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 African 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>Total specimens</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>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>
<td></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>
<td></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>
<td></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>
<td></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>
<td></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>
<td></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</td>
<td>2 MIDV</td>
<td>1 SHUV</td>
<td>3 AHSV</td>
<td>3 MIDV</td>
<td>4 MIDV</td>
</tr>
<tr>
<td></td>
<td>2 (22.2)</td>
<td>2 (33.3)</td>
<td>1 (5.6)</td>
<td>2 (16.7)</td>
<td>2 (66.7)</td>
<td>0</td>
<td>4 (17.4)</td>
<td>1 (25.0)</td>
<td>14 (17.7)</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.

![Figure 1](image-url) Seasonal occurrence of WNV in horses, South Africa, 2008–2015. Rainfall levels are indicated as a potential correlate for increases in the prevalence of the WNV mosquito vector Culex univitattus. WNV, West Nile virus.
(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, 95% CI 0.44–3.68).
The geographic distribution of WNV is mainly dependent on favorable ecology, rainfall, and competent vectors. The range of *Culex univittatus* 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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The protocol was reviewed and approved by the Department of Agriculture Forestry and Fisheries in terms of Section 20 of the Animal Diseases Act (Act No. 35 of 1984) of South Africa and approved by the University of Pretoria Animal Ethics committee, IRB 0000 2235 IORG0001762. Funding was provided by cooperative agreement 1U19GH000571-01 with the Global Disease Detection Centre, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA (2013–2015). Part of this work was funded by the National Research Foundation of South Africa and the National Health Laboratory Services (2008–2012).

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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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