REVIEW ARTICLE

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Epidemiological surveillance of West Nile virus in the world and Brazil: relevance of equine surveillance in the context of "One Health"

Vigilância epidemiológica do vírus do Nilo Ocidental no mundo e no Brasil: relevância da vigilância equina no contexto de saúde única

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ABSTRACT

West Nile virus (WNV) is a neurovirulent mosquito-borne *Flavivirus* that is maintained in nature by a zoonotic transmission cycle between avian hosts and ornithophilic mosquito vectors, mostly from the *Culex* genus. Until the 1990s, WNV was considered to be an old-world arbovirus, but in 1999, WNV emerged in the United States (US) and spread rapidly, becoming a major threat to public health. WNV adapted to the transmission cycle involving American mosquitoes and birds and reached Central and South America in subsequent years. In 2003, the National West Nile Fever Surveillance System was created in Brazil based on serological screening of animals and sentinel vectors, as recommended by the Pan American Health Organization (PAHO) and the World Health Organization (WHO). Since 2008, serological evidence of WNV infection in Brazilian horses has been reported, and the circulation of WNV has been monitored through the regular serological screening of sentinel horses and reporting of encephalomyelitis cases. Horses are highly susceptible to WNV infection, and outbreaks of neurological disease among horses often precede human cases. In this regard, equine surveillance has been essential in providing early warning to public and animal health authorities in several countries, including Brazil. This demonstrates the need for animal and public health intervention programs to allocate resources to make veterinarians aware of the role they can play in the human surveillance processes by monitoring horses. This review discusses the importance of equine surveillance and the gap that veterinarians can fill on the front line in human surveillance, in Brazil and worldwide, in the context of "One Health".

Keywords: Equine surveillance. West Nile virus. Flavivirus. Awareness. Brazil.

RESUMO

O vírus do Nilo Ocidental (WNV) é um flavivírus neuropatogênico transmitido por mosquito, mantido na natureza em um ciclo de transmissão zoonótica entre as aves e os mosquitos ornitofílicos, principalmente do gênero *Culex*. Até a década de 1990, o WNV era considerado um arbovírus do mundo antigo, mas em 1999 surgiu nos Estados Unidos da América e se espalhou rapidamente, tornando-se uma grande ameaça à saúde pública. O WNV se adaptou ao ciclo envolvendo mosquitos e pássaros americanos e chegou à América Central e do Sul nos anos subsequentes. Em 2003, o Sistema Nacional de Vigilância da Febre do Nilo Ocidental no Brasil foi criado com base na triagem sorológica de animais sentinelas e vetores, conforme recomendado pela Organização Pan-Americana da Saúde (OPAS) e pela Organização Mundial da Saúde (OMS). Desde 2008, evidências sorológicas de infecção por WNV em equinos brasileiros têm sido relatadas e a circulação do WNV monitorada por meio de triagem sorológica de cavalos sentinelas, além da notificação de casos de encefalomielite. Os equinos são altamente suscetíveis ao WNV e surtos de doenças neurológicas geralmente precedem casos humanos. Nesse sentido, a vigilância equina tem sido essencial para fornecer um alerta precoce às autoridades de saúde pública e animal em vários países, incluindo o Brasil. Isso demonstra a necessidade de programas de intervenção em saúde pública e animal para alocar recursos e conscientizar os médicos veterinários sobre seu papel em processos de vigilância humana que envolvam equinos. Nesta revisão, é discutida a importância da vigilância equina e dos médicos veterinários como linha de frente na vigilância humana no Brasil e no mundo, no contexto de saúde única.

Palavras-chave: Vigilância equina. Vírus do Nilo Ocidental. Conscientização. Flavivírus. Brasil.

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Introduction

West Nile virus (WNV) is an enveloped positive-sense single stranded RNA virus of the genus *Flavivirus*, family *Flaviviridae,* and it is classified in the Japanese Encephalitis (JE) antigen complex, with Japanese Encephalitis Virus (JE) and Saint Louis Encephalitis Virus (SLEV) (Simmonds et al., 2017; Castillo-Olivares & Wood, 2004). Currently, the virus is classified into nine lineages (L1 - L9), with lineages 1 and 2 being the most described and associated with outbreaks of neurological disease in humans and horses (Fall et al., 2017).

In nature, WNV is maintained in an enzootic cycle between birds and mosquitoes, with the first acting as a natural reservoir of the virus. Some bird orders, such as the Passeriformes, Charadriiformes and Falconiformes, are susceptible to WNV infection, acting as viral amplifiers. The virus replicates to high titers before the birds become moribund and die a few days after being infected. A study carried out with several different avian species from America reported that blue jay, common grackle, house finch, American crown and especially house sparrow are among the most important WNV amplifying avian species, while many others did not show any evident signs of WN disease (WND) (Martín-Acebes & Saiz, 2012; Chancey et al., 2015). Many avian species shed large quantities of virus in their feces or oral secretions when infected, allowing direct bird-to-bird and even bird-to-human transmission (Mclean et al., 2001; Komar et al., 2003). This demonstrates the importance of migratory birds in WNV transmission between different regions (Vilibic-Cavlek et al., 2019). Species belonging to the Galliformes order may be infected but show no clinical signs and are widely used in seroepidemiological surveillance (Martín-Acebes & Saiz, 2012; Vilibic-Cavlek et al., 2019).

Ornithophilic mosquito vectors, mainly of the *Culex* genus, are infected by WNV through blood meals from amplifying birds. Higher air temperatures influence vector competence, accelerating virus replication in mosquitoes (extrinsic incubation period) and prolonging the breeding season. Thus, higher temperatures cause an increase in vector population growth rates, shortening the interval between blood meals and increasing the efficiency of viral transmission to birds (Vilibic-Cavlek et al., 2019). Changes in climatic conditions are hypothesized to play a role in the increasing number of WNV outbreaks observed worldwide in recent years. Virus transmission occurs when competent vectors are active and abundant (Chapman et al., 2018a; Nasci et al., 2001).

Governed by favorable climatic conditions, adult female mosquitoes carrying WNV transmit the virus to humans, horses and other mammalian species that are incidental hosts. When mammalian species (primarily humans and horses) become infected, they do not develop high enough levels of viremia to efficiently transmit the virus to biting mosquitoes, so they are also referred to as "dead-end" hosts (Komar, 2000; Castillo-Olivares &Wood, 2004) (Figure 1).

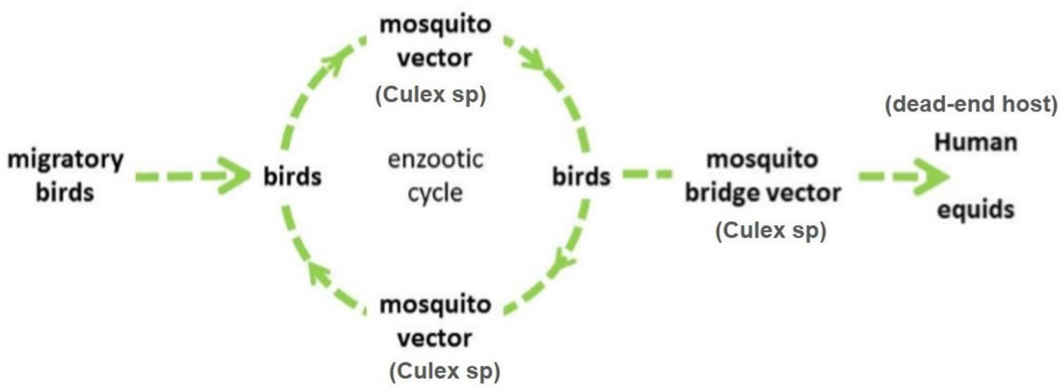

Figure 1 - WNV transmission cycle. WNV is introduced through migratory birds and can spend the winter on local bird or mosquito species. Culex sp. act as the main mosquito vector in the bird-mosquito-bird cycle (enzootic cycle) and as bridge vectors, infecting horses and humans. Birds as amplifying hosts, and mammals (primarily humans and horses) as dead-end hosts. Source: European Centre for Disease Prevention and Control (ECDC, 2013).

Clinical Manifestation, Transmission and Surveillance of WNV in Humans

In the first report of human WNV infection in the US in 1999, approximately 75% of cases were asymptomatic and 25% developed mild disease with influenza-like symptoms termed West Nile fever (WNF). In less than <1% of cases, mainly in elderly and immunocompromised people, infection resulted in severe neuroinvasive disease (WNND) (encephalitis, meningitis, or flaccid paralysis, convulsions, rigid spams), and an increase risk of death (Hayes et al., 2005).

WNF is characterized by nonspecific clinical signs such as acute onset of fever, headache, chills, backache, malaise, fatigue, weakness, muscle pain, arthralgia, myalgia, retro-orbital pain and difficulty concentrating. Approximately one-quarter to one-third of patients report vomiting, cough, sore throat, conjunctivitis, generalized lymphadenopathy, diarrhea and rash. While the symptoms disappear within a week in some cases of WNF, more severe cases have persistent fatigue and muscle weakness for a month or more. In 2003, rare cases of persistent WNV infection associated with hepatitis, splenomegaly, myocarditis, pancreatitis, kidney disease and ocular lesions (chorioretinitis, anterior uveitis, vitritis, intraretinal/vitreous hemorrhages) were reported, but these clinical manifestations have currently been more frequent (Hayes & Gubler, 2006; Murray et al., 2010; Garcia et al., 2014; Karesh et al., 2018).

In recent years, WNV epidemiology has changed worldwide, with dramatically increasing outbreaks in humans and horses. This is probably due to changes in weather conditions that alter the behavior of vector mosquitoes as well as the concomitant circulation of different WNV genetic lines (1 and 2) (Vilibic-Cavlek et al., 2019). In the USA, WNV is now endemic in most states and is responsible for over 50,830 human cases (CDCP, 2019). Of these, nearly 24,657 cases had neuroinvasive disease, and more than 2,199 were fatal. However, the true impact of WNV on human health is unknown because WNF is usually undiagnosed. In Europe, until the 1990s, WNV caused sporadic outbreaks with rare reports of encephalitis, but its epidemiological behavior changed when it resurfaced in Romania, Russia and the Mediterranean basin, causing dozens of human and equine deaths. Since 2008, WNV has spread widely throughout Central and South-Eastern Europe, becoming a serious veterinary and public health problem on the continent (Petrovic et al., 2018).

Until 2002, WNV transmission to humans was believed to occur only through the bite of infected mosquitoes, but human-human transmission of the virus began to be observed by blood transfusion in a USA outbreak. Since then, human-human transmission has been detected through organ transplantation and mother-to-child transmission (breastfeeding or transplacental) (Pealer et al., 2003). The evidence of WNV transmission by transfusion has alerted US blood centers to the need for expanded hemovigilance to prevent large-scale blood borne arbovirus epidemics (AABB, 2019). A WNV nucleic acid technology assay (NAT) was developed to detect infected donors at the earliest seronegative stages. To decrease cost and improve logistics, WNV NAT screening was implemented in minipool (MP) test formats employed for NAT screening for other viruses. MP-NAT screening involved pooling specimens from 6 to 16 donors and screening the combined specimen pool for viral RNA (Busch et al., 2005; Betsem et al., 2017).

Although MP-NAT screening of blood donors prevented hundreds of cases of WNV infection in 2003, it was unable to detect units with a low level of viremia, some of which were negative for antibodies but were infected. Most donors are asymptomatic or are in a presymptomatic stage of infection and can transmit the virus to their recipients. Therefore, a significant rate of low Viremic donations was missed by MP-NAT (Rios et al., 2007; Lustig et al.; 2016; Betsem et al., 2017; Vilibic-Cavlek et al., 2019; Young et al., 2019). Consequently, the screening of blood donors in an epidemic area was modified, with individual NAT screening (ID-NAT) rather than sample pooling (MP-NAT), until the end of the mosquito transmission season (Rios et al., 2007; Lustig et al., 2016; Betsem et al., 2017).

As cases of WNV infection in animals (horses, birds and mosquito vectors) precede human cases, "One Health" surveillance has been used to define affected areas so measurements to improve the safety of blood transfusions can be implemented. The concept of "One Health" deals with the integration between human health, animal health, the environment and the adoption of effective public policies in the prevention and control of diseases (Gossner et al., 2017; Young et al., 2019). There is no unique surveillance strategy applicable for the early detection of viral circulation in an area, and each country should adapt its surveillance to its epidemiological situation and capacity. Italy has implemented a fully integrated surveillance system that includes humans, horses, mosquitoes and birds. Although mosquito surveillance is considered effective, as virus detection in mosquitoes usually precedes human cases, this surveillance strategy is expensive. In 2010, Spain discontinued its routine WNV entomological surveillance due to insufficient funds. Surveillance of dead birds is generally not considered useful in Europe, as bird mortality due to WNV infection is rare in most European countries (Young et al., 2019). Equine surveillance is considered cheaper than mosquito and bird surveillance. As a result, Austria, France, Greece, Hungary, Italy and Spain implemented equine surveillance (Lustig et al., 2018; Young et al., 2019). In the USA, over 25,000 cases of WNV encephalitis have been reported in horses since 1999, representing 96.9% of all nonhuman mammalian cases (Lindsey et al., 2012; CDCP, 2013).

Equine Surveillance

Horses are more prone to WNV infection than humans, and 10% of infected horses show neurological signs (Castillo-Olivares & Wood, 2004). The health status of domestic horses is usually closely monitored by their owners and trainers. Therefore, signs of illness are often observed and reported quickly to veterinarians, demonstrating the importance of the horse as a sentinel of epizootic WNV activity (Vilibic-Cavlek et al., 2019). In the US and European countries, the government has prioritized investment in training veterinarians and horse owners to raise disease awareness and to improve their understanding of the information; the education strategies focus on disease risk, recognition of clinical signs in affected horses, recognition of early disease, horse protection methods and management, and detailed instructions for sending equine samples to laboratories (CDCP, 2013; Lanteri et al., 2014; Chapman et al., 2018b; Browne & Medlock, 2019).

Equine surveillance can be active or passive. Reports of new equine encephalomyelitis cases by local veterinarians give warning of increased arbovirus activity in an area. This can alert public health officials to investigate the situation. Active surveillance requires regularly contacting large-animal veterinarians and encouraging them to report clinically suspect equine cases and submit blood and autopsy samples for laboratory confirmation (CDCP, 2013; Hirota et al., 2013).

Classic clinical signs of horses infected with the WNV include fever (mild to moderate or absent), ataxia (incoordination), stumbling, hind limb weakness, depression, anorexia, recumbency with the inability to rise, muscle tremors, teeth grinding, dysphagia (inability to swallow), head pressing, signs of colic, flaccid paralysis of the lower lip, aimless wandering, excessive sweating, behavioral changes, convulsions or even coma. Up to 90% of symptomatic cases in horses result in neurologic disease with case fatality rates of 30-40% (CDCP, 2013; AAEP, 2017; USDA, 2019).

WNV infection in horses is confirmed by the detection of WNV particles, genomic material, antigens, or anti-WNV antibodies in tissue samples or body fluids. The criteria differ among countries, by testing purpose, and according to flavivirus distribution. The distribution of closely related flaviviruses in a sampling area would influence the diagnostic criteria because of their cross-reactivity in serodiagnostic tests (Hirota et al., 2013).

Because the likelihood of disease outbreaks in horses predicts the risk of disease in humans, other clinical manifestations in addition to neurological disorders have been the subject of research worldwide. An association between West Nile virus and Reproductive Loss Syndrome (MRLS) began to be questioned in the US from 2002 onwards due to the large number of aborted or deformed foals (up to 1,200 abortions and nearly 300 deformed or dummy foals) reported during the outbreak of neurological disease caused by WNV in horses. At that time, aborted cases were associated with the vaccination, as they coincided with the licensing of a WNV vaccine by the USDA Veterinary Biology Center. The Department of Agriculture's Animal and Plant Health Inspection Service analyzed alleged problems in pregnant and vaccinated mares and denied any vaccination association (AVMA, 2003). In 2010, the West Nile strain 1 virus was detected in South Africa in both the brain of a pregnant mare who succumbed to neurological diseases and in her aborted fetus, again suggesting an association of WNV with abortion in horses (Venter et al., 2011).

There are no vaccines licensed in Brazil by the Office of Veterinary Service (Ministry of Livestock Agriculture and Supply-MAPA). However, there are at least three vaccines available on the international market. According to the American Association of Equine Practitioners (AAEP) vaccination guidelines, four USDA vaccines are currently available (two are inactivated whole WN virus vaccines, one is a non-replicating live canary pox recombinant vector vaccine and one is an inactivated flavivirus chimera vaccine) (AAEP, 2019).

The importance of Equine Surveillance in Brazil

Serological evidence in horses

Following the major outbreak of neurological disease in horses and humans in the US in 2002, WNV expanded to Central and South American countries (Figure 2). (Elizondo-Quiroga & Elizondo-Quiroga, 2013). Due to the relevance of WNV to public health, the National West Nile Fever Surveillance System was created in Brazil in 2003 based on the recommendations of the Pan American Health Organization (PAHO) and the World Health Organization (WHO) (Ministério da Saúde, 2019).

The first detection of WNV in Brazil was in 2008, based on serological evidence in horses in central and western

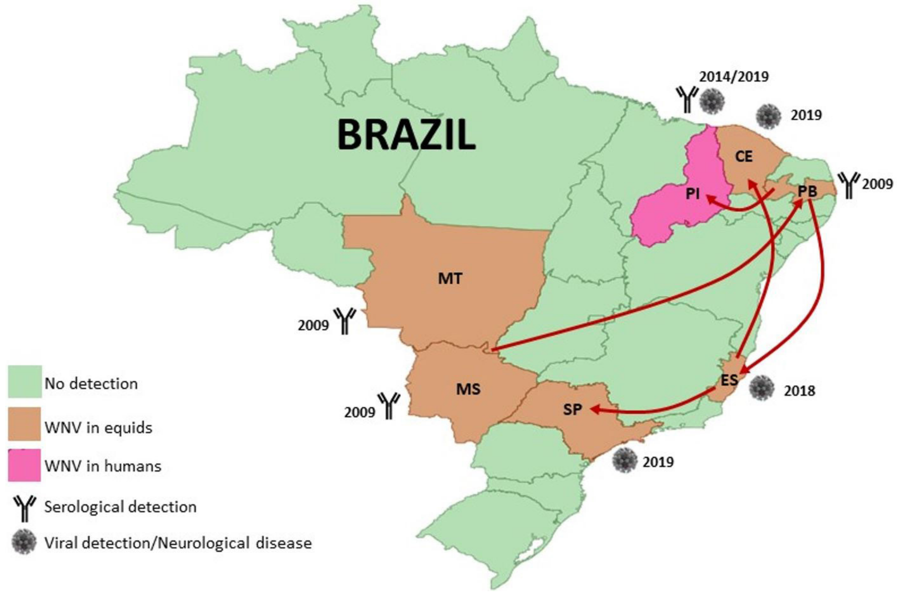

Figure 2 - West Nile virus dispersion in Brazil in horses and humans in Brazil. WNV schedule detection, diagnostic tools (serological and viral detection), State and year of sampling are represented. States are indicated by different colors according to the host species diagnosed, horses (orange), human (pink), no detection (green). The red arrows indicate the route of spread of WNV in Brazil.

Brazilian regions, particularly the Pantanal area (one of the biggest stopping places for migratory birds in Brazil) (Pauvolid-Corrêa et al., 2011). In this study, sera from 168 horses and 30 marsh alligators (*Caiman crocodilus yacare*) were evaluated by enzyme-linked immunosorbent assay (ELISA) and confirmed by plaque reduction neutralization test (PRNT). Five horses (3%) were positive, showing evidence of WNV circulation in Brazil. Following these initial studies, Melandri et al. (2012) also investigated the presence of WNV antibodies in chicken and horse serum in the Pantanal region (Mato Grosso state), with 3/38 (8%) horses and 1/31 (3,2%) chicken seropositive on ELISA and PRNT assays. Ometto et al. (2011), in a broader seroprevalence study, investigated the presence of West Nile antibodies in wild birds and equids (horse, mule and donkey). Serum was collected from 2002 to 2010 in the north, midwest, southeast, south and northeast regions. Three horses and 1 mule, from which samples were collected in 2009 in Mato Grosso state, were seropositive. Antibody detection for WNV was again confirmed in 24 horses from the Pantanal region, along with other Flavivirus including Ilheus virus (ILHV) and Saint Louis encephalitis (SLEV), proving the circulation of different arboviruses in the region (Pauvolid-Corrêa et al., 2014).

In 2013, the detection of antibodies in horse samples collected in 2009 in Paraíba state suggested that WNV was spreading toward the northeast part of Brazil (Silva et al., 2013). Subsequently, the first human case was described in 2014 in the county of Aroeiras do Itaim, Piauí state-PI (northeast region). The patient initially presented with muscle pain and fever, which progressed to flaccid paralysis. The diagnosis was confirmed by detection of WNV antibody in the cerebrospinal fluid (Vieira et al., 2015). Another three cases of WNV infection causing neurological signs in humans were reported in 2019 in Piauí state. The second case was a patient from the county of Picos who presented "neurological manifestations". Notification occurred in 2017, but confirmation by report was made in January 2019 (ISID, 2017). The *Piaui State Department of Health* (SESAPI) confirmed the third case, an elderly resident of Piripiri who died after presenting with acute encephalitis, in July 2019 (ISID, 2019). The fourth human case of WNV, a woman who was admitted to the Teresina Emergency Hospital with neurological sequelae, was confirmed by the Municipal Health Foundation (FMS, 2019). Since 2014, Piaui state has been closely monitoring cases of neurological disease and testing all suspected cases reported for the disease, both in the public and private hospitals.

Equine neurologic disease caused by WNV in Brazil

Although positive serology in horses has been described in Brazil, neurologic disease associated with WNV infection in horses had never been reported until 2018. From mid-April to May 2018, at least 12 fatal cases (nine horses and three donkeys) with acute neurological clinical signs were reported in Espírito Santo (ES) state, in the southeastern region of Brazil. The cases occurred in four counties (Nova Venecia, Boa Esperança, Baixo Guandu, and São Mateus). In all locations, there was a trace of Atlantic forest vegetation inside or near the properties. Of the 12 cases, only 4 had central nervous system (CNS) fragments sent for diagnosis. A diagnosis was made by WNV RNA detection from the cortex, brainstem, cerebellum, and spinal cord fragments by nested *reverse transcription polymerase chain reaction (RT-PCR),* followed by sequencing (Silva et al., 2019). The clinical signs observed in four equids (2 horses and 2 donkeys) included muscle tremors, dysphagia, front limb ataxia, and paddling movements within the first 24 h. After this period, pelvic limb paralysis, loss of sensitivity over the spinal column and mandibular trismus were observed. One animal died within 72 h of the onset of neurological signs, and the others were euthanized due to worsening of the clinical signs. An unusual feature of these outbreaks was the neurological manifestation of WNV-infected donkeys. Mules and donkeys may have a greater resistance to the development of diseases than horses (García-Bocanegra et al., 2012). The susceptibility of donkeys to WNV infection remains poorly documented, although there has been a report of one clinical case in a donkey in southern France (Murgue et al., 2001). Serological studies have shown that donkeys and mules have greater seroprevalence than horses (García-Bocanegra et al., 2012). This feature makes mules and donkeys a useful tool in the epidemiological monitoring of WNV infection in a given region, as they are able to act as sentinels. Antibodies have been found in clinically normal donkeys and mules in Mexico, Colombia, Spain, and Brazil (García-Bocanegra et al., 2012; Mattar et al., 2011; Pauvolid-Corrêa et al., 2014).

Three new confirmations of neurological diseases caused by WNV in horses were reported to the World Organization for Animal Health (OIE) by the veterinary service official (Ministry of Agriculture, Livestock and Supply, MAPA) in 2019. The first case occurred in the county of Boa Viagem, Ceará state (northeast region) in June 2019 (OIE, 2019a). The second case occurred in the county of João Neiva, Espirito Santo state (southeast region), in the same month (OIE, 2019b). The diagnosis was confirmed in two horses by RT-PCR and sequencing techniques by the Federal Laboratory for Agricultural Defense (LFDA/MG; National Laboratory). No additional information regarding the clinical signs and clinical course of the disease was released until the publication of this article. There are no records of the disease in humans in Espirito Santo state.

The third case occurred in the county of Suzano, São Paulo state, also in the southeast region. A 20-year-old Trotter gelding that lived in an urban area near a forest developed lameness that progressed to neurological signs (ataxia, convulsion and decubitus) over a 10-d period. The horse received anti-protozoal therapy for equine protozoal encephalomyelitis (EPM), but was unresponsive and euthanized due to poor prognosis. There were no other animals with neurological signs on the premises. The diagnosis was made through RT-PCR and sequencing by the Animal Virology Research Laboratory (LPVA) of School of Veterinary Medicine-UFMG in partnership with the Cruzeiro do Sul University (UNICSUL) and was reported immediately to the Official Veterinary Service (MAPA), which issued a technical note (Secretaria de Agricultura e Abastecimento, 2019) and immediately notified the World Organization For Animal Health (OIE, 2019c).

WNV dispersion in horses and humans in Brazil as depicted in Figure 2.

Important Topics to Consider in Brazil

Rabies should always be considered a differential diagnosis in horses with rapidly progressive or diffuse neurological signs, so appropriate precautions are taken when evaluating any horse with signs of encephalitis (Reed & Bayly, 2000). In Brazil, since 1966, the Office of Veterinary Service (MAPA) has instituted the "National Program of Control of the Rabies of Herbivores" (PNCRH), implemented by the Department of Animal Health (DSA). The objective of the PNCRH is to keep the incidence of rabies in the domestic herbivore population under control, within the concept of One Health, working in partnership with the Ministry of Health in the diagnosis of rabies and the protection of public health and the environment, respecting the fauna protection laws. Therefore, reporting cases of neurological disease in herbivores to the State Animal Defense Agency, which makes the diagnosis of rabies, is mandatory (Ministério da Agricultura..., 2009).

Notification must be made to the Official Veterinary Services of the Member States, or the Municipal Health Departments - SMS for the necessary arrangements. Notification can be made in person, by telephone or by emailing the "NOTIFICATION FORM" available on MAPA website. Neurological diseases are often fatal and are one of the most common reasons for euthanasia in horses. Necropsy is always required to confirm the diagnosis. One of the most challenging and demanding tasks for a professional is to perform a field necropsy in cases of equine neurological disease that requires removal of the brain, cerebellum, brainstem and spinal cord, which is an extremely laborious process. Sampling a horse's nervous system takes the same amount of time as evaluating all other organs during a necropsy. This is because in horses, in addition to the CNS samples required for collection in ruminants, sampling of cervical, thoracic and lumbar spinal cord segments is essential for the diagnosis of most neurological diseases, since lesions are often located in this region (Rech & Barros, 2015). If samples are not collected and stored correctly, laboratory diagnosis will not be successful, leading to inconclusive results (Pedroso et al. 2010; Peixoto et al., 2000; Rech & Barros, 2015).

Procedures for CNS collection of the National Herbivore Rabies Control Program - PNCRH are available on the MAPA website (Ministério da Agricultura..., 2016) and in Rabies Specimen Submission Guidelines available on the Centers for Disease Control and Prevention (CDCP, 2013).

Serological diagnosis is especially challenging in regions with flavivirus cocirculation. In Brazil, epidemiological studies have demonstrated the presence of other flaviviruses of public health importance in the equine population, such as dengue virus (DENV), Zika virus (ZIKV), Mayaro virus (MAYV), Saint Louis Encephalitis Virus (SLEV), Ilhéus virus (ILHV), Iguape virus (IGUV), Rocio virus (ROCV) and Cacipacore virus (CACV) (Castro-Jorge et al., 2019; Beck et al., 2019). Since horses can be infected and seroconvert against these arboviruses of human importance, they serve as sentinels for these viruses as well. Thus, the confirmation of detection of specific WNV neutralizing antibodies in Brazil is extremely challenging, due to the presence of a great diversity of flaviviruses that infect horses. Seropositive samples initially tested using blocking enzyme-linked immunosorbent assay (blocking ELISA) should be further tested using a plaque-reduction neutralization test ($PRNT_{90}$) for WNV and its most closely related flaviviruses that circulate in Brazil. Serological diagnosis of WNV in horses should always be interpreted with caution (Hirota et al., 2013).

Conclusions

Equine veterinarians play an important role in the process of epidemiological surveillance of important zoonotic diseases that are of interest to public health, such as West Nile virus, since horses are important sentinels. Therefore, the awareness and understanding of these diseases by veterinarians are essential for the early detection of these viruses, which is the most important way to prevent outbreaks and avoid epidemics. Thus, an adequate interpretation of clinical signs, collection of appropriate biological samples for diagnosis, correct interpretation of diagnostic tests and notification of suspected cases is necessary in human epidemiological surveillance programs.

Conflict of Interest

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this rapid communication.

Ethics Statement

This research project was approved by the Animal Use Ethics Committee of UFMG under the number CEUA No. 102/2018.

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