Optimization of pseudotype virus to characterize equine influenza virus and equin...

1 INTRODUCTION

This literature review introduces Equine influenza virus (EIV) and Equine herpesvirus (EHV). Both diseases can be examined by the use of various methods such as virus culture testing, antigen detection, genome detection, to name a few. However, the method of pseudotyping is of particular interest as it facilitates the analysis of highly pathogenic viruses, without the need for high containment. The subject at hand signifies the benefit in utilizing the process of pseudotyping to characterize equine influenza and equine herpes virus.

2 CHARACTERISTICS OF EQUINE INFLUENZA VIRUS

Equine influenza virus belongs to the Orthomyxoviridae family. Influenza A can affect some animals, whereas influenza B and C viruses are limited to humans. The Equine Influenza A virus is enveloped and consists of the eight-segmented single-strand RNA genome. A nucleoprotein loosely encapsidates the eight-genome virus with polymerase complexes PB1, PB2, PA located on its ends. Furthermore, the capsids surrounded by a lipid bilayer envelope that consists of two surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA) that carry out essential roles in virus entry and exit (Daly, et al., 1996). The HA is integrated as a precursor protein and divided by cellular serine proteases into the active proteins HA1 and HA2. HA is responsible for binding to host cell sialic acid receptors as it controls the entry of the virus into the cell by fusion. NA’s primary function is to facilitate the cell-to-cell spread of the virus by the removal of decoy receptors in the host’s respiratory tract. Moreover, HA is an essential target protein for neutralizing antibodies which is a necessary component of vaccinations. On

the other hand, NA reduces the severity of illness as it is the target of second-generation anti-viral drugs to inhibit its function and therefore prevent virus proliferation (Rash, et al., 2017).

In particular, influenza in horses results from infections with H7N7 and H3N8 flu A viruses. In the H and N genes of EIV, mutations occur giving rise to antigenic changes called antigenic drift which allows viruses to escape neutralization by antibody made to earlier infecting viruses (Maanen & Cullinane, 2011). Furthermore, drift consists of the accumulation of point mutations within the antibody-binding sites in the hemagglutinin and the neuraminidase that retract the binding of some antibodies (Treanor, 2004). Equine influenza viruses of the H7N7 subtype have not been isolated since 1980, whereas H3N8 viruses continue to circulate in equine populations throughout most of the world and are essentially enzootic in the USA and western Europe (Daly, et al., 1996). H3N8 has been responsible for causing significant outbreaks of disease among horses around the world, including in the UK in 1979, 1989 and 2003 as well as in South Africa in 2003, Japan and Australia in 2007, India in 2008–2009 and, more recently, in South America in 2012 (Rash, et al., 2017).

2. 1 PREVENTION

Equine influenza virus (EIV) is a highly contagious, self-limiting and rapid airborne transmitted respiratory infection which persists with symptoms of coughing, nasal discharge, and pyrexia. Consequently, the onset of such signs and symptoms should prompt isolation of horses to prevent transmission to other horses. Without effective quarantine, infected horses exhibit the capability to trigger devastating outbreaks as witnessed in

Australia in 2007. The 2007 outbreak affected thousands of horses on a continent which otherwise was previously free from EIV (Webster, 2011), this cost the Australian government over $1 billion in to control the spread of the disease. Prophylaxis against EIV is vital due to the economic implication of the disease as observed in the global equine sporting industry which has been negatively impacted by outbreaks of the disease since the late 1970s. In addition to the involvement in sport, equids are valuable assets in developing countries for agriculture, and therefore outbreaks of EIV is of concern.

2. 2 VACCINATION

Vaccination strategies are essential in the prevention and control of equine influenza, as it depends on the management regimes which decrease exposure of vulnerable horses. Numerous inactivated equine influenza vaccines are commercially available. Current vaccines contain a representative of the equine influenza H7N7 subtype and one or more strains of the H3N8 subtype, either in the form of inactivated purified whole virus or subunit viral antigens. It is necessary to maintain protection by vaccinating horses in short intervals of 4-6 months. In the past revaccination intervals of 3 to 4 months have been advised to provide clinical protection, for populations at high risk especially young horses. Therefore, vaccination strategies, particularly vaccination intervals, should not be generalized but be based on the durability of the immune response provided by the vaccine used. Although efficacious influenza vaccines are available, duration of immunity in the field remains a point of concern. Also, regular updating of vaccines in parallel with significant antigenic drift should be a continuing

focus of interest. Strategic vaccination of young horses should be encouraged, considering interference of maternally derived antibodies and decline of postvaccine antibodies (Maanen & Cullinane, 2011).

3 CHARACTERISTICS OF EQUINE HERPESVIRUS

Equine Herpesvirus (EHV) is classified under several subtypes in horses, however Equine herpesvirus-1 (EHV-1), Equine herpesvirus-3 (EHV-3) and Equine herpesvirus-4 (EHV-4) are the most severe infections as they present with serious health risks for domesticated horses and can have a significant economic impact. The different classifications of the virus affect different body systems, which in turn depicts the symptoms that are presented by the horses. Symptoms such as fever, nasal discharge, enlarged lymph nodes, decreased fitness performance, muscle weakness, anorexia, and tiredness can be evident. EHV-1 is a member of the Alphaherpesvirus subfamily (Maanen, 2002) and can cause four indications of disease in horses including neurological complications, respiratory disease, abortion and neonatal death (Frampton, et al., 2005). Additionally, equine herpesvirus myeloencephalopathy (EHM) is an alternative term for the neurological disease associated with EHV-1 infections. Neurological complications arise due to obstruction of the blood vessels in the brain and spinal cord (Kohn & Fenner, 1987). Restriction of blood supply leads to tissue damage, and subsequently, there is a loss in the normal functioning of areas in the brain and spinal cord. Signs of neurological complications associated with EHV-1 include paralysis, ataxia, seizures, and death may occur.

Similarly, EHV-4 also causes respiratory infections. However, it is not known to cause abortions and neurological deficiencies. On the other hand, EHV-3

causes equine coital exanthema, an acute infection predominately sexually transmitted, resulting in the formation of papules and ulcers on the genitals of the affected horse (Kirisawa, et al., 2017).

3. 1 PREVENTION

Equine herpesvirus infection can spread rapidly from horse to horse through direct contact and inhalation of respiratory discharge. It is vital to quarantine the infected horse and prevents the spread of the disease and because the highly contagious nature of the virus can potentially lead to an outbreak. As this is a viral infection, there is specific treatment. However, to prevent secondary bacterial infections, prophylactic antibiotics can be used. Additionally, anti-inflammatory drugs can be administered for high fever and to help ease the pain. Horses may also require parenteral hydration and nutrition due to the lack of appetite associated with the infection.

3. 2 VACCINATIONS

The first EHV-1 vaccine was generated using homogenized organs of infected equine fetuses. However, there were no sustainable results obtained. In the late ’70s and early ’80s, inactivated and live attenuated EHV-1 vaccine advancement was introduced using virus grown in cell culture (Maanen, 2002). Vaccines that are currently available are now available against EHV-1 and EHV-4 infections. They assist in infection control; however, its availability does not depict long-lasting strength and duration of immunity (Ostlund, 1993). Herpesviruses can proliferate from cell to cell without entering the extracellular space, which impedes contact between the virus and the immune system.

Furthermore, herpesviruses are considered as “ immune escape artists” as they avoid interaction with the immune system by interfering with antigen processing and producing immunosuppressive cytokines (Kydd, et al., 2006). Antibodies are usually capable of neutralizing viruses directly as they target the viral glycoproteins located on the virion envelope; in spite of this, they are unable to protect horses from the magnitude of herpesvirus infections. The cross-section between EHV-1 and EHV-4 can be established after multiple exposures to the heterologous viral antigens as it is generally not as strong as the protection produced by the introduction of homologous antigens. Vaccinated animals that come in contact with EHV-1 or EHV-4 are still infected with the virus, although the animals then shed off the infection from there nasopharynx (Kydd, et al., 2006). Primary vaccination against respiratory disease entails two initial vaccine doses to be given weeks apart, and the recommended subsequent boosters are three months to 1 year depending on the product. Pregnant horses should receive a vaccination at five, six, and nine months of their pregnancy (Maanen & Cullinane, 2011).

4 PSEUDOTYPE VIRUSES

Pseudotypes are hybrid virus consisting of a surrogate virus core which is a nucleocapsid encasing a ribonucleic acid genome, surrounded by a lipid envelope with the surface glycoproteins of another virus, such as HA in Influenza A virus. The process involves removing the genetic component of the virus being examined and substituting it with an appropriate reporter virus. Respectively, pseudotypes consist of essential elements that include cores and its associated genome, a reporter. Various pseudotypes viruses (PVs) carry foreign genes called transfer/reporter genes, configured into their

genome. This enables the binding of envelope proteins to cell receptors allowing cellular entry and consequently transfer in gene expression (Temperton, et al., 2015). Essentially, PVs enable the analysis of extremely pathogenic viruses, without the need for excessive containment. The substitution of envelope proteins expressed on the lipid surface facilitates pseudotypes to be utilized as surrogates for wildtype viruses in antibody neutralization and the study of viral interactions. In particular, PVs are progressively being used as vaccine immunogens, optimizing the antigen either on the particle surface or as a transfer gene expression (Temperton, et al., 2015).

Mainly, retroviral pseudotypes are essential to research tools as they have been manipulated as cores for pseudotyping. Their fundamental characteristic is the ability to reverse transcribe their dimeric single-stranded RNA genome into a double-stranded deoxyribonucleic acid copy, which is subsequently assimilated into the cell genome through the use of cellular and viral enzymes (Temperton, et al., 2015). If successful, this advances to the expression of the transfer gene that can be analyzed to determine the efficiency of viral interactions and whether the antibody response will interfere with the entry and replication process of the virus under observation.

Furthermore, there are two common viral cores used; gammaretrovirus and lentivirus. Gammaretroviral vectors are derived viruses such as the murine leukemia virus. They are composed of a genome without any encoded viral sequences that are responsible for the production of viral proteins.

Lentiviruses are members of the *Retroviridae* family, which can infect non-

proliferating cells making them practical for gene therapy purposes involving highly differentiated cells (Temperton, et al., 2015). The most common lentivirus vector used for pseudotyping is HIV-type 1 (HIV-1). The first genes provided by these systems are *gag* and *pol* . In HIV for instance, *gag* provides the structural proteins and *pol* provides the integrase and reverse transcriptase factors required for the cleavage and maturation of each protein (Camell, et al., 2015).

Table 1 : Advantages of using pseudotypes for the study of humoral immune responses to viral infection. (Temperton, et al., 2015)

1	The assays are serum-sparing: typically, 2-10 µL of serum is required for each replicate, 5–25 fold less than for wildtype virus assays.
2	No virus culture is

	necessary as the serological antigen is
	entirely synthetic, derived by custom synthesis or PCR.
3	There is a wide choice of available reporters depending on end-user requirement.
4	Pseudotypes are readily amenable to high-throughput platforms.

5 Using pseudotypes as a surrogate for the wildtype virus removes the need to handle dangerous pathogens in high biocontainment facilities.

6 As the pseudotyped glycoprotein is the only viral antigen routinely incorporated into the pseudotype particle, what is

measured by this assay is antibody solely directed against this protein. This assay can thus be used as a tool for dissecting out (or delineating) responses observed using wildtype virus neutralizatio n assays in parallel.

7 Multiple studies have demonstrate d high

sensitivity, specificity and reliable correlation of antibody titers when neutralizatio n assays using pseudotypes are compared with those employing wildtype viruses.

5 CONCLUSION

The pseudotype method offers the ability to promptly initiate antigenic characterization and serosurveillance studies at a low containment level, therefore supporting vaccine and antiviral development (Bentley, et al., 2015). Table 1 represents advantages in the utilization of pseudotypes. This is particularly beneficial in the analysis of highly enzootic viruses such as EIV and EHV which present significant health and economic threat (Ferrera, et al., 2015).

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