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West Nile Virus Activity in a Winter Roost of American Crows (*Corvus brachyrhynchos*): Is Bird-To-Bird Transmission Important in Persistence and Amplification?*

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ABSTRACT: Since its emergence in North America, West Nile virus (WNV) has had a large impact on equines, humans, and wild bird communities, yet gaps remain in our understanding of how the virus persists at temperate latitudes when winter temperatures preclude virus replication and host-seeking activity by mosquito vectors. Bird-to-bird transmission at large communal American crow roosts could provide one mechanism for WNV persistence. Herein, we describe seasonal patterns of crow and *Culex* mosquito abundance, WNV infection rates, and the prevalence of WNV-positive fecal samples at a winter crow roost to test the hypothesis that bird-to-bird transmission allows WNV to persist at winter crow roosts. Samples were collected from large winter crow roosts in the Sacramento Valley of California from January 2013 until August 2014, encompassing two overwintering roost periods. West Nile virus RNA was detected in local crow carcasses in both summer [13/18 (72% WNV positive)] and winter [18/44 (41% WNV positive)] 2013-2014. Winter infections were unlikely to have arisen by recent bites from infected mosquitoes because *Culex* host-seeking activity was very low in winter and all *Culex* mosquitoes collected during winter months tested negative for WNV. Opportunities existed for fecal-oral transfer at the overwintering roost: most carcasses that tested positive for WNV had detectable viral RNA in both kidney and cloacal swabs, suggesting that infected crows were shedding virus in their feces, and >50% of crows at the roost were stained with feces by mid-winter. Moreover, 2.3% of fecal samples collected in late summer, when mosquitoes were active, tested positive for WNV RNA. Nevertheless, none of the 1,119 feces collected from three roosts over two winters contained detectable WNV RNA. This study provided evidence of WNV infection in overwintering American crows without mosquito vector activity, but did not elucidate a mechanism of WNV transmission during winter.

KEY WORDS: American crow, bird-to-bird transmission, *Corvus brachyrhynchos*, *Culex*, disease, public health, West Nile virus

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