West Nile virus (WNV) lineage 2 is associated with neurologic disease in horses and humans in South Africa. Surveillance in wildlife and nonequine domestic species during 2010–2018 identified WNV in 11 (1.8%) of 608 animals with severe neurologic and fatal infections, highlighting susceptible hosts and risk for WNV epizootics in Africa.

West Nile virus (WNV) is associated with febrile disease, meningoencephalitis, and death in humans and horses (1,2). WNV infections are recognized on most continents but remain underreported in Africa. An 8-year study in horses with fever, neurologic signs, or both in South Africa described WNV lineage 2 as the cause of annual outbreaks; 93.7% of WNV-positive horses displayed neurologic signs, resulting in a 34.2% fatality rate (3). In the United States, clinical WNV disease has been reported in several nonequine species: birds, crocodiles, bats, wolves, cats, dogs, cattle, and sheep (4). The disease susceptibility of wildlife species in Africa and the role they play in amplifying the virus is unknown. We conducted surveillance for neurologic disease and death in animals other than horses in South Africa during 2010–2018 to determine potential WNV reservoir species, identify susceptible hosts, and highlight potential areas for targeted surveillance.

The Study

A total of 608 specimens comprising central nervous system tissue, visceral organs, and whole blood from wildlife; nonequine domestic animals; and birds with neurologic, febrile, or respiratory signs or sudden unexpected death were submitted to the Centre for Viral Zoonoses, University of Pretoria (Pretoria, South Africa), during February 2010–June 2018. We extracted RNA from the samples using the QIAamp viral RNA (QIAGEN, https://www.qiagen.com) (blood) or RNeasy (QIAGEN) (tissue) mini-kits under Biosafety Level 3 conditions. All specimens were subjected to 1-step nested real-time reverse transcription PCR (RT-PCR) targeting WNV (LightCycler FastStart DNA Master HybProbe; Roche Applied Science, https://www.lifescience.roche.com) (5).

Eleven (1.8% [95% CI 0.8%–2.9%]) of the 608 animals tested positive for WNV. A total of 519 (84.5%) specimens were from animals that died, of which 78 were found dead and classified as sudden unexpected death. WNV was detected in 6 (1.7% [95% CI 0.3%–3.0%]) of 361 wildlife and 5 (1.5% [95% CI 0%–3.3%]) of 196 nonequine domestic animals but in 0 of 51 birds (Table 1). We detected WNV RNA in 2 (2%) of 93 domestic cattle (Bos taurus), 1 (2%) of 54 African buffalo (Syncerus caffer), 1 (5%) of 22 domestic dogs (Canis lupus familiaris), 1 (33%) of 3 exotic fallow deer (Dama dama), 1 (9%) of 6 giraffes (Giraffa camelopardalis), 1 (9%) of 11 domestic goats (Capra aegagrus hircus), 1 (9%) of 1 domestic sheep (Ovis aries), and 2 (7%) of 28 roan antelope (Hippotragus equinus) (Table 1). Only 2 of 11 infected animals survived: 1 domestic bovid and the exotic fallow deer.

Virus isolation identified African horse sickness virus as a co-infection in the WNV-positive dog (ZRU358_17), confirmed by the Equine Research Centre (6) (Table 1). WNV neutralizing antibodies have previously been reported among dogs in South Africa, although no active infection has been described (7). The domestic bovid (ZRU181_12_1) and buffalo (ZRU161_18) had Middleburg virus co-infections, and the giraffe had Shuni virus co-infection confirmed by differential testing (8–10) at the Centre for Viral Zoonoses (Table 1). In these animals, clinical signs and death could not be attributed to any of the detected viruses alone.

Positive WNV infections were detected in the Free State (2/45, 4%), Gauteng (5/192, 3%), North West (1/47, 2%), Limpopo (2/132, 2%), and Mpumalanga provinces (1/82, 1%) (Figure 1). Most positive animals were reported during March–June, corresponding to the arbovirus season in South Africa (Appendix, https://wwwnc.cdc.gov/EID/article/25/12/19-0572-App1.pdf).

We detected WNV in lung (5/11, 45%), brain (4/11, 36%), and spleen (2/11, 18%) tissue and in blood (2/11,
18%) (Table 1). Clinical signs noted in WNV-positive animals included neurologic (4/8, 50%) and respiratory (3/8, 38%); 2 animals with neurologic signs also had pyrexia (Table 2). The lion (ZRU297_17) and giraffe (ZRU87_18) were found dead (2/11, 18%); thus, no clinical signs were reported. The WNV-positive sheep (ZRU159_18), an indigenous Dorper, was a stillborn fetus with cerebral edema. In sheep, WNV is reported to cause neurologic symptoms (11) but has not been associated with stillbirths. The roan antelope (ZRU61_16_2), the domestic bovid (ZRU181_12_1), and the sheep fetus represented WNV-positive specimens among a cluster of animals with similar signs potentially representing larger outbreaks in these areas. Despite extensive screening for arboviruses, the causative link between the clinical presentation of the various species and the evidence of WNV infection must be regarded with caution because we could not exclude all other possible infectious and noninfectious etiologies.

We subjected positive specimens to Sanger sequencing (Inqaba biotech, https://www.inqababiotec.co.za) and

![Figure 1](https://www.cdc.gov/eid)
Table 2. Clinical signs and outcomes in wildlife and nonequine domestic animals tested for WNV upon submission to Centre for Viral Zoonoses, South Africa, 2010–2018*

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. WNV positive/total no. animals (%)</th>
<th>No. WNV negative/total no. animals (%)</th>
<th>Odds ratio (95% CI)</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sign</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudden unexpected death</td>
<td>2/11 (18.2)</td>
<td>76/608 (12.5)</td>
<td>1.5 (0.3–7.2)</td>
<td>0.4</td>
</tr>
<tr>
<td>Stillborn</td>
<td>1/11 (9.1)</td>
<td>15/608 (2.5)</td>
<td>3.9 (0.5–32.3)</td>
<td>0.3</td>
</tr>
<tr>
<td>Abortion</td>
<td>0/11</td>
<td>24/608 (4.0)</td>
<td>Undefined</td>
<td>1</td>
</tr>
<tr>
<td>Congenital deformities</td>
<td>0/11</td>
<td>11/608 (1.8)</td>
<td>Undefined</td>
<td>1</td>
</tr>
<tr>
<td>Death</td>
<td>9/11 (81.8)</td>
<td>510/608 (84.4)</td>
<td>0.8 (0.2–3.6)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*WNV, West Nile virus.  
†p values <0.05 are significant.  
‡Sudden unexplained death indicates animals found dead without an obvious reason; stillborn, abortion, and congenital deformities are related to potential cross-placental transmission; death refers to sick animals that subsequently died.

Conclusions

We recorded WNV (lineages 1 and 2) in wildlife and nonequine domestic animals in South Africa. Seroconversion to WNV was demonstrated in asymptomatic white rhinoceroses from KNP. The data suggest severe disease and neurologic signs occur in species other than horses; these signs may be used for surveillance in areas of Africa where horses are less common to predict WNV outbreaks and predict spillover events into the human population. Wildlife and nonequine domestic animals are not as closely monitored as are horses, and early detection is less likely. The short viremia associated with WNV infection may result in underreporting of positive animals if only RT-PCR is used for diagnosis, but a lack of conjugates for wildlife species complicates development of IgM ELISA. The epitope-blocking ELISA and microtiter virus neutralization test can be used for seroprevalence studies in animals other than horses because they are species-independent but do not differentiate between IgM and IgG and are not quantitative.
Future work should focus on assay development for species other than horses.

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We thank all veterinarians and veterinary pathologists across South Africa who submitted samples, as well as Anna Jolles and Bryan Charleston for the buffalo serum samples.

This study was cleared by section 20 (12/11/1/1) approval through the Department of Agriculture Forestry and Fisheries, by the animal ethics committee (V057-15) (J.S.) and (H12/16) (M.V.) of the University of Pretoria and the PhD research committee. Buffalo samples were transported under a Red Cross permit (LDK2016/9/1) to the Biosafety Level 3 laboratory.

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About the Author

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West Nile Virus in Wildlife and Nonequine Domestic Animals, South Africa, 2010–2018

Appendix

Appendix Figure. Seasonality of wildlife, nonequine domestic animals, and avian specimens (N = 608) submitted and West Nile virus reverse transcription PCR–positives (n = 11), South Africa, 2012–2018. West Nile virus PCR-positive results are indicated in the graph. FSP, Free State province; GP, Gauteng province; LP, Limpopo province; MP, Mpumalanga province; NWP, North West province.