



CONSENSUS STATEMENT

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European College of Equine Internal Medicine consensus statement on equine flaviviridae infections in Europe

Jessika-M. V. Cavalleri¹  | Orsolya Korbacska-Kutasi^{1,2,3} | Agnès Leblond⁴ | Romain Paillot⁵ | Nicola Pusterla⁶ | Eike Steinmann⁷ | Joy Tomlinson⁸ 

¹Clinical Unit of Equine Internal Medicine, Department for Companion Animals and Horses, University of Veterinary Medicine Vienna, Vienna, Austria

²Department for Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine, Budapest, Hungary

³Hungarian Academy of Sciences—Szent Istvan University (MTA-SZIE) Large Animal Clinical Research Group, Üllő, Dóra major, Hungary

⁴EPIA, UMR 0346, Epidemiologie des maladies animales et zoonotiques, INRAE, VetAgro Sup, University of Lyon, Marcy l'Etoile, France

⁵School of Equine and Veterinary Physiotherapy, Writtle University College, Chelmsford, UK

⁶Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California, USA

⁷Department of Molecular and Medical Virology, Faculty of Medicine, Ruhr University Bochum, Bochum, Germany

⁸Baker Institute for Animal Health, Cornell University College of Veterinary Medicine, Ithaca, New York, USA

Abstract

Horses and other equids can be infected with several viruses of the family *Flaviviridae*, belonging to the genus *Flavivirus* and *Hepacivirus*. This consensus statement focuses on viruses with known occurrence in Europe, with the objective to summarize the current literature and formulate clinically relevant evidence-based recommendations regarding clinical disease, diagnosis, treatment, and prevention. The viruses circulating in Europe include West Nile virus, tick-borne encephalitis virus, Usutu virus, Louping ill virus and the equine hepacivirus. West Nile virus and Usutu virus are mosquito-borne, while tick-borne encephalitis virus and Louping ill virus are tick-borne. The natural route of transmission for equine hepacivirus remains speculative. West Nile virus and tick-borne encephalitis virus can induce encephalitis in infected horses. In the British Isle, rare equine cases of encephalitis associated with Louping ill virus are reported. In contrast, equine hepacivirus infections are associated with mild acute hepatitis and possibly chronic hepatitis. Diagnosis of flavivirus infections is made primarily by serology, although cross-reactivity occurs. Virus neutralization testing is considered the gold standard to differentiate between flavivirus infections in horses. Hepacivirus infection is detected by serum or liver RT-PCR. No direct antiviral treatment against flaviviridae or hepacivirus infections in horses is currently

Abbreviations: AAEP, American Association of Equine Practitioners; AST, aspartate aminotransferase; CBC, complete blood cell count; CSE, central southern European; CSF, cerebrospinal fluid; E protein, envelope protein; ECDC, European Centre for Disease Prevention and Control; ECEIM, European College of Equine Internal Medicine; EqHV, equine hepacivirus; EU, European Union; GGT, gamma glutamyl transferase; GLDH, glutamine dehydrogenase; HBLB, Horserace Betting Levy Board; HCV, hepatitis C virus; IHC, immunohistochemistry; ISH, in situ hybridization; JEV, Japanese encephalitis virus; LIPS, luciferase immunoprecipitation system; LIV, louping ill virus; MVEV, Murray valley encephalitis virus; NS3, nonstructural protein 3; OIE, World Organization for Animal Health; PRNT, plaque reduction neutralization test; qPCR, quantitative polymerase chain reaction; R/R, Romanian/Russian; RT-PCR, reverse transcriptase-PCR; SDH, sorbitol dehydrogenase; TBEV, tick-borne encephalitis virus; USUV, Usutu virus; UTR, untranslated region; VNT, virus neutralization test; WNND, West Nile neuroinvasive disease; WNV, West Nile virus.

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Correspondence

Jessika-M. V. Cavalleri, Clinical Unit of Equine Internal Medicine, Department for Companion Animals and Horses, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria.
Email: jessika.cavalleri@vetmeduni.ac.at

available and thus, treatment is supportive. Three vaccines against West Nile virus are licensed in the European Union. Geographic expansion of flaviviruses pathogenic for equids should always be considered a realistic threat, and it would be beneficial if their detection was included in surveillance programs.

KEYWORDS

equid, equine, gastroenterology, hepatitis, hepatology, microbiology, neurology, viral, zoonoses

1 | INTRODUCTION

The family *Flaviviridae* includes the genus *Flavivirus*, *Pestivirus*, *Hepacivirus*, and *Pegivirus*. All *Flaviviridae* are enveloped single-stranded RNA viruses. Known pathogens in horses have been identified in the genera flaviviruses and hepaciviruses¹⁻⁷ (Figure 1), while pestiviruses and pegiviruses do not seem to play a role as equine pathogens.^{10,11} Three neurotropic flaviviruses documented in horses suffering from meningoencephalitis are enzootic in Europe: West Nile virus (WNV), tick-borne encephalitis virus (TBEV) and Louping ill virus (LIV).¹ Among the many flaviviruses which can cause disease in mammals, West Nile virus probably has the most impact on equid health. The equine hepacivirus (EqHV) is a recently identified hepatotropic virus infecting equids (donkeys and horses).¹²⁻¹⁴ It is a close homologue to the HCV and is classified as hepacivirus A.

The goal of this consensus statement was to summarize recent knowledge on *Flaviviridae* infections relevant for equids, and to address questions of importance for the equine clinician/practitioner focusing on viruses already circulating in Europe.

2 | CLINICAL DISEASE**2.1 | West Nile virus****2.1.1 | West Nile neuroinvasive disease—WNND**

Overall, the combination, severity, and duration of clinical signs can be highly variable. Low grade fever, obtund mentation, inappetence, colic, or lameness can be among the first recognized signs in diseased animals.¹⁵ In different outbreaks the most frequent clinical signs observed are ataxia (57%-100%), weakness (30%-100%), and muscle fasciculations (42%-100%). Ataxia can be symmetrical or asymmetrical, such as weakness affecting either fore-/hindlimbs or all 4. Fewer horses present with hyper-responsiveness and cranial nerve deficits such as facial paralysis, vestibular ataxia, drooping lip and/or inability to swallow, photophobia and central blindness.¹⁶⁻²² Abnormal behavior such as obtund mentation, somnolence, disorientation, hyperexcitability and aggressive behavior as well as changes in personality have also been associated with WNV infection in equids.^{15,20,23,24} Disease presentation has been observed to be similar between WNV lineage 1 and lineage 2 infected equids.^{22,25}

2.1.2 | Risk factors of developing WNV neuroinvasive disease (WNND) and of nonsurvival of WNND

Clinical reports of case series,^{17,25,26} outbreak investigations^{20,22,27-29} and experimental infections,³⁰⁻³² contribute to our current understanding of disease manifestation and risk factors for WNND. European outbreak descriptions^{18,19} cover relatively low numbers of cases (80 at most by Murgue et al¹⁹) compared to outbreak evaluations in North America (up to 1698 cases in Texas outbreak by Ward et al²¹). Similarly, data about lineage 2 outbreaks^{22,25-27} is limited compared to lineage 1.^{18-20,28,29,33}

In line with the epidemiological evidence that the majority (>80%) of equine WNV-infections are subclinical or resulting in mild clinical signs, it has also proven difficult to experimentally replicate neurological disease in otherwise healthy horses.^{16,27,30,34} Risk is multifactorial and involves viral, host, and environmental factors. There seems to be no age, sex, or breed predisposition in horses. Mean age in different outbreaks is between 6 and 14 years (3 months-38 years).^{18-20,23,25,28,29,33,35-37}

Viral lineage: There does not seem to be a difference in the risk for clinical manifestations due to either lineage 1 or lineage 2 infections in naturally infected horses.^{22,25} Similarly, case fatality rate among WNV lineage 2-infected horses is similar to lineage 1.^{22,25,29} Based on a rodent model,³⁸ differences in pathogenicity among WNV strains do not correlate with the phylogenetic lineage, geographic origin or year of isolation. Rather, virulence appears to be an evolving phenotype acquired independently of the genetic background during virus adaptation to varying ecological niches.

Vaccination status and previous natural infection: In experimental infections vaccinated horses challenged either by the intradermal or by the intrathecal route appear to be protected against severe illness.³⁰⁻³² Large scale studies in North America have noted that equids that are not vaccinated more likely show clinical signs or die than do vaccinated equids. However, some horses respond poorly and WNND can occur in vaccinated animals.^{28,35} Natural infection theoretically results in long lasting protection, but the humoral and cellular immunity have not been assessed in a longitudinal study.

2.2 | TBEV clinical disease

Horses are prone to TBEV infection; however, they remain mostly asymptomatic. Although TBE in humans has been studied extensively,

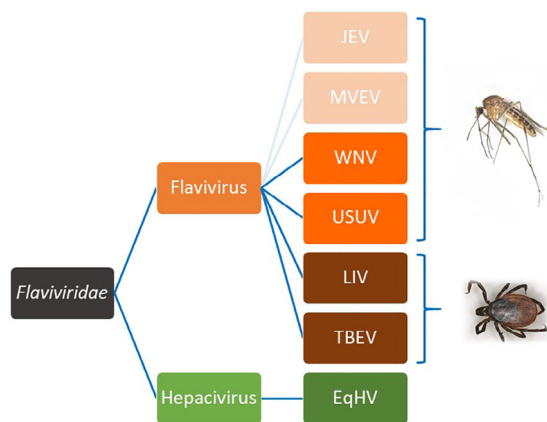


FIGURE 1 Equine pathogens in the *Flaviviridae* family: Japanese encephalitis virus (JEV), Murray Valley Encephalitis Virus (MVEV), West Nile virus (WNV) and Usutu virus (USUV) are classified in the Japanese encephalitis antigenic complex transmitted by ornithophilic mosquito species, mainly *Culex* spp.⁸ Japanese encephalitis virus and MVEV are not endemic in Europe. Tick-borne encephalitis virus (TBEV) and louping-ill virus (LIV) are closely related zoonotic flaviviruses belonging to the tick-borne encephalitis (TBE) serocomplex transmitted by the same ixodid tick vector, *Ixodes Ricinus*.⁹ The manner of equine hepacivirus (EqHV) transmission is unknown

there are only a limited number of reports on TBEV infections in animals, especially in horses. Neurological signs after TBEV infection are nonspecific and can range from peracute to acute or mild subacute courses. Signs described are: reduced general condition, inappetence, hyperreactivity, ataxia, seizures, and paralysis of neck and shoulder muscles.³⁹⁻⁴³

2.3 | USUV clinical disease

Reports on clinically apparent USUV infections are scarce and few human cases are described in the literature.^{8,44-50} Infection of humans is usually asymptomatic, but rarely serious neurological disease is observed. Pathogenicity of USUV is unclear and sometimes a case of USUV infection is wrongly identified as being caused by WNV.⁵¹ Equine USUV-related clinical disease has not been described, but horses in Poland and Croatia can be seropositive.^{52,53} Anti-USUV antibodies were detected in 2017 in horses in Austria (unpublished data).

2.4 | LIV clinical disease

Louping ill virus derives its name from an old Scottish term describing the leaping motions of encephalitic sheep. Louping ill virus causes a febrile illness in sheep, cattle, grouse and occasionally in horses and some other species, that can progress to fatal encephalitis.⁵⁴ Affected horses frequently have muscle tremors of the neck and facial area, or even generalized tremors. Paralysis, paresis of a single or all extremities, obtunded mentation, and abnormal behavior occur. Some horses are pyrexia (up to 40.5°C) and inappetent.^{55,56}

2.5 | EqHV clinical disease

Equine hepacivirus infections are mainly subclinical with mild clinicopathologic abnormalities indicating hepatopathy.^{13,14} Overt clinical hepatopathy in association with the infection is rarely described.^{4,57} Equine hepacivirus infections are usually self-limiting and resolve within weeks or months. Infected horses showed a delayed seroconversion against the nonstructural protein NS3 starting from 3 to 8 weeks after infection, which often precedes viral clearance.^{3,13,14,58,59} Additionally, successful seroconversion seems to provide a nonsterilizing immunity against reinfection, leading to lower level of viremia and shorter viremia in subsequent infections.^{13,14} Liver enzyme activities (GLDH, SDH, GGT, AST) increase around the time of viral clearance; although hepatitis lasts 1 to 4 months, peak liver enzyme activity in serum frequently remain within the reference interval.¹³ Histopathological changes are subtle, including scattered individual hepatocyte necrosis and mononuclear cell infiltrate.^{3,13,14}

Equine hepacivirus can also cause persistent infections. Experimental inoculation of naïve horses showed that adult horses were more likely to be able to clear the infection than young horses (8 months or younger) which implies an important role for the adaptive immune system in clearing the infection.^{2,59} Two case reports document persistent EqHV infection with chronic hepatitis, indicating horses might develop a similar condition of chronic hepaciviral hepatitis as is observed in humans with HCV.^{4,57} Histopathologic changes include fibrosis, hepatocyte necrosis, mononuclear inflammation, and biliary reaction.

3 | TRANSMISSION

3.1 | General

The 4 genera of the *Flaviviridae* family have diverse mechanisms of transmission. The flaviviruses are also known as arboviruses because they are arthropod-vector. In contrast, the pestiviruses are primarily transmitted through contact, such as fecal-oral transmission, or vertically transmitted. Method of transmission of the hepaciviruses and pegiviruses is unclear. Both are transmitted iatrogenically by blood or a blood-product transfer, but modes of natural horizontal transmission are not known.

3.2 | WNV transmission

Like many other flaviviruses, WNV is mosquito vectored. It is primarily an avian virus, where birds serve as amplifying hosts (Figure 2). Over 150 species of birds can be infected with WNV.^{60,61} More than 65 mosquito species transmit WNV, with *Culex* spp. being the primary vectors worldwide.^{62,63} Mosquitoes are capable of vertical transmission and can therefore sustain the virus for a period of time in the absence of infected hosts.⁶³ While mosquitoes often have a narrow host range, those that “host-switch” or feed on both birds and

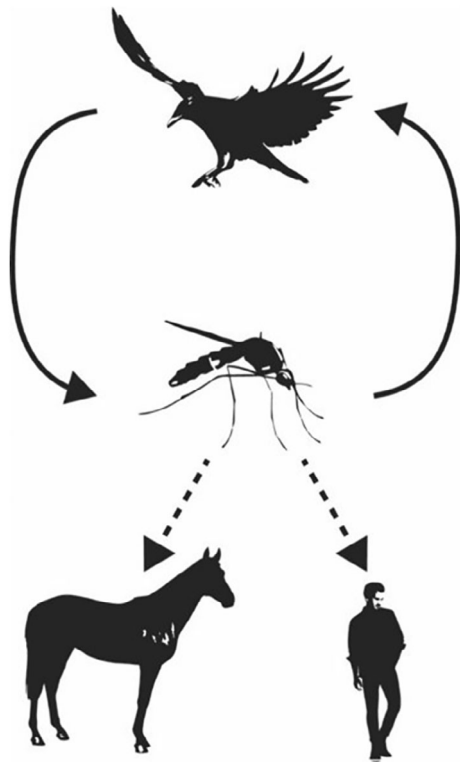


FIGURE 2 West Nile Virus (WNV) and Usutu virus (USUV) undergo sylvatic circulation between birds and mosquitoes. Birds serve as an amplifying reservoir host. *Culex* spp. are the major mosquito vectors of both viruses worldwide. Mosquitoes can transmit vertically to maintain the virus through the winter. Transmission occasionally occurs from mosquitoes to horses or humans, but mammals are dead-end hosts

mammals, such as *Culex tarsalis* in the western United States and *Culex pipiens* in the eastern United States and Europe, are responsible for the occasional transmission to horses and humans.^{63,64} Many species of birds are susceptible to infection, some with high case fatality rate.⁶¹ There however, is variation in the reservoir/amplifying species based on geographic location.⁶⁵⁻⁶⁷ Both changing bird and mosquito habitats and ecology can contribute to the spread of WNV into new geographic regions.

Unlike birds, horses and humans are both typically dead-end hosts. While they can develop clinical disease, they do not develop high enough viremia to transmit back to mosquitoes.^{61,64} In humans, there is limited horizontal transmission via blood transfusion or organ transplantation, and vertical transmission through the placenta or breast milk.⁶³ These routes are not documented in horses to date.

Because WNV is arthropod-borne, there is a seasonal transmission pattern, with cases starting in the summer and continuing through the autumn, although clustering of cases within those seasons depends on the local climate.⁶⁸ In Europe, WNV outbreaks occur during the summer and autumn seasons (July-November) when *Culex* mosquitoes are abundant. Overwintering likely occurs through winter diapause, or hibernation, of infected mosquito females, or through transovarial transmission in mosquitoes.^{69,70}

3.3 | USUV transmission

Usutu virus ecology is remarkably similar to WNV (Figure 2), and the 2 viruses cocirculate in Europe.⁷¹ Like WNV, USUV is mosquito vectored, with birds as amplifying hosts. *Culex* spp., particularly *C. pipiens*, are the major vectors, although other genera, including *Aedes*, contain competent species.^{62,71} At least 93 bird species are susceptible to infection, with blackbirds, gray owls, and house sparrows particularly susceptible to lethal infection.⁷¹ Usutu virus infects many Passeriformes that are also susceptible to WNV. Susceptible mammals, including horses and humans, are suspected to be dead end hosts, as is observed with WNV.⁷¹

3.4 | TBEV transmission

In contrast to WNV and USUV, TBEV is tick-borne (Figure 3). Although more than 14 species of ticks can be infected with TBEV, *Ixodes* spp. are the primary vectors. *Ixodes ricinus* have a broad pattern of feeding on over 300 different species including mammals, birds, and reptiles. Ticks are active while temperatures exceed 5 to 7°C, which determines the seasonal transmission to mammals.⁷² In more temperate regions such as central Europe, there are 2 peaks of activity: first in May-June and then in September-October. However, in colder regions there is only 1 peak in the middle of summer. The first clinical cases are typically observed 2 to 4 weeks after the onset of tick activity.⁷²

3.5 | LIV transmission

Louping ill virus is transmitted to sheep, deer, mountain hares, and grouse by feeding *I. ricinus*⁷³; LIV, similarly to the other TBEV subtypes, is also transmissible to offspring by goat and sheep milk and thus has a potential to be transmitted to humans.^{74,75} Naturally acquired equine infections have occurred in enzootic areas.

3.6 | EqHV transmission

Just like the closely related Hepatitis C Virus, EqHV infects hepatocytes and causes both acute and chronic infection.^{58,59,76} The virus can be transferred via blood, plasma, or serum,^{14,59} including vertical transmission to the foal.^{77,78} In contrast to other flaviviruses, hepaciviruses are not known to be insect vectored. EqHV was not detected in mosquito pools in Austria; however, this does not rule out mechanical or biological insect vectoring.⁷⁹

3.7 | Geographical distribution

West Nile virus is the most widely distributed arbovirus that induces equine encephalitis (Figure 4). It was first isolated in 1937 in Uganda,⁸¹ then detected in a few European countries between 1958 and 1970,⁸²⁻⁸⁴ reemerging in the late 1990's.

West Nile virus strikingly exemplifies how unpredictably flaviviruses can emerge in naïve populations. In the United States, WNV lineage 1 was introduced in New York City in 1999, producing large

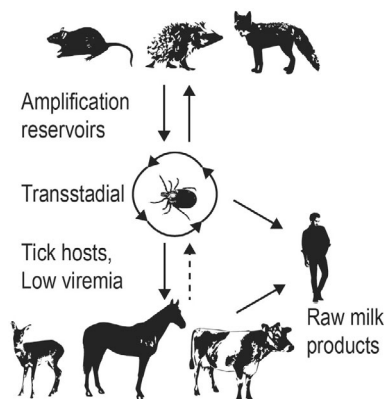


FIGURE 3 Tick-borne encephalitis virus and LIV transmission involves a complex ecology of reservoir species, ticks and indicator species that support tick populations. Small vertebrates including rodents, insectivores, and wild carnivores serve as amplifying reservoirs, developing high titer viremia and transmitting to ticks. Ticks transmit transstadially through egg, larvae, nymph, and adults, and transmit to a wide variety of vertebrate species. Large mammals such as deer and livestock maintain tick populations and are susceptible to disease, but do not transmit back to ticks due to low virus load, short duration of viremia or a combination of the 2. They are considered indicator species because they are valuable sentinel species for disease and antibody prevalence. Humans and horses are considered accidental hosts because they are not involved in sustaining transmission or feeding tick populations

and dramatic outbreaks in humans and horses. It rapidly spread throughout the country, causing more than 30 000 cases and 1200 deaths in humans and more than 24 000 cases in horses within a 10-year period.⁸² In contrast, Europe epidemics were irregular and limited in time and space from 1996 to 2010.

Until 2004, only lineages 1 and 3 WNV strains had been found in Europe. A first WNV lineage 2 strain was initially detected in Hungary in 2004,^{85,86} with a noticeable increase in WNV transmission and outbreaks in Europe since the 2010 s associated with the spread of these WNV lineage 2 strains (Table 1).^{1,88} Another WNV lineage 2 strain, first detected in 2004 in Southern Russia,⁹⁸ has also been occasionally reported in Europe.

In Europe the most important transmission wave occurred in 2018. At the end of 2018, a total of 1503 human confirmed cases were reported in 11 countries of the EU, of which some experienced the first autochthonous infections.⁹⁵ Reports from the ECDC also indicated a high transmission among horses with 285 outbreaks⁹⁹ (Table 2). Independently of the spread of the dominant lineage 2 strains, lineage 1 strains can be still responsible for local outbreaks like the 1 in Spain in 2020 (77 human and 137 equine cases).^{101,102}

Usutu virus is thought to have been introduced into Europe multiple times through bird migration, starting in the 1950s.^{71,103} In Europe, the first cases of USUV-associated large-scale deaths of wild birds were described in Italy in 1996 and in Austria in 2001.⁸⁹ Within 2 decades, USUV has spread all over Europe, except for the Baltic countries and Scandinavia, and in 2020, USUV emerged in the United Kingdom.¹⁰⁴ Viruses from African lineages were recently detected in Germany¹⁰⁵ and the Camargue area of France.¹⁰⁶

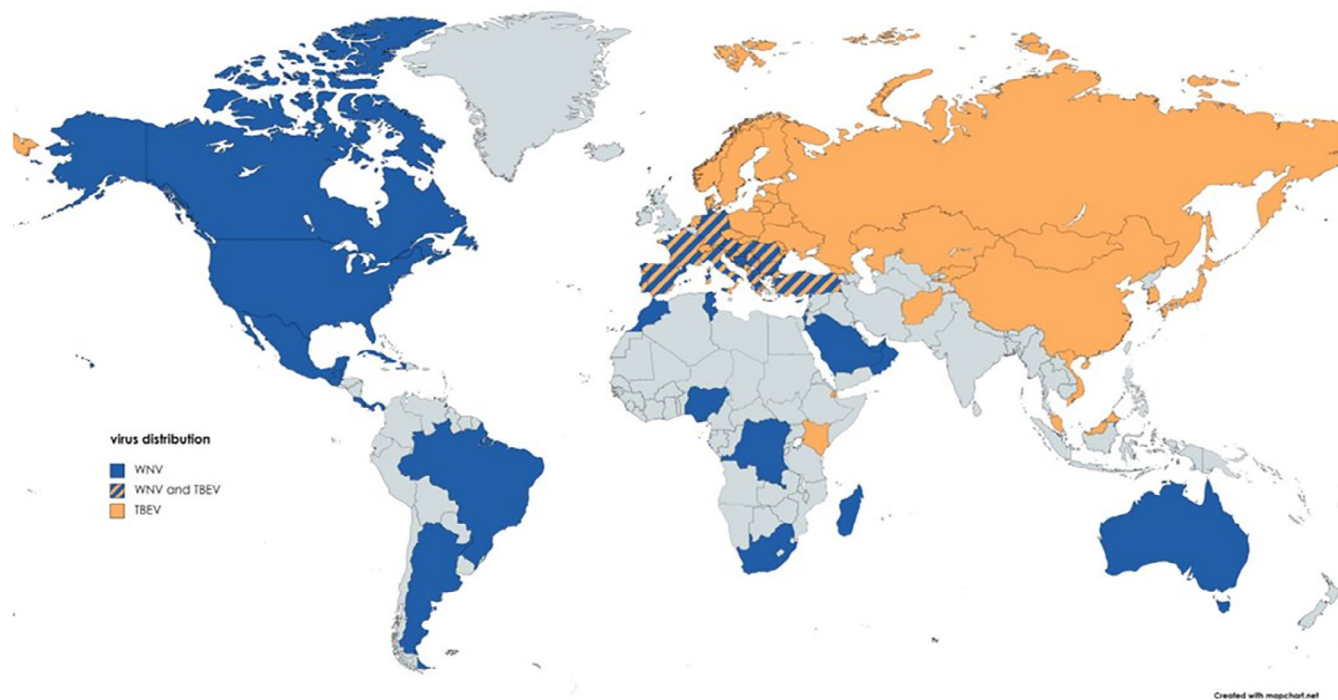


FIGURE 4 Global distribution of WNV infections reported to OIE 2005-2021 and TBEV infections according to Im et al.⁸⁰ WNV and TBEV cocirculate in Europe. Countries in gray: no data reported

TABLE 1 Spread of Lineage 2 WNV (WNV-L2) across Europe. WNV-L2 is divided in 2 clades, the Central Southern European (CSE) clade and the Romanian/Russian (R/R) clade⁸⁷ (NR: not reported)

Country	First detection of WNV	Strain detected	Hosts infected	References
Hungary	2004-2008	CSE WNV-L2 clade	Wild birds, sheep, horses, human	22,85,88
Russia	2004-2007	R/R WNV-L2 clade	Human	89
Eastern Austria	2008	CSE WNV-L2 clade	Wild birds	26,85,90
Balkan Peninsula, including Greece	2010	CSE WNV-L2 clade	Human, wild birds, mosquitoes	27,86
Italy	2010	CSE WNV-L2 clade	Human, wild birds, mosquitoes	91
Romania	2010	R/R WNV-L2 clade	Human	92
Serbia, Croatia, Bulgaria	2012	CSE WNV-L2 clade	Human	68,93
France	2017-2018	CSE WNV-L2 clade	Human, horses	87,94
Spain	2017	CSE WNV-L2 clade	Wild birds	87
Germany	2018	CSE WNV-L2 clade	Horses	95
Netherlands	2020	NR	Birds, mosquitoes, humans	96,97

Tick-borne encephalitis virus is the most important human tick-borne pathogen in Europe and Asia. Temporal and geographical distribution of seropositivity in horse populations is highly variable in Europe^{9,39,107,108} (Figure 4). Horses seem to be less sensitive to the pathogen; even with an unexpectedly high infection rate between 20% and 30% of TBEV in Austria, Germany and Lithuania,^{40,109-111} equine clinical disease is rarely identified.^{42,43,111}

Based on epidemiological investigations, LIV distribution area was initially limited to the British Isles (particularly in Scotland, Cumbria, Wales, Devon and Ireland), but seroconversion or clinical cases in sheep due to LIV-like viruses have been reported during the last decade in Norway, Denmark, and Spain.^{9,54,56,112} A limited serological survey in Ireland disclosed a positive rate of 10.6%, indicating a moderate infection rate in animals of mixed breed and of different environmental backgrounds.⁵⁶ Suspected equine neuroinvasive cases have been reported from clusters in the British Isles.¹¹³

Equine hepatitis virus is globally distributed with reported cases in all continents except for Antarctica (Figure 5).^{3,58,114-118} Its prevalence was reported to be between 2% and 40% on the nucleic acid level while the seroprevalence ranged between 22% and 84% in different equine populations.^{12,115,116,118-120} Noteworthy, EqHV, so far, does not cluster in different geographical genotypes, and all deposited sequences show high similarity, in contrast to the high diversity observed in HCV.¹²¹

4 | DIAGNOSIS

4.1 | DIAGNOSIS OF WEST NILE VIRUS INFECTION IN EQUIDS

4.1.1 | Antemortem diagnosis of WNV infection

In Europe the following diseases should be included in differential diagnosis: other flaviviruses (USUV, TBEV, LIV) which have similar seasonal (although TBE usually diagnosed early summer) and clinical

TABLE 2 Reported human and equine WNV cases according to countries in the EU during the 2018 outbreak (ECDC data: <https://www.ecdc.europa.eu/sites/default/files/documents/zoonoses-EU-one-health-2018-report.pdf>)¹⁰⁰ (NR: not reported)

Country	Number of equine cases	Number of human cases
Italy	238	610
Hungary	93	215
Greece	19	315
France	13	27
Austria	3	21
Bulgaria	0	15
Croatia	NR	58
Czech Republic	0	5
Romania	18	277
Slovenia	1	4
Germany	2	NR

presentations and cross-react in most of the diagnostic tests. Apart from equine herpesvirus myeloencephalopathy, which is endemic in Europe, rabies and Borna-virus infection can occur in certain geographical regions. Verminous meningoencephalomyelitis, bacterial meningitis, botulism, toxicosis and trauma may lead to similar acute neurologic signs as WNV. Horses with cervical vertebral malformation or equine degenerative myeloencephalopathy usually show more age-related and chronic disease progression. Autochthonous cases caused by Alphaviruses or equine protozoal myeloencephalitis have not been diagnosed so far in Europe.

A suspected diagnosis of WNV infection is generally considered based on origin of the patient (horse residing in an endemic area for WNV, Figure 4), time of year, clinical presentation of the horse and vaccination history (ie, no or inadequate vaccination history against WNV).^{17,20,122} Ancillary diagnostic tests such as complete blood count (CBC), biochemical analysis and cerebrospinal fluid (CSF) analysis could

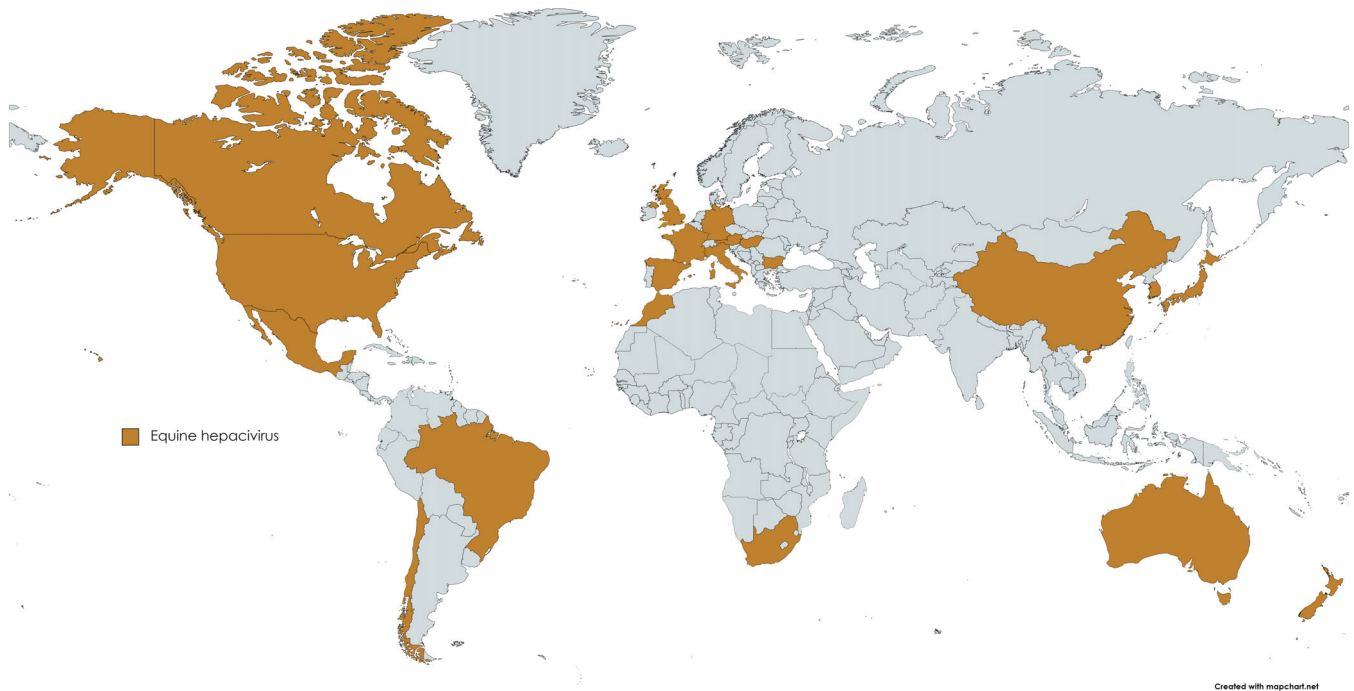


FIGURE 5 Global distribution of EqHV.^{58,79,114,116,117,119,146,169-174} Countries in gray: no data reported

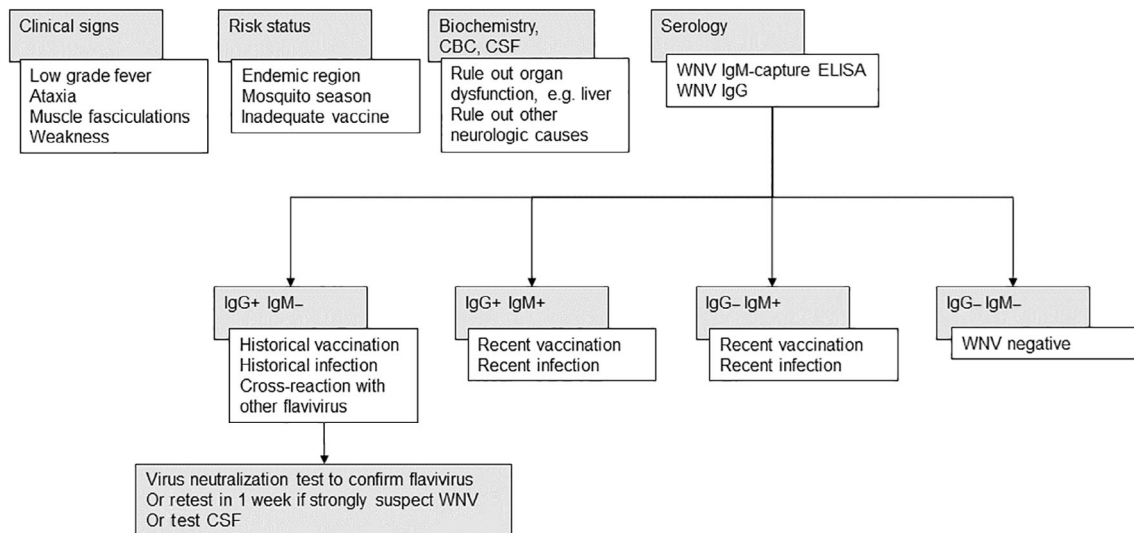


FIGURE 6 Recommended diagnostic algorithm for WNV. Results of rapid IgG tests must be interpreted in the context of IgM results, or with virus neutralization to differentiate between WNV, USUV, TBEV, and LIV infection

help rule in or rule out other neurological or systemic causes. Similar to other viral infections, horses can display a mild, absolute lymphopenia. Biochemical abnormalities might suggest organ-related conditions causing neurological deficits such as liver disease. Muscle enzymes can be elevated in horses experiencing trauma or prolonged periods of recumbency. CSF analysis is often nonspecific and variable with elevated nucleated cell count (mononuclear or neutrophilic pleocytosis) and/or protein concentration and mild xanthochromia.^{123,124} The antemortem detection of WNV in blood and/or CSF via culture and qPCR is rarely achieved because of the short-lived and low-level viremia reported in equids.^{34,125}

Neutralizing antibodies have a critical role in the long-term protection from disease and, presently, their measurement provides the best correlate of flavivirus immunity and can be used for WNV field surveillance and for clinical diagnostics.¹²⁶ The flavivirus envelope (E) protein is the major target of virus neutralizing antibodies. The flaviviruses are antigenically related and broad serological cross-reactions in general and cross-neutralizations in particular are observed. Their extent and duration are strongly dependent on amino acid similarity in the E-protein which ranges from 40% to 44% for unrelated flaviviruses and 60% to 70% within closely related flaviviruses.¹²⁷

Even though there is a problem of cross-reactions with most of the serological tests, serology still provides the mainstay of presumptive WNV antemortem diagnosis (Figure 6). West Nile virus IgM-specific antibodies are often targeted to document recent infection since these antibodies rise sharply during the first few days of clinical disease and can be detected for about 4 to 6 weeks. However, because IgM antibodies can be detected up to 52 days after vaccination with either recombinant or inactivated vaccines, the serological results always need to be interpreted in the context of recent immunization.^{128,129} From a diagnostic standpoint, the detection of intrathecally-derived IgM antibodies to WNV in CSF is even more conclusive to support a WNV infection, if there is no evidence of blood contamination or loss of integrity of the blood-brain barrier.¹³⁰ The serological test of choice offered by many veterinary diagnostic laboratories is the IgM WNV capture ELISA (MAC). The detection of anti-WNV IgM via MAC-ELISA is more specific and sensitive than the IgG assays for currently circulating European viruses, so the diagnosis of WNV infection often only requires a single test on serum, CSF or both.^{131,132} However, cross-reactivity with other viruses of the JEV group like JEV is possible and such the reliability in areas with cocirculating cross-reacting viruses is questionable.^{133,134} In Europe, circulating closely related flaviviruses like TBEV, USUV, and possibly LIV, induce antibodies that generate positive results in rapid serological diagnostic tests detecting IgG.¹³⁵ The gold standard confirmatory serological test in a nonvaccinated horse is thus based on the detection of serum neutralizing antibodies. All positive serological results using rapid assays should be confirmed by the more specific virus neutralization test (VNT) with the viruses known to circulate in the area (Figure 6). In nonvaccinated horses, a 4-fold change in paired neutralizing antibody titers is confirmatory of a diagnosis of WNND. Serum neutralizing antibodies can be measured by 1 of 2 formats, the plaque reduction neutralization test (PRNT) or the microwell (SN) neutralization test. The PRNT is the most commonly used neutralizing antibody test because the results are less influenced by individual differences between samples.¹²⁶ Since vaccination induces formation of neutralizing antibody to the E protein of the virus, the interpretation of serological results using PRNT can be more challenging. Additional tests, such as the microsphere immunoassay, hemagglutination inhibition test and indirect fluorescent antibody test, have been developed and are often used for the surveillance of WNV in various animal species.¹³⁶⁻¹³⁸

4.1.2 | Postmortem confirmation of WNV infection

West Nile virus can be isolated or detected after death in brain material of diseased horses by the use of cell cultures, through intracerebral inoculation of suckling mice with infected brain material, and by detection of specific nucleotide sequences using reverse transcriptase-polymerase chain reaction (RT-PCR) technology.¹²⁶ West Nile virus has been detected in the thalamus, hypothalamus, pons/medulla and spinal cord of infected horses.¹³⁹ The medulla contains the highest mean concentration of viral RNA, and WNV RNA could be

detected by RT-PCR in fresh tissues and in formalin-fixed neural tissue.¹⁴⁰ Immunohistochemistry (IHC) and in situ hybridization (ISH) have been used to detect WNV within formalin-fixed, paraffin-embedded brain tissue of horses. While these techniques are excellent for the detection of equine eastern encephalitis virus in the brain of affected horses, they are less reliable for the detection of WNV, mainly because of the low viral load in brain tissue of affected horses.^{141,142} It is always advised that the laboratory performing such detection methods uses several sections of brain and spinal cord in order to increase the chances to detect WNV.

4.2 | Diagnosis of TBEV and USUV

Diagnosis of infection and disease caused by TBEV and USUV follows the same algorithm as previously described in the WNV section.

4.3 | Diagnosis of LIV

In the field, serology plays a key role in confirming infection. The hemagglutination inhibition test can be used as a simple means of monitoring seroconversion in man and livestock.^{143,144} Because LIV is cytolitic in tissue culture, a plaque reduction neutralization test using LIV can also be used to measure antiviral titers.⁵⁴ There is no data available in horses whether neutralizing antibodies against LIV can cross-react with other flaviviruses but it must be assumed based on some limited data in humans.¹⁴⁵

4.4 | Diagnosis of EqHV

EqHV RNA can be isolated from serum or liver specimens and virus copy number can subsequently be quantified via quantitative RT-PCR. For reliable virus detection, highly conserved regions such as the 5' untranslated region (UTR) are targeted.¹⁴⁶ Moreover, the Luciferase Immunoprecipitation System (LIPS) allows semiquantitative serologic detection of anti-EqHV specific IgG antibodies, although this is available on a research basis only at this time. For the detection of previous hepatitis infection, the NS3 protease is a suitable target, since it is highly conserved.¹⁴⁶ Horses are seropositive during acute hepatitis and persistent infection.^{13,14}

4.4.1 | Panel opinion

Because horses can be EqHV viremic for months-to-years without hepatitis, a single positive serum or liver PCR does not confirm that EqHV is the cause of hepatitis. In acute EqHV infections, viremia should decline or clear within a few weeks after the onset of hepatitis, and hepatitis (especially elevated GGT) might continue past viral clearance.^{13,14} In chronic hepatitis, persistent infection should be confirmed by repeated serum RT-PCR, and other causes should be ruled

out by anamnesis, hematology, liver biopsy with culture, and investigation of the diet.

5 | TREATMENT

5.1 | Flaviviruses—WNV, USUV, TBEV, LIV

In horses, survival rate for WNV encephalitis is high compared with other infectious encephalitides (55%-70%).^{21,22,33,35} Treatment of WNND (and neuroinvasive diseases caused by TBEV) is mainly supportive. Nonsteroidal anti-inflammatory agents (flunixin-meglumine: 1.1 mg / kg, q12h IV) are often used for 3 to 7 days after onset of clinical signs to reduce inflammation and relieve pain. The use of corticosteroids is controversial because of their immunosuppressive effect that might increase viremia.¹⁴⁷ On the other hand, it can be hypothesized that the immunomodulatory effects of corticosteroids might hold benefits by reducing the amount of immune-mediated inflammation of the CNS. There is no scientific evidence supporting the use of corticosteroids, but also there are no contraindications and short-acting glucocorticoids (dexamethasone sodium: 0.05-0.1 mg/kg q24h IV) are often administered in cases of quickly deteriorating or recumbent animals. Antiviral agents and immunoglobulin therapy have been tested in studies mainly in humans and other species but did not gain widespread use due to limited data on their efficacy.¹⁴⁸

There is currently no treatment available against EqHV infections in equids. There are many direct-acting antiviral treatments for the closely related HCV, at least 1 of which, sofosbuvir, has been predicted to also bind EqHV by computer modeling.¹⁴⁹ Although it is possible that these treatments could be adapted for equine use, important hurdles remain, among these ethical concerns in sourcing such medications for equine use when they are not easily obtained for human patients.

6 | PREVENTION

Three WNV vaccines are available in the EU:

- inactivated chimeric Yellow Fever Flavivirus vaccine expressing the structural prM/E proteins of WNV¹⁵⁰
- whole inactivated vaccine containing a lineage 1 strain^{151,152}
- modified-live attenuated recombinant canarypox-based vaccine expressing the WNV prM/E proteins^{153,154}

All 3 vaccines induced protective antibody levels for a minimum of 6 to 12 months, and protected animals against the severe neurologic form of the disease in field and clinical trials.¹⁵³⁻¹⁵⁵

After intrathecal challenge, some vaccinated horses (in the inactivated and the modified-live vaccine groups) developed mild/moderate clinical signs, but none required euthanasia.³² Studies comparing the antibody response duration in horses vaccinated with either whole inactivated vaccines or the live canarypox-based vaccine have

reported contradictory results. The neutralizing antibody response induced by the live canarypox-based vaccine was higher and lasted longer than did that induced by whole inactivated vaccines.¹²⁸ However, an earlier publication found that the IgG response induced by the live canarypox-based vaccine had a lower magnitude and lasted for a shorter duration than did that induced by the killed WNV vaccine.¹⁵⁶

It is important to note that neutralizing antibodies are not the only factors involved in the protection against WNV infection and disease. Current evidence does not suggest a clear benefit of 1 vaccine over another.

6.1 | Vaccination schedule and recommendations

West Nile virus vaccines are usually recommended from 5 to 6 months of age, depending on the WNV vaccines used, with annual boost immunization (usually recommended in spring before the mosquito season). The frequency of boost immunization could be adjusted based on the epidemiological situation (eg, semiannual or more frequently in case of active circulation, at the start of the mosquito season). Vaccination of pregnant mares is recommended before the breeding season, with a boost-immunization a few weeks before foaling to provide passive immunity to foals through colostrum.

The impact of preexisting immunity (either maternal derived immunity or previous immunization) on subsequent immunization has not been fully determined.¹⁵⁵ Horses that have been subclinically infected or recovered from clinical disease might have long-term immunity, but there is only limited, unpublished data about the topic and thus it is recommended to vaccinate these horses.

Recommendation for WNV vaccination will vary from country to country. For example, the HBLB Code of conduct recommend WNV vaccination for horses traveling to countries with active WNV circulation¹⁵⁷ while the AAEP recommends immunization for all horses in North America.¹⁵⁸ In general, vaccination is recommended in endemic areas.

There are no vaccines licensed for horses against TBEV, USUV, LIV or EqHV. To date, there is no available scientific data that reports potential cross-protection induced by a flavivirus vaccine against infection with heterologous flaviviruses in horses.

7 | ONE HEALTH

7.1 | Syndromic surveillance of West Nile virus activity

Horses as nonhuman vertebrate hosts are particularly sensitive to WNV, and among clinically affected horses, approximately 10% present with neurological disorders as compared to 1% of humans^{159,160} rendering its detection in equids highly pertinent in a public health perspective.¹⁶¹ Considering that horses are spatially dispersed within

human populations, and equine WNV infections typically precede human infections,^{160,162,163} a syndromic surveillance system based on the analysis of these data could be especially useful in serving as early warning for possible human viral infections.¹⁶⁴ However, temporal distribution of previous outbreaks does not always support the hypothesis that clusters of equine cases precede human cases.^{27,33,165,166} This situation raises concerns about the suitability of passive WNV surveillance strategies in horses.

It is suspected that, by now, large numbers of horses have been exposed to WNV in many parts of Europe and have not developed clinical signs, while developing protective immunity. This, in combination with widespread vaccination, should eventually decrease the pool of susceptible horses and decrease their role in syndromic surveillance systems. On the other hand, clinical findings in horses might be of help for syndromic surveillance for WNV activity in countries so far considered to be free of WNV.¹⁶⁷

7.2 | EqHV as a model virus

EqHV is the closest known relative to HCV, an important human pathogen. Since HCV and EqHV share common features, EqHV is also an interesting surrogate model to study hepatitis virus immune responses and pathogenesis.

8 | CONCLUSION

The consensus statement covers information on flaviviruses currently circulating in Europe known to infect horses and induce clinical disease. The propensity of West Nile virus to emerge to new geographic areas has already led to a spread of the virus to formerly unaffected areas like Germany, potentially leading to emergence of the infection to other countries currently considered nonendemic for WNV. Similarly, related flaviviruses like JEV are currently not endemic to Europe but show a similar pattern of emergence. Recently, JEV has spread in Australia to new geographic regions.¹⁶⁸ Geographic expansion of flaviviruses pathogenic for equids should thus always be considered a realistic threat, and their detection should be included in surveillance programs.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Jessika-M. V. Cavalleri  <https://orcid.org/0000-0002-8575-7402>

Joy Tomlinson  <https://orcid.org/0000-0001-7365-3967>

REFERENCES

- Lecollinet S, Pronost S, Couplier M, et al. Viral equine encephalitis, a growing threat to the horse population in Europe? *Viruses*. 2019;12(1):23. doi:10.3390/v12010023
- Gather T, Walter S, Pfaender S, et al. Acute and chronic infections with nonprimate hepatitis virus in young horses. *Vet Res*. 2016;47:97.
- Scheel TK, Kapoor A, Nishiuchi E, et al. Characterization of nonprimate hepatitis virus and construction of a functional molecular clone. *Proc Natl Acad Sci U S A*. 2015;112:2192-2197.
- Tegtmeyer B, Echelmeyer J, Pfankuche VM, et al. Chronic equine hepatitis virus infection in an adult gelding with severe hepatopathy. *Vet Med*. 2019;5:372-378.
- McDonald E, Mathis S, Martin SW, Staples JE, Fischer M, Lindsey NP. Surveillance for West Nile virus disease—United States, 2009-2018. *MMWR Surveill Summ*. 2021;70:1-15.
- Petru PC, Aysel FS, Maria RS. West Nile virus in Central Europe—Pandora's box is wide open! *Travel med infect dis*. 2020;37. doi:10.1016/j.tmaid.2020.101864
- Castillo-Olivares J, Wood J. West Nile virus infection of horses. *Vet Res*. 2004;35:467-483.
- Pacenti M, Sinigaglia A, Martello T, et al. Clinical and virological findings in patients with Usutu virus infection, northern Italy, 2018. *Euro Surveill*. 2019;24:1900180.
- Camino E, Schmid S, Weber F, et al. Detection of antibodies against tick-borne encephalitis flaviviruses in breeding and sport horses from Spain. *Ticks Tick Borne Dis*. 2020;11:101487. doi:10.1016/j.ttbdis.2020.101487
- Stapleton JT, Fong S, Muerhoff AS, Bukh J, Simmonds P. The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus Pegivirus within the family *Flaviviridae*. *J Gen Virol*. 2011;92:233-246.
- Tomlinson JE, Wolfsberg R, Fahnøe U, et al. Equine pegiviruses cause persistent infection of bone marrow and are not associated with hepatitis. *PLoS Pathog*. 2020;16:e1008677.
- Walter S, Rasche A, Moreira-Soto A, et al. Differential infection patterns and recent evolutionary origins of equine hepatitis viruses in donkeys. *J Virol*. 2017;91(1):e01711-16.
- Tomlinson JE, Wolfsberg R, Fahnøe U, et al. Pathogenesis, MicroRNA-122 gene-regulation, and protective immune responses after acute equine hepatitis virus infection. *Hepatology*. 2021;74:1148-1163.
- Pfaender S, Walter S, Grabski E, et al. Immune protection against reinfection with nonprimate hepatitis virus. *Proc Natl Acad Sci U S A*. 2017;114:E2430-E2439.
- Long MT, Jeter W, Hernandez J, et al. Diagnostic performance of the equine IgM capture ELISA for serodiagnosis of West Nile virus infection. *J Vet Intern Med*. 2006;20:608-613.
- Angenvoort J, Brault AC, Bowen RA, Groschup MH. West Nile viral infection of equids. *Vet Microbiol*. 2013;167:168-180.
- Snook CS, Hyman SS, Del Piero F, et al. West Nile virus encephalomyelitis in eight horses. *J Am Vet Med Assoc*. 2001;218:1576-1579.
- Cantile C, Di Guardo G, Eleni C, et al. Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. *Equine Vet J*. 2000;32:31-35.
- Murgue B, Murri S, Zientara S, Durand B, Durand JP, Zeller H. West Nile outbreak in horses in southern France, 2000: the return after 35 years. *Emerg Infect Dis*. 2001;7:692-696.

20. Ostlund EN, Crom RL, Pedersen DD, Johnson DJ, Williams WO, Schmitt BJ. Equine West Nile encephalitis, United States. *Emerg Infect Dis.* 2001;7:665-669.
21. Ward MP, Scheurmann JA, Highfield LD, et al. Characteristics of an outbreak of West Nile virus encephalomyelitis in a previously uninfected population of horses. *Vet Microbiol.* 2006;118:255-259.
22. Kutasi O, Bakonyi T, Lecollinet S, et al. Equine encephalomyelitis outbreak caused by a genetic lineage 2 West Nile virus in Hungary. *J Vet Intern Med.* 2011;25:586-591.
23. Porter MB, Long MT, Getman LM, et al. West Nile virus encephalomyelitis in horses: 46 cases (2001). *J Am Vet Med Assoc.* 2003;222:1241-1247.
24. Garcia-Bocanegra I, Jaen-Tellez JA, Napp S, et al. West Nile fever outbreak in horses and humans, Spain, 2010. *Emerg Infect Dis.* 2011;17:2397-2399.
25. Venter M, Human S, Zaayman D, et al. Lineage 2 west nile virus as cause of fatal neurologic disease in horses, South Africa. *Emerg Infect Dis.* 2009;15:877-884.
26. de Heus P, Kolodziejek J, Camp JV, et al. Emergence of West Nile virus lineage 2 in Europe: Characteristics of the first seven cases of West Nile neuroinvasive disease in horses in Austria. *Transbound Emerg Dis.* 2020;67:1189-1197.
27. Bouzalas IG, Diakakis N, Chaintoutis SC, et al. Emergence of equine West Nile encephalitis in Central Macedonia, Greece, 2010. *Transbound Emerg Dis.* 2016;63:e219-e227.
28. Schuler LA, Khaitsa ML, Dyer NW, Stoltenow CL. Evaluation of an outbreak of West Nile virus infection in horses: 569 cases (2002). *J Am Vet Med Assoc.* 2004;225:1084-1089.
29. Ward MP, Levy M, Thacker HL, et al. Investigation of an outbreak of encephalomyelitis caused by West Nile virus in 136 horses. *J Am Vet Med Assoc.* 2004;225:84-89.
30. Bielefeldt-Ohmann H, Bosco-Lauth A, Hartwig AE, et al. Characterization of non-lethal West Nile virus (WNV) infection in horses: sub-clinical pathology and innate immune response. *Microb Pathog.* 2017;103:71-79.
31. Minke JM, Siger L, Cupillard L, et al. Protection provided by a recombinant ALVAC([R])-WNV vaccine expressing the prM/E genes of a lineage 1 strain of WNV against a virulent challenge with a lineage 2 strain. *Vaccine.* 2011;29:4608-4612.
32. Seino KK, Long MT, Gibbs EP, et al. Comparative efficacies of three commercially available vaccines against West Nile virus (WNV) in a short-duration challenge trial involving an equine WNV encephalitis model. *Clin Vaccine Immunol.* 2007;14:1465-1471.
33. Weese JS, Baird JD, DeLay J, et al. West Nile virus encephalomyelitis in horses in Ontario: 28 cases. *Can Vet J.* 2003;44:469-473.
34. Bunning ML, Bowen RA, Cropp CB, et al. Experimental infection of horses with West Nile virus. *Emerg Infect Dis.* 2002;8:380-386.
35. Salazar P, Traub-Dargatz JL, Morley PS, et al. Outcome of equids with clinical signs of West Nile virus infection and factors associated with death. *J Am Vet Med Assoc.* 2004;225:267-274.
36. Autorino GL, Battisti A, Deubel V, et al. West Nile virus epidemic in horses, Tuscany region, Italy. *Emerg Infect Dis.* 2002;8:1372-1378.
37. Durand B, Dauphin G, Zeller H, et al. Serosurvey for West Nile virus in horses in southern France. *Vet Rec.* 2005;157:711-713.
38. Perez-Ramirez E, Llorente F, del Amo J, et al. Pathogenicity evaluation of twelve West Nile virus strains belonging to four lineages from five continents in a mouse model: discrimination between three pathogenicity categories. *J Gen Virol.* 2017;98:662-670.
39. Müller K. *Untersuchung zum Vorkommen von Antikörpern gegen das "Tick Borne Encephalitis Virus" (TBEV) beim Pferd im Endemiegebiet Marburg-Biedenkopf.* Germany: Universitätsbibliothek Giessen; 2006.
40. Luckschander N, Kolbl S, Enzesberger O, et al. Tick borne encephalitis (TBE) in an Austrian horse population. *Tierarztl Prax Ausg Grobtiere Nutztiere.* 1999;27:235-238.
41. Fouché N, Oesch S, Ziegler U, Gerber V. Clinical presentation and laboratory diagnostic work-up of a horse with tick-borne encephalitis in Switzerland. *Viruses.* 2021;13:13.
42. Conze TM, Bago Z, Revilla-Fernandez S, et al. Tick-borne encephalitis virus (TBEV) infection in two horses. *Viruses.* 2021;13(9):1775.
43. Waldvogel A, Matile H, Wegmann C, Wyler R, Kunz C. Tick-borne encephalitis in the horse. *Schweiz Arch Tierheilkd.* 1981;123:227-233.
44. Vilibic-Cavlek T, Kaic B, Barbic L, et al. First evidence of simultaneous occurrence of West Nile virus and Usutu virus neuroinvasive disease in humans in Croatia during the 2013 outbreak. *Infection.* 2014;42:689-695.
45. Zelena H, Kleinerova J, Sikutova S, et al. First autochthonous West Nile lineage 2 and Usutu virus infections in humans, July to October 2018, Czech Republic. *Pathogens.* 2021;2021:10.
46. Simonin Y, Sillam O, Carles MJ, et al. Human Usutu virus infection with atypical neurologic presentation, Montpellier, France, 2016. *Emerg Infect Dis.* 2018;24:875-878.
47. Santini M, Vilibic-Cavlek T, Barsic B, et al. First cases of human Usutu virus neuroinvasive infection in Croatia, August-September 2013: clinical and laboratory features. *J Neurovirol.* 2015;21:92-97.
48. Pecorari M, Longo G, Gennari W, et al. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. *Euro Surveill.* 2009;14:19446.
49. Nagy A, Mezei E, Nagy O, et al. Extraordinary increase in West Nile virus cases and first confirmed human Usutu virus infection in Hungary, 2018. *Euro Surveill.* 2019;24:1900038. doi:10.2807/1560-7917.ES.2019.2824.2828.1900038
50. Domanović D, Gossner CM, Lieshout-Krikke R, et al. West Nile and Usutu virus infections and challenges to blood safety in the European Union. *Emerg Infect Dis.* 2019;25:1057. doi:10.3201/eid2506.181755
51. Vazquez A, Jimenez-Clavero M, Franco L, et al. Usutu virus: potential risk of human disease in Europe. *Euro Surveill.* 2011;16:19935.
52. Barbic L, Vilibic-Cavlek T, Listes E, et al. 20. Demonstration of Usutu virus antibodies in horses, Croatia. *Vector Borne Zoonot Dis.* 2013;13:772-774.
53. Bazanow B, Jansen van Vuren P. Survey on West Nile and Usutu viruses in horses and birds in Poland. *Viruses.* 2018;10:87.
54. Jeffries C, Mansfield K, Phipps L, et al. Louping ill virus: an endemic tick-borne disease of Great Britain. *J Gen Virol.* 2014;95:1005-1014.
55. Twomey DF, Cranwell MP, Reid HW, Tan JF. Louping ill on Dartmoor. *Vet Rec.* 2001;149:687.
56. Timoney P, Donnelly W, Clements L, et al. Encephalitis caused by louping ill virus in a group of horses in Ireland. *Equine Vet J.* 1976;8:113-117.
57. Elia G, Lanave G, Lorusso E, et al. Equine hepatitis C virus persistent infection in a horse with chronic wasting. *Transbound Emerg Dis.* 2017;64:1354-1358.
58. Pfaender S, Cavalleri JMV, Walter S, et al. Clinical course of infection and viral tissue tropism of hepatitis C virus-like nonprimate hepatitis viruses in horses. *Hepatology (Baltimore, Md).* 2015;61:447-459.
59. Ramsay JD, Evanoff R, Wilkinson TE Jr, Divers TJ, Knowles DP, Mealey RH. Experimental transmission of equine hepatitis C virus as a model for hepatitis C virus. *Hepatology.* 2015;61:1533-1546.
60. Payne S. In: Payne S, ed. *Family Flaviviridae.* Viruses: Viruses Academic Press; 2017:129-139.
61. van der Meulen KM, Pensaert MB, Nauwynck HJ. West Nile virus in the vertebrate world. *Arch Virol.* 2005;150:637-657.
62. Mancini G, Montarsi F, Calzolari M, et al. Mosquito species involved in the circulation of West Nile and Usutu viruses in Italy. *Vet Ital.* 2017;53:97-110.
63. Colpitts TM, Conway MJ, Montgomery RR, Fikrig E. West Nile virus: biology, transmission, and human infection. *Clin Microbiol Rev.* 2012;25:635-648.

64. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol.* 2006;4:e82.
65. Taieb L, Ludwig A, Ogden NH, et al. Bird species involved in West Nile virus epidemiological cycle in southern Quebec. *Int J Environ Res Public Health.* 2020;17:4517.
66. Prow NA. The changing epidemiology of Kunjin virus in Australia. *Int J Environ Res Public Health.* 2013;10:6255-6272.
67. Vasic A, Oslobanu LE, Marinov M, et al. Evidence of West Nile virus (WNV) circulation in wild birds and WNV RNA negativity in mosquitoes of the Danube Delta Biosphere Reserve, Romania, 2016. *Trop Med Infect Dis.* 2019;4:116.
68. Napp S, Petric D, Busquets N. West Nile virus and other mosquito-borne viruses present in Eastern Europe. *Pathog Glob Health.* 2018;112:233-248.
69. Rudolf I, Betasova L, Blazejova H, et al. West Nile virus in overwintering mosquitoes, Central Europe. *Parasit Vectors.* 2017;10:452.
70. Farajollahi A, Crans WJ, Bryant P, et al. Detection of West Nile viral RNA from an overwintering pool of *Culex pipiens pipiens* (Diptera: Culicidae) in New Jersey, 2003. *J Med Entomol.* 2005;42:490-494.
71. Roesch F, Fajardo A, Moratorio G, Vignuzzi M. Usutu virus: an arbovirus on the rise. *Viruses.* 2019;11:640.
72. Suss J. Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine.* 2003;21(Suppl 1):S19-S35.
73. Macleod J, Gordon WS. Studies in louping-ill (an encephalomyelitis of sheep). II. Transmission by the sheep tick, *Ixodes ricinus* L. *J Comp Pathol.* 1932;45:240-256.
74. Reid HW, Buxton D, Pow I, Finlayson J. Transmission of louping-ill virus in goat milk. *Vet Rec.* 1984;114:163-165.
75. Reid HW, Pow I. Excretion of louping-ill virus in ewes' milk. *Vet Rec.* 1985;117:470.
76. Scheel TKH, Kapoor A, Nishiuchi E, et al. Characterization of nonprimate hepacivirus and construction of a functional molecular clone. *Proc Natl Acad Sci U S A.* 2015;112:2192-2197.
77. Pronost S, Fortier C, Marcillaud-Pitel C, et al. Further evidence for in utero transmission of equine hepacivirus to foals. *Viruses.* 2019;11:11.
78. Gather T, Walter S, Todt D, et al. Vertical transmission of hepatitis C virus-like non-primate hepacivirus in horses. *J Gen Virol.* 2016;97:2540-2551.
79. Badenhorst M, de Heus P, Auer A, et al. No evidence of mosquito involvement in the transmission of equine hepacivirus (*Flaviviridae*) in an epidemiological survey of Austrian horses. *Viruses.* 2019;11:1014.
80. Im JH, Baek JH, Durey A, Kwon HY, Chung MH, Lee JS. Geographic distribution of tick-borne encephalitis virus complex. *J Vector Borne Dis.* 2020;57:14-22.
81. Smithburn E, Hughes T, Burke A, et al. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Trop Med Hyg.* 1940;20:471-492.
82. Chancey C, Grinev A, Volkova E, et al. The global ecology and epidemiology of West Nile virus. *Biomed Res Int.* 2015;2015:376230.
83. Hannoun C, Panthier R, Mouchet J, et al. Isolement en France du virus West-Nile à partir de malades et du vecteur *Culex modestus* Ficalbi. *Comptes Rendus Hebd Seances Acad Sci.* 1964;259:4170.
84. Zeller HG, Schuffenecker I. West Nile virus: an overview of its spread in Europe and the Mediterranean basin in contrast to its spread in the Americas. *Eur J Clin Microbiol Infect Dis.* 2004;23:147-156.
85. Bakonyi T, Ivanics É, Erdélyi K, et al. Lineage 1 and 2 strains of encephalitic West Nile virus, Central Europe. *Emerg Infect Dis.* 2006;12:618-623. [10.3201/eid1204.051379](https://doi.org/10.3201/eid1204.051379)
86. Papa A, Danis K, Baka A, et al. Ongoing outbreak of West Nile virus infections in humans in Greece, July–August 2010. *Euro Surveill.* 2010;15:19644.
87. Aguilera-Sepulveda P, Napp S, Llorente F, et al. West Nile virus lineage 2 spreads westwards in Europe and overwinters in north-eastern Spain (2017–2020). *Viruses.* 2022;14:569.
88. Hernandez-Triana LM, Jeries CL, Mansfield KL, et al. Emergence of West Nile virus lineage 2 in Europe: a review on the introduction and spread of a mosquito-borne disease. *Front Public Health.* 2014;2:271.
89. Bakonyi T, Haussig JM. West Nile virus keeps on moving up in Europe. *Euro Surveill.* 2020;25:2001938. doi:[10.2807/1560-7917.ES.2020.2825.2846.2001938](https://doi.org/10.2807/1560-7917.ES.2020.2825.2846.2001938)
90. Bakonyi T, Ferenczi E, Erdelyi K, et al. Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. *Vet Microbiol.* 2013;165:61-70.
91. Zehender G, Veo C, Ebranati E, et al. Reconstructing the recent West Nile virus lineage 2 epidemic in Europe and Italy using discrete and continuous phylogeography. *PLoS One.* 2017;12:e0179679.
92. Sirbu A, Ceianu CS, Panculescu-Gatej RI, et al. Outbreak of West Nile virus infection in humans, Romania, July to October 2010. *Eurosurveillance.* 2011;16:19762.
93. Merdic E, Peric L, Pandak N, et al. West Nile virus outbreak in humans in Croatia, 2012. *Coll Antropol.* 2012;37:943-947.
94. Beck C, Leparc Goffart I, Franke F, et al. Contrasted epidemiological patterns of West Nile virus lineages 1 and 2 infections in France from 2015 to 2019. *Pathogens.* 2020;9(11):908. doi:[10.3390/pathogens9110908](https://doi.org/10.3390/pathogens9110908)
95. Bergmann F, Trachsel DS, Stoeckle SD, et al. Seroepidemiological survey of West Nile virus infections in horses from Berlin/Brandenburg and North Rhine-Westphalia, Germany. *Viruses Basel.* 2022;14:14.
96. Vlaskamp D, Thijsen S, Reimerink J, et al. First autochthonous West Nile virus infections in The Netherlands; from one to six cases, July to August 2020. *Euro Surveill.* 2020;25:2001904. doi:[10.2807/1560-7917.ES.2020.25.46.2001904](https://doi.org/10.2807/1560-7917.ES.2020.25.46.2001904)
97. Sikkema RS, Schrama M, Tvd B, et al. Detection of West Nile virus in a common whitethroat (*Curruca communis*) and *Culex* mosquitoes in The Netherlands, 2020. *Euro Surveill.* 2020;25:2001704. doi:[10.2807/1560-7917.ES.2020.2825.2840.2001704](https://doi.org/10.2807/1560-7917.ES.2020.2825.2840.2001704)
98. Platonov AE, Karan LS, Shopenskaia TA, et al. Genotyping of West Nile fever virus strains circulating in southern Russia as an epidemiological investigation method: principles and results. *Zhurnal Mikrobiol Epidemiol Immunobiol.* 2011;2:29-37.
99. ECDC. *Epidemiological Update: West Nile Virus Transmission Season in Europe.* 2018. <https://www.ecdc.europa.eu/en/news-events/epidemiological-update-west-nile-virus-transmission-season-europe-2018>. Accessed October 16, 2022.
100. ECDC. The European Union one health 2018 Zoonoses report. *EFSA J.* 2019;17.
101. ECDC. *Communicable Disease Threats Report Week 23, May 31-June 6, 2020.* 2020. <https://www.ecdc.europa.eu/sites/default/files/documents/Communicable-disease-threats-report-6-june-2020-PUBLIC.pdf>. Accessed October 16, 2022.
102. MoH S, CdCdAyE S. *Evaluación rápida de riesgo: Meningoencefalitis por el virus del Nilo occidental en España (1ª actualización).* [Rapid risk assessment: Meningoencephalitis caused by West Nile virus in Spain (1st update)]. Madrid: Ministry of Health; In Spanish. https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/docs/20201009_ERR_Nilo_Occidental.pdf. Accessed October 9, 2020
103. Engel D, Jost H, Wink M, et al. Reconstruction of the evolutionary history and dispersal of Usutu virus, a neglected emerging arbovirus in Europe and Africa. *MBio.* 2016;7:e01938-01915.
104. Folly AJ, Lawson B, Lean FZ, et al. Detection of Usutu virus infection in wild birds in the United Kingdom, 2020. *Euro Surveill.* 2020;25:2001732. doi:[10.2807/1560-7917.ES.2020.2825.2841.2001732](https://doi.org/10.2807/1560-7917.ES.2020.2825.2841.2001732)

105. Ziegler U, Fast C, Eiden M, et al. Evidence for an independent third Usutu virus introduction into Germany. *BMC Vet Res.* 2016;192:60-66.
106. Eiden M, Gil M, Ziegler U, et al. Emergence of two Usutu virus lineages in *Culex pipiens* mosquitoes in the Camargue, France, 2015. *Infect Genet Evol.* 2018;61:151-154.
107. Csank T, Drzewniokova P, Korytar L, et al. A serosurvey of flavivirus infection in horses and birds in Slovakia. *Vector Borne Zoonotic Dis.* 2018;18:206-213.
108. Pautienius A, Armonaite A, Simkute E, et al. Cross-sectional study on the prevalence and factors influencing occurrence of tick-borne encephalitis in horses in Lithuania. *Pathogens.* 2021;10:2001732.
109. Rushton JO, Lecollinet S, Hubalek Z, et al. Tick-borne encephalitis virus in horses, Austria, 2011. *Emerg Infect Dis.* 2013;19:635-637.
110. de Heus P, Kolodziejek J, Hubálek Z, et al. West Nile virus and tick-borne encephalitis virus are endemic in equids in eastern Austria. *Viruses.* 2021;13:1873.
111. Klaus C, Hörügel U, Hoffmann B, Beer M. Tick-borne encephalitis virus (TBEV) infection in horses: clinical and laboratory findings and epidemiological investigations. *Vet Microbiol.* 2013;163:368-372.
112. Balseiro A, Royo L, Martinez C, et al. Louping ill in goats, Spain, 2011. *Emerg Infect Dis.* 2012;18:976-978.
113. Hyde J, Nettleton P, Marriott L, et al. Louping ill in horses. *Vet Rec.* 2007;160:532. doi:10.1136/vr.1160.1115.1532
114. Gemaque BS, de Souza AJS, Soares MCP, et al. Hepacivirus infection in domestic horses, Brazil, 2011-2013. *Emerg Infect Dis.* 2014;20:2180.
115. Lyons S, Kapoor A, Schneider BS, et al. Viraemic frequencies and seroprevalence of non-primate hepacivirus and equine pegiviruses in horses and other mammalian species. *J Gen Virol.* 2014;95:1701-1711.
116. Tanaka T, Kasai H, Yamashita A, et al. Hallmarks of hepatitis C virus in equine hepacivirus. *J Virol.* 2014;88:13352-13366.
117. Reuter G, Maza N, Pankovics P, Boros Á. Non-primate hepacivirus infection with apparent hepatitis in a horse—short communication. *Acta Vet Hung.* 2014;62:422-427.
118. Lu G, Sun L, Xu T, et al. First description of hepacivirus and pegivirus infection in domestic horses in China: a study in Guangdong Province, Heilongjiang Province and Hong Kong District. *PLoS One.* 2016;11:e0155662.
119. Reichert C, Campe A, Walter S, et al. Frequent occurrence of nonprimate hepacivirus infections in thoroughbred breeding horses—a cross-sectional study for the occurrence of infections and potential risk factors. *Vet Microbiol.* 2017;203:315-322.
120. Date T, Sugiyama M, Lkhagvasuren D, Wakita T, Oyunsuren T, Mizokami M. Prevalence of equine hepacivirus infection in Mongolia. *Virus Res.* 2020;282:197940.
121. Bartenschlager R, Lohmann V. Replication of hepatitis C virus. *J Gen Virol.* 2000;81:1631-1648.
122. Trock SC, Meade BJ, Glaser AL, et al. West Nile virus outbreak among horses in New York state, 1999 and 2000. *Emerg Infect Dis.* 2001;7:745-747.
123. Wamsley HL, Alleman AR, Porter MB, Long MT. Findings in cerebrospinal fluids of horses infected with West Nile virus: 30 cases (2001). *J Am Vet Med Assoc.* 2002;221:1303-1305.
124. Kutasi O, Feher O, Sardi S, et al. Characterisation of the cerebrospinal fluid of horses with West Nile virus neuroinvasive disease. *Acta Vet Hung.* 2020;68:177-185.
125. Ozkul A, Ergunay K, Koysuren A, et al. Concurrent occurrence of human and equine West Nile virus infections in Central Anatolia, Turkey: the first evidence for circulation of lineage 1 viruses. *Int J Infect Dis.* 2013;17:e546-e551.
126. Hirota J, Shimizu S, Shibahara T. Application of West Nile virus diagnostic techniques. *Expert Rev Anti Infect Ther.* 2013;11:793-803.
127. Kaaijk P, Luytjes W. Are we prepared for emerging flaviviruses in Europe? Challenges for vaccination. *Hum Vaccin Immunother.* 2018;14:337-344.
128. Monaco F, Purpari G, Di Gennaro A, et al. Immunological response in horses following West Nile virus vaccination with inactivated or recombinant vaccine. *Vet Ital.* 2019;55:73-79.
129. Joo K, Bakonyi T, Szenci O, et al. Comparison of assays for the detection of West Nile virus antibodies in equine serum after natural infection or vaccination. *Vet Immunol Immunopathol.* 2017;183:1-6.
130. Porter MB, Long M, Gosche DG, et al. Immunoglobulin M-capture enzyme-linked immunosorbent assay testing of cerebrospinal fluid and serum from horses exposed to west nile virus by vaccination or natural infection. *J Vet Intern Med.* 2004;18:866-870.
131. Martin DA, Biggerstaff BJ, Allen B, Johnson AJ, Lanciotti RS, Roehrig JT. Use of immunoglobulin m cross-reactions in differential diagnosis of human flaviviral encephalitis infections in the United States. *Clin Diagn Lab Immunol.* 2002;9:544-549.
132. Klaus C, Ziegler U, Kalthoff D, Hoffmann B, Beer M. Tick-borne encephalitis virus (TBEV)—findings on cross reactivity and longevity of TBEV antibodies in animal sera. *BMC Vet Res.* 2014;10:78.
133. Endale A, Medhin G, Darfiro K, Kebede N, Legesse M. Magnitude of antibody cross-reactivity in medically important mosquito-borne flaviviruses: a systematic review. *Infect Drug Resist.* 2021;14:4291-4299.
134. Yeh JY, Lee JH, Park JY, et al. A diagnostic algorithm to serologically differentiate West Nile virus from Japanese encephalitis virus infections and its validation in field surveillance of poultry and horses. *Vector Borne Zoonotic Dis.* 2012;12:372-379.
135. Beck C, Lowenski S, Durand B, Bahuon C, Zientara S, Lecollinet S. Improved reliability of serological tools for the diagnosis of West Nile fever in horses within Europe. *PLoS Negl Trop Dis.* 2017;11:e0005936.
136. Balasuriya UB, Shi PY, Wong SJ, et al. Detection of antibodies to West Nile virus in equine sera using microsphere immunoassay. *J Vet Diagn Invest.* 2006;18:392-395.
137. Vasil'ev AV, Shchelkanov M, Dzharfenov AF, et al. West Nile virus infection of agricultural animals in the Astrakhan region, as evidenced by the 2001-2004 serological surveys. *Vopr Virusol.* 2005;50:36-41.
138. Sutherland LJ, Cash AA, Huang YJ, et al. Serologic evidence of arboviral infections among humans in Kenya. *Am J Trop Med Hyg.* 2011;85:158-161.
139. Toplu N, Oguzoglu TC, Ural K, et al. West Nile virus infection in horses: detection by immunohistochemistry, in situ hybridization, and ELISA. *Vet Pathol.* 2015;52:1073-1076.
140. Kleiboeker SB, Loiacono CM, Rottinghaus A, Pue HL, Johnson GC. Diagnosis of West Nile virus infection in horses. *J Vet Diagn Invest.* 2004;16:2-10.
141. Pennick KE, McKnight CA, Patterson JS, et al. Diagnostic sensitivity and specificity of in situ hybridization and immunohistochemistry for Eastern equine encephalitis virus and West Nile virus in formalin-fixed, paraffin-embedded brain tissue of horses. *J Vet Diagn Invest.* 2012;24:333-338.
142. Cantile C, Del Piero F, Di Guardo G, et al. Pathologic and immunohistochemical findings in naturally occurring West Nile virus infection in horses. *Vet Pathol.* 2001;38:414-421.
143. Williams H, Thorburn H. Serum antibodies to louping-ill virus. *Scott Med J.* 1962;7:353-355.
144. Laurenson MK, McKendrick IJ, Reid HW, et al. Prevalence, spatial distribution and the effect of control measures on louping-ill virus in the Forest of Bowland, Lancashire. *Epidemiol Infect.* 2007;135:963-973.
145. Mansfield KL, Horton DL, Johnson N, et al. Flavivirus-induced antibody cross-reactivity. *J Gen Virol.* 2011;92:2821-2829.

146. Burbelo PD, Dubovi EJ, Simmonds P, et al. Serology-enabled discovery of genetically diverse hepaciviruses in a new host. *J Virol*. 2012; 86:6171-6178.
147. Bowen RA, Rouge MM, Siger L, et al. Pathogenesis of West Nile virus infection in dogs treated with glucocorticoids. *Am J Trop Med Hyg*. 2006;74:670-673.
148. Nath A, Tyler KL. Novel approaches and challenges to treatment of central nervous system viral infections. *Ann Neurol*. 2013;74: 412-422.
149. de Albuquerque P, Santos LHS, Antunes D, et al. Structural insights into NS5B protein of novel equine hepaciviruses and pegiviruses complexed with polymerase inhibitors. *Virus Res*. 2020;278:197867.
150. EMA. *CVMP Assessment Report for Equilis West Nile (EMEA/V/C/002241/0000)*. 2013. https://www.ema.europa.eu/en/documents/assessment-report/equilis-west-nile-epar-public-assessment-report_en.pdf. Accessed June 23, 2022.
151. EMA. *Equip WN Annex I Summary of Product Characteristics*. 2013. https://www.ema.europa.eu/en/documents/product-information/equip-wnv-epar-product-information_en.pdf. Accessed June 23, 2022.
152. Chaintoutis SC, Diakakis N, Papanastassopoulou M, Banos G, Dovas CI. Evaluation of cross-protection of a lineage 1 West Nile virus inactivated vaccine against natural infections from a virulent lineage 2 strain in horses, under field conditions. *Clin Vaccine Immunol*. 2015;22:1040-1049.
153. EMA. *Proteq West Nile (West Nile recombinant canarypox virus [vCP2017])*. 2011. https://www.ema.europa.eu/en/documents/overview/proteq-west-nile-epar-medicine-overview_en.pdf. Accessed June 23, 2022.
154. EMA. *Proteq West Nile Annex I Summary of Product Characteristics*. 2016. https://www.ema.europa.eu/en/documents/product-information/proteq-west-nile-epar-product-information_en.pdf. Accessed June 23, 2022.
155. El Garch H, Minke JM, Rehder J, et al. A West Nile virus (WNV) recombinant canarypox virus vaccine elicits WNV-specific neutralizing antibodies and cell-mediated immune responses in the horse. *Vet Immunol Immunopathol*. 2008;123:230-239.
156. Khatibzadeh SM, Gold CB, Keggan AE, et al. West Nile virus-specific immunoglobulin isotype responses in vaccinated and infected horses. *Am J Vet Res*. 2015;76:92-100.
157. HBLB. *International Codes of Practice: West Nile Fever*. 2022. <https://codes.hblb.org.uk/index.php/page/174>. Accessed June 23, 2022.
158. AAEP. *AAEP West Nile Virus Vaccination Guidelines*. 2012. <https://aaep.org/issue/west-nile-virus-vaccination-guidelines>. Accessed June 23, 2022.
159. Petersen LR, Roehrig JT. West Nile virus: a reemerging global pathogen. *Emerg Infect Dis*. 2001;7:611-614.
160. Leblond A, Hendrikx P, Sabatier P. West Nile virus outbreak detection using syndromic monitoring in horses. *Vector Borne Zoonotic Dis*. 2007;7:403-410.
161. Saegerman C, Alba-Casals A, Garcia-Bocanegra I, et al. Clinical sentinel surveillance of equine West Nile fever, Spain. *Transbound Emerg Dis*. 2016;63:184-193.
162. Ward MP, Scheurmann JA. The relationship between equine and human West Nile virus disease occurrence. *Vet Microbiol*. 2008;129: 378-383.
163. Kulasekera VL, Kramer L, Nasci RS, et al. West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. *Emerg Infect Dis*. 2001;7:722-725.
164. Faverjon C, Vial F, Andersson MG, et al. Early detection of West Nile virus in France: quantitative assessment of syndromic surveillance system using nervous signs in horses. *Epidemiol Infect*. 2017; 145:1044-1057.
165. Corrigan RL, Waldner C, Epp T, et al. Prediction of human cases of West Nile virus by equine cases, Saskatchewan, Canada, 2003. *Prev Vet Med*. 2006;76:263-272.
166. Control ECfDPa. *Historical Data by Year—West Nile Virus Seasonal Surveillance*. 2021. <https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical>. Accessed October 16, 2022.
167. van Galen G, Calozet L, Leblond A, et al. Can horses be clinically screened for West Nile fever? *Vet Rec*. 2013;172:101.
168. van den Hurk AF, Skinner E, Ritchie SA, et al. The emergence of Japanese encephalitis virus in Australia in 2022: existing knowledge of mosquito vectors. *Viruses*. 2022;14:1208.
169. Abbadi I, Lkhider M, Kitab B, et al. Non-primate hepacivirus transmission and prevalence: novel findings of virus circulation in horses and dogs in Morocco. *Infect Genet Evol*. 2021;93:104975.
170. Lyons S, Kapoor A, Sharp C, et al. Nonprimate hepaciviruses in domestic horses, United Kingdom. *Emerg Infect Dis*. 2012;18:1976-1982.
171. Drexler JF, Corman VM, Muller MA, et al. Evidence for novel hepaciviruses in rodents. *PLoS Pathog*. 2013;9:e1003438.
172. Matsuu A, Hobo S, Ando K, et al. Genetic and serological surveillance for non-primate hepacivirus in horses in Japan. *Vet Microbiol*. 2015;179:219-227.
173. Pronost S, Hue E, Fortier C, et al. Prevalence of equine hepacivirus infections in France and evidence for two viral subtypes circulating worldwide. *Transbound Emerg Dis*. 2017;64:1884-1897.
174. Badenhorst M, Tegtmeyer B, Todt D, et al. First detection and frequent occurrence of equine hepacivirus in horses on the African continent. *Vet Microbiol*. 2018;223:51-58.

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