Virology 597 (2024) 110164

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Role of pseudotyped viruses in understanding epidemiology, pathogenesis and immunity of viral diseases affecting both horses and humans

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ARTICLE INFO

Handling Editor: Dr. Jasmine Tomar

Keywords:
Pseudo
Virus
Pseudotyped

ABSTRACT

In this review, we explore how pseudotyped viruses (PVs) are being applied to the study of viruses affecting both humans and horses. For the purposes of this review, we define PVs as non-replicative viruses with the core of one virus and the surface protein(s) of another and encapsulating a reporter gene such as luciferase. These 'reporter' PVs enable receptor-mediated entry into host cells to be quantified, and thus can be applied to study the initial stages of viral replication. They can also be used to test antiviral activity of compounds and measure envelope protein-specific antibodies in neutralisation tests.

1. Introduction

Similarities in immune responses, including to viral infections, have led to the suggestion that the horse is a good model for human disease (Horohov, 2015). For some of the viruses that affect both horses and humans, the horse is a good model for vaccine development. Indeed, West Nile virus (WNV) vaccines have become available for equine use before human vaccines. Furthermore, for arboviral infections such as West Nile fever, horses can act as sentinels for circulation of the virus in a region, thus providing an early warning of increased risk of human infection (Gothe et al., 2023). The aim of conducting this review was to identify potential synergies between studies of equine and human viruses using pseudotyped viruses.

2. Pseudotyped viruses

Pseudotyped viruses (PVs), 'pseudotypes' or 'pseudotype particles' are chimeric viruses consisting of a virus core surrounded by a lipid envelope with the surface protein(s) of another virus (reviewed in Wang et al., 2023). In this review, we focus on PVs that package a reporter gene providing a surrogate readout for entry of the particle into a target cell. They are a valuable tool to study viruses for which there is no reliable cell culture system or that are difficult to handle because of the requirement for a high level of containment.

Initiation of host cell infection by an enveloped virus requires a viral-to-host cell membrane fusion event, which is mediated by at least one of the viral transmembrane or 'envelope' proteins (reviewed in Barrett and Dutch, 2020). In broad terms, this involves two steps (priming and triggering) to bring the viral and cell membranes together prior to fusion and release of the viral genome. Proteolytic cleavage by a host cell protease exposes a highly hydrophobic region of the protein, the fusion peptide or fusion loop, to prime either the envelope protein responsible for fusion (a class I fusion protein) or an accessory viral protein (a class II fusion protein). The class I fusion proteins (e.g. of influenza A virus, IAV, and human immunodeficiency virus, HIV) have been most extensively studied. Different triggering events include exposure of the fusion protein to the low pH environment of an intracellular compartment such as an endosome. Class II fusion proteins were separately classified in 2001 in recognition of their structural differences; class II fusion proteins contain more β-sheet secondary structures than class I fusion proteins, which have mainly α-helical secondary structure. The class I proteins often form homotrimers, whereas the class II proteins usually initially assemble as homodimers but also form heterodimers with a second viral protein. This 'companion' protein is proteolytically cleaved before it then primes the class II fusion protein homodimers to dissociate into monomers in the low pH environment of the endosome, exposing the fusion loop. Members of the *Bunyaviridae* and *Flaviviridae* have class II viral fusion proteins. Finally, in 2006, after the ectodomain structures of the vesicular stomatitis virus glycoprotein (VSV-G) and herpes simplex virus type 1 glycoprotein B (HSV-1 gB) were solved, a third class of fusion protein was created due to their structures being similar (despite a lack of sequence homology) but distinct from the structures of class I and class II fusion proteins. A key feature of the class III fusion proteins is that they undergo a reversible conformational change.

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https://doi.org/10.1016/j.virol.2024.110164
Received 12 May 2024; Received in revised form 24 June 2024; Accepted 27 June 2024
Available online 28 June 2024

Pseudotyped viruses are often based on lentiviruses including HIV and murine leukaemia virus (MLV), a gammaretrovirus. 'Third generation' lentiviral vectors offer safety advantages over earlier systems and are currently the most frequently used approach for generating PVs. Typically, 293 T cells are co-transfected with plasmids expressing (i) the envelope protein (or proteins) of the virus being studied, (ii) a reporter gene (e.g. luciferase) flanked by packaging signals and (iii) the minimal lentiviral proteins to package the virus particle – the core or backbone (Fig. 1A). Earlier core plasmids such as pNL4-3.Luc.R-E−, in which the firefly luciferase reporter gene was inserted into *nef* and two frameshifts prevent expression of Vpr and Env, are also only competent for a single round of replication (He et al., 1995). However, it only requires co-transfection with an HIV *env* expression vector to reconstitute infectious virus. Reporter lentivirus PVs can transduce appropriate target cells (i.e. cells with receptors recognised by the envelope protein) leading to integration of the reporter gene into the genome of the target cell.

Another virus commonly used to generate PVs is vesicular stomatitis virus (VSV), which can accept a diverse range of envelope proteins and can also bud (albeit less efficiently) without an envelope protein. In early studies, cells were co-infected with VSV and other enveloped viruses to generate PVs. The most popular approach currently was described by Whitt (2010). In this system, cells are co-transfected with a plasmid encoding full-length VSV genome with the VSV glycoprotein (G) gene replaced with a reporter gene (e.g. green fluorescent protein or luciferase) under the control of a T7 promoter and the four plasmids encoding the VSV nucleoprotein (N), phosphoprotein (P), G and large (L) viral polymerase proteins. The virus produced is propagated, plaque

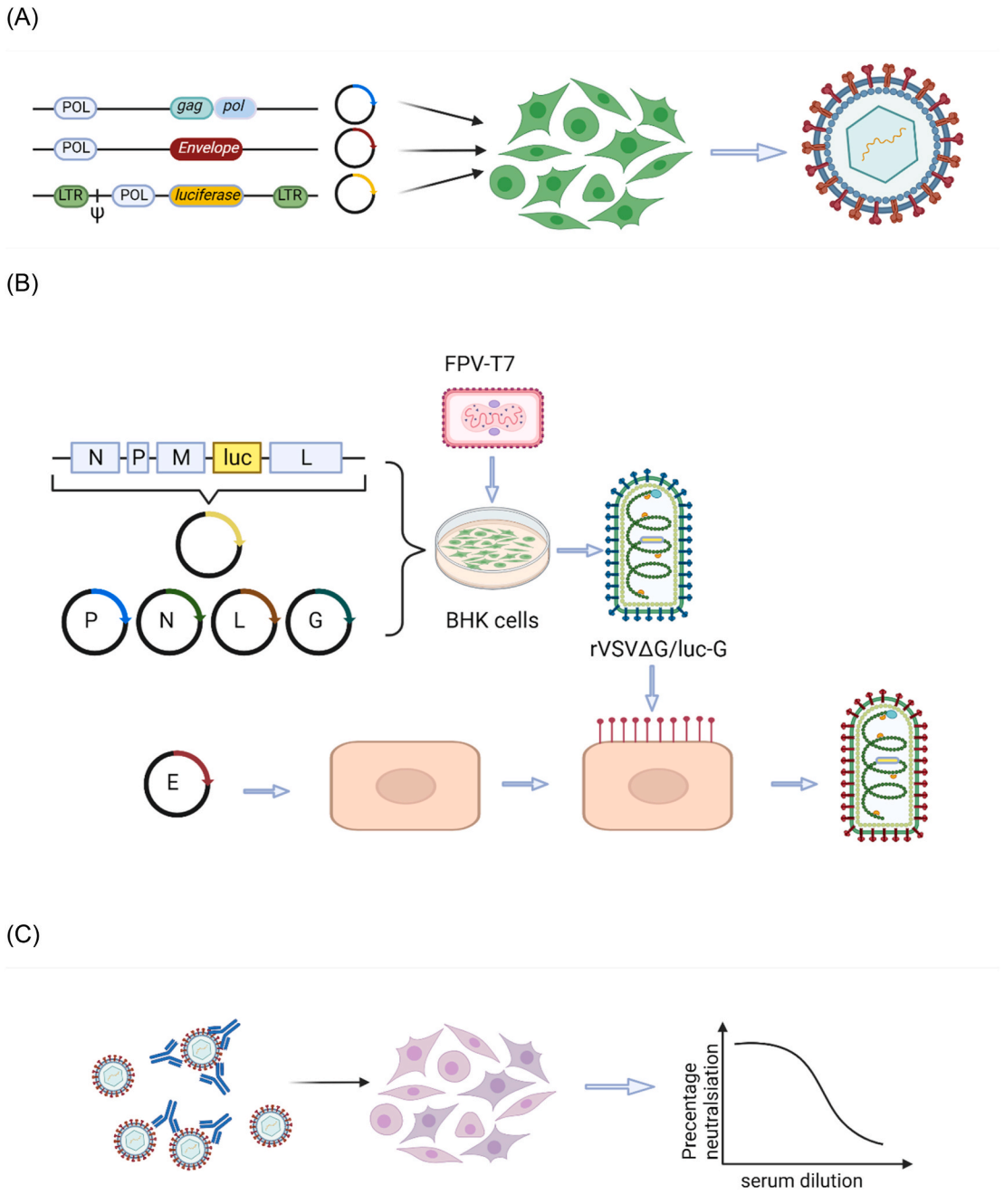

Fig. 1. Schematic of production of pseudotyped viruses based on a lentiviral core (A) or a vesicular stomatitis core (B) and their use in neutralisation tests (C). Created with BioRender.com.

purified and working stocks of the G-complemented rVSV-ΔG-reporter virus made (e.g. rVSV ΔG/luc-G). The G-complemented virus stocks are titrated and then used to infect cells transiently or stably transfected to express a heterologous envelope protein (E) for production of PVs (Fig. 1B).

Expression of the reporter gene provides a readout of the ability of the PV to enter the target cell enabling studies of viral entry mechanisms and reduction of reporter gene expression enables inhibition of entry by antiviral compounds or antibodies to be measured (Fig. 1C). The use of PVs for determining factors involved in virus entry and measuring neutralising antibodies really came to the fore during the COVID-19 pandemic.

3. Viruses reported to infect both humans and equids

A recent systematic review of the literature concluded that horses are one of the three domesticated species (the others being pigs and cattle) with which humans share the highest number of viruses (Johnson et al., 2020). However, there are relatively few viruses that are transmitted directly from horses to humans (or *vice versa*). This may reflect that zoonotic transmission of equine viruses capable of adapting to the human host already occurred historically. To identify publications describing the application of PVs to study viruses shared between horses and humans, a systematic literature search was conducted for each of the viruses listed in the supplementary table provided by Johnson et al. (2020). This table lists 33 of 139 zoonotic viruses as infecting species of the genus Equus but notably omits *Equus* spp. as a species infected by influenza A virus (IAV), the addition of which gives 34 viruses that infect both humans and equids. As nomenclature is not standardized, the search was conducted using the broad terms 'pseudo' and 'virus', which encompasses a range of non-replicative virus particles (Supplementary file 1). Due to the volume of articles found for IAV, rabies virus and VSV, a separate search was performed to limit the articles found to those that included the MesH terms horses, horse, equine or Equidae (Supplementary file 1). The refined search after removing duplicates and reviews identified 143 articles relevant to the use of 'pseudo viruses' applied to 17 viruses identified as affecting both horses and humans (Table 1). Selected articles on development and application of reporter PVs highlighted in later sections of this review are presented in Table 2.

Table 1
Classification of viruses (Walker et al., 2022) identified in the initial literature search for "pseudo virus" together with the name of each of the viruses listed by Johnson et al. (2020) as infecting both humans and equids (and influenza A virus).

Virus order	Virus Family/Genus	Viruses
Amarillovirales	*Flaviviridae,* Flavivirus	Japanese encephalitis (JEV) Tick-borne encephalitis (TBEV) West Nile (WNV) Zika (ZIKV)
Articulavirales	*Orthomyxoviridae,* Alphainfluenzavirus	Influenza A (IAV)
Bunyavirales	*Phenuiviridae,* Phlebovirus *Nairoviridae,* Orthonairovirus	Rift Valley fever (RVFV) Crimean Congo haemorrhagic fever (CCHF)
Martellivirales	*Togaviridae*, Alphavirus	Eastern equine encephalitis virus (EEEV) Getah (GETV) Ross River (RRV) Sindbis (SINV) Venezuelan equine encephalitis (VEEV) Western equine encephalitis (WEEV)
Mononegavirales	*Bornaviridae,* Bornavirus *Paramyxoviridae,* Henipavirus *Rhabdoviridae,* Lyssavirus *Rhabdoviridae,* Vesiculovirus	Borna disease (BDV) Hendra (HeV) Rabies (RABV) Vesicular stomatitis (VSV)

Table 2
Selected articles describing development and application of reporter pseudotyped viruses.

Citation	Core	Reporter protein	Virus/ Envelope protein(s)	Application
Aljofan et al. (2009)	VSV	RFP	HeV F & G	Antiviral screening
Bae et al. (2019)	MLV	β-gal	HeV F & G	Neutralisation test
Bradel-Tretheay et al. (2019)	VSV	rLuc	HeV F & G	Host-pathogen interaction
Bukbuk et al. (2014)	VSV	Luc	RVFV Gn/Gc	Neutralisation test
Di Genova et al. (2024)	HIV	GFP/Luc	EHV-1 gB, gD, gH, gL	Neutralisation test
Elshabrawy et al. (2014)	HIV	Luc	HeV F & G	Antiviral screening
Feeley et al. (2011)	MMLV HIV VSV	β-gal	IAV HA VSV G	Host-pathogen interaction
James et al. (2021)	HIV	Luc	SARS-CoV-2 S	Neutralisation test
Kaku et al. (2009)	VSV	GFP	NiV F & G	Neutralisation test
Kinsley et al. (2020)	HIV	Luc	IAV HA	Neutralisation test
Kwak et al. (2020)	MLV	β-gal	JEV E	Neutralisation test
Lee et al. (2009)	MLV	β-gal	JEV prM/E or E	Development of PV
Lee et al. (2014)	MLV	β-gal	JEV E	Development of PV Neutralisation test
Lee et al. (2020)	MLV	β-gal	JEV E	Neutralisation test
Li et al. (2018)	HIV	β-gal	RVFV Gn/Gc	Neutralisation test
Liu et al. (2017)	HIV	GFP & Luc	JEV prM/E	Development of PV
Ma et al. (2019)	VSV	FLuc	RVFV	Neutralisation test
Markosyan and Cohen (2013)	MLV	DiD/GFP	VSV G	Host-pathogen interaction
Negrete et al. (2007)	VSV	Luc	HeV F, G	Host-pathogen interaction
Pan et al. (2018)	HIV	Luc	ZIKV E	Antiviral screening
Perez et al. (2001)	VSV	GFP	BDV p56	Host-pathogen interaction
Porotto et al. (2007)	VSV	RFP	HeV F, G	Antiviral screening
Porotto et al. (2009)	VSV	RFP	HeV F, G	Antiviral screening
Porotto et al. (2011)	VSV	RFP	HeV F, G	Antiviral screening
Ruiz-Jimenez et al. (2021)	HIV MLV	Luc	JEV E	Development of PV
Scott et al. (2012)	HIV	Luc	IAV HA	Neutralisation test
Scott et al. (2016)	HIV	Luc	IAV HA	Neutralisation test
Shtanko et al. (2014)	VSV	FLuc	CCHFV Gn/ Gc	Host-pathogen interaction
Suzuki et al. (2020)	HIV	FLuc	CHIKV E3-E2-6 K-E1	Diagnosis
Tani et al. (2010)	VSV	Luc	JEV E	Host-pathogen interaction
Wright et al. (2009)	HIV	b-gal/GFP	Lyssa G	Neutralisation test
Zhang et al. (2022)	VSV	FLuc	RVFV	Neutralisation test

β-gal, β-galactosidase; BDV, Borna disease virus; CCHFV, Crimean Congo haemorrhagic virus fever glycoprotein; CHIKV, chikungunya virus; FLuc, firefly luciferase; GFP, green fluorescent protein; HIV, human immunodeficiency virus; IAV, influenza A virus; JEV, Japanese encephalitis virus; Lyssa, lyssaviruses;

MLV, murine leukaemia virus; MMLV, Moloney murine leukaemia virus; RFP, red fluorescent protein; RVFV, Rift Valley fever virus; VSV, vesicular stomatitis virus; ZIKV E, Zika virus envelope protein.

3.1. Viruses affecting horses and humans for which 'pseudo virus' articles identified.

Most of the viruses identified in our literature search are arboviruses, which tend to have a wide host range and tissue tropism because their infectious cycle requires infection of both insects and their non-insect hosts. Several arboviral diseases have been increasing in geographic range, with climate change implicated as one driver of disease emergence and spread (Whitehorn and Yacoub, 2019).

Six of the identified viruses are mosquito-borne arboviruses in the Alphavirus genes of the *Togaviridae*. As their name suggests, the equine encephalitides (Eastern (EEEV), Western (WEEV) and Venezuelan equine encephalitis virus (VEEV)) can infect the brain and cause neurological disease. Other viruses in the same genus (including chikungunya virus, CHIKV) are rarely reported to infect horses, with the notable exception of Ross River virus (RRV) in Australia (Yuen and Bielefeldt-Ohmann, 2021). In both humans and horses, RRV can cause chronic, long-term debilitating arthritogenic infection, similar to CHIKV. In their review, Yuen and Bielefeldt-Ohmann (2021) suggest that an equine model for RRV may provide better understanding of immunopathogenesis than the currently used murine model. They also recently reported that, as for WNV, horses could act as sentinels to provide early warning of potential outbreaks in human populations (Yuen et al., 2024). The epidemiology and ecology and common clinical signs of these viruses in horses (including Getah and Sindbis) are reviewed in Chapman et al. (2018) and from the human perspective, viral-host interactions and therapeutics development are reviewed in Guerrero-Arguero et al. (2021).

Both horses and humans are incidental hosts of members of the family *Flaviviridae* in the order *Amarillovirales* including Japanese encephalitis virus (JEV) and West Nile virus (WNV), which are transmitted by mosquitoes. One study found Zika virus (ZIKV) neutralising antibodies in 4.3% (7/163) of horses sampled 2015/2016 in New Caledonia and 15.4% (20/130) in French Polynesia (Beck et al., 2019) indicating exposure to ZIKV, although clinical disease has not been reported. Two tick-borne flaviviruses can infect horses and humans. Tick-borne encephalitis virus (TBEV) causes potentially fatal disease of the central nervous system in humans but while TBEV-specific neutralising antibodies have been detected in horses in more than one study (e.g. Rushton et al. (2013)), infection is apparently asymptomatic. Louping ill virus (LIV) on the other hand causes a febrile illness that can progress to fatal encephalitis in both humans and horses (reviewed in Jeffries et al. (2014)).

Two viruses in the order Bunyavirales, Rift Valley fever virus (RVFV) and Crimean-Congo haemorrhagic fever virus (CCHFV) were identified by Johnson et al. (2020) as affecting both horses and humans. Transmission of RVFV is by mosquitoes and of CCHFV is via tick bites, but both viruses can also be transmitted by close contact with the blood, secretions, organs or other bodily fluids of infected individuals. While CCHFV causes outbreaks of severe haemorrhagic disease in humans, with up to 30% mortality, it does not cause significant clinical disease in horses. However, high levels of seroprevalence have been detected and infected horses infected become viraemic, which means they could play an important role in the transmission of CCHFV (Fanelli and Buonavoglia, 2021). Although RVFV was identified by Johnson et al. (2020) as a virus that also infects horses and antibodies can be detected in horses where RVFV is endemic, seroprevalence is low (3–10%). Furthermore, experimental infection of Shetland ponies with a high dose of virus did not produce any clinical signs and there was only a low level of viraemia suggesting that horses are resistant to RVFV infection (Bird et al., 2009). Human infection with RVFV usually results in self-limiting febrile illness, but in 1–2% of cases can progress to more severe disease resulting in blindness or death (Bird et al., 2009).

Borna disease virus (BDV) is somewhat enigmatic. It was known for many years as a neurotropic virus that primarily infects horses and sheep, sporadically causing polioencephalomyelitis in horses in Austria, Germany and Switzerland (Richt et al., 2000). It is not clear why disease does not occur in other European countries, Australia, Iran, Israel, Japan and the United States where detection of antibodies suggests that the virus does circulate. The discovery in the 1980s that human patients with various psychiatric diseases had BDV-specific antibodies suggested that it can also infect and cause disease in people.

Hendra (HeV) was first identified during an outbreak involving racehorses and two human cases in Australia in 1994 (Halpin and Rota, 2015). The natural host was soon identified as fruit bats. It appears only horses are directly infected by bats and human infection occurs where there is close contact with infected horses; in other words, horses act as bridging hosts. Although relatively few people are reported to have been infected with HeV, over half of them died as a result, the first death being in someone who initially recovered but had a relapse and developed fatal encephalitis just over a year later (O'Sullivan et al., 1997). Although horses can survive infection, it is policy in Australia to euthanise infected horses to prevent relapsing infection and possible further transmission.

Rabies and VSV (now known as Indiana vesiculovirus) are in different genera (*Lyssavirus* and *Vesiculovirus*, respectively) of the *Alpharhabdovirinae* subfamily of the *Rhabdoviridae* family, which have distinctive bullet-like structures. The glycoprotein (G) proteins of both viruses can direct entry into a broad range of cells from different species, hence they have been widely used in the context of viral pseudotypes and the high number of papers identified for VSV and RABV in the literature search. As well as infecting and being transmitted by insects (including phlebotomine sand flies), VSV primarily infects cattle and horses, in which species it causes blister-like lesions (Munis et al., 2020). It is important in cattle because its clinical presentation can be confused with the more economically important foot-and-mouth disease. Humans can be infected by close contact with diseased animals, leading to flu-like illness. Rabies lyssavirus is a neurotropic virus that causes behavioural changes rabies in animals, including humans (Fooks et al., 2017). Rabies transmission can occur through the saliva of animals, often from a bite, and less commonly through contact with human saliva.

Influenza A viruses also have a wide host range (Krammer and Palese, 2020). Influenza A viruses are classified into subtypes based on the surface glycoproteins haemagglutinin (HA) and neuraminidase (NA). Two subtypes (H18N10 and H19N11) were recently discovered only in bats. Most subtypes of IAV (H1–H16 and N1–N9) are found in wild aquatic birds but some subtypes can be transmitted to mammalian species and have become established. In equids, IAV of the H7N7 subtype was first isolated in 1956, followed by isolation of the H3N8 subtype in 1963. The two subtypes co-circulated for some years, but the H7N7 subtype is now regarded as extinct. Different subtypes of IAV circulate in people, currently H1N1 and H3N2, with emergence of a novel subtype in the human population usually associated with a pandemic. Although clinical disease is similar in both species, primarily fever and cough, there is scant evidence for transmission between them. Interspecies transmission is limited due to differences in receptor specificity of the equine and human viruses (alpha 2,3 and alpha 2,6 linked sialic acid receptors, respectively) and distribution of those receptors on their respiratory tracts.

3.1. Viruses affecting horses and humans for which pseudo viruses not reported to be available

Viruses affecting both horses and humans for which no articles referencing the application of pseudo viruses were found were Barmah Forest, Bhanja, Bwamba, Cache Valley, Dhori, hepatitis E, Ilheus, Jamestown Canyon, Kairi, Kokobera, louping ill, Muray Valley encephalitis, rotavirus A, sandfly fever, St. Louis encephalitis, Usutu, and

Wesselsbron. Most of these are arboviruses with relatively limited geographic range and/or human health impact. Nonetheless, several are of concern as potential emerging viruses.

Barmah Forest virus, an alphavirus, is limited to Australia but, along with RRV, it is one of the most common mosquito-borne infections of people there, causing fever, rash and sore joints. A serosurvey identified higher seropositivity in horses (1.2%) than in people (0.9%) in south-west Australia (Johansen et al., 2005).

Cache Valley virus (CVV), Jamestown Canyon virus (JCV) and Kairi virus (KRIV) are mosquito-borne orthobunyaviruses found in the Americas. Cache Valley virus mainly infects sheep in North America, but horses and humans are frequently exposed (for example, seroprevalence of 69% was reported for horses in Saskatchewan, Canada (Uehlinger et al., 2018). Disease is usually mild, but CVV can cause life-threatening encephalitis. Kairi virus, which circulates in South America, has been isolated from a febrile horse (Calisher et al., 1988). Bwamba virus (BWAV) is also an orthobunyavirus present in large parts of Africa, but infections have only been detected in wild equine species not in the domesticated horse *Equus caballus* (Johnson et al., 2020). Similarly, Bhanja virus infection has only been detected in wild equids (*Equus africanus, E. ferus, E. quagga*).

Louping ill virus (LIV), a tick-borne flavivirus closely related to tick-borne encephalitis virus, again has a limited geographic range; historically, it was the only flavivirus endemic in the UK. Usutu is a mosquito-borne flavivirus in the Japanese encephalitis (JE) complex, which is an increasing cause for concern in Europe because of its similar ecology to related viruses such as WNV. St. Louis encephalitis virus (SLEV) is another member of the JE serocomplex transmitted between mosquitoes and birds and found in the Americas, mainly North America. Murray Valley encephalitis virus (MVEV) is also a mosquito-borne flavivirus but is limited in range to northern Australia and Papua New Guinea. Wesselsbron is another mosquito-borne flavivirus; it mainly affects sheep in sub-Saharan Africa. Kokobera virus is a mosquito-vectored virus found in Australia and Papua New Guinea. Finally, Ilheus virus was one of the earliest isolated flaviviruses. It causes mainly asymptomatic infections in humans with rare reports of encephalitis in South America (e.g. Milhim et al., 2020) and there is serological evidence of infection of horses (e.g. Pauvolid-Correa et al., 2014).

Dhori virus (DHOV) is in the same family as IAV but in the Thogoto virus genus. It has a very broad host range and is transmitted to mammalian hosts by ticks. Sandfly fever (also known as pappataci or phlebotomus fever) is caused by three serotypes (Naples virus, Sicilian virus and Toscana virus) of phlebovirus.

Hepatitis E virus, which was first identified in 1980, is a significant cause of acute viral hepatitis with an estimated 20 million human cases annually that is becoming increasingly recognised as a zoonotic infection in developed as well as developing countries (Iqbal et al., 2023). It is transmitted by the faecal–oral route and has a broad host range. Although pigs are regarded as a major source of human infection, there is evidence of spillover infection of horses with seroprevalence of 11–16% in apparently healthy horses (Yoon et al., 2022). Contact with horses has also been identified as a risk factor for human seropositivity (Christensen et al., 2008).

Finally, rotaviruses are a leading cause of diarrhoeal disease in young animals including children and foals (Crawford et al., 2017). There are ten species of rotavirus (A–J), with rotavirus species A being the most common cause of human infection. Rotavirus A was thought to be the only species to infect horses until a novel equine rotavirus B was identified in 2021 (Uprety et al., 2021). Not only do rotaviruses infect both horses and humans, there is also evidence of zoonotic transmission. For example, a new strain of rotavirus species A with genetic sequences suggesting an equine origin was identified in 2013 (Kirkwood and Roczo-Farkas, 2014) and became the predominant strain children in 2015–2016 (Utsumi et al., 2018). Rotaviruses are non-enveloped with three concentric capsids. The capsid proteins of non-enveloped viruses lack transmembrane domains required for incorporation into the membrane bilayers of VSV or lentivirus cores, which probably explains the lack of any report of successful PV production, if indeed it has been attempted.

4. Applications of pseudotyped viruses to the study of viruses infecting both humans and equids

In this review, we are focussing on articles describing the use of 'reporter pseudotyped viruses', defined as a non-replicative virus having the core of one virus with the surface proteins of another and encapsulating a reporter gene. Most of the 143 articles identified in the search describe studies using pseudo viruses as gene delivery tools, for example cancer gene therapy, and for vaccination as opposed to directly studying the viruses from which the envelope protein is obtained. In these contexts, a reporter gene is not necessarily required. Lentiviral vectors are considered promising vaccine vectors due to their ability to transduce dendritic cells and the foreign envelope can be changed to modify the pseudo virus tropism. For example, the VSV-G can be used to produce lentiviral vectored vaccines from stable packaging cells but is cytotoxic, due to its fusion activity (Hoffmann et al., 2010), therefore, Lopes et al. (2011) investigated using the envelope from Ross River virus.

As mentioned, most studies using PVs without reporter genes were using envelope proteins from various viruses to target gene therapy vectors according to their tropism. For example, neurotropism is conferred by CHIKV (Eleftheriadou et al., 2017), RABV (Federici et al., 2009; Mazarakis et al., 2001), RRV (Jakobsson et al., 2006), VEEV (Trabalza et al., 2013), VSV (VandenDriessche et al., 2002) or ZIKV (Kretschmer et al., 2020) envelope proteins; ocular targeting by VSV or VEEV (Lipinski et al., 2014) and haematopoietic cell targeting by Sindbis (Morizono et al., 2006), RRV and VSV (Kahl et al., 2005). Borna disease virus (BDV) has been explored as a potential virus vector for delivering genes to the central nervous system by inserting a foreign gene (GFP) into an intercistronic noncoding region between the phosphoprotein (P) and matrix (M) genes (Daito et al., 2011).

Interestingly in the context of this review, equine infectious anaemia virus (EIAV) has been explored as a core for generating PVs with VSV or rabies glycoproteins for gene therapy (Azzouz et al., 2004; Balaggan et al., 2006; Beutelspacher et al., 2005; Mazarakis et al., 2001; Mitrophanous et al., 1999; Teng et al., 2005; Wong et al., 2004). However, no articles describing the use of reporter PVs to study EIAV were identified.

4.1. Development of flavivirus reporter pseudotyped viruses

Efforts have been made to generate flavivirus PVs for many years, but there has been limited success at reproducibly making PVs based on a core virus from a different virus family such as the lentivirus core systems. In early attempts, TBEV pseudo viruses with TBEV envelope proteins were made by co-culture of TBEV with VSV (Dragúnová and Greśíková, 1986) or recombinant vaccinia viruses (Konishi and Mason, 1993).

Several papers describe the development and use of JEV PVs. Lee et al. (2009) described the development of a JEV PV using the packaging cell line TELCeB6, which expresses a non-infectious (Env^-) Moloney MLV (MMLV) gag-pol gene construct and a β-galactosidase gene with an MMLV-derived packaging signal (MFG-nlslacZ). After transduction of different cell types, individually transduced cells, which stained blue with X-Gal, were counted as an infectious unit (IFU). These were counted at higher dilutions of PV at which it is more likely that each blue-stained cell is infected by a single PV particle. They used expression plasmids expressing the prM and envelope (prM/E) or only envelope (E) of two JEV strains and showed that the IFUs obtained of all four PVs were comparable to the infectivity of wild-type JEV in different target cell lines. The highest IFU/ml obtained with the PVs were in Vero (4–5 x 10^4) and Crandall-Rees feline kidney (around 1×10^5) cells. In a later paper, Lee et al. (2014) showed that results obtained in neutralisation tests with the PV correlated well with plaque reduction neutralisation

tests performed with native virus. The same approach was used to perform serosurveys in subsequent articles (Kwak et al., 2020; Lee et al., 2020).

Tani et al. (2010) made recombinant VSV-JEV prM/E particles with no reporter gene by replacing the VSV-G gene with the JEV prM/E gene, but they also made reporter PVs by infecting cells transiently expressing JEV prM/E with rVSVΔG/luc-G as described in Fig. 1B. However, they only present the percentage residual infectivity, for example after neutralisation by an *anti*-E polyclonal antibody, and not the titrated luciferase activity of the PVs. The same approach was applied to study the role of the host factor cyclosporin A (CsA) in replication of JEV (Kambara et al., 2011).

Liu et al. (2017) compared using the pcDNA3.1 expression plasmid with JEV prM/E with the native signal peptide, which was predicted to be weak using SignalP, replaced with the signal peptide from VSV-G. They found that the VSV-G signal peptide significantly enhanced the production of PV particles. They used a dual reporter (GFP and luciferase) lentiviral vector (pCDH-CMV-Luc–copGFP with psPAX2.1), which enabled them to quantify 'transducing units (TU)' by determining the percentage of GFP-expressing cells for a specific dilution. When input virus was normalised according to quantification of reporter gene copies, the three PVs were equally able to transduce target cells. However, they noted that the TU (GFP readout) and RLU/ml (luciferase readout) of the JEV-E PVs were 100-fold lower than those of the VSV-G positive control and that more research is needed to improve the infectivity of lentiviral JEV-E PVs.

Pan et al. (2018) generated Zika virus PVs by co-transfecting pHIV-luciferase, pCMV-ΔR8.2 and Env-expressing plasmids. However, the luciferase activity of the ZIKV PVs was low (<300) compared to the VSV-G PV, which gave signals of around 10,000. For use in antiviral screening, A549 cells in a 96-well-plate were transduced with 200 μl of the PV and 10 μg/ml of polybrene. Polybrene (hexadimethrine bromide) is a cationic polymer that enhances HIV-1 infection by reducing the electrostatic repulsion between the virus particle and target cell surface, enhancing receptor-independent adsorption (Davis et al., 2002). The challenges of developing Zika virus PVs are described by Ruiz-Jiménez et al. (2021) who attempted to make PVs using different glycoprotein constructs, different lenti- or retro-viral cores (pNL4.3.Luc.R (−)E (−) or MLV), and different ratios of envelope to core plasmids.

Because of the difficulties of consistently generating reporter PVs based on lenti- or retro-viral cores, there has been greater focus on development of reporter virus particles (RVPs). For example, as part of the response to the 2015–2016 emergence of Zika in South America, Whitbeck et al. (2020) adapted their previously developed DENV RVP to generate 'pseudo-infectious' ZIKV RVPs. These RVPs are generated by transfecting a plasmid expressing the CprM/E structural genes into a stable cell line that expresses the full-length DENV2 replicon with the CprM/E genes replaced by a gene for Renilla luciferase (BHK-DRRZ).

4.2. Development of influenza A reporter pseudotyped viruses

For influenza A viruses, there was such a large volume of literature found in the initial search that a more focussed search was performed (Supplementary file 1); this yielded 17 primary research articles, 4 of which refer specifically to the generation of equine IAV PVs. These describe the initial generation of equine influenza PVs using an HIV core (Scott et al., 2012) and their optimisation (Scott et al., 2016). The successful generation of IAV PVs requires activation of the haemagglutinin (HA) surface glycoprotein by protease cleavage of HA0 into the HA1 and HA2 subunits and addition of neuraminidase (NA) to allow release of newly formed pseudotype particles from the producer cells. Both the protease and neuraminidase can be provided exogenously in the form of trypsin and bacterial neuraminidase, respectively or by transfection of additional plasmids during production of the pseudotyped virus. Scott et al. (2016) found that the efficiency of plasmids expressing different proteases varied with the HA protein with human airway trypsin (HAT) cleaving the HA0 more efficiently than TMPRSS2 for A/equine/Richmond/1/2007 (H3N8) whereas for the HA from an earlier isolate (A/equine/Newmarket/79), the proteases were equally efficient. The comparison with addition of exogenous trypsin to the cell culture medium was not made. However, they showed that addition of exogenous neuraminidase from *Clostridium perfringens* was more efficient than co-transfection of plasmid encoding the viral (N8) NA. Subsequently, (Kinsley et al., 2020) applied reporter PVs to measure neutralising antibodies in ponies experimentally vaccinated and infected with IAV. They reported good agreement between traditionally used antibody tests (the single radial haemolysis assay and haemagglutination inhibition test) but noted that the PVNT is more sensitive. They suggested that further studies should be conducted to determine the inter-laboratory reproducibility of the PVNT. Interestingly, in a comparison of two ELISAs, virus neutralisation test (VNT) and lentivirus-based PVNT to measure antibodies against SARS-CoV-2, James et al. (2021) concluded that there was strong correlation between the infectious virus and pseudotyped virus neutralisation tests and that there was good concordance between results obtained with the PVNT when the PVs were generated and tests performed independently in three different institutions.

4.3. Reporter pseudotyped viruses for immunoassays and vaccine development

Use of reporter pseudotyped viruses for neutralisation tests was the most frequent application identified in this review. Bukbuk et al. (2014) describe development of a RVFV glycoprotein PV based on the VSV system, although the details are limited. They showed a positive correlation (Spearman's rank correlation coefficient of 0.77) between the titres of neutralising antibodies obtained using the PV and those obtained using a conventional neutralisation test with the attenuated MP12 strain. Li et al. (2018) provide more detail about their approach to generate a RVFV PV using the third-generation lentiviral transfer plasmid pLV-eGFP-C and helper plasmid PH1, both from Invitrogen. Ma et al. (2019) also generated a RVFV VSV-PV, which they used to determine whether individual amino acid differences affect protein function. They found that introduction of 42 single amino acid substitutions had little impact on neutralisation by post-immunisation guinea pig sera. They also used bioluminescence imaging in mice 'infected' with the RVFV PV to study dissemination and distribution of the PV. They then vaccinated mice with a RVFV DNA vaccine and challenged them with the RVFV PV and assessed vaccine efficacy by the reduction of bioluminescence, which they could correlate with the neutralising antibody response to the vaccine. The RVFV PVNT established by Ma et al. (2019) was also used to measure the immune response of mice to an experimental vaccine by Zhang et al. (2022).

The most used reporter in PV production is luciferase, but the substrate is relatively expensive and requires a luminometer to obtain a readout. Green fluorescent protein provides an alternative reporter that does not need addition of a substrate but requires a fluorescent microscope for visualisation. Wright et al. (2009) cloned the lacZ gene, which encodes β-galactosidase (β-gal) into the reporter plasmid and to produce a rabies virus PV. Entry of the PV into target cells could be detected by the addition of different β-gal substrates (X-gal, CPRG or ONPG) that turn blue, red or yellow, respectively, in the presence of the enzyme and can be visualised under a light microscope. A further advantage of the lacZ gene-containing PVs was that they were highly stable despite freeze-thaw cycles and storage at room temperature. Finally, the lacZ gene was used as the reporter for JEV PVs in several reports (Kwak et al., 2020; Lee et al., 2009, 2014, 2020), which raises the possibility that this readout is advantageous with difficult to pseudotype envelope proteins. Low intrinsic stability is a desirable property in reporter genes used to monitor transcriptional dynamics and luciferase genes have been modified to reduce their half-life, but this is not necessarily optimal for applications using pseudotyped viruses. Mammalian codon-optimisation of the lacZ gene, which originates from *E. coli*, was

shown to enhance expression in mammalian cells due to enhanced transcript stability and increased translational efficiency (Anson et al., 2004).

Pseudotyped viruses can also be used as surrogates for dangerous pathogens in immunoassay development. For example, Suzuki et al. (2020) used a CHIKV PV to evaluate the sensitivity, specificity, and cross-reactivity of a rapid immunochromatographic test for detection of CHIKV E1-antigen using new monoclonal antibodies.

4.4. Reporter pseudotyped viruses for studying host–pathogen interactions

Reporter PVs can be used to study interactions between viruses and host cells in the early stages of infection. For example, Perez et al. (2001) used the VSV core system to shed more light on the role of BDV glycoprotein in receptor recognition and cell entry. The glycoprotein is cleaved by furin, generating *N*-terminal and *C*-terminal fragments of 41 kDa and 43 kDa (gp41 and gp43), respectively. The *C*-terminal gp43 had already been shown to be involved in viral entry. By generating chimeric proteins with gp41 and the transmembrane and carboxyl terminus of VSV G, they showed that gp41 could also independently mediate virus receptor recognition and cell entry.

The bunya viruses CCHFV and RVFV have three segments (small, medium and large) of single-stranded RNA genome in negative orientation. The medium segment, M, encodes the viral glycoprotein polyprotein, which is co-translationally cleaved and post-translationally modified to generate two structural transmembrane proteins, Gc and Gn. These form complexes on the virion surface, bind the cellular receptors and are responsible for membrane fusion (Schmaljohn and Nichol, 2007). CCHFV is known to enter host cells through clathrin-mediated endocytosis. Shtanko et al. (2014) generated a VSV PV to study uptake of CCHFV and identify host factors controlling the movement of CCHFV to the acidified compartment where membrane fusion takes place. They found that CCHFV passes through early endosomes before being delivered to multivesicular bodies (MVBs). They demonstrated that preventing vesicular acidification trapped pseudoparticles in the MVBs, suggesting that they are the sites of virus-endosome membrane fusion.

In order to study fusion mediated by class II and III viral proteins, Markosyan and Cohen (2013) generated PVs with Semliki Forest virus (SFV) E1/E2 proteins or VSV G with the fluorescent lipid dye DiD incorporated in the viral envelope and the core labelled by fusing GFP at the *C*-terminus of the MLV nucleocapsid protein. They could determine whether fusion occurred in an endosome or the plasma membrane by differences in the pattern of fluorescence. When fusion occurs within an endosome, the fluorescence from the DiD remains the same because endosomes are sub-microscopic, but the GFP fluorescence disperses due to the release of the viral core after fusion. On the other hand, if fusion occurs at the plasma membrane, neither fluorescence signal is detectable.

As previously mentioned, horses appear to be resistant to RVFV infection. Potential mechanisms involved in this resistance could be investigated using PVs. For example, Feeley et al. (2011) used PVs to demonstrate that IAV is inhibited by interferon inducible transmembrane protein 3 (IFITM3) in late endosomes. Similarly to IAV, RVFV entry involves virus–endosome membrane fusion, therefore a similar mechanism of resistance to RVFV infection in horses may be investigated using PVs.

4.5. Reporter pseudotyped viruses for antiviral screening

Hendra and the closely-related Nipah virus require the highest level of biosecurity, hence several papers report the use of PVs to study host-pathogen interactions (Bradel-Tretheway et al., 2019; Mathieu et al., 2015; Negrete et al., 2007) and for neutralisation tests (Bae et al., 2019; Kaku et al., 2009; Khetawat and Broder, 2010). Also notable is the number of publications describing the use of PVs for high throughput screening for antivirals targeting Hendra and Nipah G and F envelope proteins (Aljofan et al., 2009; Elshabrawy et al., 2014; Porotto et al., 2007, 2009, 2011).

5. Conclusions and future perspectives

A cross-species or comparative approach is particularly relevant for studying viral disease that naturally infect different species. Furthermore, the equine and human immune systems share more similarities than between the human immune system and common species of laboratory animals. In this review, we have highlighted several applications of PVs, including furthering our understanding of host–pathogen interactions by identification of proteins that mediate viral entry and the mechanisms of early stages of viral replication. Of comparative interest is understanding early restriction mechanisms in different species, such as interactions with IFITM proteins, which could potentially lead to identification of opportunities for prevention and treatment of disease. The ability to study highly hazardous viruses using PVs has made them a key tool in studying emerging viruses such as SARS-CoV-2. Development of neutralisation tests using PVs that can be used to monitor seroprevalence in equine populations can help provide early warning of expansion of zoonotic viruses into new geographic regions. Not only are PVs useful in vaccine development by enabling safe measurement of virus neutralising antibodies but they can also be used to challenge vaccinated animals to monitor vaccine efficacy as described by Ma et al. (2019). This could enable horses to be used as the most relevant animal model to evaluate vaccines against viruses such as Hendra, which would not be feasible using the infectious wild-type virus.

Challenges remain in the development of PVs. For example, it appears to be more difficult to make PVs with viruses that have class II fusion proteins. However, refinements continue to be made, with surprising recent success in generating PVs against equine herpesvirus-1 (EHV-1), a DNA virus (Di Genova et al., 2024), the entry of which is complex due to the presence of 12 glycoproteins. The EHV-1 PVs were successfully made with glycoproteins B, D, H and L.

CRediT authorship contribution statement

Rebecca L. Sedgwick: Writing – original draft, Formal analysis, Data curation. **Ola ElBohy:** Writing – review & editing. **Janet M. Daly:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

RLS was supported by the Academy of Medical Sciences INSPIRE scheme funded by the Wellcome Trust.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2024.110164.

References

ALJOFAN, M., SGANGA, M.L., LO, M.K., ROOTES, C.L., POROTTO, M., MEYER, A.G., SAUBERN, S., MOSCONA, A., MUNGALL, B.A., 2009. Antiviral activity of gliotoxin, gentian violet and brilliant green against Nipah and Hendra virus in vitro. Virol. J. 6, 187.

ANSON, D.S., LIMBERIS, M., 2004. An improved beta-galactosidase reporter gene. J. Biotechnol. 108, 17–30.

AZZOUZ, M., LE, T., RALPH, G.S., WALMSLEY, L., MONANI, U.R., LEE, D.C., WILKES, F., MITROPHANOUS, K.A., KINGSMAN, S.M., BURGHES, A.H., MAZARAKIS, N.D., 2004. Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy. J. Clin. Invest. 114, 1726–1731.

BAE, S.E., KIM, S.S., MOON, S.T., CHO, Y.D., LEE, H., LEE, J.Y., SHIN, H.Y., LEE, H.J., KIM, Y.B., 2019. Construction of the safe neutralizing assay system using pseudotyped Nipah virus and G protein-specific monoclonal antibody. Biochem. Biophys. Res. Commun. 513, 781–786.

BALAGGAN, K.S., BINLEY, K., ESAPA, M., IQBALL, S., ASKHAM, Z., KAN, O., TSCHERNUTTER, M., BAINBRIDGE, J.W., NAYLOR, S., ALI, R.R., 2006. Stable and efficient intraocular gene transfer using pseudotyped EIAV lentiviral vectors. J. Gene Med. 8, 275–285.

BARRETT, C.T., DUTCH, R.E., 2020. Viral membrane fusion and the transmembrane domain. Viruses 12.

BECK, C., LEPARC-GOFFART, I., DESOUTTER, D., DEBERGÉ, E., BICHET, H., LOWENSKI, S., DUMAREST, M., GONZALEZ, G., MIGNÉ, C., VANHOMWEGEN, J., ZIENTARA, S., DURAND, B., LECOLLINET, S., 2019. Serological evidence of infection with dengue and Zika viruses in horses on French Pacific Islands. PLoS Neglected Trop. Dis. 13, e0007162.

BEUTELSPACHER, S.C., ARDJOMAND, N., TAN, P.H., PATTON, G.S., LARKIN, D.F., GEORGE, A.J., MCCLURE, M.O., 2005. Comparison of HIV-1 and EIAV-based lentiviral vectors in corneal transduction. Exp. Eye Res. 80, 787–794.

BIRD, B.H., KSIAZEK, T.G., NICHOL, S.T., MACLACHLAN, N.J., 2009. Rift Valley fever virus. J. Am. Vet. Med. Assoc. 234, 883–893.

BRADEL-TRETHEWAY, B.G., ZAMORA, J.L.R., STONE, J.A., LIU, Q., LI, J., AGUILAR, H. C., 2019. Nipah and Hendra virus glycoproteins induce comparable homologous but distinct heterologous fusion phenotypes. J. Virol. 93.

BUKBUK, D.N., FUKUSHI, S., TANI, H., YOSHIKAWA, T., TANIGUCHI, S., IHA, K., FUKUMA, A., SHIMOJIMA, M., MORIKAWA, S., SAIJO, M., KASOLO, F., BABA, S.S., 2014. Development and validation of serological assays for viral hemorrhagic fevers and determination of the prevalence of Rift Valley fever in Borno State, Nigeria. Trans. R. Soc. Trop. Med. Hyg. 108, 768–773.

CALISHER, C.H., ORO, J.G., LORD, R.D., SABATTINI, M.S., KARABATSOS, N., 1988. Kairi virus identified from a febrile horse in Argentina. Am. J. Trop. Med. Hyg. 39, 519–521.

CHAPMAN, G.E., BAYLIS, M., ARCHER, D., DALY, J.M., 2018. The challenges posed by equine arboviruses. Equine Vet. J. 50, 436–445.

CHRISTENSEN, P.B., ENGLE, R.E., HJORT, C., HOMBURG, K.M., VACH, W., GEORGSEN, J., PURCELL, R.H., 2008. Time trend of the prevalence of hepatitis E antibodies among farmers and blood donors: a potential zoonosis in Denmark. Clin. Infect. Dis. 47, 1026–1031.

CRAWFORD, S.E., RAMANI, S., TATE, J.E., PARASHAR, U.D., SVENSSON, L., HAGBOM, M., FRANCO, M.A., GREENBERG, H.B., O'RYAN, M., KANG, G., DESSELBERGER, U., ESTES, M.K., 2017. Rotavirus infection. Nat. Rev. Dis. Prim. 3, 17083.

DAITO, T., FUJINO, K., HONDA, T., MATSUMOTO, Y., WATANABE, Y., TOMONAGA, K., 2011. A novel borna disease virus vector system that stably expresses foreign proteins from an intercistronic noncoding region. J. Virol. 85, 12170–12178.

DAVIS, H.E., MORGAN, J.R., YARMUSH, M.L., 2002. Polybrene increases retrovirus gene transfer efficiency by enhancing receptor-independent virus adsorption on target cell membranes. Biophys. Chem. 97, 159–172.

DI GENOVA, C., SUTTON, G., PAILLOT, R., TEMPERTON, N., PRONOST, S., SCOTT, S.D., 2024. Studying longitudinal neutralising antibody levels against Equid herpesvirus 1 in experimentally infected horses using a novel pseudotype based assay. Virus Res. 339, 199262.

DRAGŮNOVÁ, J., GREŠÍKOVÁ, M., 1986. Vesicular stomatitis virus pseudotype with neutralization antigen of tick-borne encephalitis virus. Acta Virol. 30, 346–348.

ELEFTHERIADOU, I., DIERINGER, M., POH, X.Y., SANCHEZ-GARRIDO, J., GAO, Y., SGOUROU, A., SIMMONS, L.E., MAZARAKIS, N.D., 2017. Selective transduction of astrocytic and neuronal CNS subpopulations by lentiviral vectors pseudotyped with Chikungunya virus envelope. Biomaterials 123, 1–14.

ELSHABRAWY, H.A., FAN, J., HADDAD, C.S., RATIA, K., BRODER, C.C., CAFFREY, M., PRABHAKAR, B.S., 2014. Identification of a broad-spectrum antiviral small molecule against severe acute respiratory syndrome coronavirus and Ebola, Hendra, and Nipah viruses by using a novel high-throughput screening assay. J. Virol. 88, 4353–4365.

FANELLI, A., BUONAVOGLIA, D., 2021. Risk of Crimean Congo haemorrhagic fever virus (CCHFV) introduction and spread in CCHF-free countries in southern and Western Europe: a semi-quantitative risk assessment. One Health 13, 100290.

FEDERICI, T., KUTNER, R., ZHANG, X.Y., KURODA, H., TORDO, N., BOULIS, N.M., REISER, J., 2009. Comparative analysis of HIV-1-based lentiviral vectors bearing lyssavirus glycoproteins for neuronal gene transfer. Genet. Vaccine Ther. 7, 1.

FEELEY, E.M., SIMS, J.S., JOHN, S.P., CHIN, C.R., PERTEL, T., CHEN, L.M., GAIHA, G.D., RYAN, B.J., DONIS, R.O., ELLEDGE, S.J., BRASS, A.L., 2011. IFITM3 inhibits influenza A virus infection by preventing cytosolic entry. PLoS Pathog. 7, e1002337.

FOOKS, A.R., CLIQUET, F., FINKE, S., FREULING, C., HEMACHUDHA, T., MANI, R.S., MULLER, T., NADIN-DAVIS, S., PICARD-MEYER, E., WILDE, H., BANYARD, A.C., 2017. Rabies. Nat. Rev. Dis. Prim. 3, 17091.

GOTHE, L.M.R., GANZENBERG, S., ZIEGLER, U., OBIEGALA, A., LOHMANN, K.L., SIEG, M., VAHLENKAMP, T.W., GROSCHUP, M.H., HÖRÜGEL, U., PFEFFER, M., 2023. Horses as sentinels for the circulation of flaviviruses in eastern–Central Germany. Viruses 15, 1108.

GUERRERO-ARGUERO, I., TELLEZ-FREITAS, C.M., WEBER, K.S., BERGES, B.K., ROBISON, R.A., PICKETT, B.E., 2021. Alphaviruses: host pathogenesis, immune response, and vaccine & treatment updates. J. Gen. Virol. 102.

HALPIN, K., ROTA, P., 2015. A review of Hendra virus and Nipah virus infections in man and other animals. In: SING, A. (Ed.), Zoonoses - Infections Affecting Humans and Animals: Focus on Public Health Aspects. Dordrecht: Springer Netherlands.

HE, J., CHOE, S., WALKER, R., DI MARZIO, P., MORGAN, D.O., LANDAU, N.R., 1995. Human immunodeficiency virus type 1 viral protein R (Vpr) arrests cells in the G2 phase of the cell cycle by inhibiting p34cdc2 activity. J. Virol. 69, 6705–6711.

HOFFMANN, M., WU, Y.J., GERBER, M., BERGER-RENTSCH, M., HEIMRICH, B., SCHWEMMLE, M., ZIMMER, G., 2010. Fusion-active glycoprotein G mediates the cytotoxicity of vesicular stomatitis virus M mutants lacking host shut-off activity. J. Gen. Virol. 91, 2782–2793.

HOROHOV, D.W., 2015. The equine immune responses to infectious and allergic disease: a model for humans? Mol. Immunol. 66, 89–96.

IQBAL, H., MEHMOOD, B.F., SOHAL, A., ROYTMAN, M., 2023. Hepatitis E infection: a review. World J. Virol. 12, 262–271.

JAKOBSSON, J., NIELSEN, T.T., STAFLIN, K., GEORGIEVSKA, B., LUNDBERG, C., 2006. Efficient transduction of neurons using Ross River glycoprotein-pseudotyped lentiviral vectors. Gene Ther. 13, 966–973.

JAMES, J., RHODES, S., ROSS, C.S., SKINNER, P., SMITH, S.P., SHIPLEY, R., WARREN, C.J., GOHARRIZ, H., MCELHINNEY, L.M., TEMPERTON, N., WRIGHT, E., FOOKS, A.R., CLARK, T.W., BROOKES, S.M., BROWN, I.H., BANYARD, A.C., 2021. Comparison of serological assays for the detection of SARS-CoV-2 antibodies. Viruses 13.

JEFFRIES, C.L., MANSFIELD, K.L., PHIPPS, L.P., WAKELEY, P.R., MEARNS, R., SCHOCK, A., BELL, S., BREED, A.C., FOOKS, A.R., JOHNSON, N., 2014. Louping ill virus: an endemic tick-borne disease of Great Britain. J. Gen. Virol. 95, 1005–1014.

JOHANSEN, C.A., MACKENZIE, J.S., SMITH, D.W., LINDSAY, M.D.A., 2005. Prevalence of neutralising antibodies to Barmah Forest, Sindbis and Trubanaman viruses in animals and humans in the south-west of Western Australia. Aust. J. Zool. 53, 51–58.

JOHNSON, C.K., HITCHENS, P.L., PANDIT, P.S., RUSHMORE, J., EVANS, T.S., YOUNG, C.C.W., DOYLE, M.M., 2020. Global shifts in mammalian population trends reveal key predictors of virus spillover risk. Proc. Biol. Sci. 287, 20192736.

KAHL, C.A., POLLOK, K., HANELINE, L.S., CORNETTA, K., 2005. Lentiviral vectors pseudotyped with glycoproteins from Ross River and vesicular stomatitis viruses: variable transduction related to cell type and culture conditions. Mol. Ther. 11, 470–482.

KAKU, Y., NOGUCHI, A., MARSH, G.A., MCEACHERN, J.A., OKUTANI, A., HOTTA, K., BAZARTSEREN, B., FUKUSHI, S., BRODER, C.C., YAMADA, A., INOUE, S., WANG, L. F., 2009. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J. Virol Methods 160, 7–13.

KAMBARA, H., TANI, H., MORI, Y., ABE, T., KATOH, H., FUKUHARA, T., TAGUWA, S., MORIISHI, K., MATSUURA, Y., 2011. Involvement of cyclophilin B in the replication of Japanese encephalitis virus. Virology 412, 211–219.

KHETAWAT, D., BRODER, C.C., 2010. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. Virol. J. 7, 312.

KINSLEY, R., PRONOST, S., DE BOCK, M., TEMPERTON, N., DALY, J.M., PAILLOT, R., SCOTT, S., 2020. Evaluation of a pseudotyped virus neutralisation test for the measurement of equine influenza virus-neutralising antibody responses induced by vaccination and infection. Vaccines (Basel) 8.

KIRKWOOD, C.D., ROCZO-FARKAS, S., 2014. Australian rotavirus surveillance program annual report, 2013. Commun Dis Intell Q Rep 38, E334–E342.

KONISHI, E., MASON, P.W., 1993. Proper maturation of the Japanese encephalitis virus envelope glycoprotein requires cosynthesis with the premembrane protein. J. Virol. 67, 1672–1675.

KRAMMER, F., PALESE, P., 2020. Orthomyxoviruses. In: HOWLEY, P.M., KNIPE, D.M., WHELAN, S. (Eds.), Fields Virology: Emerging Viruses, 7 ed. Lippincott Williams & Wilkins (LWW).

KRETSCHMER, M., KADLUBOWSKA, P., HOFFMANN, D., SCHWALBE, B., AUERSWALD, H., SCHREIBER, M., 2020. Zikavirus prME envelope pseudotyped human immunodeficiency virus type-1 as a novel tool for glioblastoma-directed virotherapy. Cancers 12.

KWAK, B.O., KWON, Y.S., HONG, Y.J., NAHM, C.H., JANG, W., UH, Y., CHO, Y.G., KIM, J., KIM, M., KIM, D.H., 2020. Seroprevalence of neutralizing antibodies against Japanese encephalitis virus among adolescents and adults in korea: a prospective multicenter study. Vaccines (Basel) 8.

LEE, H.J., CHOI, H., PARK, K.H., JANG, Y., HONG, Y.J., KIM, Y.B., 2020. Retention of neutralizing antibodies to Japanese encephalitis vaccine in age groups above fifteen years in Korea. Int. J. Infect. Dis. 100, 53–58.

LEE, H.J., MIN, K.I., LEE, J., KANG, S.H., JEON, W., NAM, J.H., JU, Y.R., KIM, Y.B., 2009. The prM-independent packaging of pseudotyped Japanese encephalitis virus. Virol. J. 6, 115.

LEE, H.J., MIN, K.I., PARK, K.H., CHOI, H.J., KIM, M.K., AHN, C.Y., HONG, Y.J., KIM, Y. B., 2014. Comparison of JEV neutralization assay using pseudotyped JEV with the conventional plaque-reduction neutralization test. J. Microbiol. 52, 435–440.

LI, Y., ZHAO, Y., WANG, C., ZHENG, X., WANG, H., GAI, W., JIN, H., YAN, F., QIU, B., GAO, Y., LI, N., YANG, S., XIA, X., 2018. Packaging of Rift Valley fever virus pseudoviruses and establishment of a neutralization assay method. J. Vet. Sci. 19, 200–206.

LIPINSKI, D.M., BARNARD, A.R., CHARBEL ISSA, P., SINGH, M.S., DE SILVA, S.R., TRABALZA, A., ELEFTHERIADOU, I., ELLISON, S.M., MAZARAKIS, N.D., MACLAREN, R.E., 2014. Vesicular stomatitis virus glycoprotein- and Venezuelan equine encephalitis virus-derived glycoprotein-pseudotyped lentivirus vectors differentially transduce corneal endothelium, trabecular meshwork, and human photoreceptors. Hum. Gene Ther. 25, 50–62.

LIU, H., WU, R., YUAN, L., TIAN, G., HUANG, X., WEN, Y., MA, X., HUANG, Y., YAN, Q., ZHAO, Q., CAO, S., WEN, X., 2017. Introducing a cleavable signal peptide enhances

the packaging efficiency of lentiviral vectors pseudotyped with Japanese encephalitis virus envelope proteins. Virus Res. 229, 9–16.
LOPES, L., DEWANNIEUX, M., TAKEUCHI, Y., COLLINS, M.K., 2011. A lentiviral vector pseudotype suitable for vaccine development. J. Gene Med. 13, 181–187.
MA, J., CHEN, R., HUANG, W., NIE, J., LIU, Q., WANG, Y., YANG, X., 2019. In vitro and in vivo efficacy of a Rift Valley fever virus vaccine based on pseudovirus. Hum. Vaccines Immunother. 15, 2286–2294.
MARKOSYAN, R.M., COHEN, F.S., 2013. The transmembrane domain and acidic lipid flip-flop regulates voltage-dependent fusion mediated by class II and III viral proteins. PLoS One 8, e76174.
MATHIEU, C., DHONDT, K.P., CHÂLONS, M., MÉLY, S., RAOUL, H., NEGRE, D., COSSET, F.L., GERLIER, D., VIVÈS, R.R., HORVAT, B., 2015. Heparan sulfate-dependent enhancement of henipavirus infection. mBio 6, e02427.
MAZARAKIS, N.D., AZZOUZ, M., ROHLL, J.B., ELLARD, F.M., WILKES, F.J., OLSEN, A.L., CARTER, E.E., BARBER, R.D., BABAN, D.F., KINGSMAN, S.M., KINGSMAN, A.J., O'MALLEY, K., MITROPHANOUS, K.A., 2001. Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. Hum. Mol. Genet. 10, 2109–2121.
MILHIM, B., ESTOFOLETE, C.F., ROCHA, L.C.D., LISO, E., BRIENZE, V.M.S., VASILAKIS, N., TERZIAN, A.C.B., NOGUEIRA, M.L., 2020. Fatal outcome of Ilheus virus in the cerebrospinal fluid of a patient diagnosed with encephalitis. Viruses 12.
MITROPHANOUS, K., YOON, S., ROHLL, J., PATIL, D., WILKES, F., KIM, V., KINGSMAN, S., KINGSMAN, A., MAZARAKIS, N., 1999. Stable gene transfer to the nervous system using a non-primate lentiviral vector. Gene Ther. 6, 1808–1818.
MORIZONO, K., RINGPIS, G.E., PARIENTE, N., XIE, Y., CHEN, I.S., 2006. Transient low pH treatment enhances infection of lentiviral vector pseudotypes with a targeting Sindbis envelope. Virology 355, 71–81.
MUNIS, A.M., BENTLEY, E.M., TAKEUCHI, Y., 2020. A tool with many applications: vesicular stomatitis virus in research and medicine. Expet Opin. Biol. Ther. 20, 1187–1201.
NEGRETE, O.A., CHU, D., AGUILAR, H.C., LEE, B., 2007. Single amino acid changes in the Nipah and Hendra virus attachment glycoproteins distinguish ephrinB2 from ephrinB3 usage. J. Virol. 81, 10804–10814.
O'SULLIVAN, J.D., ALLWORTH, A.M., PATERSON, D.L., SNOW, T.M., BOOTS, R., GLEESON, L.J., GOULD, A.R., HYATT, A.D., BRADFIELD, J., 1997. Fatal encephalitis due to novel paramyxovirus transmitted from horses. Lancet 349, 93–95.
PAN, T., PENG, Z., TAN, L., ZOU, F., ZHOU, N., LIU, B., LIANG, L., CHEN, C., LIU, J., WU, L., LIU, G., PENG, Z., LIU, W., MA, X., ZHANG, J., ZHU, X., LIU, T., LI, M., HUANG, X., TAO, L., ZHANG, Y., ZHANG, H., 2018. Nonsteroidal anti-inflammatory drugs potently inhibit the replication of Zika viruses by inducing the degradation of AXL. J. Virol. 92.
PAUVOLID-CORREA, A., CAMPOS, Z., JULIANO, R., VELEZ, J., NOGUEIRA, R.M., KOMAR, N., 2014. Serological evidence of widespread circulation of West Nile virus and other flaviviruses in equines of the Pantanal, Brazil. PLoS Neglected Trop. Dis. 8, e2706.
PEREZ, M., WATANABE, M., WHITT, M.A., DE LA TORRE, J.C., 2001. N-terminal domain of Borna disease virus G (p56) protein is sufficient for virus receptor recognition and cell entry. J. Virol. 75, 7078–7085.
POROTTO, M., CARTA, P., DENG, Y., KELLOGG, G.E., WHITT, M., LU, M., MUNGALL, B. A., MOSCONA, A., 2007. Molecular determinants of antiviral potency of paramyxovirus entry inhibitors. J. Virol. 81, 10567–10574.
POROTTO, M., OREFICE, G., YOKOYAMA, C.C., MUNGALL, B.A., REALUBIT, R., SGANGA, M.L., ALJOFAN, M., WHITT, M., GLICKMAN, F., MOSCONA, A., 2009. Simulating henipavirus multicycle replication in a screening assay leads to identification of a promising candidate for therapy. J. Virol. 83, 5148–5155.
POROTTO, M., YI, F., MOSCONA, A., LAVAN, D.A., 2011. Synthetic protocells interact with viral nanomachinery and inactivate pathogenic human virus. PLoS One 6, e16874.
RICHT, J.A., GRABNER, A., HERZOG, S., 2000. Borna disease in horses. Vet. Clin. N. Am. Equine Pract. 16, 579–595.
RUIZ-JIMÉNEZ, F., PÉREZ-OLAIS, J.H., RAYMOND, C., KING, B.J., MCCLURE, C.P., URBANOWICZ, R.A., BALL, J.K., 2021. Challenges on the development of a pseudotyping assay for Zika glycoproteins. J. Med. Microbiol. 70.
RUSHTON, J.O., LECOLLINET, S., HUBÁLEK, Z., SVOBODOVÁ, P., LUSSY, H., NOWOTNY, N., 2013. Tick-borne encephalitis virus in horses, Austria, 2011. Emerg. Infect. Dis. 19, 635–637.
SCHMALJOHN, C.S., NICHOL, S.T., 2007. Bunyaviridae. In: KNIPE, D.M., HOWLEY, P.M. (Eds.), Fields Virology. Lippincott Williams & Wilkins, Philadelphia.
SCOTT, S., MOLESTI, E., TEMPERTON, N., FERRARA, F., BÖTTCHER-FRIEBERTSHÄUSER, E., DALY, J., 2012. The use of equine influenza pseudotypes for serological screening. J. Mol. Genet. Med. 6, 304–308.
SCOTT, S.D., KINSLEY, R., TEMPERTON, N., DALY, J.M., 2016. The optimisation of pseudotyped viruses for the characterisation of immune responses to equine influenza virus. Pathogens 5.
SHTANKO, O., NIKITINA, R.A., ALTUNTAS, C.Z., CHEPURNOV, A.A., DAVEY, R.A., 2014. Crimean-Congo hemorrhagic fever virus entry into host cells occurs through the multivesicular body and requires ESCRT regulators. PLoS Pathog. 10, e1004390.
SUZUKI, K., HUITS, R., PHADUNGSOMBAT, J., TUEKPRAKHON, A., NAKAYAMA, E.E., VAN DEN BERG, R., BARBÉ, B., CNOPS, L., RAHIM, R., HASAN, A., IWAMOTO, H., LEAUNGWUTIWONG, P., VAN ESBROECK, M., RAHMAN, M., SHIODA, T., 2020. Promising application of monoclonal antibody against chikungunya virus E1-antigen across genotypes in immunochromatographic rapid diagnostic tests. Virol. J. 17, 90.
TANI, H., SHIOKAWA, M., KANAME, Y., KAMBARA, H., MORI, Y., ABE, T., MORIISHI, K., MATSUURA, Y., 2010. Involvement of ceramide in the propagation of Japanese encephalitis virus. J. Virol. 84, 2798–2807.
TENG, Q., GARRITY-MOSES, M., FEDERICI, T., TANASE, D., LIU, J.K., MAZARAKIS, N. D., AZZOUZ, M., WALMSLEY, L.E., CARLTON, E., BOULIS, N.M., 2005. Trophic activity of Rabies G protein-pseudotyped equine infectious anemia viral vector mediated IGF-I motor neuron gene transfer in vitro. Neurobiol. Dis. 20, 694–700.
TRABALZA, A., GEORGIADIS, C., ELEFTHERIADOU, I., HISLOP, J.N., ELLISON, S.M., KARAVASSILIS, M.E., MAZARAKIS, N.D., 2013. Venezuelan equine encephalitis virus glycoprotein pseudotyping confers neurotropism to lentiviral vectors. Gene Ther. 20, 723–732.
UEHLINGER, F.D., WILKINS, W., GODSON, D.L., DREBOT, M.A., 2018. Seroprevalence of Cache Valley virus and related viruses in sheep and other livestock from Saskatchewan, Canada. Can. Vet. J. 59, 413–418.
UPRETY, T., SREENIVASAN, C.C., HAUSE, B.M., LI, G., ODEMUYIWA, S.O., LOCKE, S., MORGAN, J., ZENG, L., GILSENAN, W.F., SLOVIS, N., METCALFE, L., CARTER, C.N., TIMONEY, P., HOROHOV, D., WANG, D., EROL, E., ADAM, E., LI, F., 2021. Identification of a ruminant origin group B rotavirus associated with diarrhea outbreaks in foals. Viruses 13.
UTSUMI, T., WAHYUNI, R.M., DOAN, Y.H., DINANA, Z., SOEGIJANTO, S., FUJII, Y., JUNIASTUTI, YAMANI, L.N., MATSUI, C., DENG, L., ABE, T., SOETJIPTO, LUSIDA, M.I., ISHII, K., SHIMIZU, H., KATAYAMA, K., SHOJI, I., 2018. Equine-like G3 rotavirus strains as predominant strains among children in Indonesia in 2015-2016. Infect. Genet. Evol. 61, 224–228.
VANDENDRIESSCHE, T., NALDINI, L., COLLEN, D., CHUAH, M.K., 2002. Oncoretroviral and lentiviral vector-mediated gene therapy. Methods Enzymol. 346, 573–589.
WALKER, P.J., SIDDELL, S.G., LEFKOWITZ, E.J., MUSHEGIAN, A.R., ADRIAENSSENS, E. M., ALFENAS-ZERBINI, P., DEMPSEY, D.M., DUTILH, B.E., GARCIA, M.L., CURTIS HENDRICKSON, R., JUNGLEN, S., KRUPOVIC, M., KUHN, J.H., LAMBERT, A.J., LOBOCKA, M., OKSANEN, H.M., ORTON, R.J., ROBERTSON, D.L., RUBINO, L., SABANADZOVIC, S., SIMMONDS, P., SMITH, D.B., SUZUKI, N., VAN DOORSLAER, K., VANDAMME, A.M., VARSANI, A., ZERBINI, F.M., 2022. Recent changes to virus taxonomy ratified by the international committee on taxonomy of viruses (2022). Arch. Virol. 167, 2429–2440.
WANG, Y., ZHOU, Z., WU, X., LI, T., WU, J., CAI, M., NIE, J., WANG, W., CUI, Z., 2023. Pseudotyped viruses. In: WANG, Y. (Ed.), Pseudotyped Viruses. Springer Nature Singapore, Singapore.
WHITBECK, J.C., THOMAS, A., KADASH-EDMONDSON, K., GRINYO-ESCUER, A., STAFFORD, L.J., CHENG, C., LIAO, G.C., HOLTSBERG, F.W., AMAN, M.J., SIMMONS, G., DAVIDSON, E., DORANZ, B.J., 2020. Antigenicity, stability, and reproducibility of Zika reporter virus particles for long-term applications. PLoS Neglected Trop. Dis. 14, e0008730.
WHITEHORN, J., YACOUB, S., 2019. Global warming and arboviral infections. Clin. Med. 19, 149–152.
WHITT, M.A., 2010. Generation of VSV pseudotypes using recombinant ΔG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. J. Virol Methods 169, 365–374.
WONG, L.F., AZZOUZ, M., WALMSLEY, L.E., ASKHAM, Z., WILKES, F.J., MITROPHANOUS, K.A., KINGSMAN, S.M., MAZARAKIS, N.D., 2004. Transduction patterns of pseudotyped lentiviral vectors in the nervous system. Mol. Ther. 9, 101–111.
WRIGHT, E., MCNABB, S., GODDARD, T., HORTON, D.L., LEMBO, T., NEL, L.H., WEISS, R.A., CLEAVELAND, S., FOOKS, A.R., 2009. A robust lentiviral pseudotype neutralisation assay for in-field serosurveillance of rabies and lyssaviruses in Africa. Vaccine 27, 7178–7186.
YOON, J., PARK, T., SOHN, Y., LEE, S.-K., PARK, B.-J., AHN, H.-S., GO, H.-J., KIM, D.-H., LEE, J.-B., PARK, S.-Y., SONG, C.-S., LEE, S.-W., CHOI, I.-S., 2022. Surveillance of hepatitis E virus in the horse population of Korea: a serological and molecular approach. Infect. Genet. Evol. 103, 105317.
YUEN, K.Y., BIELEFELDT-OHMANN, H., 2021. Ross River virus infection: a cross-disciplinary review with a veterinary perspective. Pathogens 10.
YUEN, N.K.Y., BIELEFELDT-OHMANN, H., COYLE, M.P., HENNING, J., 2024. Exposure dynamics of Ross River virus in horses - horses as potential sentinels (a One Health approach). Epidemiol. Infect. 152, e67.
ZHANG, S., YAN, F., LIU, D., LI, E., FENG, N., XU, S., WANG, H., GAO, Y., YANG, S., ZHAO, Y., XIA, X., 2022. Bacterium-like particles displaying the Rift Valley fever virus Gn head protein induces efficacious immune responses in immunized mice. Front. Microbiol. 13, 799942.