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Equine Influenza Virus: Mode of Transmission, Epidemiology, Control and Prevention Measures

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Abstract: Equine influenza virus is important pathogens of animals though it is rarely zoonotic. The virus has been reported worldwide with the exceptions of New Zealand and Iceland's. Equine Influenza is encompassed under the genus of *Influenza A virus.* Equine influenza is caused by H7N7 and H3N8 strains. The recurring pattern of the influenza virus is due to its ability to exhibit variations in the surface antigen as a result of the genetic shift and drift activities of the organism. Influenza virus particles are irregularly shaped which are 80-120nm in diameter. Viral replication begins with the binding of hemagglutinin to sialic acid structures on the cell surface glycoproteins and it is one of the most important viral diseases of the upper and lower respiratory tract and rarely the nervous, gastrointestinal as well as the musculoskeletal system of different equine breeds. Out breaks are associated with the movement and assembly of horses for shows, sales, racing or trainings. The disease is highly contagious and aerogenous route is the most common means of transmission. The spread of pathogens is relatively very high after the onset of clinical diseases to susceptible animals. It causes a significant economic loss due to the disruption of major equestrian activities. There is no specific treatments available for equine influenza. The common antiviral drugs of choices are amantadines, rimantadines, oseltamivirs and zanamivirs. There are different prevention and control mechanisms of this disease which includes vaccination, management, isolation and quarantines.

Key words: Equine Influenza · Equine Influenza Virus · Pathogenesis · Prevention and Control · Review

INTRODUCTION

More than half of the human population is dependent on the power produced by draft animals and off which 90 million are equines. The world equine population has been estimated as 44 million donkeys, 15 million mules and 65 million horses [1]. Ethiopia has the highest equine population in Africa and 3rd in the world with around 9 million horses, donkeys and mules [2].

Influenza virus is caused by zoonotic viruses that occur in different species of animals. Influenza virus is belongs to the orthomyxoviridae family of ribonucleic acid (RNA) gnomes. These viruses are enveloped, pleomorphic, segmented and single stranded structures. Influenza virus is one of the most contagious diseases of the respiratory system. Itis divided in to 5 genera; *Influenza A virus*, *Influenza B virus, Influenza C virus, Thogoto virus* and *Isa virus* [3].

Equine Influenza is encompassed under the genus of *Influenza A virus*. *Influenza A virus* has not been isolated since 1980. It was first recognized in 1963 as cause of wide spread epidemics and has subsequently become endemic in many countries [4]. Based on the antigenic properties of hemagglutinin and neuraminidase, *Influenza A virus* is divided in to subtypes. To date, 16haemagglutininand 9 neuraminidase subtypes have been described. Although viruses of all 16 hemagglutinin and 9 neuraminidase subtypes of *Influenza A virus* has been isolated from wild water fowl, only a limited numbers of subtypes have been associated with infection of mammals. *Influenza A virus* affects a variety of animals such as horses, mules, donkeys, sea mammals, pigs, man and various species of birds[5].

Of the different subtypes, equine influenza disease is caused by equine influenza1(H7N7) and equine influenza 2(H3N8). The recurring pattern of Influenza viruses is due

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to their ability to exhibit variations in their surface antigens. The viral hemagglutinin and neuraminidase are the most important in the classification system of these viruses. The virion's envelope is derived from the plasma membrane of host cells and both the hemagglutinin and neuraminidase glycoprotein's attach to the viral envelope. Viral replication starts at the binding of hemagglutinin in to specific sialic acid structures on the cell surface glycoprotein's[6].

Although the mortality rate associated with equine Influenza virus infection is very low, it is considered the most important respiratory virus of horses. This is because it is highly contagious and has a potential to cause significant economic losses due to the disruption of major equestrian events. Unlike mortality the morbidity rate of equine influenza virus is very high[7]. Currently the virus has been reported worldwide with the exception of New Zealand and Iceland's. It is endemic in almost all parts of the world. This virus is relatively susceptible to environmental factors and chemical disinfectants. The most common source of infection and cause of an outbreak disease is the introduction of new animals in to the herd. Immunocompromized horses are at a great vulnerable of developing the disease. Out breaks of equine influenza occurs at any period of time and it produces a high risk in young ages. It is fatal for foals and pregnant mares[8].

In fully susceptible equidae, the critical clinical signs include serous runny nasal discharge, harsh dry cough in early cases, fever and pneumonia in secondary bacterial complications. Even though thus signs are imperative, it is confusing with many other viral and bacterial disease of the respiratory tract. The treatment applied against this disease is supportive and prophylactic therapeutics and other encouraging practices. Besides, vaccination, isolation and quarantine measures are major strategies to control the disease prevalence and incidences [9].

Aetiology: Equine influenza virus is one of the commonest, highly contagious, self-limiting upper and lower respiratory tract infections of horses. Equine influenza virus (EIV)has high morbidity but low mortality rates. Usually, the equine influenza is mild but sometimes can cause severe Broncho-interstitial pneumonia with pulmonary oedema [10,11]. Equine influenza is caused by the virus under the family of Orthomixoviridae and the genus *influenza A virus*. This virus is an enveloped and negative sense single stranded RNA[12].

Equine influenza viruses are type A viruses. They have divided in to two subtypes; equine influenza 1(H7N7) and equine influenza 2 (H3N8) based on antigenic differences in their hemagglutinin(HA) and neuraminidase[13].These viral strains are considered to be the most important causes of respiratory diseases of horses [14]. The recurring pattern of the influenza viruses is due to their ability to exhibit variations in the surface antigen [15]. These viruses can be propagated in the amniotic cavity of embryonated chicken eggs, bovine kidney and chick embryo kidney [13].

Classification: The classification of influenza viruses are influenced greatly by the practical need for assessment of the risk represented by the emergence of new variant viruses and the question of whether herd immunity against previously circulating strains will dampen the spread where existing vaccines will need to be reformulated. The emergence variant virus not only depends on the genetic drift but also on the genetic shift. So, as the viral HA and neuraminidase (NA)are the most important in the classification system, *influenza A virus* is classified in to 16 HA and 9NA types. These viruses are also further classified by their host, geographic distribution and strain numbers. So that equine influenza viruses are H7N7 and H3N8based on their strain fragments[16].

Morphology and Replication Cycles: Influenza virus particles are irregularly shaped particles which are80-120nm in diameter. The virion's envelope is derived from the plasma membrane of host cells. So, EIV have 2 distinct types of spikes (Peplomers) like other influenza viruses. The one is a road shaped and corresponds to HA and the other is a mushroom shaped which possesses to NA activity [4]. Both the HA and the NA are like proteins that attach to the viral envelope by short sequences of hydrophobic amino acids. The viral envelope surrounds a matrix protein shell which in turn surrounds eight single stranded (Segment) RNA molecules along with the nucleoprotein and these codes for 7 structural and three large (Nonstructural)proteins. These proteins are responsible for RNA replication and transcription[12].

Viral replication begins with the binding of HA to sialic acid structures on the cell surface glycoproteins. The virus is then internalized in to a coated vesicle and transferred to an endosome. Acidification of the endosome causes the HA to bend and exposes

hydrophobic fusion promoting regions of proteins. The viral envelope then fuses with the endosome membrane. The influenza transcriptase (RNA dependent RNA polymerase) uses host cell messenger (m) RNA as a primer for viral mRNA synthesis. In doing so, it seals the methylated cap region of the RNA; the sequences required for sufficient binding to ribosomes [6].

Epidemiology: Equine influenza is an acute disease of the respiratory system common in young unvaccinated horses [16]. Out breaks are associated with the movement and assembly of horses for shows, sales, racing or trainings. The initial source of infection is often partially immuned horses which can able to shed the virus without showing clinical signs. Equine influenza is a highly contagious erogenous disease and spreads rapidly among susceptible horses. Large quantities of viruses shed in aerosols by the frequent coughing of affected animals. Infections can be acquired at a distance up to30 meters [6].

An epidemic disease of EI is associated with the antigenic drift or antigenic shift of existing viruses and resultant inefficacy of extant vaccines. Out brakes of EIV infection may cause clinical diseases in nearly all horses in a susceptible population. The mortality rate of EI is usually very low which is reported as about 1% with most deaths associated with the secondary bacterial infections, but the morbidity rate is 100%. The virus is endemic in almost all parts of the world except in New Zealand and Iceland's. The profile of an epidemic can vary from explosive with a large proportion of a small group of susceptible horses housed in close proximity to much more prolonged out breaks lasting several weeks housed in multiple barns. In short epidemics arise when one or more acutely infected horses are introduced into susceptible groups[17].

Origin of Infection and Host Ranges: Equine influenza virus is relatively susceptible to environmental condition which is killed by heat($56^{O}C$) per 30minutes, desiccation and disinfectants. During an outbreak, the infection must originate from infected horses although the proximate source of the virus can be contaminated equipment's and fomites [18, 19].

Horses, donkeys, mules and other equids can be naturally affected by equine influenza viruses. Exceptional transmission to man has been reported on occasions and recent transmission to dogs with severe signs in some cases also was reported. Mice and ferrets can be infected intranasal and develop minor clinical signs [9,20].

Risk Factors: Immunocompromized horses are at higher risk of developing the disease. Outbreak of equine influenza diseases can occur at any period time of the year and their timing periods probably depends on husbandry and management practices such as yearling sales transport of horses for racing, movement for shows and breeding animals [15].

Animal factors: all age groups of horses including new borne foals are prevalent. The greatest risk appears between the ages of 2 to 6monthssince serum levels of passively acquired antibodies are being lost in foals at 2 months of ages. Generally, the virus is mostly affect horses of 2 months and below ages. The reason is due to less exposure to the diseases. Immunity depends on the means of exposures or vaccinations. Protective immunity induced by natural infections is characterized by production of immunoglobulin Ain nasal secretions and immunoglobulin G in serum. Immunity after vaccination lasts for a short period of time (3-4 months) and it is specific for the serovars of the strains [16].

Environmental Factors: housing large numbers of horses in a close contact or in a closed environment like large stables (Studs) provide an optimum condition for spreading of the virus through aerosols from infected to healthy horses [22]. Shed barns characterized by poor ventilations and greater stocking density is a four-fold to increase the risk of influenza diseases than the pale barns. Presence of small numbers of horses with access to large numbers of at risk horses may impact the course of an epidemic disease [18].

Pathogen Factors: molecular biology has now revealed the antigenic diversity of the virus. This is achieved mainly by the antigenic shift of the strains and results in a failure of vaccination unless a new specific vaccine is produced. Several different HA and NA antigens have been identified and grouped on the basis of serological tests[5].

Modes of Transmission: Equine influenza virus is most commonly transmitted via aerogenous routes of infections and can spread extremely due to the frequent and violent cough [12]. The virus is excreted during the incubation periods (IP) and horses remain infectious for

Table 1: Prevalence of equine influenza by Sex and Ages [21].

	Sex		Age				
Samples	M	F	Gelding	Foals	Yearling	Adult	Aged
No. of samples collected	159	173	59	23	143	187	38
No. of positive samples	19	23	5	0	25	18	4
Prevalence (%)	11.95	10.3	88.47	17.48		9.63	10.52

at least 5 days after clinical diseases has been started. Close contact between horses like nose to nose and grooming facilitate transmission. However, contaminated clothing of stable personnel's, equipment and transport vehicles can also contribute to the viral disseminations. Equine populations that are moved frequently such as race horses, breeding stocks, show jumpers and horses sent to sale are at special risk. A carrier state has not been reported for equine influenza [13,19].

Pathogenesis: The disease is principally caused an inflammation of the upper and lower respiratory tract. The virus is first inhaled, attaches to the respiratory epithelial cells with its HA spikes, fuses with the cells and it is released in to the cytoplasm of nucleated cells where it replicates [23]. Initial viral infection and replication occurs mainly in the nasopharyngeal mucosa, but after3-7 days of infection the virus can be recovered from the cell throughout the respiratory system. Infection of the respiratory mucosa results in death of the tracheal and bronchial epithelial cells, inflammation, oedema and loss of the protective mucocilliary clearance [24]. The most important changes occur in the lower respiratory tract and includes laryngitis, tracheitis, bronchiolitis and interstitial pneumonia accompanied by congestion and alveolar oedema [7]. Combined viral-bacterial pneumonia is common. Proliferations of the epithelial cells by bacteria can occur because of the disruption of normal clearance mechanisms and may cause bronchopneumonia [17].

Clinical signs: In fully susceptible horses, equine influenza virus spreads rapidly and causes diseases of high morbidity. The rapid spread is a valuable diagnostic indicator.

Forms of Clinical Signs: *Acute form*: the typical clinical signs include high fever (40-41 ^{O}C) after the incubation period of 1-3 days, accelerated breathing and pulse, dry cough and runny nasal discharge. Other clinical signs are reddening of nasal mucosa, conjunctivitis, myocaditis, muscularsore, swelling of limbs and later mucopurulent nasal discharge due to growth of opportunistic bacteria. Bacterialpneumonia is a complication in this form and leads to death. H3N8 seldomly cause enteritis and encephalitis [6].

Sub Acute Form: Here, clinical signs are less intense than the acute form and frequently only loss of appetite, coughing and slight nasal discharge arises suspicion of the disease [4].

Unapparent Form: This has been frequent in vaccinated or partially immune horses. So, this form of clinical sign is the main source of infection since infected animals excrete the virus through nasal secretions during the incubation periods. Transient viral excretion also common in horses suffering from the unapparent form. These animals are generally a major threat for other horses due to the absence of clinical diseases [9].

Complicated Form: Respiratory tract epithelium takes about 3 weeks to regenerate. During this time horses are susceptible for the development of secondary bacterial complications [8]. Horses which are worked, transported or exposed to adverse conditions can be experienced a worsening cough, severe bronchitis and pneumonia. Secondary bacterial growth result in mucopurulent nasal discharge, persistent fever and markedly abnormal lung sounds because of pharyngitis, bronchopneumonia, chronic pulmonary diseases and strangles. Equine influenza virus has also been speculated for predisposing of horses to the development of recurrent air way obstruction and exercise induced pulmonary hemorrhage [3].

Diagnosis: Diagnosis of equine influenza is first based on clinical signs such as pyrexia; dry cough and asthenia are typical when associated with high morbidity and rapid spread of the disease on infected premises. The age of the animals and their vaccination status are also important elements. Another essential method to diagnose clinically are the recent introduction of an animal that has not been quarantined and movement of animals in shows [6,7].

Laboratory Diagnosis

Cell Culture: Isolation of the virus can be attempted on cell cultures. Trypsin has to be added to cell cultures for the activation of viruses. It induces the proteolytic cleavage of the viral HA. Confulent MDCK cell cultures are inoculated and examined for cythopathic effect and Supernatant fluids are tested for HA. Cell cultures are suitable for haemadsorption tests using a 50% of chick red blood cell suspensions. Viral replication is detected by the demonstration of hemagglutinating activity in the harvested cell culture fluids and isolates are identified by HI, using a panel of subtype specific reference anti-sera [25]. *Polymerase chain reaction (PCR*): a nested reverse transcriptase polymerase chain reaction (RT-PCR) is used for the rapid diagnosis and characterization of EIV directly from the clinical specimens has been described [26]. PCR test is important for rapid diagnosis and routine surveillance but viral isolation remains essential in cases of large-scale out breaks. The disadvantage of PCR is the result of false positive [27,28].

Haemagglutinin Inhibition Test (HT): the HI test is performed by using ether treated antigens in order to increase the sensitivity of the test. Heat inactivated anti-sera is pre-treated by different chemicals like potassium periodate to remove non-specific HA and non-specific inhibitors of the hemagglutinations. Therefore, for positive result no agglutination is occurred [12].

Indirect Enzyme Linked Immunosorbent Assay: An indirect ELISA has been described for the detection of anti-equine influenza Abs in horse sera. By using the HI test as a reference standard test, indirect ELISAs have good specificity and sensitivity and can be used for rapid screening tests [9,29].

Differential Diagnosis: Equine influenza can be confused with strangles, equine viral rhino pneumonitis and equine viral arteritis disease. Both equine viral rhino pneumonitis and equine viral arteritis can cause abortion in pregnant mares. Furthermore, equine viral arteritis can cause ventral oedema, anasarca, diarrhoea and jaundice. Strangles causes copious mucopurulent nasal discharge, pharyngeal obstruction, supra orbital abscess and abscessation of the lymphoid tissues of upper respiratory tract and mesenteries [8,17].

Treatment: Equine influenza infections are generally self-limiting and there is no specific treatment sexist. Owners should provide general supportive cares. This includes encouraging of diseased horses to eat and drink and administering of non-steroidal anti-inflammatory drugs as prescribed by veterinarians to control the high fever. Resting of infected horses for at least one week is imperative. If the fever persists for three or more days and if mucopurulent nasal discharge is observed, they should be treated aggressively with antibiotics. Antibiotic selection is generally based on culture and sensitivity tests [6].

The commonly used antiviral prophylactic drugs are amantadines and rimantadines which are effective for the inhibition of viral HA. So that attachments of the virus will be prohibited. In addition to this, zanamivirs (Relenza) and oseltamivirs (Tamizlu) are used for the inhibition of the NA of the virus. So that, release of the virus will be inhibited. Hence, release of the virus from one host to another host has to be minimized [30].

Prevention and Control: The major strategies required for effective control of the disease is strict quarantine. Besides, controlled movement of equine influenza infected animals with in the state as well as outside the state is important. Interstate movement of equines should be avoided completely for 6 weeks in an outbreak cases. Quarantine of new horses for 14 days before mingling them with the resident horses is vital. Newly infected animals can keep shading the virus until 21 days and the OIE recommends for 28 days isolation of the diseased horses [17]. Infected horses should be kept away at a minimum distance of 100m from the healthy animals and continuous surveillance of the antigenicity of EIV strains is essential in order to monitor the viral changes [13].

Vaccination: Vaccination of EI is common in countries where the disease occurs. It may be effective in limiting the severity of clinical illness and morbidity during an outbreak. Vaccination of clinically normal horses in the face of an outbreak may enhance the immunity and is probably safe. New generations of vaccines based on viral attenuation and genetic engineering are available nowadays. A good and durable individual immunity is still a difficult challenge especially in young animals. The recommended vaccine for foals is at 4 and 6 months of ages for those borne from sero negative mares and sero positive mares respectively because of the inability to provide protective immune responses for foals with maternally derived antibodies [8,31].

An inactivated vaccine contains a whole virus of aqueous adjutants such as aluminum phosphate or aluminumhydroxide. The activity and efficacy of these

vaccines are not excellent. The substances such as carbomers have been introduced and have better activities than the former adjuvants. Oil adjutants are generally not used because of local reactions. These vaccines are administered intramuscular form[13].

An intranasal modified live vaccine has been designed to induce mucosal (local) antibodies. Since this vaccine is highly sensitive for temperature, it is not able to replicate beyond the nasal passage.

Therefore, animals administered with this cold vaccine are protected against the virulent challenges of homologous or heterologous strains [9].

Zoonotic Importance: Equine influenza virus could infect occupationals rarely although such infections are unusual and subclinical [17].

Economic Importance: Equine influenza is not a serious disease in itself but it can cause much inconvenient in racing horses since it occurs in an explosive outbreaks [32]. For example, during 2007, the Australian Bureau of Agricultural and Economics estimated that costs during the initial response to an equine influenza outbreak in Australian were $ 560,000 per day for disease control and $ 3.35 million per day in lost income for equestrian, farming and recreation industries[33]. Although the mortality rate associated with EIV infection is very low it is considered to be the most important respiratory diseases of horses. This is because it is highly contagious and has the potential to cause significant economic loss due to the disruption of major equestrian events. An additional cost has been incurred because of the restriction in movement of horses and associated quarantine periods [4,6].

CONCLUSION

Equine influenza (H7N7 and H3N8) viruses are highly contagious and pathogenic agents which are mostly common diseases of the upper and lower respiratory tract of equine and these viruses have economic as well as public health significance. They are made up of a negative RNA genome and have the feature of genetic variability (Instability) that results in epidemicity and pandemicity worldwide. Unless the disease is diagnosed early, these viruses have the ability to erode the epithelium and cilia of the respiratory tract. Because of the slough of the epithelial cells, they provide an optimum environment for the growth of opportunistic bacteria and later on they evoke a fulminant pneumonia which leads to death. When an outbreak occurs; vaccination, quarantine, isolation of infected animals, application of disinfectants on premises and continuous close observation among infected horses is the most preferred control and prevention options

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