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

Jessika-m V Cavalleri, Orsolya Korbacska-Kutasi, Agnès Leblond, Romain Paillot, Nicola Pusterla, Eike Steinmann, Joy Tomlinson

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CONSENSUS STATEMENT

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

Jessika-M. V. Cavalleri[1] | **Orsolya Korbacska-Kutasi**[1,2,3] | **Agnès Leblond**[4] | **Romain Paillot**[5] | **Nicola Pusterla**[6] | **Eike Steinmann**[7] | **Joy Tomlinson**[8]

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

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

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

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

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

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

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

[8]Baker 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 flavi- 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, immunehistochemistry; 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.

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 hepaciviruses[1-7] (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 al[19]) compared to outbreak evaluations in North America (up to 1698 cases in Texas outbreak by Ward et al[21]). 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,

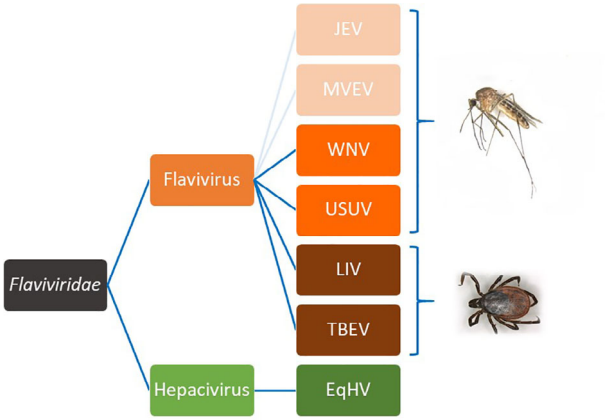

FIGURE 1 Equine pathogens in the *Flaviviridae* family: Japanese encephalitis virus (JEV), Murray Valley Encephalitis Virus (MVEV), West Nile virus (WNV) and Usutu virus (USUV) are classified in the Japanese encephalitis antigenic complex transmitted by ornithophilic mosquito species, mainly *Culex* 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 pyrexic (up to 40.5°C) and inappetent.[55,56]

2.5 | EqHV clinical disease

Equine hepaciviraus 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-vectored. 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

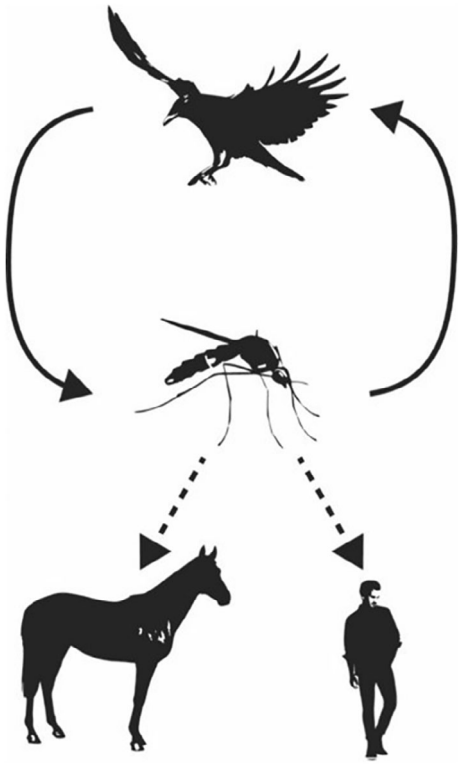

FIGURE 2 West Nile Virus (WNV) and Usutu virus (USUV) undergo sylvatic circulation between birds and mosquitoes. Birds serve as an amplifying reservoir host. *Culex* spp. are the major mosquito vectors of both viruses worldwide. Mosquitoes can transmit vertically to maintain the virus through the winter. 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 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.

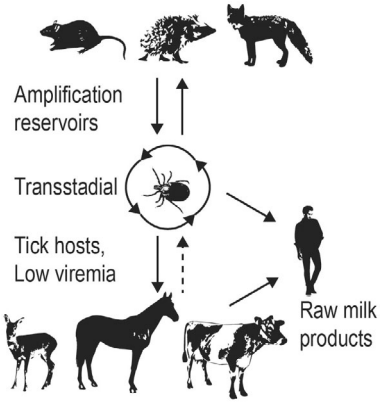

FIGURE 3 Tick-borne encephalitis virus and LIV transmission involves a complex ecology of reservoir species, ticks and indicator species that support tick populations. Small vertebrates including rodents, insectivores, and wild carnivores serve as amplifying reservoirs, developing high titer viremia 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

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 outbreaks[99] (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 Germany[105] and the Camargue area of France.[106]

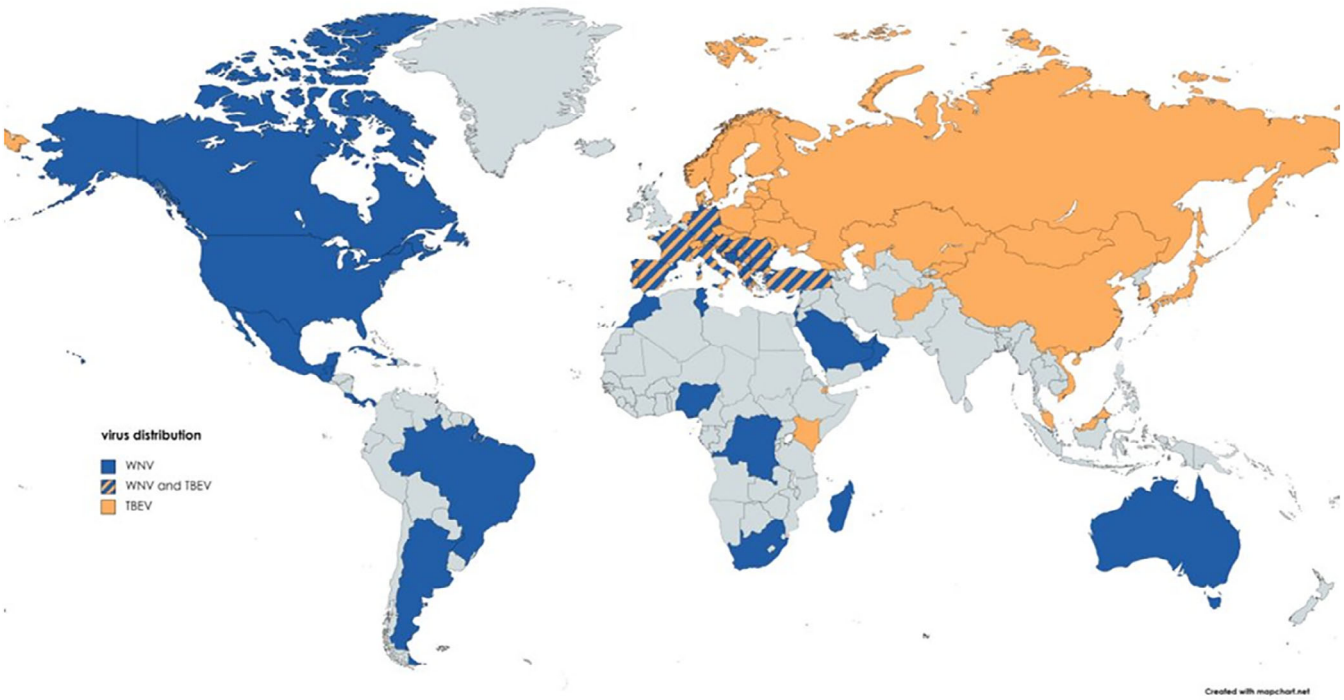

FIGURE 4 Global distribution of WNV infections reported to OIE 2005-2021 and TBEV infections according to Im et al.[80] WNV and TBEV cocirculate in Europe. Countries in gray: no data reported

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) clade[87] (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 hepacivirus 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]

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

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 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 WNND. 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

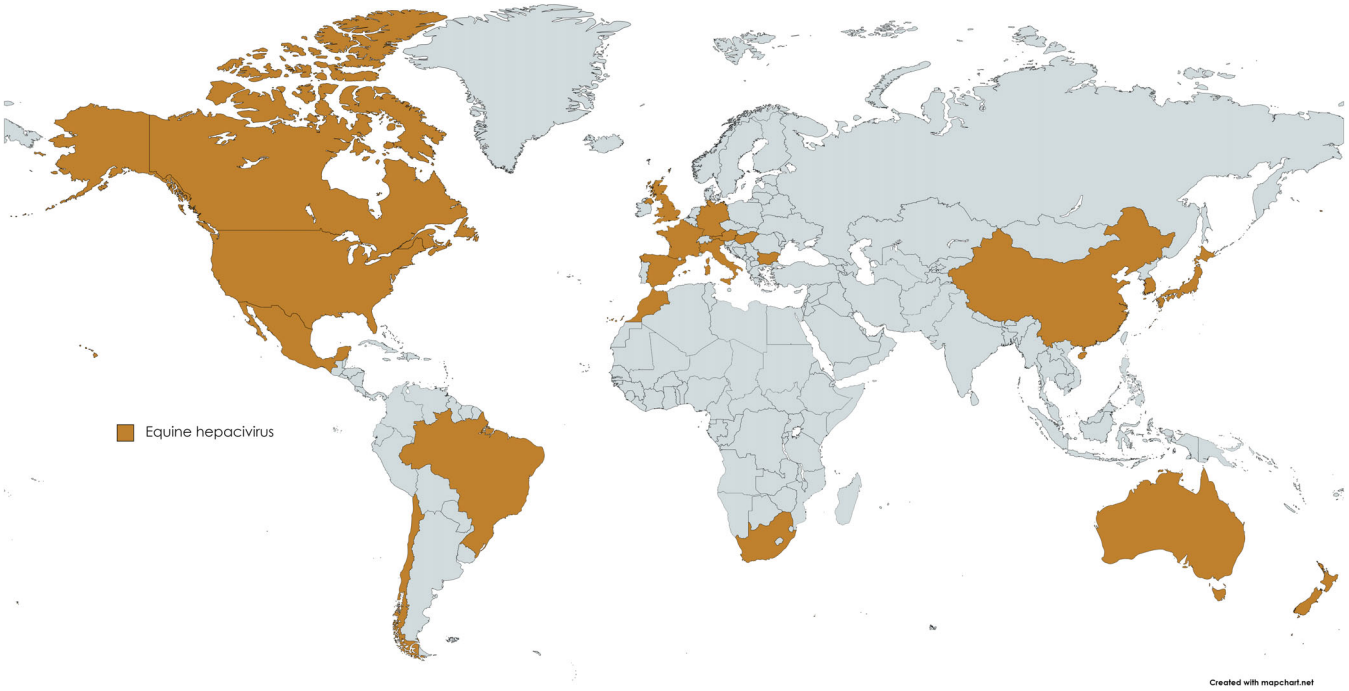

FIGURE 5 Global distribution of EqHV.[58,79,114,116,117,119,146,169-174] Countries in gray: no data reported

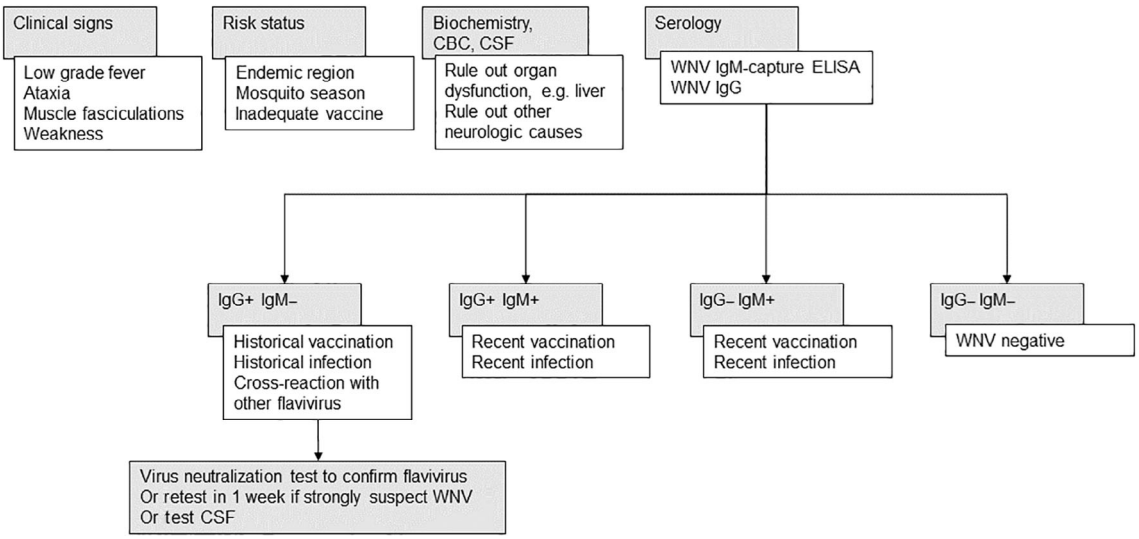

FIGURE 6 Recommended diagnostic algorithm for WNND. Results of rapid IgG tests must be interpreted in the context of IgM results, or with virus neutralization to differentiate between WNV, USUV, TBEV, and LIV infection

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 cytolytic 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 hepacivirus 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 WNV[150]
- 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, nonpublished 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 circulation[157] 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 hepacivirus 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

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