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Source: Journal of Wildlife Diseases, 60(3) : 745-752

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/JWD-D-23-00158>

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Genetic Tracking of a Rabid Coyote (*Canis latrans*) Detected beyond a Rabies enzootic Area in West Virginia, USA

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ABSTRACT: Wildlife translocation and cross-species transmission can impede control and elimination of emerging zoonotic diseases. Tracking the geographic origin of both host and virus (i.e., translocation versus local infection) may help determine the most effective response when high-risk cases of emerging pathogens are identified in wildlife. In May 2022, a coyote (*Canis latrans*) infected with the raccoon (*Procyon lotor*) rabies virus variant (RRV) was collected in Lewis County, West Virginia, USA, an area free from RRV. We applied host population genomics and RRV phylogenetic analyses to determine the most likely geographic origin of the rabid coyote. Coyote genomic analyses included animals from multiple eastern states bordering West Virginia, with the probable origin of the rabid coyote being the county of collection. The RRV phylogenetic analyses included cases detected from West Virginia and neighboring states, with most similar RRV sequences collected in a county 80 km to the northeast, within the oral rabies vaccination zone. The combined results suggest that the coyote was infected in an RRV management area and carried the RRV to Lewis County, a pattern consistent with coyote local movement ecology. Distant cross-species transmission and subsequent host movement presents a low risk for onward transmission in raccoon populations. This information helped with emergency response decision-making, thereby saving time and resources.

Key words: Coyote, cross-species transmission, phylogenetics, raccoon rabies virus variant, single-nucleotide polymorphisms, translocation.

The emergence and reemergence of zoonotic pathogens can include spread to new reservoir hosts or geographic areas, which may lead to outbreaks in areas previously free of disease,

with potential negative health consequences for humans, domestic animals, and wildlife. Detection of an emerging pathogen in a new geographic location may indicate that local transmission is already established, making it more challenging to contain. When the detection is in a nonreservoir species, there are two possibilities for management to consider: 1) the case has been translocated from an endemic area and was an isolated cross-species transmission (i.e., spillover event) with a low probability of further transmission; and 2) the case is evidence of a local transmission cycle. These two scenarios lead to different management responses. Rapid host and virus genomic characterizations may differentiate between these scenarios and inform management and public health response, thereby saving time, money, and lives.

Determination of the probable geographic origin and location of host infection may help to estimate the risk of onward transmission when an infected animal is found in an area previously known to be free of a specific pathogen. Specifically, this knowledge can inform decisions about the scale of early intervention and management response (Bird and Mazet 2018; Martel et al. 2020). Genomic tools are highly effective at tracking both pathogen and wildlife host movement across landscapes (Barton et al. 2010; Biek and Real 2010; Szanto et al. 2011; Bruncker et al. 2020; Gigante et al. 2020). With sufficient sampling, genomic data can be used to estimate the geographic origin

of both the host and the infecting virus, with the potential to distinguish a translocated rabid animal versus local host movement and pathogen spread.

The raccoon rabies virus variant (RRV; family Rhabdoviridae, genus *Lyssavirus*) was first reported in raccoons (*Procyon lotor*) in Florida, US, during the late 1940s; by the late 1970s, the virus had spread to nearby states of the southeastern US (McLean 1971). An unintentional human-mediated translocation of rabid raccoons to the border shared between Virginia and West Virginia, US, resulted in one of the largest epizootics of wildlife rabies in the US (Rupprecht and Smith 1994; Rupprecht et al. 1995; Szanto et al. 2011). The RRV is now the most commonly detected rabies virus in raccoons and other wild carnivores of the eastern US and is one of the predominant exposure risks for domestic animals and humans (Ma et al. 2023). The US Department of Agriculture, Wildlife Services, National Rabies Management Program (NRMP) provides federal leadership and multiagency coordination to prevent the spread of and eventually eliminate RRV through oral rabies vaccination (ORV) combined with enhanced rabies surveillance (ERS; active, targeted surveillance that complements passive public health surveillance; Slate et al. 2009; Elmore et al. 2017).

Long-distance host movements, whether naturally dispersing or human-mediated translocation events, and virus spillover into new reservoir species may threaten rabies control efforts and result in negative health and economic consequences (Rosatte and MacInnes 1989; Russell et al. 2005; Chipman et al. 2008; Slate et al. 2009; Singh et al. 2018; Grome et al. 2022). For example, repeated translocations of RRV into southeastern Canada have diverted resources from RRV elimination programs to containment of the new epizootics; this hinders management program elimination goals (Trewby et al. 2017; Lobo et al. 2018; Nadin-Davis et al. 2020).

In March 2022, a rabid juvenile male coyote (*Canis latrans*) was collected in Lewis County, West Virginia, a region free of RRV (Fig. 1). The coyote, collected through public health surveillance, was diagnosed with RV

infection by using the direct fluorescent antibody test (Genevie et al. 2003). The RV was determined to be RRV by antigenic typing at the Centers for Disease Control and Prevention, Atlanta, Georgia, US; this was later confirmed by sequencing (see Supplementary Material). When RRV is found outside the enzootic area, a contingency action is initiated by the NRMP, including ERS and more intensive ORV management. This approach is costly, so an accurate estimation of risk of onward RRV spread can help tailor contingency action response planning for effective control of RRV, while minimizing the resources necessary to reestablish control. We combined host and virus population genetic and phylogenetic methods to assess probable origins of the West Virginia coyote and the infecting RRV to help with the emergency response.

Population genomic analyses were used to determine the origin of the rabid West Virginia coyote. Ear tissue samples from the rabid coyote (ID E22R007878-01) and 54 additional coyotes were combined with coyote genotypes from Heppenheimer et al. (2018). We obtained genotype data from 107,888 single-nucleotide polymorphisms (SNPs) determined to be statistically neutral and unlinked across the genomes of 318 coyotes. We then conducted two unsupervised cluster analyses at different sampling resolutions to provide multiple geographic perspectives, and a supervised population assignment to estimate the probable ancestry of the rabid coyote (for details of the analyses, see Supplementary Material).

Initial clustering revealed that E22R007878-01 had high assignment probability (>88%) to the genetic cluster containing coyotes from Kentucky and West Virginia (Fig. 2; Table 1 and Supplementary Material Table S1). Given that population structure is often hierarchical, we repeated the maximum likelihood cluster analysis at a finer geographic scale to determine whether we could locate a more precise point of origin by using only coyotes from Kentucky and West Virginia and sample E22R007878-01 for $K=2-7$ genetic partitions. The most likely number of partitions was $K=2$ and $K=3$ and the rabid coyote assigned to West Virginia (Supplementary Material Fig. S1A).

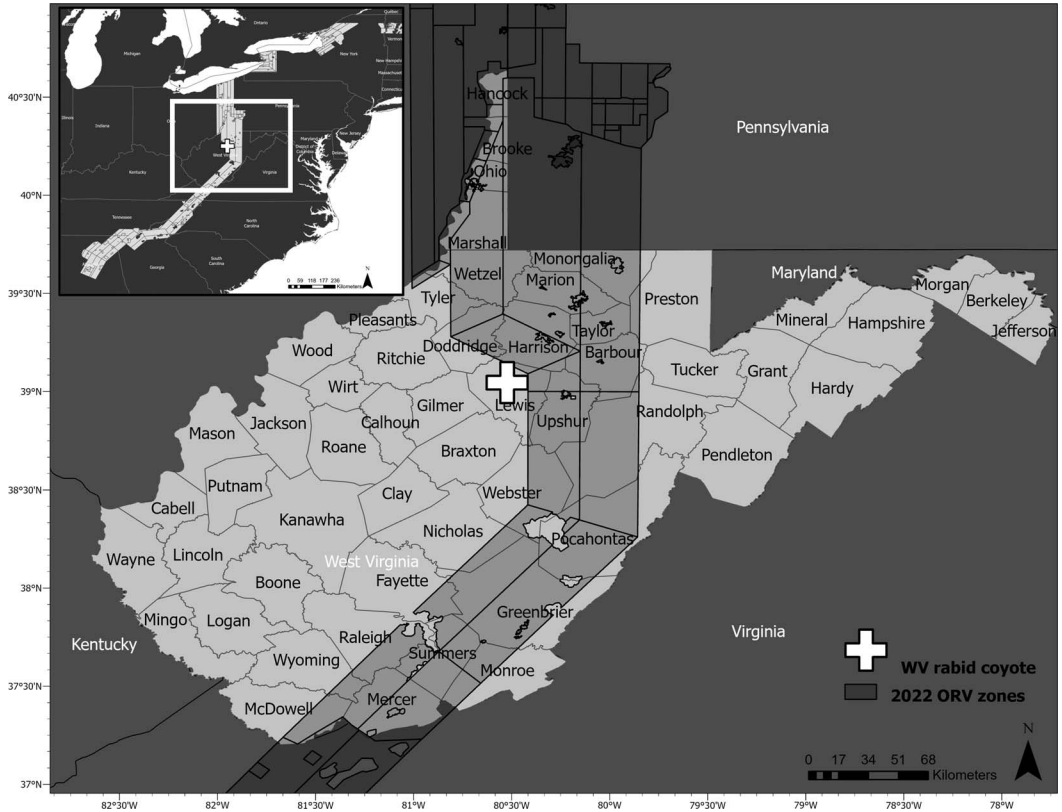


FIGURE 1. Map of West Virginia in the eastern US. The light gray areas are the oral rabies vaccination zones, and the plus sign is the collection location of the rabid coyote (*Canis latrans*; sample E22R007878-01). The inset is to provide perspective on the animal's distance from the oral rabies vaccination zone. East of the vaccination zone is the raccoon rabies virus variant enzootic area.

Although we identified 18,145 alleles private to Kentucky coyotes and 20,019 to West Virginia coyotes, sample E22R007878-01 did not carry any of these private alleles. We then analyzed

sample E22R007878-01 with coyotes sampled from West Virginia, considering the hypothesis that the sample would have relatively comparable assignment proportions across all sampled

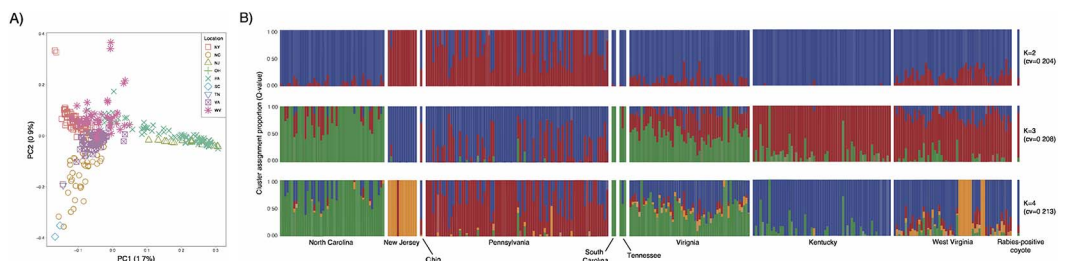


FIGURE 2. Population cluster analysis of 107,888 single-nucleotide polymorphism (SNP) genotypes from eastern US coyotes (*Canis latrans*) by using (A) a principal component analysis (PCA) and (B) the unsupervised maximum likelihood clustering algorithm ADMIXTURE (Alexander et al. 2009) for 2–4 genetic partitions (K). The cross-validation (cv) value per K is provided. The farthest right sample (single bar) is the rabies-positive coyote (sample E22R007878-01). As K increases, West Virginia and Kentucky, USA, form a distinct cluster and the rabid Lewis County, West Virginia, coyote groups with these states.

TABLE 1. ADMIXTURE (Alexander et al. 2009) supervised assignment proportions based on single-nucleotide polymorphisms from a rabid coyote (*Canis latrans*; sample E22R007878-01) collected in West Virginia, USA, to genetic clusters ($K=2-4$) and associated US states in parentheses.

K and US states included in each training cluster	Assignment proportion E22R007878-01
K=2	
Cluster 1 (Kentucky, North Carolina, South Carolina, Tennessee, Virginia, West Virginia)	0.897
Cluster 2 (New Jersey, Ohio, Pennsylvania)	0.103
K=3	
Cluster 1 (Kentucky, West Virginia)	0.900
Cluster 2 (North Carolina, South Carolina, Tennessee, Virginia)	0.000
Cluster 3 (New Jersey, Ohio, Pennsylvania)	0.100
K=4	
Cluster 1 (Kentucky, West Virginia)	0.885
Cluster 2 (North Carolina, South Carolina, Tennessee, Virginia)	0.000
Cluster 3 (New Jersey)	0.000
Cluster 4 (Ohio, Pennsylvania)	0.115

localities if it did not originate from West Virginia. We found that coyotes sampled in Lewis County clustered with the target sample E22R007878-01 at every genetic partition between $K=3$ and 10 (Table 2), suggesting that the target sample likely originated in Lewis County.

To identify which sampled coyotes were most closely related to the rabid coyote, we filtered the SNP dataset more stringently to infer interindividual relatedness, producing 1,630 SNPs for 116 coyotes from Kentucky and West Virginia, which included sample E22R007878-01. We obtained 6,670 pairwise relatedness estimates with a mean $r=0.01$ ($SD\pm 0.035$). Only eight pairs had $r>0.5$, with none spanning the boundaries of West Virginia and Kentucky. A focused analysis of sample E22R007878-01 with 53 comparisons with other West Virginia coyotes and 61 comparisons with Kentucky coyotes revealed that mean relatedness to both states was comparable (West Virginia: mean= 0.009 ± 0.01 , range= $0-0.62$; Kentucky: mean= 0.008 ± 0.01 , range= $0-0.70$; Welch two-sample t -test: $t=0.03$, $df=111.42$, $P=0.978$). Six relatedness values fell within the top 95th percentile of the pairwise relatedness distribution: two were with Kentucky coyotes ($r=0.070$ in Metcalfe County, 395 miles [636 km] from Lewis County; $r=0.042$ in Pike County, 214

miles [344 km] away), and four were with West Virginia coyotes ($r=0.062$ in Wirt County, 64 miles [103 km] away; $r=0.049$ in Marshall County, 124 miles [200 km] away; $r=0.045$ in Wetzel County, 69 miles [111 km] away; and $r=0.041$ in Wirt County, 64 miles [103 km] away). These relatedness levels are probably indicative of proximal relationships (i.e., more than two generations apart).

To determine the possible origin of the RRV, we performed a comparative analysis of RRV sequences from the rabid coyote brain to RRV sequences from West Virginia and neighboring US states: West Virginia [65], Pennsylvania [20], Virginia [14], and Ohio [1] (see Supplementary Material Table S2). Rabies virus from E22R007878-01 had the highest percent nucleotide identity to three RV sequences from Monongalia County, West Virginia, with identical glycoprotein gene sequences and two synonymous nucleotide changes in the nucleoprotein gene (Fig. 3; Supplementary Material Tables S3 and S4). Two additional sequences from Monongalia County had two synonymous nucleoprotein gene changes and either one non-synonymous or one synonymous glycoprotein gene change.

Phylogenetic analysis of RRV sequences revealed support for three clusters of RRV in West Virginia based on the available sequences

TABLE 2. Coyote (*Canis latrans*) genetic clusters identified by ADMIXTURE (Alexander et al. 2009) in West Virginia, USA. X represents the West Virginia counties that clustered with the rabid coyote (sample E22R007878-01) at $Q > 0.90$ for genetic cluster (K)=3–10. In bold is Lewis County, the only county that clustered with the rabid coyote at all values of K .

County	K3	K4	K5	K6	K7	K8	K9	K10
Barbour					X			
Braxton	X				X			
Doddridge	X	X			X	X		X
Gilmer	X		X	X		X		
Grant	X	X		X			X	
Greenbrier	X							
Hardy				X				
Harrison	X	X		X				
Lewis	X	X	X	X	X	X	X	X
Monongalia	X						X	
Marion	X		X		X			
Marshall		X						
Mason		X	X					
Mercer	X							
Mineral		X						
Nicholas		X	X	X	X			
Pendleton	X	X	X					X
Pleasants	X			X			X	
Preston			X			X		
Pocahontas	X	X						
Roane	X					X		X
Randolph		X	X					
Upshur					X	X		
Wetzel	X	X					X	
Wirt		X						
Wood								X

(Fig. 4; see Supplementary Material Fig. S2 for maximum likelihood tree). Grant and Monroe counties contained samples that came from multiple clades. The RRV from E22R007878-01 belonged to a large clade of RRV sequences from across Pennsylvania, eastern Ohio, northern West Virginia, southwestern West Virginia, and western Virginia (Fig. 4). Within this large clade, E22R007878-01 belonged to a subclade with high support that included six sequences from Monongalia County.

These host and virus genomic analyses provided high confidence that the rabid coyote originated in Lewis County, the same county where it was collected, and that the origin of RRV infection was most likely from northern West Virginia, possibly in or near Monongalia

County, which is an active RRV management area. However, samples were not available from all counties; thus, origin in a nearby county cannot be discounted. It is very unlikely that the coyote was infected with RRV in Kentucky or western West Virginia, because RRV has never been detected in these regions.

The complete explanation of how and where this animal was infected will never be known; however, based on the combined genomic data, we can confidently say it was not a long-distance translocation of RRV (e.g., from Maine or Florida, US) or a migrant animal. Although the rabid coyote showed the strongest genetic association with the county where it was collected, RRV is not enzootic in Lewis County, and the most similar virus was from northern

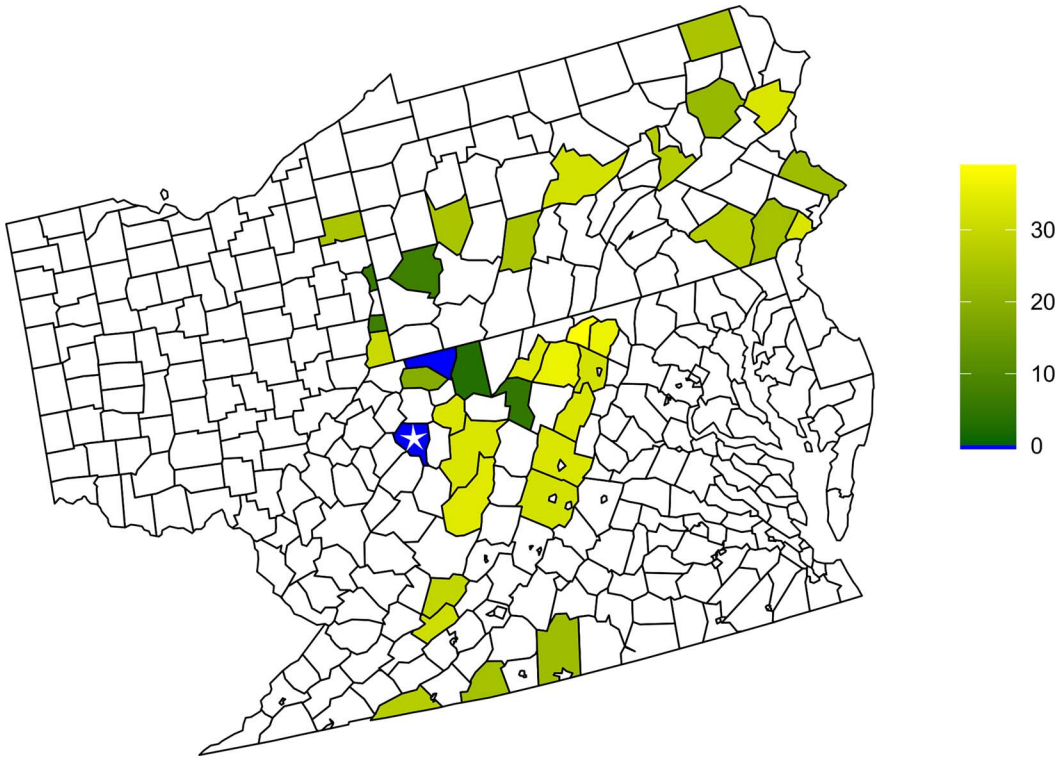


FIGURE 3. Rabies virus sequence similarity to Lewis County, West Virginia, USA, coyote (*Canis latrans*) rabies virus (raccoon, *Procyon lotor*, variant) glycoprotein gene. Color indicates number of nucleotide changes (blue = identical, dark green = one nucleotide change, yellow = most diverged, white = no data). Lewis County is highlighted with a white star, whereas the other blue county is Monongalia County, West Virginia. Nucleotide differences are shown for the most similar sequence from that county when more than one sequence was available (minimum distance).

West Virginia, approximately 80 km away. Studies have suggested that coyotes in the Appalachian plateau, particularly transient individuals, can range $>100 \text{ km}^2$, occasionally $>500 \text{ km}^2$ (Crawford 1992; Mastro et al. 2019). Given that this individual was a juvenile male, there is a possibility that it did not have an established territory and may have been exhibiting natural dispersal or exploratory movements into areas of northern West Virginia where it was infected with RRV before returning to Lewis County. The incubation time of RV can vary from a few weeks to a few months, depending on host species, infectious dose, and variant (Müller and Freuling 2020); thus, it is possible that the coyote moved across the landscape before displaying clinical signs of rabies virus infection.

Based upon the combined host and viral molecular data from this study, program

managers did not implement a full emergency response, which would have included multiple years of intensive ORV management and ERS. Determining that the rabid coyote was a single case of cross-species transmission within a RRV enzootic area, with a low chance of onward local transmission in raccoons, saved both time and resources. Since the detection of the rabid coyote, no additional RRV-infected animals have been found in Lewis County or surrounding counties, despite ERS efforts in the region, thereby supporting the hypothesis of a wandering juvenile coyote.

By combining host and pathogen genomic data, we were able to infer more information about the origin of infection and risk of RRV establishment in a new area than if we had performed only host or viral analysis alone. This approach has potential applications to

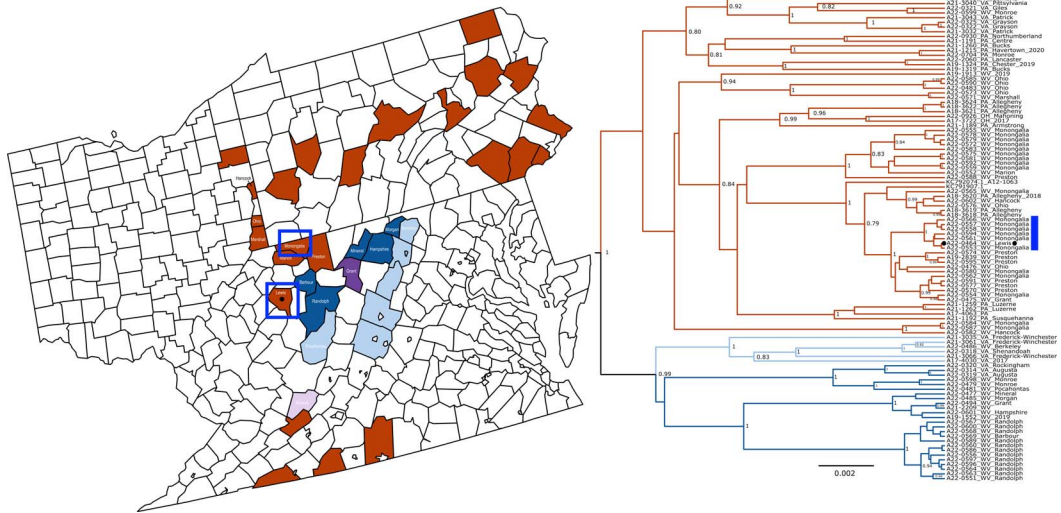


FIGURE 4. Phylogenetic analysis of raccoon (*Procyon lotor*) rabies virus variant glycoprotein gene sequences from West Virginia, Virginia, Pennsylvania, and Ohio, USA. The geographic distributions of major clade are indicated by colored branches on the tree that match colors on the map to the left. Two counties had multiple major clade: Monroe County, West Virginia (pink = light blue + red) and Grant County, West Virginia (purple = dark blue + red). Lewis County is highlighted by the black dot on the phylogeny and map with the blue boxes (map) and bar (phylogeny) representing the clade to which the Lewis County coyote sequence (sample E22R007878-01) belongs. Phylogenetic analysis was performed using BEAST v1.10.4 (Suchard et al. 2018). Posterior support >0.7 is indicated at the branch points. West Virginia counties are labeled on the map; sequences without county information are not included on the map. South central skunk rabies virus variant sequences JQ685938.1 and JQ685968.1 were used to root the tree.

zoonotic pathogens beyond rabies. Nevertheless, the success of our investigation was dependent on the early detection of RRV in a RRV-free county. In this case, a strong, coordinated rabies surveillance system was able to quickly identify this case through rapid, routine diagnostic testing, variant typing, and reporting. The resolution of geographic inference is also dependent on sampling breadth for both host species and pathogen genomic analyses, and data must be available rapidly to inform management decisions in a meaningful way. Establishment of geographically curated and genetically diverse genomic databases for both hosts and pathogens, collected through interdisciplinary efforts among geneticists, disease ecologists, and wildlife managers provides a backdrop for linking epizootiology and management of wildlife diseases in real-time during a high-consequence event.

We thank the following individuals without whom this work would not have been possible: C. Mankowski, A. Barbee, A. Piaggio, Immunology/

Virology Group at the Virginia Department of General Services, Division of Consolidated Laboratories, and West Virginia Department of Health Rabies Laboratory. We are thankful for US Department of Agriculture (USDA) Wildlife Services and Centers for Disease Control and Prevention (CDC) staff for collection and rapid confirmatory testing and typing during the initial investigation of this case. Comments from the associate editor and two anonymous reviewers greatly improved this manuscript. This work was supported in part by the USDA, Animal and Plant Health Inspection Service and the CDC. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA, CDC, or US Government determination or policy.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/JWD-D-23-00158>.

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Submitted for publication 29 September 2023.

Accepted 13 March 2024.