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Controlling equine influenza: Traditional to next generation serological assays

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ABSTRACT

Serological assays provide an indirect route for the recognition of infectious agents via the detection of antibodies against the infectious agent of interest within serum. Serological assays for equine influenza A virus can be applied for different purposes: diagnosing infections; subtyping isolates; surveillance of circulating strains; and to evaluate the efficacy of vaccines before they reach the market. Haemagglutination inhibition (HI) and single radial haemolysis (SRH) assays are most commonly used in the equine field. This review outlines how both these assays together with virus neutralization (VN) and ELISA are performed, interpreted and applied for the control of equine influenza, giving the limitations and advantages of each. The pseudotyped virus neutralization assay (PVNA) is also discussed as a promising prospect for the future of equine influenza virus serology.

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1. Equine influenza

Equine influenza (EI) is a respiratory disease of equids that causes morbidity amongst unvaccinated and in some cases vaccinated horses worldwide. The equine influenza A virus, which belongs to the *Orthomyxoviridae* family, is enveloped and contains a single-stranded, eight-segmented RNA genome. Prophylaxis against EI is of great importance due to the economic burden of the disease. The global equine sporting industry has been detrimentally impacted by outbreaks of the disease since the late 1970s, and cancellation of race meetings still occur today. As well as participation in sport, equids remain a very valuable working animal in developing countries and thus outbreaks of EI in such areas are a concern (Virmani et al., 2010).

Transmission of EI is rapid, particularly amongst stabled horses; with large quantities of virus expelled during coughing episodes, EI is highly infectious. Naive equids typically present with clinical signs such as nasal discharge, coughing and pyrexia. Upon the onset of such signs, horses should be quarantined immediately in an attempt to prevent transmission to other individuals. Vaccinated horses with subclinical infections are problematic when trying to prevent the spread of disease. Therefore, when considering the regional, national or global transport of horses, quarantine before introduction to a new population is essential (Morley et al., 2000). Without effective quarantine, sub-clinically infected horses have the potential to cause devastating outbreaks such as that in Australia in 2007, which affected thousands of horses on a continent previously free from EI (Webster, 2011). Efforts by the Australian government to control the spread of the outbreak cost in excess of $1 billion (Callinan, 2008).

2. Control of equine influenza

2.1. Diagnosis

Diagnosis of an EI infection is usually achieved through identification of viral antigens or genetic material contained in nasal swab samples. However, the virus can be indirectly detected through serological assays that highlight the presence of antibodies to EI within an individual's serum. Comparisons between acute and convalescent samples (taken upon the onset of clinical signs and around 2 weeks later) are necessary to determine recent seroconversion. Serological diagnosis is a useful adjunct to directly testing for viral antigens or genetic material to confirm whether influenza is the cause of disease. Virus replication is transient and therefore the virus may be cleared by the time clinical signs are observed, particularly in animals that are partially protected by vaccination. Where vaccination is employed, serological assays can indicate possible cases of vaccine failure.

2.2. Vaccination

Vaccines are available for EI, and regulatory equine bodies including the BHA (British Horseracing Authority), British Show Jumping Association (BSJA) and FEI (Federation Equestrian International) impose a mandatory vaccination programme for horses competing on national and international circuits, respectively. However, as with other domesticated species such as cats and dogs, vaccination is only recommended and not required for the native and 'everyday' working horse.

Both cell-mediated and humoral immunity are necessary to combat influenza infection. Consequently, a desirable attribute of vaccines is the induction of cytotoxic T lymphocyte responses (CTL) as well as specific antibody responses (Slater and Hannant, 2000). However, cell-mediated responses do not prevent infection. When challenged with an influenza virus, neutralizing antibodies against the haemagglutinin (HA) protein on the virus surface provide subtype-specific protection by inhibiting viral entry into host cells. Different approaches to producing effective vaccines are constantly under development, with serological assays of great importance in efficacy testing procedures (Paillot et al., 2006).

2.3. Surveillance

Surveillance of circulating EI virus strains is necessary to monitor the spread of disease, and to identify the most prevalent strains for inclusion in vaccines.

H7N7, the first equine influenza A virus to be isolated in 1956 in Prague, is presumed extinct as it has not been isolated for over 30 years and consequently is no longer recommended as a vaccine strain (Chambers, 2014). Equine H3N8 viruses were first identified in Miami 1963 and in the late 1980s diverged into Eurasian and American lineages (Daly et al., 1996). The American lineage has since diverged into South American, Kentucky and Florida sub-lineages with further divergence of the Florida sub-lineage into Clades 1 and 2 (Lai et al., 2001). The H3N8 subtype continues to circulate globally with very few nations (e.g. New Zealand and Iceland) that have not experienced EI (Cullinane and Newton, 2013).

Unless vaccines are periodically reviewed and updated, outbreaks amongst vaccinated populations are probable. The UK and Japan are two countries to have experienced outbreaks of EI in the last 15 years amongst vaccinated racehorses due to the use of an out-dated vaccine (Newton et al., 2006; Yamanaka et al., 2008). EI vaccines were introduced in the late 1960s, but a major outbreak in 1989 demonstrated the need for vaccine strains to be updated. Furthermore, after the divergence of the original single H3N8 lineage into American and Eurasian lineages, it was demonstrated that vaccines were more likely to be effective if they included a representative strain from both lineages (Daly et al., 2004; Yamanaka et al., 2014; Woodward et al., 2015). Ideally, vaccine strain efficacy would be determined through sequence data to avoid the need for pony challenge studies when deciding on vaccine updates (Daly and Elton, 2013). However, the requirement to update vaccine strains is not simply dependent on the number of amino acid changes in the HA protein (Yamanaka et al., 2014; Woodward et al., 2015). Understanding which amino acid changes are antigenically critical is key to optimising protection.

At present in the UK, there is one vaccine available that meets the recommendations of the OIE (World Organisation for Animal Health), i.e. it contains two H3N8 strains, one each from the Florida sub-lineage Clades 1 and 2.

3. Current serological assays

3.1. Haemagglutination inhibition assay (HI)

3.1.1. Principles of assay

Developed in the 1940s, the HI assay uses the agglutination effect of the HA protein binding sialic acid receptors on erythrocytes (red blood cells; RBCs) to identify the presence of

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