Transmission of West Nile Virus during Horse Autopsy

To the Editor: West Nile virus (WNV) circulates mainly in birds and ornithophilic mosquitoes. Humans and horses are considered incidental, dead-end hosts (1). Fever, rash, arthralgia, and myalgia develop in ~20% of cases in humans; severe neurologic disease may develop in <1% (1). In horses, 20% of infections result in clinical disease, of which ~90% involve neurologic disease with ataxia, weakness, recumbency, muscle fasciculation, and high death rates (30%) (2).

Genetic variants of WNV include lineage 1 found in the Northern Hemisphere and Australia; lineage 2 found mainly in southern Africa and Madagascar (3); lineages 3 and 4 found in central and eastern Europe (4); and lineage 5 found in India (5). Differences in neuroinvasiveness and pathogenic potential are functions of individual genotypes, not lineage (3, 6–8).

We recently reported WNV lineage 2 in several cases of neurologic disease in horses in South Africa (most cases were fatal) (7). We report a case of zoonotic transmission to a veterinary student during the autopsy of a horse. The study was reviewed and approved by the Ethics Committee of the University of Pretoria, and informed consent was provided by the veterinary student.

On April 9, 2008, a 4-month-old Welsh pony from Gauteng in South Africa had fever, Schiﬀ-Sherrington signs, and a leukocyte count of 32 × 10^9 cells/L. He was treated with dimethyl sulfoxide, dexamethasone, and chloramphenicol and responded well. He was able to stand with help, and did not show neurologic signs at this stage. On May 9, he was sent home and was able to walk with support. On May 12, he had a relapse with neurologic deterioration and rectal prolapse, and was treated with antiinﬂammatory agents. Symptoms worsened and he was humanely killed on May 15 using ketamine and MgSO_4. The carcass was sent to the Faculty of Veterinary Sciences, University of Pretoria, for autopsy because of unusual neurologic signs in the pony. Autopsy was performed by a veterinary pathologist and 2 students on May 16, 2008.

Macroscopic findings included moderate intermuscular, fascicular, perineural edema, severe diffuse pulmonary edema, mild hydropericardium, and rectal prolapse resulting in marked submucosal edema and mucosal hyperaemia, i.e., traumatic proctitis. The spinal cord up to C1 showed marked Wallerian degeneration of the peripheral white matter from the median fisure, which extended along the ventral funiculus up to the most dorsal sector of the lateral funiculus. Changes were characterized by white matter spongiosis with numerous digestion chambers containing phagocytosing myelinophages and scattered interstitial gemistocytes. No inﬂammatory reaction was detected. We also observed septal edema and moderate multifocal perivascular and peribronchial lymphocytic inﬁltration with occasional apoptosis in the lungs.

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LETTERS
The brain, which was removed by 1 of the students, was sent to the Department of Medical Virology, University of Pretoria for WNV reverse transcription-PCR (RT-PCR). The lungs were sent to Onderstepoort Veterinary Institute for African horse sickness RT-PCR. WNV-specific real-time RT-PCR showed positive results. DNA sequencing and phylogenetic analysis identified WNV lineage 2 in several sections of the brain (HS23/2008, GenBank accession no. FJ464376). Results of African horse sickness RT-PCR on lung tissue specimens were inconclusive and could not be confirmed by culture.

On May 22, six days after the autopsy on the horse, fever, malaise, myalgia, stiff neck, and severe headache developed in the veterinary student who had handled the horse brain. A rash appeared 2 days later. Symptoms persisted for \( \approx 10 \) days. The patient was treated symptomatically by an infectious disease specialist and prescribed bed rest. Because cases of Rift Valley fever were recently reported in veterinarians in South Africa, serum was sent to the National Institute for Communicable Diseases, where a virus isolate was obtained in suckling mice and identified as WNV by RT-PCR.

After diagnosis of WNV infection in the pony, RNA extracted from the original human serum and from the suckling mouse isolate was sent to the Department of Medical Virology, University of Pretoria, for DNA sequencing and phylogenetic analysis of the virus. Comparison of part of the nonstructural protein 5 gene identified identical sequences from the student’s serum, the virus isolate, and the pony’s brain. All sequences clustered with lineage 2 WNV and were closely related to isolates obtained from horses diagnosed with fatal WNV encephalitis in South Africa in 2008 (7) (Figure).

Human infections with WNV have been described after bird autopsies and needle stick injury in laboratory workers (9,10). The case acquired by our patient suggests a zoonotic risk exists for infection with WNV during autopsy of horses that died from neurologic disease. Although humans and horses are considered to have low-grade viremia, virus levels may be higher in nerve tissue.

The patient wore latex gloves, his only protection during the autopsy, and had removed the spinal cord and brain. No protective inhalation or eye equipment was worn. No autopsy assistants or other students who worked with or were near the carcass became sick or seroconverted. The most likely route of infection may have involved exposure of mucous membranes to droplets. After the incident, biosafety measures were improved and included wearing of masks and eye protection gear during autopsies at the facility.

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Breeding Sites of Bluetongue Virus Vectors, Belgium

To the Editor: Bluetongue (BT) is an emerging disease of ruminants in northern Europe (1,2). This disease was reported in August 2006 in the Netherlands and a few days later in Belgium. In 2006, animals in the Netherlands, Belgium, and Germany were affected. In contrast to 2006, when BT virus (BTV) was identified in ≈2,000 enclosures on farms, BTV was identified in >40,000 farm buildings containing ruminants in 2007; many infected animals had severe disease. In addition, the virus expanded its range to include France, Denmark, the United Kingdom, Switzerland, and the Czech Republic (2).

In 2008, BTV serotype 8 (BTV-8) continued its spread across Europe and showed virulence in France where 26,925 BTV-8 outbreaks were reported (3). This observation indicates possible overwintering of the vector from year to year. However, the mechanism of overwintering is not clear. The biting midges responsible for transmission of BTV in northern Europe belong to the genus Culicoides, but only few species are vectors of this virus (2).

During the winter of 2006–2007, Losson et al. (1) monitored the presence of biting midges inside farm buildings. Zimmer et al. (4) observed potential vectors of BTV inside a sheepfold during the winter of 2007–2008 and in farm buildings in 2008. These authors suggested that Culicoides spp. may be more abundant indoors than outdoors when animals are kept in these buildings. Breeding sites of bluetongue vector species have been found near farms (silage residues) (5) and in neighboring meadows (overwintering cattle dung and silt along a pond) (5,6) but not inside sheds.

We conducted a study on 5 cattle farms in Belgium during February–October 2008. Three samplings were performed: the first in late February, the second in mid-June, and the third in late October. Soil samples (15 biotopes) were collected inside cowsheds. These samples were incubated at 24°C to enable adult midges to emerge. All Culicoides specimens were identified by sex and to the species level by using the morphologic key of Delécolle (7).

Among 15 soil biotopes obtained from farm buildings, only 1 showed the emergence of adult Culicoides biting midges. At a cattle farm in Spy (50°28′31″N, 4°40′39″E), we found that dried dung adhering to walls inside animal enclosures and used animal litter was a breeding site for the C. obsoletus/scoticus complex (Table). Only 25% of emerging Culicoides midges were females.

We observed that C. obsoletus/scoticus complex midges are more prevalent in soil samples with a high carbon:nitrogen (C:N) index; this index indicates the amount of organic matter in soil. C:N indices between 15 and 30 support production of humus and ensure good microbial growth. In addition, larvae of Culicoides spp. feed on organic material and microorganisms in soil (8).

Our observations suggest that biting midges can complete their life cycle in animal enclosures. This finding is consistent with the high capture

References


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LETTERS