REVIEW ARTICLE

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Scientific review on unpredictable virus: EQUINE INFLUENZA

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ABSTRACT

Equine influenza is an acute respiratory infection of horses, donkeys, mules and zebras caused by two distinct subtypes (H7N7, formerly equi-1, and H3N8, formerly equi- 2) of influenza A virus within the genus Influenza virus A of the family Orthomyxoviridae. They are related to but distinct from the viruses that cause human and avian influenza. In fully susceptible equidae, clinical signs include pyrexia and a harsh dry cough followed by a mucopurulent nasal discharge. Vaccinated infected horses can still shed the virus and serve as a source of virus to their cohorts. Characteristically, influenza spreads rapidly in a susceptible population. The disease is endemic in many countries with substantial equine populations like Australia, Japan, South Africa. to date New Zealand and Iceland are reported to be free of equine influenza virus. This review paper consists of introduction ,identification of the agent, etiology and epidemiology, transmission, clinical signs, diagnosis, serological tests ,treatment, prognosis, requirements for vaccines ,vaccination against equine influenza, prevention and conclusion.

Keywords: - influenza, H7N7, Orthomyxoviridae, pyrexia.

INTRODUCTION

Equine influenza is a common respiratory infection of horses caused by an orthomyxovirus of the genus influenza A. Influenza A viruses can be divided into sub-types on the basis of the antigen I creactivity of the surface glycoproteins, the haemagglutinin (H1–H16) and neuraminidase (N1–N9) molecules [1]. The viruses currently circulating in horses are of the H3N8sub-type. Although influenza viruses were first isolated in the1930s, we still have much to learn about this pathogen .Influenza A viruses have only eight RNA gene segments encoding between them 11

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or 12 proteins, yet the existence of two of these proteins was only reported in this decade [2,3].

Equine influenza is caused by a type 'A' orthomyxovirus. The viruses are 80 to 120 nanometer (nm) in diameter and consists of a core of eight separate segments of single stranded RNA surrounded by a spiked arrangement of glycoproteins. These viruses are classified based on the relative numbers of haemagglutinin (H) and neuraminidase (N) glycoproteins in the lipid outer layer[4].

Equine influenza viruses are believed to be of avian ancestry, and more recent transmission of avian viruses to horses and donkeys has been recorded. The sequence analysis of an H3N8 virus isolated in 1989 from horses during a limited influenza epidemic in North Eastern China (People's Rep. of) established that the virus was more closely related to avian influenza viruses than to equine influenza viruses[5] . Avian H5N1 has been associated with respiratory disease of donkeys in Egypt[6]. Horses become infected by inhaling the influenza A virus that is shed by infected, coughing horses or via equipment such as feed buckets, tack, and grooming aids that were contaminated by an infected horse. The virus then replicates in the lining (epithelial cells) of the upper respiratory tract. These infected cells die, and there is increased production of a watery discharge as well as swelling and inflammation of the respiratory tract.

Identification of the Agent

Embryonated hens' eggs and/or cell cultures can be used for virus isolation from nasopharyngeal swabs or nasal and tracheal washes. Isolates should always be sent immediately to an OIE Reference Laboratory. Infection may also be demonstrated by detection of viral nucleic acid or antigen in respiratory secretions using the reverse-transcription polymerase chain reaction (RT-PCR) or an antigen-capture enzyme-linked immunosorbent assay (ELISA), respectively.[7][8]

Etiology and Epidemiology

Equine influenza is highly contagious and spreads rapidly among naive horses. Horses 1–5 yr old are the most susceptible to infection. Orthomyxovirus A/equine-2 was first recognized in 1963 as a cause of widespread epidemics and has subsequently become endemic in many countries, except for New Zealand and Iceland. China, Japan, and Australia experienced devastating epidemics of equine influenza affecting tens of thousands of horses in 2007. Equine influenza had not been reported in China since 1993, in Japan since 1972, and had never been reported in Australia.[9].

Endemicity is maintained by sporadic clinical cases and by in apparent infection in susceptible horses introduced into the population by birth,

through waning immunity, or after movement from other areas or countries. A carrier state is not recognized for equine influenza. The clinical outcome after viral exposure largely depends on immune status; clinical disease varies from a mild, in apparent infection to severe disease in susceptible animals. Influenza is rarely fatal except in donkeys, zebras, and debilitated horses. Transmission occurs by inhalation of respiratory secretions. Epidemics arise when one or more acutely infected horses are introduced into a susceptible group. The epidemiologic outcome depends on the antigenic characteristics of the circulating virus and the immune status of a given population of horses at time of exposure. Frequent natural exposure or regular vaccination may contribute to the degree of antigenic drift seen with specific strains of A/equine-2 virus in some parts of the world.

Transmission

Highly contagious, EI is spread by contact with infected animals, which in coughing excrete the virus. In fact animals can begin to excrete the virus as they develop a fever before showing clinical signs. It can also be spread by mechanical transmission of the virus on clothing, equipment, brushes etc carried by people working with horses. Once introduced into an area with a susceptible population, the disease, with an incubation period of only one to three days, spreads quickly and is capable of causing explosive outbreaks. Crowding and transportation are factors that favour the spread of EI.[10]

Clinical Signs

Equine influenza is characterized by a very high rate of transmission among horses, and has a relatively short incubation time of one to three days. Clinical signs of equine influenza include fever (up to 106°F [41.1°C]), nasal discharge, have a dry, hacking cough, depression, loss of appetite and weakness. Secondary infections may include pneumonia. Horses that are mildly affected will recover within 2-3 weeks; however, it may take up to 6 months for recovery for severely affected horses. Horses that become immune may not show sings but will still shed the virus.[11]

The virus has a very short incubation period of only one to three days, and the clinical signs of influenza are obvious three to five days after initial exposure to the virus. Classic clinical signs associated with equine influenza include a sudden on set of a high fever (up to 106°F), coughing, a serous (clear, runny) nasal discharge, and sometimes mild swelling of the submandibular(under the jaw) lymph nodes. Rarely, veterinarians might note edema(swelling) of the distal limbs and trunk in horses with influenza, which is referred to as epizootic cellulitis.1,3In some horses a secondary bacterial infection can develop, resulting in pneumonia. The nasal discharge in these horses usually

changes from serous to mucoid (green,yellow, and thick), signifying a secondary bacterial pneumonia on the heels of the influenza, which can be fatal if untreated. It is important to remember that the severity of clinical signs is highly variable depending on the immune status of the horse. Sometimes horses can be infected and contagious without showing any clinical signs .of infection. These horses, in particular, risk circulating the virus throughout the horse population. Since the clinical signs of influenza are nonspecific, distinguishing influenza from other causes of upper respiratory tract infections can be challenging based on presentational one. Other diseases that influenza could be mistaken for include equine herpesvirus-1 and4,rhinovirus,*Streptococcus equi* infection (strangles),equine viral arteritis, bacterial pneumonia, chronic obstructive pulmonary disease, pharyngitis (inflammation of the throat),and a multitude of other less commonconditions.1Influenza is most commonly diagnosed in unvaccinated horses and in young horses between one and five years of age, especially those that frequent areas with large groups of transient horses (such as race tracks and show grounds).1,3Older horses can also become infected with influenza, but they generally experience a milder disease than their younger counterparts.

Diagnosis

A diagnosis of influenza A used to be presumed based on history, clinical presentation, and by ruling out other causes off ever, cough, and nasal discharge (i.e., strangles ,bacterial pneumonia). Now experts recommend using the available technology to identify the exact cause of the infection. Accurately diagnosing the cause of respiratory disease is an important step in implementing proper management practices and controlling disease spread.4 An accurate diagnosis also is important for the industry to determine efficacy of the existing vaccines. Virus isolation, serology (determination of acute and convalescent antibody titers) and stall side immunoassay kits that detect the influenza A virus can be used to diagnose affected horses. At the 2010 American Association of Equine Practitioners'(AAEP) Annual Convention ,researchers described the use of a polymerase chain reaction (PCR) test to diagnose equine influenza(as well as equine herpesviruses-1and -4 and *Streptococcus equi* subsp *equi*).According to the study authors, the PCR test quickly (i.e., within 24 hours) and accurately diagnosed respiratory infections in 761 horses.[12]

Serological tests

Diagnosis of influenza virus infections is usually only accomplished by tests on paired sera; the first sample should be taken as soon as possible after the onset of clinical signs and the second approximately 2 weeks later. Antibody levels are

determined by haemagglutination inhibition (HI) or single radial haemolysis (SRH).[13]

Treatment

When a horse contracts the equine influenza virus, rest and supportive care is advised so that complications do not occur. Veterinarians recommend at least one week of rest for every day that the fever persists with a minimum of three days rest. This allows the damaged mucocilliary apparatus to regenerate. Non-steroidal anti-inflammatory drugs are administered if the fever reaches greater than 104°F (40°C). If complications occur, such as the onset of pneumonia, or if the fever last more than 3-4 days, antibiotics are often administered.

Equine influenza infections are generally self-limiting, and no specific treatment exists. Owners should isolate affected horses from healthy horses and institute general supportive care. This includes encouraging the horse to eat and drink and administering on-steroidal anti-inflammatory drugs as prescribed by a veterinarian to control high fevers. Resting affected horses (typically one week for every day of fever) is imperative. Do not resume training until the horse's coughing has subsided completely. If the fever persists for three or more days and the nasal discharge becomes mucopurulent(containing pus and mucus),then the horse should be re -examined for development of a bacterial pneumonia. Horses with a suspected pneumonia are treated aggressively with antibiotics. Antibiotic selection is generally based on the culture and sensitivity results performed on a tracheal aspiration (a fluid sample collected from the trachea using a long, thin tube and saline).

Prognosis

For uncomplicated cases, horses should completely recover and return to exercise within three to six weeks of infection.1,3 In more severe cases horses might require up to 100 days of rest .Horses that develop secondary bacterial infections require longer recovery periods and have a more conservative prognosis for return to previous athletic function due to damage to the lung tissues.

Requirements for vaccines

Spread of infection and severity of disease may be reduced by the use of potent inactivated equine influenza vaccines containing epidemiologically relevant virus strains. Inactivated equine influenza vaccines contain whole viruses or their subunits. The vaccine viruses are propagated in embryonated hens' eggs or tissue culture, concentrated, and purified before inactivation with agents such as formalin or beta-propiolactone. Inactivated vaccines provide protection by inducing humoral antibody to the haemagglutinin protein. Responses are generally short-lived and multiple doses are required to maintain protective levels of antibody. An adjuvant is usually required to stimulate durable

protective levels of antibody. Live attenuated virus and viral vectored vaccines have been licensed in some countries.

Vaccine breakdown has been attributed to inadequate vaccine potency, inappropriate vaccination schedules, and out dated vaccine viruses that are compromised as a result of antigenic drift. An in-vitro potency test (single radial diffusion) can be used for in-process testing of the antigenic content of inactivated products before addition of an adjuvant. In process testing of live and vectored vaccines relies on titration of infectious virus. International surveillance programmes monitor antigenic drift among equine influenza viruses and each year the Expert Surveillance Panel (ESP) for Equine Influenza makes recommendations for suitable vaccine strains. Following a change in recommendations, vaccines should be updated as quickly as possible to ensure optimal protection. This is particularly important for highly mobile horse populations and for any horse travelling internationally.[14]

Vaccination against equine influenza

The main targets of virus-neutralising antibodies are the surface glycoproteins[15]: haemagglutinin (HA) is responsible for viral entry into host cells by binding to receptors on the cell surface; neuraminidase (NA) enzymatically releases newly synthesise d virus particles from the cell. Vaccine strategies against influenza have traditionally focussed on generating robust antibody responses against the surface glycoproteins ,particularly HA. During the last century a number of improvements were made to methods for standardising in activated virus vaccines against equine influenza[16]. Adjuvants and antigen presentation systems were also enhanced to extend the duration of immunity induced by inactivated virus vaccines, although high levels of antibody are required for protection against field infection by these vaccines[17].Alternatively, infection induced immunity in horses can afford some protection against re-infection even in the absence of high levels of circulating antibody [18,19]. Live attenuated or vectored equine influenza vaccines, which may better mimic the immunity generated by influenza infection than inactivated virus vaccines, are now available for use in horses. The various approaches to vaccination against equine influenza are the subject of a recent extensive review[20].

Prevention

Vaccination plays an important role in prevention of influenza A infections. The AAEP recommends vaccinating all at-riskhorses.5 Vaccination schedules vary depending on current vaccine status, age, broodmare status, and potential exposure. For example, the AAEP recommends administering a series of three boosters to adult, unvaccinated horses followed by semi-annual vaccination. Discuss vaccination

protocols with your veterinarian to develop a custom risk-based vaccine schedule most suitable for your horse. Like all influenza viruses, equine influenza viruses mutate (change) overtime, and the vaccines must periodically be updated to keep up with the changing viruses in circulation. To accomplish this, surveillance and laboratory diagnosis of equine influenza cases is essential. Management also is important in disease prevention. Quarantine new horses for 14 days prior to mixing them with resident horses to minimize the chance of introducing the influenza virus to your herd. Don't share equipment or supplies between horses, especially if one spikes a fever, has nasal discharge, or is coughing.

Conclusions

Equine influenza A H3N8 viruses continue to cause wide spread problems in horses despite control measures including quarantine and vaccination, and international spread of the virus occurs as horses travel for racing and breeding purposes. The first decade of this century has highlighted the unpredictable nature of equine influenza viruses and provided salient reminders that there is no room for complacency in the arena of influenza surveillance and research.

The figure-1 shows the structure of the influenza A virus. It has a segmented RNA genome, which is coated in protein. On the surface are the two glycoproteins haemagglutinin (HA) and neuraminidase (NA) that are recognized by the host immune system. These proteins are involved in virus entry and exit of host cells and are used to subtype influenza viruses.

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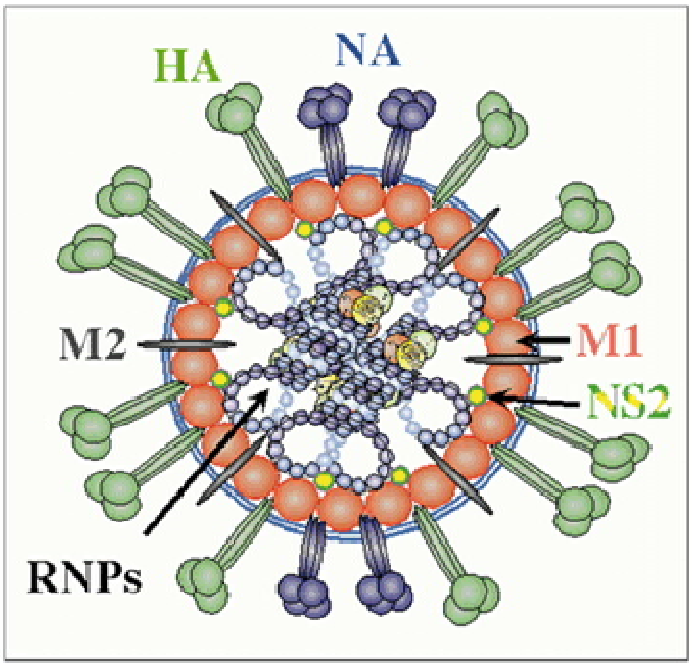

Figure 1: The structure of influenza A virus