 2016). EIV is mostly transmitted by respiratory droplet via aerosol between infected and susceptible equids in close proximity (Khan *et al*., 2021). The equine influenza virus has a lipid envelope and does not survive for long outside its hosts making it is fragile and easily inactivated by exposure to ultraviolet light, however, the virus will survive on skin, fabrics, and the surfaces of contaminated equipment for 12 – 24 hours (Whitlock *et al*., 2022). In naïve and immunocompromised equids, the short incubation period and persistent coughing releases large amounts of virus into the environment contribute to the rapid spread of the infection while personnel and fomites also contribute to the spread of the virus in the absence of infected equids in quarantine (Chambers, 2022). International trade and traffic also lead to spread of disease-to-disease free zones of the world because virus can survive for 3 days in the environment leading to the spread in other animals through fomites (Whitlock *et al*., 2022).

Apart from fomites, other environmental factors such and the wind, humidity (<60%) and temperature (20 – 25°C) play a very crucial role in the transmission of EIV (Karech *et al*., 2022). This is evident during the outbreak of equine influenza viral disease in Australia in 2007 when the wind speeds of greater than 30 kph from the direction of infected horses correlated with an increased risk for infection for horses downwind. Further analysis in Queensland, Australia, found east to west spread of EIV with distances of 1–2 km consistent with wind patterns; colder and drier air aid the fast rate of transmission; and water can also be a source of infection as some influenza virus strain can persist and remain infectious for more than 2 months in water (Sack *et al*., 2019). Similarly, environment with low humidity aid the transmission and persistence of EIV in the air as the virus can be easily inactivated by ultraviolet light and cannot survive in environment with high humidity (Laabassi, 2016).

Horse to horse transmission of EIV can be rapid and faster than other respiratory infections in the equine species therefore equids were thought to be the dead-end-hosts of EIV, but equine influenza viral disease

has been identified in race dog, camels, cats, pigs and human though with no evident clinical manifestation of the disease (Khan *et al.*, 2021). Isolated of EIV from pigs in China revealed that pigs served as vehicle for the viral rearrangement with the emergence of a new strain of equine influenza virus (Dionísio *et al.*, 2021). It is important to note that Influenza virus has restrictions on cross-infection because of HA which is a viral receptor-binding protein, it has the function of binding to the sialic acid of the host cell receptor (Landolt, 2014). Bidirectional transmission between dogs and horses is made possible by the virus' simultaneous circulation in both species. The variations in the EIV isolated from dogs and horses also allow the viruses to match the unique target cell receptors of each species (Morens and Taubenberger, 2010).

Pathogenesis of equine influenza

The first phase of EIV replication in host after the inhalation of the viral particle is adhesion of the viral particles to the ciliated epithelial cells of the upper and lower respiratory tracts with the aid of Hemagglutinin glycoprotein spikes present on the viral particle making it difficult for the infected animal to eliminate foreign substances in their respiratory tracts (Whitlock *et al.*, 2022). Horses have a mucus layer in the nasal cavity that can prevent HA virus binding, thereby inhibiting the virus from entering the cell but the HA adhesion to the sialic acid receptors on the cell surface aided by low pH allows the viral particle to enter the cytoplasm and remain in an endosome to continue replication (Dionísio *et al.*, 2021). The change in HA, the opening of the ion channel known as M2, and the acidification of the virus nucleus, which allows viral RNA to enter the target cell's nucleus, are all significantly impacted by the low acidic pH (Dionísio *et al.*, 2021). As a RNA virus, EIV utilise RNA dependent RNA polymerase (RdRp) to initiates the RNA synthesis internally after completion of the virions, viral particles will be released from the cell through budding into the outer environment from one cell to enter another cell in the airway, subsequently damaging the respiratory tract leading to necrosis of the respiratory epithelial cells, protein rich fluid exudation into the airways, cilia getting clumped and impairing the muco-ciliary apparatus (Singh *et al.*, 2018).

Horse respiratory epithelium possess high concentration of Neu5Gc2- 3Gal molecular complex essential for viral replication and rapid release of virions from viral replication (Dionísio *et al.*, 2021). EIV-infected cells suffer apoptosis, and non-structural protein 1 (NS1) of the virus plays a significant role in the pathophysiology of the disease and the changes in disease severity that are seen (Chambers, 2022). In addition to aiding in viral multiplication, NS1 inhibits the host's antiviral defence mechanisms by blocking the host's RNA processing machinery and then using these host components for the preferred transcription of viral RNA, NS1 promotes viral replication, also by blocking the activation of several host defence components, including NF-kB, interferon regulatory factor 3 (IRF-3), and other transcription factors, NS1 further suppresses the host's anti-viral response (Landolt, 2014). *In vitro* research of EIV in Madin-Darby canine kidney cells (MDCK), several pathogenic processes were discovered. The infected MDCK cells increase stress-related transcription factors such c-jun/Ap-1, which is linked to apoptosis and cell death, and cause cellular oxidative stress. Additionally, EIV triggers the production of TGF-β1 in cells, a cytokine that, at greater expression levels, can cause apoptosis. The JNK/SAPK pathway is activated, which is how TGF-β inhibits growth. TGF-β activates JNK and stimulates JNK phosphorylation of c-JUN, which induces apoptosis. Inhibition of TGF-B1 by neutralizing antibodies attenuates apoptosis induced by EIV through attenuated c-JUN/AP1 upregulation. The role of c-JUN and TGF-β1 in the induction of apoptosis was evaluated by antioxidant NAC, which leads to overexpression of BCL2 (anti-apoptotic gene) (Virmani *et al.*, 2020). Viral shedding can be seen for 7–10 days following a successful infection, and PCR can identify the viral genome (RNA) for at least 15 days throughout this time (Oishi *et al.*, 2018). Although the primary infection in equines is usually not fatal, the infection worsens as a result of a concurrent secondary bacterial infection because the respiratory epithelium takes around three weeks to heal (Chambers, 2022).

Clinical sign and diagnosis of equine influenza

EIV epidemic is defined by a noticeable and quick spread of the illness, peaking one week after the first case is discovered and ending when fresh cases can no longer be identified after 21–28 days and EIV diagnosis is made based on the clinical symptoms presented by an exposed equid (Dionísio *et al.*, 2021). Although EIV could be self-limit in previously exposed equid populations, naive populations have morbidity rates ranging from 60–90 % and fatality rates with

confirmed infection ranging from 1 – 20 % (Virmani *et al.*, 2020). EIV is characterised by a maximum incubation period of 5 days and an infective period of 14 days, also, during severe epidemics, susceptible horse populations will exhibit an incubation period of 2-3 days, and populations of naive horses with persistent viral excretion for 7–10 days may exhibit an incubation period of less than 24 hours (Laabassi, 2016). The common clinical signs observed in infected horses are; dry cough, fever, lethargy, anorexia, enlarged submandibular lymph nodes, tachycardia, hyperemia of the airways and conjunctival mucous membranes, serous nasal discharge, edema of the limbs, pain, muscle stiffness, and abortions (Câmara *et al.*, 2020).

Furthermore, the age and susceptibility of the animal to the equine influenza is essential to how the clinical signs will be presented (Dionísio *et al.*, 2021). A common clinical indication that follows a temperature rise and lasts for one to three weeks is a dry, harsh cough that is easily triggered by manual compression of the trachea's cranial portion, additionally, probing of the submaxillary lymph nodes may reveal pain, especially in younger animals with mucous nasal discharge during lung auscultation which may reveal altered sounds in the lung, such as wheezing, crackles, and increased breathing intensity in a horse suffering from secondary bacterial pneumonia (Virmani *et al.*, 2020).

Laboratory diagnosis is crucial for effective management of equine influenza virus disease for the provision of rapid and accurate diagnosis to improve medical management by allowing timely provision of antiviral therapy and prophylaxis including implementation of appropriate infection control strategies, public health responses to outbreaks, and limitation of unnecessary investigations or antibiotic therapy (Dwyer *et al.*, 2006). The first step toward accurate laboratory diagnosis of EIV is specimen collection. According to the guideline given by WHO, the most appropriate specimens for the detection of equine influenza are upper respiratory tract specimens. Samples are to be taken from the deep nostrils (nasal swab), throat (oropharyngeal swab) and nasopharynx (nasopharyngeal swab) while, nasopharyngeal aspirate and bronchial aspirate are also useful (WHO, 2021). It is important to note that appropriate precautions should be taken in collection of specimens since this may expose the collector to respiratory secretions from infected animals, therefore, sample collection from suspected infected animals should be conducted using appropriate personal protective equipment (PPE) and transported in appropriate virus transport media.

Equine influenza virus isolation was a traditional method of laboratory diagnosis conducted in an embryonated chickens' eggs or Madin-Darby canine kidney cell cultures for the observation of shrinking and rounding cytopathic effect that generally manifest with 5 days (OIE, 2019). EIV infection can also be diagnosed in the laboratory by the detection of its nucleic acid and antigen from the nasopharyngeal swab using the reverse-transcription polymerase chain reaction (RT-PCR) or Indirect and direct immunofluorescence which is 60–100 % sensitive compared with cell culture, and antigen-capture enzyme-linked immunosorbent assay (ELISA) using commercial type-specific H3N8 and H7N7 monoclonal antibodies (Singh *et al.*, 2018).

Serological detection methods are fundamental for identifying infected horses, assessing the immune response, and implementing control measures through surveillance. Serological laboratory diagnosis for the detection of equine influenza is performed on paired sera. Sera specimens for serology are collected during the acute phase within 7–10 days from onset of symptoms and the convalescent phase 14–21 days after the onset of symptoms (WHO, 2021). Serological tests are done to demonstrate steep rise of 4-fold or more in specific antibodies usually when specimens for virus isolation or antigen detection are negative, inadequate, or unavailable (Whitlock *et al.*, 2022). Serological diagnosis of influenza is retrospective; therefore, it is not providing information on the antigenic composition of circulating strains but very useful in delayed diagnosis or surveillance research (Dwyer *et al.*, 2006). Complement fixation, hemagglutinin inhibition assay, single radial haemolysis, neutralisation, immunofluorescence, and enzyme immunoassay are common serological tests used in the diagnosis of EIV. Hemagglutinin inhibition assay can differentiate subtype-specific and strain-specific serological responses, but the test interpretation can be complicated with the presence of inhibitors of hemagglutination (such as α-2 macroglobulin) in equine sera (Singh *et al.*, 2018). This complication can be overcome by pre-treating the sera with Tween-80 and ether or Kaolin while single radial hemolysis (SRH) test very useful for identification of susceptible population of horses and for the purpose of disease investigations related to

outbreak in immunized horses (Chambers, 2022). In order to improve the sensitivity and specificity of HI test, modifications to the traditional HI assay by the use of recombinant hemagglutinin proteins enhance the accuracy of the HI assay, particularly in detecting antibodies against emerging EIV strains (Sugiura *et al*., 2001).

Reverse transcription-polymerase chain reaction, or real-time RRT-PCR, is a sensitive and targeted molecular technique for finding influenza viruses (Mirzaei *et al*., 2020). The capacity to recognise and carry out influenza virus care programs has improved with the implementation of these nucleic acid-based approaches, particularly in surveillance operations (Sandybayev *et al*., 2023). The efficacy of multiplex rRT-PCR assays in veterinary diagnostics has been enhanced by recent research that have concentrated on their ability to concurrently detect EIV and other respiratory pathogens which is essential for controlling co-infections (Lee *et al*., 2021). Next-generation sequencing (NGS) is another molecular method that enables the simultaneous sequencing of millions of small DNA fragments in parallel, offering advantages over conventional molecular methods for higher yield, faster turnaround time, and more comprehensive genomic information (Buermans and Den Dunnen, 2014). Three main methods based on NGS technologies are currently used to sequence viral genomes: PCR amplicon sequencing, target enrichment sequencing, and metagenomics sequencing (Wang *et al*., 2022). Each method has its strengths, enabling researchers to better understand equine virus genomes for various purposes, such as disease tracking and surveillance, discovery of novel pathogens, and disease surveillance (Quer *et al*., 2022).

Point-of-care testing (POCT) is an additional diagnostic method that can be utilised for the prompt identification of equine influenza antibodies. One measurement, quick turnaround times, no sample preparation or pipetting, the use of pre-made reagents, user-friendly specialised analytical tools, and immediate, result-deduced therapy are some of POCT's benefits (Luppa *et al*., 2011). Example of POCT is Lateral flow assays (LFA) and Loop-Mediated Isothermal Amplification (LAMP). Lateral flow assays (LFA), which are utilised for bio sensing and measuring a variety of analytes, including several viruses, are an example of POCT (Di Nardo *et al*., 2021). Since the detection of human chorionic gonadotropin in the urine of pregnant women first popularised the LFA principle, LFAs have found extensive application in the fields of medicine, veterinary medicine, agriculture, bio warfare, food safety, and environmental health and safety (O'Farrell, 2009). Using gold nanoparticles on a nitrocellulose strip that have been coupled with an antibody, LFAs are quick tests that can identify virus antigens. Due to their ability to identify low virus loads, LFAs are well suited for early diagnosis in field settings. This is especially helpful in outbreak scenarios where prompt decision-making is essential. Their ease of use and portability make them valuable tools for veterinarians in the field (Sadeghi *et al*., 2021).

The loop-mediated isothermal amplification method (LAMP) is a fast and simple method for screening diseases and pathogens, including avian viruses. It uses at least four different primers to recognize six distinct regions on the target nucleotide sequence, and is facilitated by unique Bst polymerase with strand displacement activity (Padzil *et al*., 2021). The LAMP reaction can be performed at a constant temperature of 60˚C to 70˚C in a single step, and positive results can be recorded in 20-30 minutes (Parida *et al*., 2008). RNA detection can also be achieved by adding reverse transcriptase or using isothermal polymerase with reverse transcription activity for reverse-transcription LAMP (RT-LAMP) (Notomi *et al*., 2015). The LAMP method is proposed as a convenient solution for point-of-care testing, as it is simpler, cost-efficient, fast, accurate, reliable, and easy to operate even by non-technical personnel. Many common avian viruses have been successfully screened through clinical studies with good detection limits and efficient sensitivity (Padzil *et al*., 2021).

Scientists and veterinarians now have a comprehensive toolkit for identifying EIV because of the recent developments in diagnostic technologies. The selection of a diagnostic test is contingent upon the particular circumstances, such as the requirement for prompt outcomes during an outbreak or comprehensive genetic data for vaccine research. To ensure efficient management and control of equine influenza, more research and development are necessary to improve these instruments and solve their shortcomings.

Prevention and control of equine influenza

There is currently no specific antiviral medication available for EIV infection, so the condition must be

managed by isolation and allowing afflicted horses to rest (Madhwal *et al.*,2020). Amantadine and Baloxavir marboxil have been tested for the management of EIV, however, the usage of this antiviral agent has an ability to reduce in long term the susceptibility of the virus to the treatment, because this agent induces mutations in EIV, at position 38 in polymerase acidic protein (Dionísio *et al.*, 2021). Therefore, vaccine surveillance and updating programs remain the best way to prevent and control equine influenza. Vaccination has been practiced since 1960s, however, its efficacy is still a matter of debate due to the use of less potent vaccines, improper vaccination schedule, also use of outdated virus strains and continuous drift in the viral genome (Singh *et al.*, 2018).

Vaccination is most effective when it uses the virus from the most recent outbreak, with subsequent vaccine boosters after initial vaccination (Madhwal *et al.*, 2020). Vaccination plays an important role in controlling replication, that is, limiting the severity of clinical signs and their morbidity during outbreaks but do not eliminate the chances of successful infection (Whitlock *et al.*, 2022). Nevertheless, the use of vaccine was successful in prevention EIV H7N7 subtype and not H3N8 subtype because of constant antigenic drift of the prevailing subtype leading to subclinical infection, which is followed by viral shedding from vaccinated animals. Therefore, continuous checks and monitoring through surveillance programs and updating of vaccines with recent strains remains the best and effective way in prevention and control of this disease (Virmani *et al.*, 2020).

The World Organization for Animal Health (WOAH) is the only official organization that decides the strain EIV to be used in the preparation of commercial vaccines. This selection is conducted every year based on the circulating infective stain of EIV. New emerging strains are included only in cases where previously recommended strains are not providing optimum protection. The setback to this system is the exclusivity of only OIE approved reference laboratory are in charge of the update on the circulating strain of EIV thereby neglecting several other countries with previous or current outbreak without access to the reference laboratories (Cullinane *et al.*, 2010). Commercial vaccines available for the control of EIV include the whole inactivated virus, subunit, live attenuated and viral vector-based vaccines, while, there are other vaccine types under development, such as the case of the vaccine based on reverse genetics and others that have not shown benefits in relation to the existing ones, such as a DNA vaccine (Madhwal *et al.*, 2020; Dionísio *et al.*, 2021).

An inactivated whole virus EI vaccine consists of a whole EIV strain or strains that have been grown in mammalian cell culture or in the amniotic cavity of fertile chicken's eggs and then denatured either by physical (heat or irradiation) or chemical (formalin treatment) means. Virus particles in this vaccine are not able to replicate since they are destroyed, but the proteins retain antigenic epitopes recognizable by the host immune system and are able to evoke adaptive immunity. A major setback of inactivated vaccine is the requirement for the addition of an adjuvant because they tend to be less immunogenic (Oladunni *et al.*, 2021). An adjuvant is a molecule of an inactivated vaccine that potentiates the immune responses to interact either physically or chemically with the antigenic component of the vaccine. Several chemical ingredients have been used as adjuvants in inactivated EI vaccine compositions, but Aluminium-based adjuvants are predominately used particularly Aluminium Hydroxide. Inactivated vaccines provide protection to horses without releasing the virus making them the most suitable for the vaccination for pregnant mares (Dionísio *et al.*, 2021). Typically, inactivated whole virus EI vaccines need to be administered multiple times for a stable protective adaptive immune response to be induced. In EIV naïve horses, a complete primary course of vaccination requires three doses based on American Association of Equine Practitioners (AAEP) guidelines, consisting of a priming dose, a booster within 3–6 weeks, and a second booster within 5 months. Thereafter, annual boosters are recommended for most horses and twice-annual boosters for horses at risk of exposure (Cullinane *et al.*, 2020).

Subunit EI vaccine unlike the inactivated vaccines are made of only EIV antigenic fragments (HA or NA) that are encapsulated in colloidal particles as an antigen delivery system to the host immune system to elicit a protective immunity instead of the entire virus (Oladunni *et al.*, 2021). Subunit vaccines are presented to the host immune system using immuno-stimulating complexes (ISCOMs) and ISCOMATRIXTM (Cullinane *et al.*, 2020). The response obtained with ISCOM in this type of vaccine is more prominent, observing the induction of strong antibody response

with high levels of IFN-γ while immunity is usually short-lived, primarily an antibody-based response. When administered as a vaccine booster, the animal presents high levels of immunoglobulin A (IgA) specific to the virus. This type of vaccine has a longer response duration when administered in a protocol combining vaccines for intramuscular administration (Virmani *et al*., 2020).

The live-attenuated vaccine is obtained through the process of manipulating a live virus under laboratory conditions such that it can no longer cause harmful effects but retains its immunogenicity upon immunization. Modified live-attenuated vaccines can induce strong, long-lasting cross-protection by eliciting local mucosal immunity as well as systemic B- and T-cell proliferation (Oladunni *et al*., 2021). The attenuated vaccines aim to simulate a natural infection, and they are able to generate local and systemic immune responses because of the strain's ability to replicate only in the upper respiratory tract, making the development of more severe clinical signs is avoided (Dionísio *et al*., 2021).

Viral vectors are viruses that can be genetically modified to encode gene segments of another virus that is immunogenic for the purpose of vaccine production. Viral vectors have the potential for applications in both gene therapy and vaccine production. Unlike the inactivated whole virus and subunit EI vaccines that predominantly stimulate the humoral arm of the host adaptive immune response, the recombinant viral vector vaccines deliver antigens to the intracellular compartment in their targets, stimulating a robust and long-lasting cytotoxic T-lymphocytes response in the process, leading to the elimination of virus-infected cells (Oladunni *et al*., 2021).

Other preventive measures aside vaccination include strict biosecurity measures, proper quarantine practices, restricted movement and traffic, and post vaccination surveillance programs so as to update the new emerging viral strains according to the latest OIE guidelines (Madhwal *et al*., 2020). An experiment in New Zealand demonstrated vaccination combined with complete movement restriction was very effective in a control program for EIV outbreak (Rosanowski *et al*., 2019). In the event of outbreak, adoption of biosecurity measures can provide protection to horses while standard protocol of hygienic practices should be devised and adhered to by all veterinarian and first responders involved with infected animals. The implementation of High-health, High Performance (HHP) provided by OIE is also a great mitigation measures for the spread of equine diseases including EI (Singh *et al*., 2018).

Zoonotic potentials of equine influenza virus

Equine influenza has little implication on public health (Virmani *et al*., 2020). Though historically, from 1658 to the 20th century human influenza occurred three weeks after the outbreak of EIV and most scholars believed the 1889 human influenza pandemic was caused by EIV H3N8 (Xie *et al*., 2016). There are serological and clinical evidences to support EIV infection in human also, the virus has been successfully isolated from volunteered individual in an experiment study done by Alford *et al*. (1966), (Xie *et al*. (2016), and Khan *et al*. (2021). Human infection should not be possible because, EIV binds to specific host respiratory epithelial cell surface receptors containing sialic acid but the distribution of these sialic acid receptors within the respiratory tract is different in horses compared to humans. EIV binds to α 2,3-sialic acid receptor on the epithelial cell surface while the human Influenza virus binds to sialic acid-α2,6-galactose on the epithelial cells for entry (Whitlock *et al*., 2022). Therefore, the absence of specific receptors on the cell surface does not exclude the possibilities of cross-species transmission as observed during highly pathogenic avian influenza (HPAI) H5N1 outbreak in human beings (Li *et al*., 2019). However, H5 of these HPAI has special multi-basic amino acids at the cleavage site of HA, which is not the case with HA protein of H3 viruses which maybe the reason why in human volunteers demonstrated the possibilities of human infection with the equine H3N8 virus. Though seroconversion has been observed in humans in contact with equine influenza infected equids in the natural settings outside of experimental studies, few clinical signs were observed, and the virus was not isolated (Virmani *et al*., 2020). In 1958–1963, human serum samples were tested in the Netherlands for EIV antibodies and less than 0.5% of people 60 years of age had elevated EIV antibodies, with >40% EIV antibody elevation among people >70 years of age, therefore, it was summarized that a virus resembling the 1963 EIV strain infected humans during 1896–1900 (Xia *et al*.,2016).

A study conducted in exposed humans to EIV H3N8 infection during the outbreak in Australia (2007),

found 10% of people had serologic reaction against EIV H3N8, all at a low level, suggesting cross-reactivity with human influenza strain (Burnell *et al.*, 2014). A similar study conducted in Mongolia found 4.8% of people tested to have elevated EIV H3N8 antibodies, all at low titre levels that could be explained by cross-reactivity with seasonal human influenza virus infection or vaccine (Khurelbaatar *et al.*, 2014). Scientists thought that the equine influenza virus H3N8 infection caused the 1889 pandemic in human influenza history (Khan *et al.*, 2021). Since the isolation of equine influenza H3N8 and H7N7 in humans has never been proven, it has been classified under Animal Influenza A Viruses with Low Zoonotic Potentials (Abdelwhab and Mettenleiter 2023).

Economic importance of equine influenza virus

Equine influenza is one of the most economically important respiratory diseases of horses in most parts of the world, due to its highly contagious nature and rapid spread among susceptible hosts (Miño *et al.*, 2019). Equine influenza infection is associated with high economic loss because of mortality in equids, also the high morbidity in susceptible farm donkeys and racing horses which are left inactive after infection, thus, accumulate great financial losses for their respective owners (Sack *et al.*, 2019). Losses resulting from outbreaks affect industry and those who depend on racehorses and breeding, government, and individuals because the affected household will be in quarantine and the borders closed to suppress the virus (Dionísio *et al.*, 2021). The economic impact of the viral infection was highly demonstrated during the outbreak of the disease in Australia in 2007, involving 70,000 horses living on >9,000 properties, that resulted in a 5% mortality rate therefore, forcing the implementation of a contingency plan that cost the government of that country with about one billion Australian dollars (Rosanowski *et al.*, 2019).

In response to the major outbreaks of the diseases in 1989, the OIE Biological Standards Commission initiated the formal global Equine Influenza Surveillance Programme in 1995 (Sack *et al.*, 2019). Currently, OIE has reference laboratories in Ireland, the United Kingdom, and the United States with additional laboratories in Asia, Europe, and South America collect data on outbreaks of EIV and strain characterization, which the Expert Surveillance Panel on Equine Influenza Vaccine Composition reviews annually (Cullinane *et al.*, 2014). The panel, composed of OIE and World Health Organization representatives. The level of active or passive EIV surveillance in each country depends on the nature of the horse industry, status of the disease, laboratory capability and financial resources available for veterinary intervention, which are major challenges to evaluate the public health and economic significance of EI outbreak. Also, many laboratories involved in EIV surveillance experience difficulty obtaining sufficient samples because horse owners seldom request a confirmatory diagnosis for influenza. Surveillance is compounded by the failure of some diagnostic laboratories to characterize virus or to submit positive real-time PCR samples to an OIE reference laboratory for virus isolation and antigenic characterization (Sack *et al.*, 2019).

Current situation of equine influenza virus in Nigeria

Equine influenza (EI) is a highly contagious viral disease that affects horses and other equids, leading to significant economic losses in the equine industry. The first detection of equine influenza in Nigeria was reported in Ibadan Polo club during a tournament (Adeyefa and McCauley, 1994). Polo tournaments and other equestrian activities such as the Durbar festival are popular in Nigeria often attracting horses from far and near creating opportunities for cross infection. In a study conducted by Olusa *et al.* (2010) during the annual polo tournament in Lagos provided evidence of equine influenza subtype H7N7 in polo horses in Nigeria though H7N7 has not been isolated from horses since the 1970s. Nigeria saw an outbreak of equine influenza virus subtype H3N8 Florida clade 1 in 2018 which was the first of its kind to be reported from West Africa (Shittu *et al.*, 2020). Further studies have shown that the virus is present in Nigeria, with sporadic outbreaks and ongoing transmission reported in various regions (Olufemi *et al.*, 2022). Wide-ranging serological surveys conducted by Olufemi *et al.* (2022), Omoniwa *et al.* (2023), and Alaba *et al.* (2024), spanning from North Central, South West, and all states in Nigeria, have proven the extent of the deadly outbreak. The combined results of these studies highlight the ongoing potential threat that equine influenza presents in Nigeria. Diagnosis relies on clinical signs and serological tests, but the lack of advanced molecular diagnostic tools and limited access to veterinary services in Nigeria contributes to underreporting and delayed response to outbreaks. Vaccination is one of the key strategies in the prevention

and control of EI, but in Nigeria, the availability and use of vaccines is not available or encourage by the Nigerian government policy. Inconsistency in vaccination and the constant changes in antigenic make up of equine influenza virus can also lead to massive outbreak of the virus in previously vaccinated horses as observed in outbreak of the disease in vaccinated horses in Argentina (Olguin-Perglione *et al*., 2020). There is a need for continuous monitoring of EIV strains and improved biosecurity measures to reduce the risk of EI transmission. The social impact of EI is notable, as horses hold cultural significance in many Nigerian communities. Surveillance and molecular characteristics of the virus have advanced, but vaccine coverage, outbreak control, and economic effect still face major obstacles. In order to overcome these obstacles, veterinary authorities, researchers, and horse owners must work together to improve vaccination efficacy, strengthen surveillance systems, and lessen the financial impact of the disease.

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Novelty Statement

This review provides a comprehensive synthesis of the evolving global landscape of equine influenza, highlighting the interplay between viral evolution, vaccine limitations, and emerging One Health concerns. It integrates recent global epidemiological data, offering actionable insights for surveillance and control strategies in both equine populations and potential zoonotic interfaces

Author's Contribution

Oluwasheun Agnes Abioje: Conceived the idea, led the literature search, and drafted the initial manuscript. Martha Echioda-Ogbole: Contributed to data collection, critical analysis, and review of each sections. Olatunde Hamza Olabode: Revised and edited the manuscript for technical accuracy and coherence.
All authors read and approv

Conflict of interest

The authors have declared no conflict of interest.

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