West Nile Virus Lineage 2 in Horses and Other Animals with Neurologic Disease, South Africa, 2008–2015

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During 2008–2015 in South Africa, we conducted West Nile virus surveillance in 1,407 animals with neurologic disease and identified mostly lineage 2 cases in horses (7.4%, 79/1,069), livestock (1.5%, 2/132), and wildlife (0.5%, 1/206); 35% were fatal. Geographic correlation of horse cases with seropositive veterinarians suggests disease in horses can predict risk in humans.

West Nile virus (WNV) circulates between ornithophilic mosquitoes and birds. Birds are used as WNV sentinels, but in Africa, birds rarely die of infections, probably because of genetic resistance (1). Therefore, another animal is needed to predict risk for WNV disease in Africa. Humans and horses are considered incidental dead-end hosts for WNV (2). Although ≈20% of infections in these species are symptomatic, 1%–10% of human cases involve neurologic disease, with 1%–10% fatality rates (3), but 90% of horse cases involve neurologic disease, with 30%–40% fatality rates (4). Monitoring equine populations living near human populations might enable prediction of human outbreaks in Africa (5).

WNV is endemic to South Africa (1). The largest human outbreak occurred in 1974 in Karoo (1); during 1983–1984, a smaller epizootic outbreak occurred in Witwatersrand (Gauteng Province) (6). During 2010–2011, screening of the cerebrospinal fluid of patients in hospitals in Gauteng Province indicated WNV was present in 3.5% of unsolved cases of neurologic disease, suggesting severe WNV cases might be missed because of a lack of clinical awareness of the pathogenic potential of this virus (7).

WNV isolates are divided into 2 lineages (8): lineage 1, which predominates in the Northern Hemisphere and Australia, and lineage 2, which is endemic to southern Africa and Madagascar and started emerging in central Europe in 2008 (9). Lineage 2-associated encephalitis outbreaks have been occurring in Greece since 2010, causing hundreds of neurologic cases in humans (fatality rate 17%) and horses (3). Lineage 2 was first associated with severe neurologic disease in horses in South Africa in 2009 (8). Neurologic signs of infection in horses are similar for both lineages and include ataxia, weakness, recumbence, seizures, and muscle fasciculation (8,10). However, epidemiologic data are lacking.

South Africa conducts active surveillance for infectious pathogens in horses, such as African horse sickness virus (AHSV), to help the horse industry maintain disease-free status, as required by the World Organisation for Animal Health. Equine WNV is also a World Organisation for Animal Health–listed disease, enabling researchers to use horses for WNV surveillance internationally. To assist with predicting WNV human cases and managing outbreaks in susceptible animals, we sought to define the epidemiology of WNV in horses in South Africa.

The Study

We prospectively investigated horses and other animals with fever or neurologic signs during 2008–2015 and compared the geographic range of WNV-positive animals with that of WNV-seropositive veterinarians involved in equine, wildlife, and livestock disease management during 2011–2012 (11). A total of 210 veterinarians from all 9 South Africa provinces submitted blood, neurologic tissue, and visceral tissue specimens from horses (acquired during 2008–2015) and wildlife and livestock (acquired during 2010–2015) that displayed acute fever, neurologic disease, or other signs of acute infection, accompanied by their demographic and disease data, to the Centre for Viral Zoonoses, University of Pretoria (Pretoria, South Africa). A total of 1,407 samples (64% blood/serum, 25% tissue, 6% both blood/serum and tissue, 4% viral RNA) were received.

We tested all specimens for WNV, Shuni virus, alphaviruses, and equine encephalitis virus and submitted samples for rabies virus testing, if suspected, to Onderstepoort Veterinary Research, Onderstepoort, South Africa, as previously
described (12). All WNV-positive cases were tested for AHSV (13). We screened all equine serum and plasma specimens for WNV IgM (WNV IgM Capture ELISA Test; IDEXX Laboratories, Montpellier, France) and confirmed by neutralization assay (8). Reverse transcription PCR–positive cases were sequenced (GenBank accession nos. KY176717‒36) and subjected to maximum likelihood analysis (online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/12/16-2078-Techapp1.pdf). We compared WNV positivity with clinical signs in horses by logistic regression using crude odds ratios (ORs) and adjusted ORs (aORs) with 95% CIs (Stata 14; StataCorp LLC, College Station, Texas, USA) (online Technical Appendix Table).

Most clinical cases were in horses (76.0%, 1,069/1,407), followed by wildlife (14.6%, 206/1,407) and livestock (9.4%, 132/1,407). We detected most WNV cases in horses (7.3%, 79/1,069; p<0.001), and 1 (0.5%) case in wildlife (imported North American white-tailed deer [Odocoileus virginianus]), and 2 in (1.5%) livestock (locally bred Ayreshire cow [Bos taurus], boer goat [Capra aegagrus hircus]).

Real-time PCR results were positive for 24 cases; 20 isolates could be sequenced, and 18 clustered with lineage 2 (online Technical Appendix Figure). A mare and her miscarried fetus were the only animals infected with lineage 1 viruses (14). We detected 14 (17.7%) co-infections in WNV-infected horses (Table 1), with high fatality rates for most co-infecting viruses: MIDV (100%, 4/4); AHSV (66.7%, 2/3); SINV (100%, 3/3); Shuni virus (33.3%, 1/3); and equine encephalitis virus (0%, 0/1).

Most (77.2%) WNV cases occurred in Southern Hemisphere autumn (March‒May) (Figure 1), 2–3 months after peak precipitation. The interannual detection rate among horses was 2.1–12.7% (Table 1). Most specimens came from Gauteng (n = 400) and Western Cape (n = 296) Provinces (Figure 2, panel A). Most WNV-positive cases were from Gauteng Province (7.3%, 29/400), but detection rates were highest in Northern Cape (10.2%) and Eastern Cape

### Table 1. WNV infection, co-infection, disease, and death in horses, by year, South Africa, 2008–2015*

<table>
<thead>
<tr>
<th>Category</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total specimens</td>
<td>71</td>
<td>76</td>
<td>150</td>
<td>164</td>
<td>89</td>
<td>138</td>
<td>193</td>
<td>188</td>
<td>1,069</td>
</tr>
<tr>
<td>Confirmed WNV positive†</td>
<td>9 (12.7)</td>
<td>6 (7.9)</td>
<td>18 (12.0)</td>
<td>12 (7.3)</td>
<td>3 (3.4)</td>
<td>4 (2.9)</td>
<td>23 (11.9)</td>
<td>4 (2.1)</td>
<td>79 (7.4)</td>
</tr>
<tr>
<td>WNV PCR positive†</td>
<td>5 (7.0)</td>
<td>3 (3.9)</td>
<td>8 (5.3)</td>
<td>2 (1.2)</td>
<td>0 (0)</td>
<td>1 (0.7)</td>
<td>4 (2.1)</td>
<td>1 (0.5)</td>
<td>24 (2.2)</td>
</tr>
<tr>
<td>WNV IgM positive†</td>
<td>5 (7.0)</td>
<td>3 (3.9)</td>
<td>12 (8.0)</td>
<td>10 (6.1)</td>
<td>3 (3.4)</td>
<td>3 (2.2)</td>
<td>20 (10.4)</td>
<td>3 (1.6)</td>
<td>59 (5.5)</td>
</tr>
<tr>
<td>Deaths‡</td>
<td>5 (55.6)</td>
<td>3 (50.0)</td>
<td>8 (44.4)</td>
<td>3 (25.0)</td>
<td>1 (33.3)</td>
<td>1 (25.0)</td>
<td>5 (21.7)</td>
<td>1 (25.0)</td>
<td>27 (34.2)</td>
</tr>
<tr>
<td>Any neurologic signs‡</td>
<td>8 (88.9)</td>
<td>6 (100.0)</td>
<td>16 (88.9)</td>
<td>11 (91.7)</td>
<td>2 (66.7)</td>
<td>4 (100.0)</td>
<td>21 (91.3)</td>
<td>4 (100.0)</td>
<td>72 (91.1)</td>
</tr>
<tr>
<td>Fever‡</td>
<td>2 (22.2)</td>
<td>2 (33.3)</td>
<td>3 (16.7)</td>
<td>6 (50.0)</td>
<td>1 (33.3)</td>
<td>1 (25.0)</td>
<td>10 (43.5)</td>
<td>3 (75.0)</td>
<td>28 (35.4)</td>
</tr>
<tr>
<td>Co-infections‡ and co-infecting viruses</td>
<td>2 AHSV</td>
<td>2 SINV</td>
<td>1 SHUV</td>
<td>2 MIDV</td>
<td>1 AHSV, 1 SINV</td>
<td>2 MIDV, 1 SHUV</td>
<td>3 AHSV, 3 SINV, 1 EEV</td>
<td>3 SHUV, 4 MIDV, 1 EEV</td>
<td></td>
</tr>
</tbody>
</table>

*AHSV, African horse sickness virus; EEV, equine encephalitis virus; MIDV, Middleburg virus; SHUV, Shuni virus; SINV, Sindbis virus; WNV, West Nile virus.
†Percentage of total number of specimens tested.
‡Percentage of total number of confirmed WNV-positive cases. Confirmed cases were those that tested positive by PCR plus those that tested positive by WNV IgM Capture ELISA (IDEXX Laboratories, Montpellier, France) followed by neutralization assay.
(10.5%) Provinces. Of 152 samples from Limpopo and Mpumalanga Provinces, 99 of which came from wildlife species, none were WNV positive. The geographic distribution of WNV-seropositive veterinarians (11) was similar to that of WNV-positive horses (Figure 2 panel B).

Older horses (≥15 y) were least likely (4.9%, 3/61) and 1–4-year-old horses most likely (41.0%, 25/61) to test WNV positive (Table 2); 35.4% of WNV-positive horses were febrile, 93.7% displayed neurologic signs, and 34.2% died. WNV-associated signs included ataxia, paralysis, paresis, seizures, and tongue paralysis. Multiple logistic regression models (online Technical Appendix Table) confirmed neurologic signs as a strong predictor (aOR 4.12, 95% CI 1.59–10.70) and fever a weak predictor (aOR 1.25,
mosquitoes, the predominant WNV vector, correlated with the geographic distribution of equine cases in South Africa (6). The distribution of WNV exposure among horses correlated with that among humans (Figure 2, panels A, B), suggesting horses could serve as sentinels for human risk for WNV disease in South Africa. Horses have low WNV viremia, precluding them from transmitting infections and establishing epidemics in humans; however, those handling horse central nervous system tissue should do so with caution (15). Vaccination before the start of the rainy season could reduce the risk for WNV in horses.

In summary, surveillance for neurologic disease in animals across South Africa showed WNV lineage 2 as the primary cause of annual outbreaks, with high fatality rates in horses. Horses proved to be good sentinels for WNV in Africa and can be used to determine geographic and seasonal risk patterns for human WNV disease.

Acknowledgments
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References

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World AIDS Day, December 1

December 1 is World AIDS Day, an opportunity for people to work actively and collaboratively with partners around the world to raise awareness about HIV and help us move closer to the goal of an AIDS-free generation. This year’s theme, “The Time to Act Is Now,” calls us to act with urgency to implement the latest high-impact, evidence-based HIV prevention strategies.

http://wwwnc.cdc.gov/eid/page/world-aids

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