

The challenges posed by equine arboviruses

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Summary

Equine populations worldwide are at increasing risk of infection by viruses transmitted by biting arthropods including mosquitoes, biting midges (*Culicoides*), sandflies and ticks. These include the flaviviruses (Japanese encephalitis, West Nile and Murray Valley encephalitis), alphaviruses (eastern, western and Venezuelan encephalitis) and the orbiviruses (African horse sickness and equine encephalosis). This review provides an overview of the challenges faced in the surveillance, prevention and control of the major equine arboviruses, particularly in the context of these viruses emerging in new regions of the world.

Introduction

The rate of emergence of infectious diseases, in particular vector-borne viral diseases such as dengue, chikungunya, Zika, Rift Valley fever, West Nile, Schmallenberg and bluetongue, is increasing globally in human and animal species for a variety of reasons [1]. These include increased movement of animals and people worldwide, environmental and climate change, and human encroachment into natural habitats (taking domestic species with them). Equine arboviruses are no exception to this trend, and a number of authors have highlighted the potential for the introduction of various equine arboviruses to Europe [2-4].

Arboviruses are defined as viruses transmitted by biting arthropods, which include mosquitoes, biting midges (*Culicoides*), sandflies and ticks. Arboviruses replicate in the body of the insect and are, therefore, distinct from viruses with a mechanical mode of transmission (e.g. equine infectious anaemia virus is transmitted on or in the mouthparts of insects without replication). The equine arboviruses discussed in this review are listed, with their abbreviations, in Table 1. Although there are similarities in the transmission cycles of some of these viruses, the details are virus-specific and some are particularly complex. Similarities in clinical presentation and cross-reactivity of antibodies between related viruses can lead to initial misdiagnosis and delayed identification of an emerging arbovirus. For example, the first human cases of WNV infection in the USA were initially thought to be

caused by SLEV infection [5]. In this review, the major challenges presented by equine arboviruses in relation to vector roles, surveillance, control and risk management are summarised.

Equine Arboviruses

Alphavirus and Flavivirus Disease

The main flavi- and alpha-viruses that affect horses share some similarities: they are transmitted by mosquitoes, and viruses of both genera cause encephalitic disease in both horses and humans. Major flaviviruses known to affect equids include Japanese encephalitis virus (JEV), WNV and Murray Valley encephalitis virus (MVEV) (Table 1). Major alphaviruses of horses include eastern, western and Venezuelan equine encephalitis viruses (EEEV, WEEV and VEEV, respectively), Ross River virus (RRV) and Getah virus (GETV).

Equine morbidity and mortality information for mosquito-borne viruses affecting horses is presented in Chapman et al. [6]. Inapparent infections with limited clinical signs (e.g. transient pyrexia) are common with the encephalitogenic viruses, as a result of which, the proportion of deaths per diagnosed cases (case-fatality rate) can be very high. For example, the average fatality rate reported for cases of West Nile encephalitis in the United States between 1999 and 2006 was 30–40% [7]. However, retrospective estimates suggest that less than 10% of infected horses develop encephalitis. This figure was confirmed in a prospective study involving 37 unvaccinated horses in which only 2 of 25 animals (8%) that seroconverted developed encephalopathy, which was fatal in one case [8]. Although around 80% of surviving horses recover in 3–4 weeks, a small proportion have residual neurological deficits [9]. In addition to inapparent infections, strain-dependent differences in virulence can lead to wide variation in morbidity and mortality or case fatality estimates. However, in contrast to WNV, JEV, EEEV and VEEV, morbidity rates of MVEV and RRV are low and they rarely cause fatal disease, and GETV is often subclinical with affected horses usually recovering completely [10].

Clinical signs in horses that are infected with the encephalitogenic viruses (WNV, JEV, MVEV, EEEV, WEEV, and VEEV) include a variety of neurological abnormalities. However, there is a significant degree of overlap between clinical presentation of these diseases (clinical signs of ataxia and paresis are common to all; Table 2), and it is this that can present a challenge in terms of clinical diagnosis, particularly where there is overlapping distribution.

Flavivirus epidemiology and ecology

The flaviviruses that affect horses share characteristics in their transmission cycles. In general, these viruses are maintained in an enzootic cycle (i.e. they are transmitted between wild animals, usually birds) and horses (and humans) are infected as “incidental” or “dead-end” hosts (Figure 1). Dead-end hosts usually do not produce sufficiently high viraemia to infect mosquitoes, and therefore are not involved in ongoing transmission. Although the reservoir hosts are avian, large outbreaks of JEV may be associated with efficient amplification of virus in pigs, which also produce high levels of viraemia [11].

West Nile virus has the most widespread geographical distribution (Figure 2a) and the largest known vector and host range of all mosquito-borne flaviviruses [12]. Its ability to occupy a wide geographical area is due to its broad host and vector range. In contrast, JEV and MVEV have more restricted ranges (Figure 2b, 2c). However, it is not clear whether host or vector range may be more important in restricting their distribution.

Alphavirus epidemiology and ecology

Although like the flaviviruses, alphaviruses are transmitted by mosquitoes, their life cycles tend to be more complex. The eastern, western and Venezuelan equine encephalitic viruses (EEVs) are all restricted to the Americas. Eastern EEV affects horses, swine and humans as dead-end hosts [13]. It was traditionally thought to be maintained in an enzootic cycle between passerine birds and mosquitoes. However rodents are now thought to be epidemiologically significant hosts in South

America, and possibly in Florida [14; 15]. The ecology of South American EEEV is poorly understood [16]. Western EEV is maintained in an enzootic cycle between mosquitoes and birds, from Canada to Argentina [3], with horses and humans affected as dead-end hosts [17; 18]. Venezuelan EEV circulates in enzootic cycles between rodent hosts and mosquito vectors in Mexico, Central and South America [19]. The virus is antigenically complex with six antigenic subtypes within which there are antigenic variants. The E2 envelope glycoprotein determines equine viraemia and virulence, and mutations in the E2 gene can cause avirulent strains to be more efficiently amplified in horses. This results in an epizootic cycle during which virus amplification in the horse is sufficient to result in mosquito infection (i.e. the horse is no longer a dead-end host) and this is thought to significantly increase the risk of human infection [20]. Epizootics of VEEV have generally occurred in South America, although an epizootic occurred in Texas in 1971, affecting an estimated 10% of the equine population in the region and 1,500 equids died [21; 22]. Humans are typically considered dead-end hosts, but there is some recent evidence to suggest that humans may develop high enough virus titre to continue epidemic transmission in urban environments [23].

Ross River virus is active each year in most regions of Australia and has also caused epidemics in Papua New Guinea, the Solomon Islands, Fiji, New Caledonia and the Cook Islands. Epidemic polyarthrititis due to RRV infection is the most common arboviral disease in humans in Australia [24]. The major enzootic cycle of RRV involves members of the macropod (i.e. kangaroo) family as the vertebrate host, although other mammals have been suggested as reservoir hosts [25]. There is evidence that both horses and humans are able to infect vectors and at least one outbreak thought to be due to human air travel has occurred [26-29].

Getah virus is found from Eurasia to Australasia (Figure 3e). The natural transmission cycle is not well described or studied, although swine are thought to play an important role in amplification [30]. Getah virus appears to have a wide host range, although the main enzootic cycle is thought to be between mammals (particularly rodents) and mosquitoes, as birds show lower seroprevalence rates [10; 30].

Horses produce a high enough viraemic titre during an epidemic to infect mosquitoes and direct horse-to-horse transmission has been demonstrated experimentally, although it is unlikely to be a common mechanism for natural infection [31].

Orbiviruses

African horse sickness virus

African horse sickness (AHS) is a disease almost exclusively of equids. It is endemic in sub-Saharan Africa, but outbreaks have occurred elsewhere including the Middle East and India, and the western Mediterranean (reviewed in [32]).

The virus infects capillary endothelial cells in the myocardium, lung, spleen and liver. A mild form (horse sickness fever) involves oedema of the supraorbital fossae and fever, and occurs in donkeys and zebra and horses that have some immunity or are infected with a less virulent strain [33]. The pulmonary form causes rapid respiratory failure and is associated with case fatality rates that can exceed 95% [34]. The cardiac form causes oedema of the head and neck, progressing to dyspnoea, cyanosis, abdominal pain and heart failure and case fatality rates are around 50%. A mixed form of AHS is common with a mortality rate of around 70% [33].

Transmission occurs via the bite of infected midges of the genus *Culicoides*. All recorded outbreaks of AHS have occurred within the geographic range of the primary vector, *Culicoides imicola*, although in recent years a second vector, *C. bolitinos*, has been identified in South Africa [35]. The transmission cycle of AHSV involves only equids as hosts; other infected mammals (dogs) appear to be dead-end hosts [36; 37]. Species that produce a prolonged viraemia and have low disease mortality, such as zebra and donkeys, act as reservoir hosts [38]. As there is no carrier state, persistence of AHSV is believed to rely on sufficient density and renewal of susceptible reservoir hosts [32; 39].

Equine encephalosis virus

Equine encephalosis virus (EEV) usually causes subclinical or mild disease in horses. Clinical signs include oedema of the back legs, eyelids and lips. As for AHSV, transmission is via *C. imicola* and the

disease is endemic in temperate regions of Africa [40; 41], although there has been a recent outbreak in Israel [42]. Veterinarians and horse owners in at-risk regions should be aware that mild clinical signs including oedema of the eyelids and lips may be due to EEV rather than AHSV.

Others

Several other viruses belonging to the families described here and some belonging to other families may infect horses but their impact is more limited due to the sporadic nature of infection or geographic restriction (Table 1) [43-45]. Others are only just being recognised as potential causes of disease in horses. Since 2013, Bunyamwera virus, an Orthobunyavirus, has emerged as a cause of neurological disease and possibly abortion in horses in Argentina [46; 47]. Clinical signs include apparent disorientation, weakness, visual deficits, tongue protrusion, recumbency and death. Transmission cycles are poorly characterised, although mammals are considered to be amplifying hosts and most isolates have been recovered from mosquitoes. Seroprevalence among birds in Argentina indicate that they could be involved as endemic hosts [48].

Meeting the challenges of diagnosis and control of equine arboviruses

Diagnosis and surveillance

The overlapping geographical ranges of the flavi- and alphaviruses and the overlap in clinical signs between these and other viruses can make clinical diagnosis challenging, particularly in some regions of the world. For example, differential diagnosis for infections causing non-suppurative encephalitis in horses in Australia include MVEV, RRV, WNV (Kunjin), a strain of WNV, and Hendra virus [49]. Similarly, various combinations of WNV, EEEV, WEEV and VEEV overlap in some regions of the Americas, particularly in Mexico and Central America. A recent serosurvey of 246 horses in Costa Rica revealed prevalence of 57% for WNV, 62% for EEEV, 43% for VEEV, and 17% for WEEV [50]. In many

regions of the world, only outbreaks of WNV have been reported in horses, but an early and accurate diagnosis is important to rule out emergence of other flavi- or alpha-viruses.

Although challenging, clinical diagnosis remains the cheapest approach to surveillance for equine arbovirus outbreaks and can be enhanced by adopting a ‘multivariate syndromic surveillance’ system in which data are compiled from various sources. For example, taking weekly time series data on occurrence of nervous signs consistent with encephalitic disease in horses and mortality in both horses and wild birds has been used to identify outbreaks of WNV in France [51]. Laboratory confirmation is still required, and to be effective, this approach requires communication of data between various agencies including veterinary bodies and equine industry stakeholders.

Diagnostic tests for equine arboviruses have a number of disease-specific limitations. For many diseases, detection of virus nucleic acid is now favoured over traditional virus isolation. Some arboviruses are readily detected in blood using polymerase chain reaction (PCR)-based techniques, for example, for VEEV, viraemia occurs with the onset of pyrexia and lasts for 5–6 days. For AHSV, the World Organization for Animal Health (OIE) recommends that virus is isolated for serotyping so that a vaccine containing the relevant serotype can be implemented, although this could be achieved by direct sequencing [52]. However, as already described, the horse is a ‘dead-end’ host for some equine arboviruses such as the flaviviruses WNV and JEV. As a result of the limited and transient viraemia, even sensitive PCR-based diagnostic tests may yield false-negative results for these viruses. Therefore, although virus can be detected in post mortem tissues, particularly the brain and spinal cord [52], diagnosis frequently relies on serological tests.

Although diagnosis of equine and human flavivirus infections relies heavily on antibody detection, this is complicated by high levels of serological cross-reactivity among flaviviruses, particularly in rapid assays such as enzyme-linked immunosorbent assay (ELISA). Testing for IgM antibodies, which indicate the most recent infection, is preferable as IgG antibodies can remain in circulation long after the acute phase of infection. A positive diagnosis by ELISA usually requires confirmation by the more specific

virus neutralisation test; this is time-consuming and costly due to the requirement for BSL-3 containment to handle infectious virus. To address these issues, new approaches are being developed, including rapid neutralisation tests based on pseudotyped viruses that do not require high levels of biosafety containment (reviewed in [53]). Multiplex serological assays (e.g. using micro-bead technology [54]) are desirable to speed up identification of pathogens and enable a syndromic approach to diagnosis to be undertaken (e.g. for neurological disease).

Vaccine challenges

Equine vaccines are available for most of the mosquito-borne equine arboviruses described in regions where they are endemic, with the notable exceptions of RRV and MVEV. In the USA, multivalent vaccines are available that include WNV, EEEV, WEEV and VEEV. Although effective vaccines against WNV are available, there may be a desire to prohibit vaccination of horses in some countries so that they can fulfil the role of a sentinel species [55; 56]. As vaccines become available, tests with differentiation of infected from vaccinated animals (DIVA) potential are desirable in order to determine whether an animal has seroconverted in response to natural infection or vaccination. This is particularly the case when attempting to control an outbreak of an emerging virus to prevent it from becoming established. For example, using a DIVA approach enables ring vaccination to be performed whilst still allowing any spread of the virus to be monitored. Three different vaccines for WNV are commercially available including the multivalent inactivated (killed virus) vaccine, a live canarypox virus vectored vaccine and a DNA vaccine. The latter two vaccines only contain a portion of the viral genome (expressing the prM/E proteins) and therefore have the potential to be used in a DIVA approach in combination with an assay that detects antibodies to other viral proteins (e.g. the NS1 non-structural protein [57]).

The existence of nine serotypes of AHSV presents a particular challenge for vaccination. The control of AHSV in South Africa depends on a live-attenuated AHSV vaccine produced by Onderstepoort Biological Products. This vaccine contains seven of the serotypes delivered as two separate injections,

and it requires two or three doses to generate an adequate immune response to all of the serotypes in the vaccine. Reassortment (i.e. exchange of gene segments between vaccine and field strains) and reversion to virulence have been demonstrated [58]. Consequently, the live-attenuated vaccine is not licensed for use in countries where the virus is not endemic and alternative recombinant vaccines are being actively developed.

Arbovirus emergence: challenges in risk prediction

In order to understand the potential impacts of globalization and climate change on the global disease patterns of equine arboviral disease, knowledge of the complexities of transmission cycles, vector life cycle and the effects of climate change on the vector and vector infection dynamics are vital. In general, it is difficult to predict whether (or when) vector-borne diseases could become established in new areas. Due to the involvement of multiple different host types and vectors for some of the equine arboviruses, risk prediction for epidemic transmission and for virus establishment and endemicity is challenging. There is often a lack of information on the presence of vectors, their vectorial capacity, and the competence of potential reservoir hosts, which may not be present (and therefore studied) in endemic areas.

Virus introduction

Equine arboviruses cannot be controlled solely by animal trade policies as some can be introduced into new regions by movement of viraemic people, migratory birds, or incidental transport of infected vectors as well as by animal movements. It is important to remember that movement of infected individuals does not pose the same risk if they are a 'dead-end' host, as is the case for WNV. Therefore, disease introduction may occur without risk of onward transmission due to the absence or low titre of virus in the blood at the time of importation. For example, a horse imported into the UK from Europe had neurological signs and antibodies against WNV but no detectable viraemia at the time of

testing [59]. If local vectors are infected, then clinical cases in horses or humans may occur. Occurrence of a small number of autochthonous (locally-acquired) infections could occur without local reservoir host involvement, where all vectors are infected from imported hosts. This type of locally-acquired infection may occur without being detected as many infections with equine arboviruses are subclinical. In certain cases, a small number of onward transmission events could occur without a vector contacting the importation host, for example by blood transfusion, as has been recorded in humans [60]. It has also been suggested that bird-to-bird transmission of WNV may occur in some species and contribute to overwintering of the virus [61]. A competent vector, however, is required for epizootic spread.

Ongoing transmission in the case of a VEEV epizootic strain or RRV would require only susceptible equids or humans and competent vectors, and in this case equids could represent a public health risk. In the case of other equine mosquito-borne pathogens, competent local reservoir hosts are required for ongoing transmission, as with the introduction of WNV into the USA.

Mathematical and epidemiological modelling can help to identify subpopulations, regions and periods when there is a higher risk of entry and spread of specific diseases, enabling the occurrence of outbreaks to be anticipated through targeted surveillance. For example, a study to identify hotspots for potential introduction of EEEV, WEEV, VEEV and JEV to Europe through live animal trade demonstrated that the risk was higher for EEEV than the other three viruses, which was mainly associated with trade in exotic pet species such as rodents, reptiles or caged birds [2]. The risk was greatest in Belgium, the Netherlands and northern Italy, highlighting that managing the risk of introduction of exotic arboviruses to Europe as a result of animal trade requires a transboundary approach.

265

266 **Vector presence**

267 The distribution of equine arboviruses is dependent on the presence of the relevant insect vector
268 (Table 1). Many mosquito species act as vectors of WNV, hence the global distribution of this disease.
269 For a number of the arboviruses, the distribution of outbreaks does not extend to all regions of vector
270 occurrence. Therefore, these areas may be considered at risk of emergence.

271 The distribution of the main vector of AHSV (*Culicoides imicola*) extends from South Africa to Southern
272 Europe and across the Middle East to South East Asia and Southern China [62]. African horse sickness
273 virus was introduced into Spain in 1987 when a group of zebra were brought by ship from Namibia
274 and were quarantined outdoors near Madrid. At the time, it was not known that *C. imicola*, was
275 present across the southern half of Spain. The ensuing epizootic resulted in the death of at least 1000
276 equids over a 4-year period, and affected three countries (Spain, Portugal and Morocco) [63]. This
277 emphasises the need for vector surveillance in order to accurately predict the risk of introduction of
278 equine arboviruses into different regions. Some countries, such as Italy, also have AHSV vector
279 populations but have not recorded outbreaks of disease. As *C. imicola* is also the main vector of EEV
280 and the transmission cycle is similar, the same regions that are at risk of AHSV emergence may also
281 be considered at risk of EEV emergence.

282 The presence of an alternative species to the main known vector species can have a profound impact
283 on the epidemiology of incursion, but this is difficult to predict. For example, the main insect vector
284 of JEV in Asia is *Culex tritaeniorhynchus*, which can be infected when there are very low levels of
285 viraemia in the host. However, when there was an incursion of JEV in northern Australia, the main
286 vector was *Culex annulirostris* [64; 65], which is thought to require higher titres to become infected.
287 It is not clear whether JEV failed to become endemic in northern Australia for this reason or due to
288 the presence of other flaviviruses conferring cross-protection to hosts, or because *Cx. annulirostris*

preferentially feeds on marsupials and not wading birds or pigs, the reservoir and amplifying hosts, respectively, of JEV [66].

For some other viruses, such as EEEV, it is not clear whether virus introduction into new regions (e.g. Europe) would find adequate populations of competent vectors for ongoing transmission. Of the major vectors of EEEV in the Americas, only *Cs. morsitans* is present in Europe [67]. There are many other species of mosquito implicated as possible vectors of EEEV. However, without further information such as vector competence and ecological information on vector populations and host–vector interaction, it is not possible to accurately predict whether mosquito populations present in Europe would be capable of facilitating epidemic or endemic transmission. Similarly, the major vector of WEEV (*Cx. tarsalis*) and the major vectors of enzootic VEEV belonging to the genus *Cx. melanoconion* are only found in the Americas [19; 68; 69]. For the epidemic form of VEEV, many other mosquito species in the genera *Psorophora*, *Aedes* (*Ochlerotatus*) and *Culex* act as vectors [3], and species of these genera are found worldwide.

The major vector of MVEV (*Cx. annulirostris*) is confined to Oceania [68; 70] (not including New Zealand), and the distribution of outbreaks reflects this. RRV is active each year in most regions of Australia. Important vectors include *Aedes camptorhyncus*, *Ochlerotatus vigilax* and *Cx. annulirostris* [24]. The distributions of *Cx. annulirostris* and *Oc. vigilax* extend to South East Asia; for *Oc. vigilax*, this includes Japan and Taiwan [68].

For BUNV and GETV, transmission is not well studied, but *Culex* spp. are implicated in GETV transmission and the virus has been isolated from *Aedes* spp. in Siberia [71].

Vector introduction

The risk of arbovirus vector introduction is important in both endemic and non-endemic regions. Invasive vectors, i.e. introduced species that have increased in number and regional range [72], include vectors of equine arboviruses such as *Aedes albopictus*, *Culex pipiens*, *Culex quinquefasciatus*

and *Culicoides imicola* [72; 73]. The introduction of an invasive vector with high vector competence could have significant ecological impact, even in endemic areas of virus transmission, and in disease-free regions increase the risks posed by virus introduction. Surveillance for invasive mosquito species is therefore carried out by governmental organisations, for example by Public Health England in the UK.

Introduction may occur through human activity such as the movement of people, livestock or goods. The used tyre trade has been implicated in long distance sea transport of mosquito eggs of *Aedes* mosquitoes [74] and desiccation-resistant eggs are associated with increased probability of species introduction [72]. Mathematical modelling applied to assess the risk of WNV-infected mosquitoes being introduced to the UK from America led to the conclusion that each summer there is a high risk of at least one WNV-infected mosquito arriving at Heathrow airport, which has a moderate density of susceptible vector and bird species that could allow local dissemination of the virus [75]. The airport is also in a region with a high density of horses [76].

Some barriers to expansion of vector range by natural dispersal include non-vegetated corridors, mountain ranges and large bodies of water [77]. However, *Culicoides*, being extremely small and light, can be transported long distances by the wind, and it is thought that plumes of wind prevailing from continental Europe were responsible for the introduction of both Schmallenberg and bluetongue viruses into the UK [78]. Interestingly, an outbreak of African horse sickness in 1966 occurred just across the Strait of Gibraltar, which separates southern Spain from North Africa [79].

Vector–host interaction

The details of vector–host interaction are complex and vary depending on the ecosystem. For some of the equine arboviral diseases, such as GETV, Bunyamwera and to an extent EEEV in South America, they have not been well studied. This lack of knowledge creates major challenges in predicting potential outcomes of an arboviral disease outbreak in a non-endemic region.

Apart from AHSV and epidemic VEEV, in which the amplification host is the equid, multiple hosts are necessary for completion of the transmission cycle of the arboviral diseases described in this review. Therefore, vectors must necessarily feed from more than one host species in order to infect susceptible hosts. Many of the equine encephalitis viruses have avian reservoir hosts. Vectors that blood-feed from both birds and mammals are therefore required for equine infection to occur. Vectors that bite multiple host types are commonly referred to as ‘bridge vectors’. Some of these bridge vectors are indiscriminate feeders whereas others mainly feed on avian species, and occasionally feed on large mammals. This can therefore lead to infection in these species, for example in the case of *Culex pipiens* and WNV [80] or *Culiseta melanura* and EEEV [81], both of which cause infections in horses and humans.

Vector life cycle and climate dependence

In order to appreciate how climate change might alter the ability of equine arboviruses to become established in temperate regions, it is necessary to consider how it may affect vectorial capacity, which is a measure of the efficiency of vector-borne disease transmission by a vector population.

Vectorial capacity is usually expressed as the number of infective bites received daily by a single host. It is comprised of the average daily vector biting rate (the daily probability of a vector feeding on a susceptible host), vector competence (the proportion of vectors capable of being infected; this may be determined genetically), the extrinsic incubation period, and vector lifespan [82; 83], all of which depend on temperature to a greater or less extent.

Adult female mosquitoes and *Culicoides* feed on blood to enable egg production. They become infected when they feed on a viraemic host and, following virus replication in the vector and spread to the salivary glands, transmit the virus when they subsequently feed on a susceptible host. Therefore, the vector biting rate depends on the time between laying egg batches (the gonotrophic

cycle length). As the ambient temperature increases, the gonotrophic cycle decreases, leading to more frequent feeding, and creating more opportunities for onward transmission of virus.

The extrinsic incubation period (EIP) is defined as the time between a vector obtaining an infective blood meal and being able to infect a susceptible host [84]. The EIP for arboviruses has been shown to be temperature dependent; EIP shortens with increasing environmental temperature up to a maximum transmission efficiency, though some studies detect a subsequent decrease as the temperature rises further [85; 86]. There may also be strain differences in the relationship between temperature and EIP. For example, WNV is thought to have expanded rapidly across the US because of genome changes in the strain that became established resulting in a shorter EIP in *Culex* mosquitoes than the strain that was originally introduced [87; 88]. This advantage is also greater with increased temperatures [89].

Increases in temperature generally accelerate mosquito development [90], thereby reducing longevity. The impact of raising temperature is greater on some species than others, but the reduction in longevity will act to reduce vectorial capacity [91].

The vector biting rate also depends on the density of vectors in relation to the density of the host. Increases in vector populations may result from other consequences of climate change, for example decreased rainfall has been associated with a higher incidence of WNV infection in horses [92].

Overwintering

A characteristic of equine arboviruses in temperate regions is that transmission tends to stop, or at least not be observed, during the cold season when the vectors are not active, but they then reappear the following year. Potential mechanisms by which an arbovirus is able to 'overwinter' are virus–vector specific and are unknown or poorly understood in many cases. In mosquitoes, transovarial (vertical) transmission has been proposed as an over-wintering mechanism for several viruses, for example RRV [93]. Alternatively, some mosquito species hibernate as adults and may harbour virus over winter; for

example, WNV has been demonstrated in overwintering adult *Culex pipiens* [94; 95]. In contrast, EEEV has not been found in hibernating mosquitoes in endemic areas [96]. *Culicoides* species do not hibernate, but overwinter in the larval stage. However, vectors of AHSV may be active in some temperate regions throughout the year and therefore low level virus transmission is proposed as a potential overwintering mechanism [97]. Laboratory studies suggest that at 10°C, low levels of AHSV may persist in *Culicoides*, and these vectors can develop transmissible infections when the temperature rises to 25°C [98]. The zebra also represents an overwintering opportunity for AHSV, as virus has been isolated from zebra up to 7 weeks post infection [38; 99]. Other vertebrate hosts that maintain a prolonged viraemia, such as bats and reptiles, have been suggested as potential overwintering hosts for JEV [100; 101], WNV [102], WEEV [103; 104] and EEEV [105]. Whilst it is apparent that many arboviruses overwinter in temperate regions, uncertainty about the underlying mechanisms presents a challenge when trying to determine whether a virus with expanding range will become endemic in a new region. For example, detection of persistent viral RNA in a vector or host species does not necessarily imply that there is sufficient infectious virus to subsequently re-establish an active transmission cycle.

Conclusion

Equine populations worldwide are at increasing risk of arboviral disease. Better understanding of factors that alter the likelihood of emergence and transmission of these viruses are important for accurate surveillance and control, enabling timely evidence-based information about disease risk and control to be conveyed to appropriate veterinary and equine industry stakeholders. In addition, it is important that equine veterinary surgeons are aware of the clinical signs of arboviral diseases and that different equine arboviral diseases may present with similar clinical signs. There is also the potential for emergence of new arboviruses in the future. Further advances in the development of diagnostic tests for rapid and accurate confirmation of disease and development of effective vaccines against some equine arboviruses are also required.

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| Family / genus | Virus | Abbr. | Major vectors | Zoonotic | Major hosts involved in transmission |
|----------------|---------------------------------------|-------|---|----------|--------------------------------------|
| Toga~/Alpha~ | Eastern equine encephalitis | EEEV | <i>Culiseta melanura</i> , <i>Aedes taeniorhynchus</i> [113] | Y | Passerine birds, rodents |
| | Getah | GETV | <i>Aedes vexans niponii</i> , <i>Culex</i> spp. [10; 24; 114] | N | Swine |
| | Ross River | RRV | <i>Aedes camptorhynchus</i> , <i>Culex annulirostris</i> [24] | Y | Marsupials |
| | Western equine encephalitis | WEEV | <i>Culex tarsalis</i> , <i>Culiseta melanura</i> [113] | Y | Passerine birds |
| | Venezuelan equine encephalitis | VEEV | Enzootic form, <i>Culex melanoconion</i> spp. Epizootic form – wide vector range including <i>Psorophora</i> and <i>Ochlerotatus</i> spp. [3] | Y | Rodents |
| | Highlands J | HJV | <i>Culiseta melanura</i> [115] | Y | Passerine birds |
| | Sindbis | SINV | <i>Culex</i> spp. <i>Culiseta</i> spp. [116] | Y | Birds |
| Flavi~/ Flavi~ | Japanese encephalitis | JEV | <i>Culex tritaeniorhynchus</i> , <i>vishnu complex</i> spp., <i>gelidus</i> [117; 118] | Y | Waterbirds, swine |
| | Murray Valley encephalitis | MVEV | <i>Cx. annulirostris</i> [70] | Y | Waterbirds |
| | West Nile virus | WNV | Many <i>Culex</i> spp. some of the most important include <i>Culex pipiens</i> , <i>tarsalis</i> , <i>modestus</i> , <i>quinquefasciatus</i> [95; 119; 120] | N | Birds (rodents & reptiles) |
| | Usutu | USUV | <i>Culex pipiens</i> [121] | (Y) | Birds |
| | Powassan | POWV | | (Y) | Small mammals |
| | Louping ill | LIV | <i>Ixodes ricinus</i> [122] | (Y) | Sheep, red grouse |
| Reo~/Orbi~ | African horse sickness | AHSV | <i>Culicoides imicola</i> , <i>bolitinos</i> [123] | N | Equids |
| | Equine encephalosis | EEV | <i>Culicoides imicola</i> [41] | N | Equids |

| | | | | | |
|--------------------|-------------------------|------|--|-----|---------------|
| Bunya~/Orthobunya~ | Bunyamwera virus | BUNV | | | Small mammals |
| | Shuni | SHUV | <i>Culicoides</i> , <i>Culex theileri</i> ? [124] | (Y) | ? |
| | Main Drain | MDV | <i>Culicoides variipennis</i> , other <i>culicoides</i> spp.? [125; 126] | (Y) | Small mammals |
| | Snowshoe hare | SSHV | <i>Aedes</i> spp., <i>Culiseta</i> spp. [127; 128] | Y | Small mammals |

776

777 **Table 2.** Common and important clinical signs of mosquito-borne arboviral diseases in horses

| Clinical sign | Virus | | | | | | | |
|----------------------------------|------------------|------------------|-------------------|------------------|-------------------|-------------------|------------------|--------------------|
| | WNV ¹ | JEV ² | MVEV ³ | EEV ⁴ | WEEV ⁴ | VEEV ⁵ | RRV ⁶ | Getah ⁷ |
| Pyrexia | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Gait abnormalities / ataxia | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |
| Paresis | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | |
| Paralysis | ✓ | ✓ | ✓ | ✓ | | ✓ | | |
| Hyperexcitability | | ✓ | | ✓ | ✓ | ✓ | | |
| Impaired vision / blindness | ✓ | ✓ | | ✓ | | ✓ | | |
| Seizures | ✓ | | | ✓ | | ✓ | | |
| Dromomania / circling | | ✓ | ✓ | | | | | |
| Muscle fasciculations | ✓ | ✓ | ✓ | | | | | |
| Inability to swallow | | ✓ | | | | ✓ | | |
| Head pressing | | | | ✓ | | ✓ | | |
| Colic-like signs | ✓ | | ✓ | | | | | |
| Stiffness | | | | | | | ✓ | ✓ |
| Oedema | | | | | | | ✓ | ✓ |
| Lymphadenopathy | | | | | | | ✓ | ✓ |
| Flaccid lips / tongue protrusion | | ✓ | | ✓ | | | | |
| Urticaria / skin eruptions | | | | | | | | ✓ |
| Hypersensitivity | ✓ | | | | | | | |
| Cataplexy / narcolepsy | ✓ | | | | | | | |
| Cranial nerve deficits | ✓ | | | | | | | |
| Neck rigidity | | ✓ | | | | | | |
| Radial paralysis | | ✓ | | | | | | |

Profuse sweating



Teeth grinding



Low head carriage



Drooping ears



Swollen eyelids



778 ¹Reference [129]; ²References [129-132]; ³Reference [49]; ⁴Reference [17]; ⁵References [17; 133; 134];

779 ⁶Reference [135]; ⁷Reference [110].

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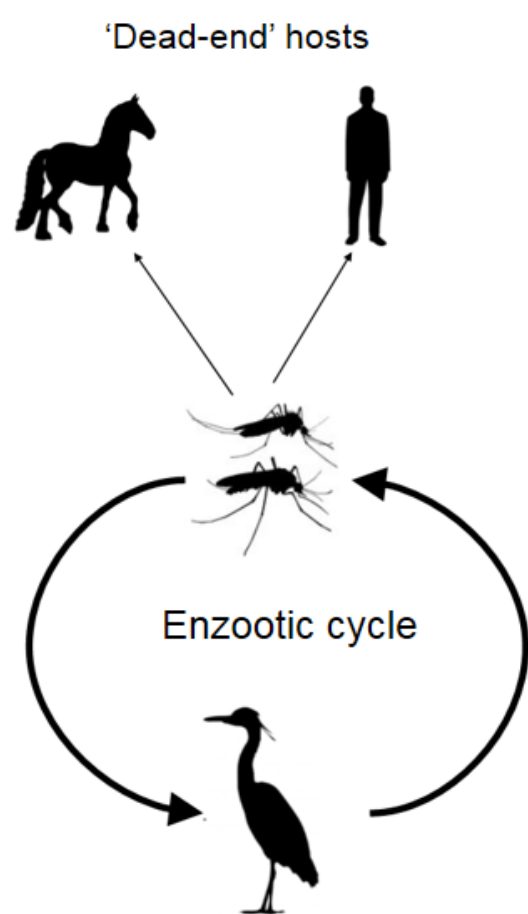
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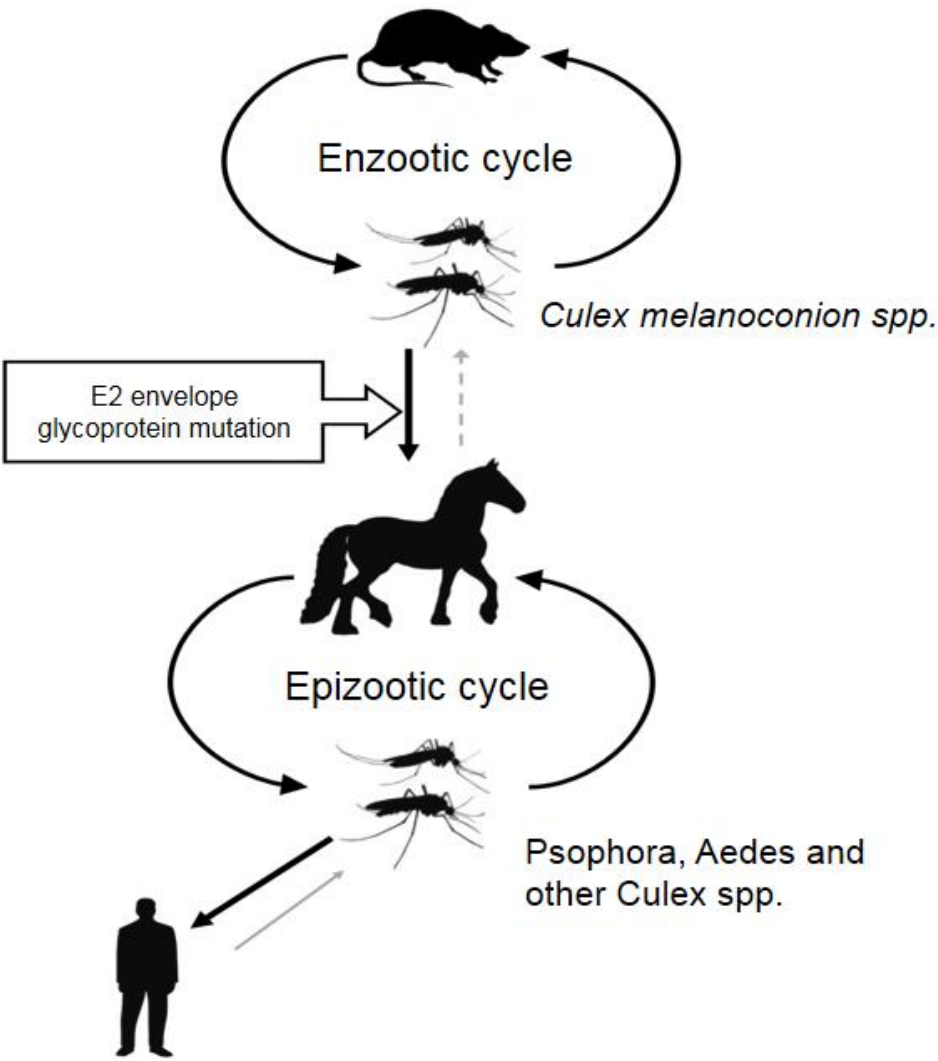
784 **Figure legends**

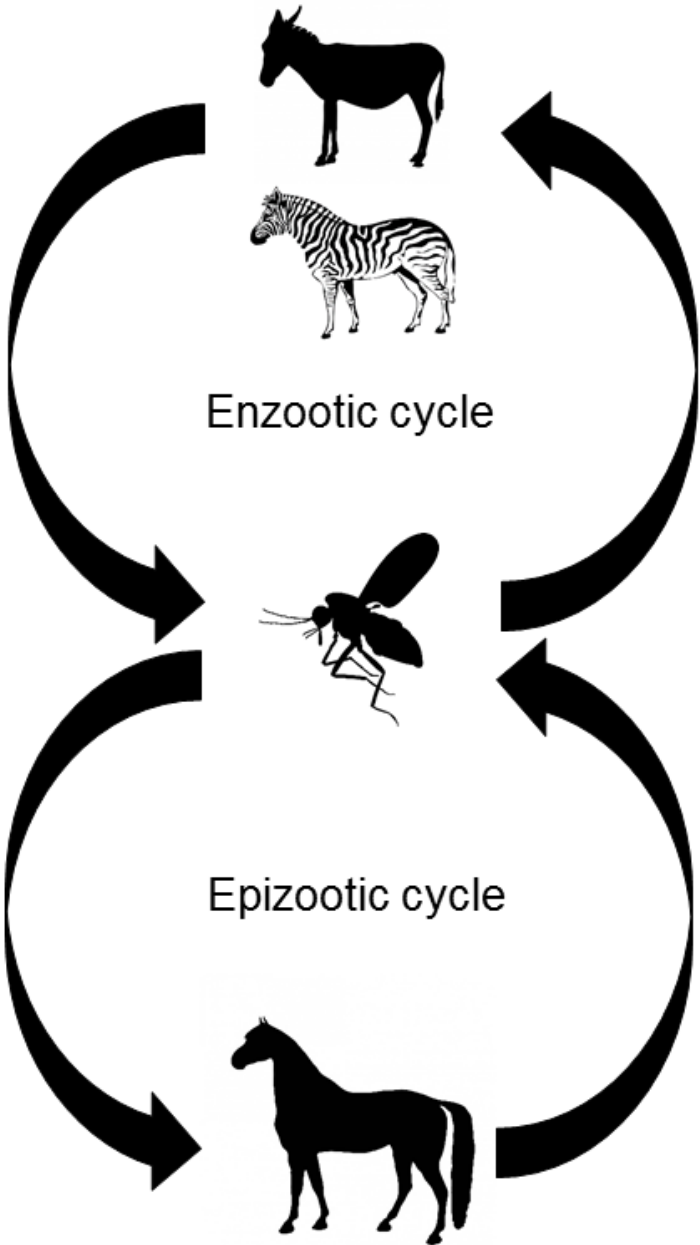
785 **Figure 1.** Example transmission cycles of equine arboviruses: (a) Murray Valley encephalitis virus,
786 which has a simple transmission cycle in which the horse is a 'dead-end' host similar to other
787 arboviruses such as West Nile virus; (b) Venezuelan encephalitis virus, which has enzootic and
788 epizootic cycles; and (c) African horse sickness virus.

789 **Figure 2.** Global distribution, by country, of equine flaviviruses: (a) West Nile virus [95; 106-109]; (b)
790 Japanese encephalitis virus [110; 111]; (c) Murray Valley encephalitis virus [112].

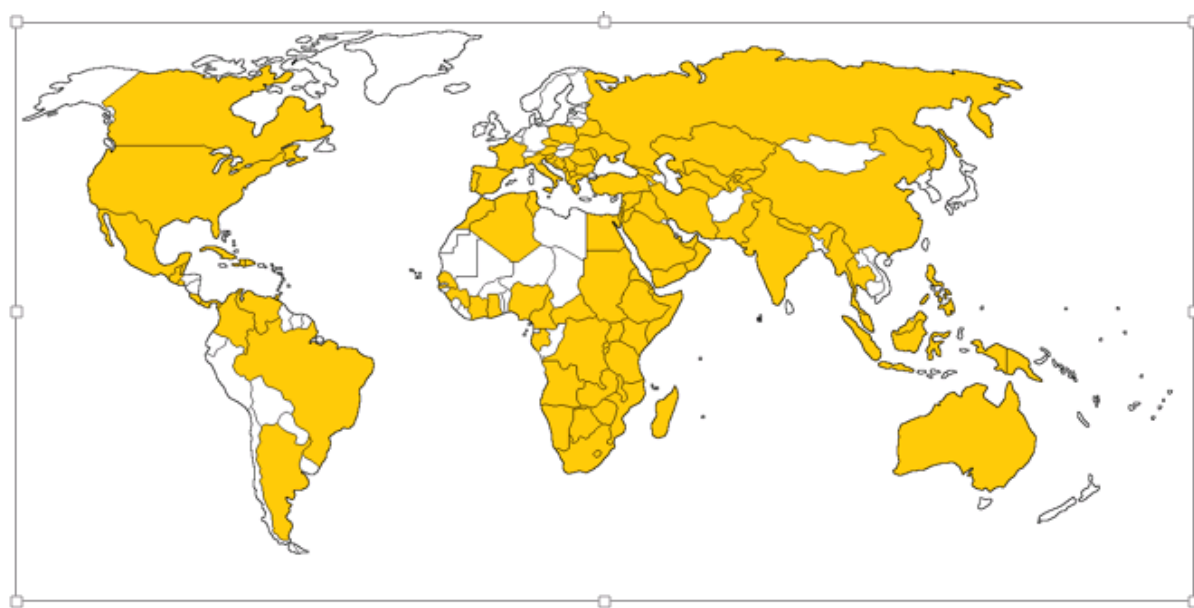
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801 Fig 2a



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804 Fig 2b



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808 Fig 2c



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