Horse Owner Practices and Equine and Human Arboviral Encephalitis in North Carolina

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Abstract **Researchers have long studied comparative medicine related to the One Health approach (Centers for Disease Control and Prevention, 2021a). An element of the One Health approach suggests that animals can serve as early-warning sentinels for infectious diseases in humans. In this study we compare cases of human and equine Eastern equine encephalitis (EEE), human West Nile neuroinvasive disease (WNND), and equine West Nile fever (WNF)/WNND reported during 2008–2018 in North Carolina. West Nile is a priority zoonotic disease in need of more investigation. We documented year-to-year variation in human and equine cases and noted a relative increase in WNND in 2016–2018. We detected a correlation between numbers of human and equine cases of EEE. We also surveyed North Carolina equine owners to assess vaccination practices, knowledge, and concern about mosquitoes and EEE and WN virus transmission. Most owners (93%) reported vaccinating their horses against these viruses. Equine owners and those who work with horses were minimally concerned about their own health risks related to mosquitoes and associated diseases. Mosquitoes were considered a nuisance during some types of farm activities. Respondents occasionally protected themselves from mosquito exposure by wearing long-sleeved shirts/pants and/or permethrin-treated clothing, self-applying repellent, and/or applying insecticides to properties via barrier treatments. There remains a clear need to effectively communicate the risks of arboviral diseases and the benefits of personal protection measures.**

Introduction

Exotic and native zoonoses continue to threaten human and animal health, changing the landscape of mosquito-borne disease in the U.S. over the last 20 years. There are frequent human outbreaks associated with exotic mosquito-borne pathogens such as the zoonotic West Nile virus (WNV), as well as intermittent focal outbreaks of anthroponotic chikungunya, dengue, and Zika viruses (Rosenberg et al., 2018). Native mosquito-borne viruses—such as La Crosse virus and particularly Eastern equine encephalitis virus (EEEV), one of the deadliest mosquito-borne pathogens—continue to cause substantial human morbidity and mortality. EEEV and WNV can infect equines, causing significant mortality. Although these diseases are vaccine-preventable in equines, cases are regularly reported each year.

WNV (family Flaviviridae) was first isolated in 1937 in Uganda. There were sporadic and major outbreaks during the 1990s, mainly in the Mediterranean Basin and Europe (Fall et al., 2017). This virus emerged in the U.S. in 1999 and has since spread throughout the Americas (Grinev et al., 2017; Kramer et al., 2019). Subsequent studies have revealed that nonhuman hosts include horses, dogs, cats, chickens, and livestock (e.g., cattle); however, these animals, like humans, are "dead-end" hosts for WNV, as their viremias do not support secondary transmission (Bosco-Lauth & Bowen, 2019).

Culex spp. of mosquitoes are the primary enzootic and epizootic vectors of WNV. In late summer after key bird species (i.e., American robins) migrate, *Culex* spp. become more opportunistic in their blood feeding habits, feeding on mammals, including humans (Hamer et al., 2009). WN fever (WNF) and WN neuroinvasive disease (WNND; encephalitis) cases, however, peak in humans and equines in late summer and early fall in some areas; this pattern could be due to amplification of WNV in *Cx. pipiens* populations in July and August (Hamer et al., 2009; Kilpatrick et al., 2006).

In the U.S., equines account for approximately 97% of reported cases of WNND in nonhuman mammalians (American Association of Equine Practitioners, 2021; Centers for Disease Control and Prevention [CDC], 2021b). Overt clinical manifestations of WNV infection in equines include weakness, ataxia, and muscle fasciculation. WNV infections, however, can also result in exclusively neurological symptoms, with approximately 10% of infected equines having neurological disorders (Bunning et al., 2002; Castillo-Olivares & Wood, 2004). Since 1999, there have been multiple WNF/WNND outbreaks in equines in the U.S. In 2002, there were approximately 15,000 laboratory-confirmed equine cases in the U.S. (CDC, 2002). In 2003, however, the U.S. Department of Agriculture reported WNF/WNND in approximately 4,000 equines from 41 states, with the lower rate that year attributed to widespread vaccination (Castillo-Olivares & Wood, 2004).

EEEV (family Togaviridae) also causes neuroinvasive disease in humans and equines. This virus is maintained in nature by a mosquito–bird enzootic transmission cycle in freshwater hardwood swamps that involves the mosquito *Culiseta melanura* Coquillett as the primary enzootic vector (CDC, 2021c; Soghigian et al., 2018). In the U.S., EEEV was first recognized in 1931 following the deaths of 75 equines after encephalitic illnesses, although reports of epidemics of equine deaths like EEE have been reported since the early 19th century (Giltner & Shahan, 1933; Kumar et al., 2018). EEEV is endemic in the U.S. and generally found east of the Mississippi River (Calisher, 1994; Heiberlein-Larson et al., 2019). In North Carolina, EEE was first reported in 1955 in veterinary cases (i.e., pheasants) (Alexander & Murray, 1958). Infection from EEEV can result in morbidity and high mortality rates in equines (80–90% mortality, long-term neurological sequelae in 66% of survivors) and humans (30–70% mortality, long-term neurological sequelae in 30% of survivors) (Goodman et al., 2015; Porter et al., 2017; Young et al., 2008).

Human EEE cases are rare in the U.S. (approximately 11 cases/year); however, veterinary infections are common, with equines averaging 169 cases/year between 2005 and 2019 (CDC, 2021c; U.S. Department of Agriculture, 2021). Unvaccinated equines are vulnerable to EEEV infection and are likely to die within a few days after symptoms begin. Clinical symptoms include muscle fasciculation, sleepiness, and a weak or staggering gait (Louisiana State University Agricultural Center, 2005).

While there are no commercially available vaccines to protect humans against WNV or EEEV infection, there are equine vaccines (Bosco-Lauth & Bowen, 2019). More than 300,000 equines live in North Carolina and this industry is valued at approximately $2 billion/year (www.horsecouncil.org).

Overreliance on insecticides, resulting in the emergence of widespread insecticide resistance, has advanced the need for integrated mosquito management programs to control vector species (Rose, 2001). Integrated mosquito management programs should make control decisions based on evidence obtained from surveillance to protect public and veterinary health from diseases such as WNF, WNND, and EEE. In states such as North Carolina where funding may be limited for arboviral and mosquito surveillance, however, effective and timely communication between mosquito control programs, agriculture officials, and public/environmental health personnel can be lacking. One Health recognizes that humans, animals, and the environment are interconnected (CDC, 2020). Thus, using existing or systematic placement of animals as sentinels could help in predicting, controlling, and preventing human cases of WNF, WNND, and EEE.

In this study, we assess whether equine and human cases of these diseases are correlated in time. As equine vaccines are available and could affect the relationship between equine and human cases, we also measured knowledge, concern, and vaccination practices of North Carolina equine owners with respect to mosquito-borne diseases.

Methods

Data for EEE, WNF, and WNND for 2008–2018

We gathered data for EEE, WNF, and WNND in North Carolina for 2008–2018. Reports from the North Carolina Department of Health and Human Services (NC DHHS) were analyzed for veterinary (equine) and human (neuroinvasive only) cases. Deidentified county/city level data (including onset dates) were obtained. We obtained human surveillance data for EEE and WNND from the North Carolina Electronic Disease Surveillance System (NCEDSS) via a data use agreement (DUA) established between East Carolina University and the Division of Public Health within NC DHHS (UMCIRB 18-001987). Human cases are reported using standard case definitions (https://ndc.services.cdc.gov). In North Carolina, only neuroinvasive human cases (WNND) attributed to WNV infection are reported, unlike some other states that also report non-neuroinvasive human cases (i.e., WNF). We also obtained veterinary data from NC DHHS but a DUA was not required. Veterinary cases in North Carolina do not follow the same case definitions as human cases (www.ncagr.gov/vet/vetdis.htm), as any veterinary case is reportable (not solely neuroinvasive cases).

We also analyzed the publicly available Centers for Disease Control and Prevention (CDC) ArboNET human and equine surveillance data and compared the human data to the NCEDSS databases to check reporting accuracy for WNND and EEE cases. Human and veterinary cases were deidentified to show onset dates and city/county information only.

Survey of Equine Farms

We developed an 18-question survey (UCMIRB 18-001987; see the Supplemental Appendix at www.neha.org/jeh/supplemental for the survey) in Qualtrics for distribution via email, Facebook, and/or postal mail to North Carolina equine farms. Equine farms in North Carolina are not required to register with the North Carolina Department of Agriculture and Consumer Services, thus we developed a contact list for North Carolina equine farms using internet searches and other publicly available databases (e.g., North Carolina Horse Council). We defined an equine farm as any operation with at least one equine and tracked the number of equines at each farm in the survey.

Respondents of surveys administered via email or other online delivery method entered their responses directly into Qualtrics. Investigators manually entered survey data received by postal mail into Qualtrics. Results were tabulated and displayed graphically to evaluate trends for specific survey questions of interest.

We offered each participant a $10 gift card to encourage participation. There were 416 surveys deployed to equine farms in North Carolina (112 to the western region, 136 to the central region, and 168 to the eastern region) and 314 of these were delivered successfully (i.e., not returned due to inaccurate contact information). Of the 416 total surveys, 260 surveys were sent via email. A total of 232 surveys were emailed successful, with 16 emails bounced back due to inactive email accounts and 12 emails delivered to multiple individuals from the same farm. An additional 94 surveys were sent via postal mail (12 were returned as undeliverable) and 62 surveys were sent via Facebook or other social media platforms.

Statistical Analyses

Statistical analyses were carried out using SAS with an α = .05. Temporal trends in case incidence were visualized in bar charts between months (month of disease onset), years, and counties. Pearson correlation analyses were conducted to determine the extent to which equine and human cases were correlated in each year. Fisher's exact tests were used to analyze gender differences between human cases of EEE and WNND.

Results

EEE, WNF, and WNND in North Carolina, 2008–2018

For 2008–2018, 26 North Carolina counties (3 from the western region, 10 from the central region, and 13 from the eastern region) experienced at least one human case attributed to infection with either EEEV or WNV, with WNND cases observed statewide and EEE more common in central and eastern regions (Figure 1). In North Carolina, only neuroinvasive cases are reported in humans; thus, case data obtained refer to WNND. Reported human cases of WNND ranged from 0–10 cases/year during 2008–2018 (10-year mean = 3.6 cases/year; Figure 2). In contrast, reported cases of WNF/WNND in equines were lower during 2008–2018 (10-year mean = 1.3 cases/year; Figure 2). While equine cases were reported between September and October, reported human WNND cases ranged from May–November. Humans and equines experienced an increase in cases of WNND and WNF/WNND, respectively, over the last 3 years of the study (i.e., 2016–2018). Most of the time, reported human cases of WNND occurred during the same year of reported equine cases of WNF/WNND, except for 2 years (i.e., 2011 and 2014) when no human WNND cases were reported.

FIGURE **1**

Distribution of Human West Nile Neuroinvasive Disease (WNND) and Eastern Equine Encephalitis (EEE) in North Carolina, 2008–2018

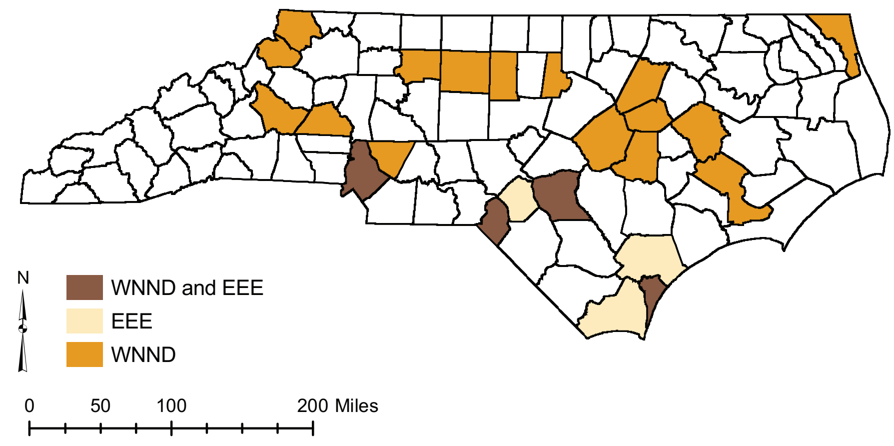
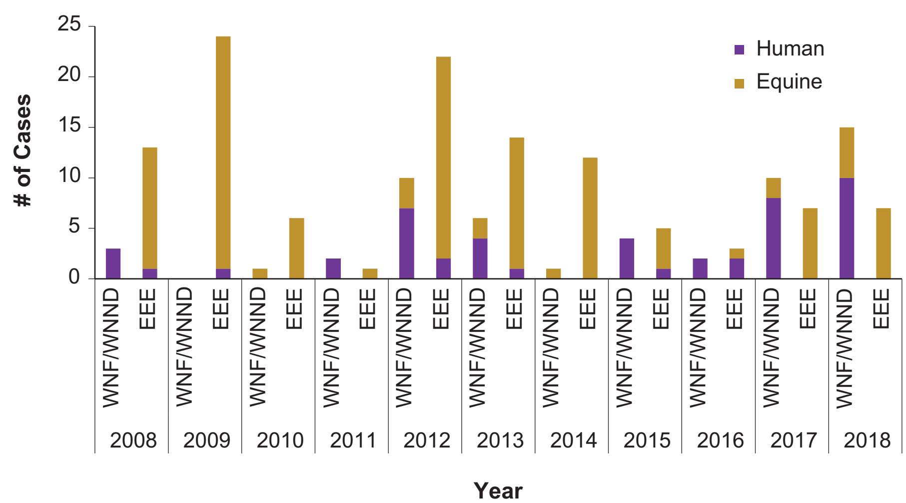


FIGURE **2**

Eastern Equine Encephalitis and West Nile in North Carolina, 2008–2018



Note. EEE = Eastern equine encephalitis; WNF = West Nile fever; WNND = West Nile neuroinvasive disease. For humans, only WNND cases are reported. For equines, both WNND and WNF cases are reported.

Similar to WNF/WNND, both humans and equines had reported cases of EEE between 2008 and 2018 (Figure 2). The mean number of reported human EEE cases, however, was lower (10-year mean = 0.73 cases/year). Few demographic patterns were associated with human cases of WNND and EEE, although there was a significant gender difference in human EEE cases (100% men, 0% women, $p = .014$). Equine EEE cases were significantly higher (10-year mean = 9.67 cases/year) than human cases. For 2008–2018, there was a significant correlation between equine and human EEE cases ($r = .30$, $p = .002$, 95% confidence interval [0.29, 0.31]). In general, years in which at least one human EEE case was

TABLE 1

Actions Taken by Survey Respondents to Protect Against Mosquitoes

Action	Respondents # (%)	95% CI
Removal of empty containers such as tires, flowerpots, and bird baths	62 (83)	[72.6, 89.6]
Cleaning gutters or removing leaves, pine needles, and other debris	55 (73)	[62.4, 82.1]
Use of drainage system for stormwater such as ditches	52 (69)	[58.2, 78.6]
Personal protection by wearing repellent	52 (69)	[58.2, 78.6]
Personal application of insecticides targeting mosquitoes	37 (49)	[38.3, 60.4]
Personal protection by wearing appropriate clothing	34 (45)	[34.6, 56.6]
Hiring professional mosquito control services to conduct pesticide treatments*	4 (5)	[2.1, 12.9]

Note. CI = confidence interval.
* For example, Pest Arrest, Mosquito Tek, and county mosquito control programs.

reported experienced at least 3 times as many equine EEE cases—with the exception of 2016, when there were more reported human cases of EEE than equine cases by 2:1 (Figure 2). See the Supplemental Figures (S1–S4) and Table (S1) Appendix at www.neha.org/jeh/supplemental for additional information.

Comparison of ArboNET and NCEDSS

CDC maintains a database (ArboNET) of arbovirus surveillance for human and veterinary cases, mosquitoes, dead birds, and other sentinel animals (wwwn.cdc.gov/arbonet/Maps/ADB_Diseases_Map/index.html). Analysis of human WNND data retrieved from NCEDSS and ArboNET showed a discrepancy in reporting for North Carolina in 2013 during the period of study—in 2013 NCEDSS reported WNND human cases in four counties (Nash, Wilson, Mecklenburg, Johnston) but ArboNET showed WNND human cases in three counties (Nash, Wilson, Mecklenburg). Nevertheless, data sets reported in the two surveillance systems were comparable.

Survey of Equine Farms

Surveys (N = 84, 26.7% response rate) were used to determine equine vaccination knowledge and use in North Carolina. We received 84 surveys, of which 9 respondents answered only the consent question. Hence, we calculated an overall response rate of 24% (75 responses/314 successfully delivered surveys). Most respondents (n = 48, 64%) stated they had >15 equines, while others had 11–15 equines (n = 14, 19%), 5–10 equines (n = 9, 12%), or ≤4 equines (n = 4, 5%).

Awareness of equine vaccines for the prevention of WNF/WNND and EEE was reported by most respondents (n = 73, 97%). Most (n = 72, 96%) reported their equines had not experienced illness associated with WNF/WNND and EEE. For those indicating equine illness, two equines died due to EEEV infection and one equine contracted WNV and lived, but with long-term health issues. Most respondents vaccinated equines against WNV and EEEV each year via a veterinarian (n = 50, 67%) or by administering the vaccine on their own (n = 20, 27%). Some (n = 5, 7%), however, did not vaccinate their equines for the following reasons: too expensive, vaccine does not work, mosquito-borne diseases are not an issue, one equine previously had an adverse reaction to the vaccine.

Many indicated it was very important (n = 39, 52%) or important (n = 30, 40%) to protect equines from mosquitoes, whereas only 6 (8%) stated this protection was unimportant. Thus, respondents and equines were protected from mosquito bites using various means (Table 1).

Respondents were concerned about the health of equines related to mosquito-borne disease with most (n = 39, 52%) reporting in the agree or strongly agree category. Some were not concerned with equine health related to mosquito-borne disease, with 20 (27%) reporting in the disagree or strongly disagree categories. See the Supplemental Figures (S5–S8) Appendix at www.neha.org/jeh/supplemental for additional information.

Discussion

We found a correlation between the year-to-year numbers of human and equine cases of EEE during the period of study. Onset of EEE symptoms in humans typically occur within 4–10 days of a mosquito bite, while onset occurs within 5 days for equines (CDC, 2021c; Louisiana State Agricultural Center, 2005). A previous study identified use of clinical signs along with month of occurrence as indicators of prevalence of WNV infections (Leblond & Lecollinet, 2017; Saegerman et al., 2014). Hence, unvaccinated equines might be good sentinels for human EEE cases. A few weeks of warning lead time could elicit a mosquito-control response by public health and veterinary health agencies, possibly preventing or limiting human cases.

Case data in North Carolina for these diseases should be monitored and compared with national trends. It is possible that information from North Carolina could be used to help other jurisdictions or states that conduct similar analyses. A multiyear study on EEEV in New York, for example, showed four adjacent counties with similar patterns of transmission over time (Oliver et al., 2020). Communication with mosquito control programs (MCPs) and public health and veterinary health agencies should be facilitated for timely response to potential mosquito-borne disease threats.

In 2018, WNV was the most common cause of human neuroinvasive arboviral disease (92% of cases) in the U.S. (McDonald et al., 2019). The same study showed the number of U.S. WNND cases in 2018 was approximately 25% higher than annual cases reported from 2008–2017. Historical and real-time data can be used to help develop action thresholds applied in operational MCPs at the local level (Nasci & Mutebi, 2019); however, complex interactions of factors contributing to WNV and EEEV transmission and epidemiology can complicate risk predictions. It has been suggested that Florida, which has year-round EEEV transmission, is a major source of EEE epizootics in the Northeastern U.S. (Heberlein-Larson et al., 2019). North Carolina and other states should take advantage of wide-scale monitoring of arboviral disease in other states, such as Florida, to improve its own risk predictions.

Adequate response in an early warning system requires organized and effective mosquito control infrastructure. There is, however, a general lack of funding for mosquito surveillance and control programs in many areas of the U.S., including North Carolina (Del Rosario et al., 2014; Vazquez-Prokopec et al., 2010) and this gap in resources could prevent the effective use of an early warning arboviral disease system in North Carolina. A survey by the National Association of County and City Health Officials (2017) assessed 1,906 MCPs in the U.S. and classified 84% of the programs as needing improvement. Lack of sustained funding for North Carolina MCPs remains a significant public health issue that should be addressed to protect public health (Del Rosario et al., 2014). Reactive rather than proactive approaches in mosquito control can be costly and usually occur after human and/or animal cases have happened (Nasci & Mutebi, 2019). Long-term surveillance systems should be instituted to monitor a) mosquito abundance and b) virus presence in mosquitoes and/or sentinel animals with an action plan implemented when threshold levels are reached (Nasci & Mutebi, 2019).

Another element to an early warning system can be arbovirus surveillance in mosquitoes. Due to the lack of sustained and structured funding for MCPs in North Carolina, however, only a few programs routinely submit mosquito samples for arbovirus testing to NC DHHS and there has not been a standardized tracking method for reporting and using these data to inform operational control decisions. Therefore, these incomplete data are not included, and the tracking method should be improved for the future. Currently in North Carolina, no MCPs use sentinel chickens for arbovirus surveillance (e.g., WNV, EEEV), although many used them in the early 2000s.

We acknowledge limitations of this study, including the relatively low response rate, which potentially could be improved in future studies by increasing the number of surveys administered and providing additional incentives for completion of the surveys. Furthermore, as North Carolina reports only neuroinvasive human cases of WN, data for non-neuroinvasive human cases is underreported and this issue could limit comparisons in areas where both WNF and WNND are jointly occurring. Data on mosquito pool testing for WNV and EEEV were not available and would have improved the study's spatiotemporal comparisons.

Conclusion

The survey of equine owners in North Carolina that we conducted demonstrates a lack of concern for mosquito exposure and arboviral disease for the equine owners personally. Equine vaccination rates reported were high (93%), thereby possibly reducing some concern for disease and likely decreasing the sensitivity of an equine-triggered early warning system. Further studies could conduct mosquito surveillance at equine farms across North Carolina to determine seasonality and abundance of mosquitoes likely to be involved in WNV and EEEV transmission.

Supplemental assessment of blood-fed mosquitoes could be utilized in determining blood-feeding hosts. In this study, equine EEE cases were significantly higher than human cases and equine and human EEE cases were significantly correlated in 2008–2018. Our study findings indicate that, in years when human EEE cases were reported, there are at least 3 times as many equine EEE cases. We analyzed a span of 10 years in this study; however, analyses of additional years would also be beneficial to examine trends over time. Taken together, these tools can be used to underscore the importance of equine vaccinations.

In summary, we found: a) numbers of human and equine cases of EEE were correlated in most years of the study (2008–2018), but this correlation was not found for WNF/WNND; b) communication with MCPs and public health and veterinary health agencies should be facilitated for timely response to potential mosquito-borne disease threats; and c) most equine owner participants reported vaccinating equines against EEEV and WNV and were minimally concerned about their own health risks related to mosquitoes and associated diseases.

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